Available online on 15.05.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Antiretroviral drug resistance in HIV-1 patients on first-line therapy or untreated across five treatment centers in Yaounde's Centre Region, Cameroon

Bello Djoda [1,5,6], Patrick Valere Tsouh Fokou [2,*], Patrick Achiangia Njukeng [1], Jerome Fru-Cho [1,*], Rodrigue Kamga Wouambo [8], Rosine Yemetio Fouotsa [8], Martin Daumer [6], Pierre Kamtsap [1], Alain Nantchouang [4], Charles Kouanfack [8], Klaus Korn [3], Josef Eberle [3], Oliver Bosnjak [5]

[1] Department of Microbiology and Parasitology, Faculty of Science, University of Buea, Buea, South West Region, Cameroon

[2] Department of Biochemistry, Faculty of Science, University of Bamenda, Bambili, North West Region, Cameroon

[3] National Reference Centre for Retroviruses, Max von Pettenkofer-Institute, Ludwig-Maximilians-Universität München, München, Germany

[4] National Reference Centre for Retroviruses, Universitat Erlangen-Nurnberg, Erlangen-Nurnberg, Germany

[5] BIOMEX GmbH, Heidelberg, Germany

[6] Institute of Immunology and Genetics Kaserslautern, Medical Laboratory, Kaiserslautern, Germany

[7] Yaounde Central Hospital, Ministry of Public Health, Yaounde, Cameroon

[8] Institute des Sciences Appliquées à la Santé de l'IUEs/INSAM de Yaoundé, Cameroun

Article Info:

Article History:

Received 25 Feb 2025
Reviewed 07 April 2025
Accepted 04 May 2025
Published 15 May 2025

Cite this article as:

Djoda B, Tsouh Fokou PV, Njukeng PA, Fru-Cho J, Kamga Wouambo R, Yemetio Fouotsa R, Daumer M, Kantsap P, Nantchouang A, Kouanfack C, Korn K, Eberle J, Bosnjak O, Antiretroviral drug resistance in HIV-1 patients on first-line therapy or untreated across five treatment centers in Yaounde's Centre Region, Cameroon, Journal of Drug Delivery and Therapeutics. 2025; 15(5):86-99
DOI:
http://dx.doi.org/10.22270/jddt.v15i5.7126

*Address for Correspondence:
Patrick Valere Tsouh Fokou, Department of Biochemistry, Faculty of Science, University of Bamenda, PO Box 39, Bambili, Bamenda, Cameroon,

Jerome Fru-Cho, Department of Microbiology and Parasitology, Faculty of Science, University of Buea, Buea, Cameroon

Abstract

Antiretroviral therapy (ART) improves HIV survival. However, as ART programs expand in resource-limited settings like Cameroon toward UNAIDS 95-95-95 goals, monitoring drug resistance is critical. This study investigated HIV-1 subtypes and resistance mutations in Yaoundé, Cameroon. From 2017 to 2021, 231 HIV-positive individuals (treatment-naïve and ART-experienced patients with virological failure [viral load >1,000 copies/mL]) were enrolled across five clinics. Plasma samples were sequenced for reverse transcriptase (RT) and protease (PR) genes. Participants were predominantly female (67.5%), aged 21–35 years. Over half (58.5%) received ART (median duration: 6 months). High median viral load (536,263 copies/mL) indicated poor suppression. CRF02_AG dominated (64.5%), followed by A1 (11.7%) and G (6.9%). Resistance mutations were detected in 18.2% of ART-experienced and 13.4% of treatment-naïve participants, indicating acquired and transmitted resistance. NNRTI resistance occurred in 6.1% (ART-experienced) and 1.3% (naïve); NRTI mutations were rare (0.4%). Key mutations included M184V (NRTI) and K103N (NNRTI). Protease inhibitor (PI) resistance was prevalent (19.5%), with I54V most common. Notably, PI resistance was detected in treatment-naïve individuals. CRF02_AG dominance and high resistance rates underscore challenges to ART efficacy. Significant PI resistance in untreated patients suggests transmission of resistant strains. These findings highlight urgent needs for enhanced resistance surveillance and optimized ART strategies in Cameroon.

Keywords: First-line antiretroviral therapy, HIV-1 drug resistance, viral subtype, transmitted drug resistance, Centre, Transversal study

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a global health problem affecting approximately 38 million people worldwide [1]. Since the introduction of antiretroviral therapy (ART), the goal is to suppress HIV replication below the detection limit, there is growing evidence that treatment programs in resource-limited settings can obtain results comparable to those of developed countries [2]. Antiretroviral therapy ultimately offers the greatest potential for immune reconstitution.

Despite the increasing availability of antiretroviral (ARV) drugs, genetic diversity has posed a major challenge to the overall management of HIV infection. The use of highly active antiretroviral therapy (HAART) has been shown to be very effective, but treatment failure remains a common phenomenon among patients. In addition to adherence issues [3], the emergence of drug-resistant variants has been identified as a major barrier to the effectiveness of antiretroviral therapy (ART) and as one of the main causes of treatment failure [4].

The emergence of drug resistance variants of HIV-1 has been attributed to mutations within the HIV-1 pol genes that encode the molecular targets of major antiretroviral drugs [5]. A number of factors are thought to contribute to the acquisition of drug resistance in Africa, including lack of plasma viral load monitoring [6], drug interactions [7], treatment interruptions due to drug stock-outs [8] and the use of substandard antiretroviral regimens [9]. Available data show that the efficacy of ARV therapy is also influenced by viral subtype and pre-existing mutations [10, 11]. Additionally, it has been postulated that drug resistance pathways may be affected by pre-existing polymorphisms among different HIV-1 subtypes [12].

Following the revision of treatment guidelines by the WHO, first in 2010 [13], 2013 [14] and most recently in 2016 [15], it is now recommended that infected people start antiretroviral treatment, regardless of or the WHO clinical stage and the number of CD4 cells, contrary to what was the case before 2010 (≤200 cells/mm3). This has dramatically increased the number of patients starting first-line ART. There are concerns that resistance to antiretroviral drugs among people on treatment could spread to those newly infected and compromise current regimens, which would lead to early treatment failure in people on ART. Adequate knowledge of drug resistance mutations and polymorphisms in the reverse transcriptase and protease gene of circulating strains is therefore necessary to help optimize the selection of treatment regimens and limit the acquisition of cross-resistance. This study was conducted with the aim of establishing the prevalence of drug resistance mutations in HIV-1 infected patients seeking care and treatment.

MATERIALS AND METHODS

Study design and population

This study was conducted between 2017 and 2021 at five routine HIV care and treatment clinics (CTC) (Obala district hospital, Djoungolo district hospital, Efoulan district hospital, Cité verte district hospital and Yaoundé central hospital) located in the Centre Region of Yaoundé, Cameroon. We employed a non-probability convenience sampling method whereby patients with suspected virological failure, who were attending these clinics, were enrolled based on their accessibility throughout the study period.

Participant enrolment and sample collection

After providing informed consent (or assent where applicable, depending on the participant's age of 21 years), participants were assessed against the following inclusion criteria: 1) confirmed positive HIV status; 2) suspected virological treatment failure, defined as having a viral load >1000 copies/mL at any time during their treatment; and 3) acceptance to participate in the study by providing informed consent or assent.

From 231 enrolled participants, baseline plasma samples were collected. For each participant, whole blood was collected in two EDTA tubes of 4 ml each by venipuncture, and plasma was separated by centrifugation at 2000 g for 10 minutes. Plasma aliquots of 1 ml were prepared and stored at -80°C until further analysis. This cohort included both treatment-naïve and previously treated patients experiencing suspected virological failure.

Sample size

A total of 231 baseline plasma samples were collected and analysed from participants meeting the inclusion criteria. This sample size was determined by the number of eligible patients with suspected virological failure who were conveniently accessible and consented to participate during the study period between 2017 and 2021.

Viral load measurement

Viral load of the 231 clinical specimens was determined in the diagnostic labs of the Max von Pettenkofer Institute Virology department according to their accredited routine diagnostic procedures using the Abbott m2000sp/rt system and the Abbott RealTime HIV-1 assay (art. no. 02G31-010). Accordingly, two parts (ca. 1053 and 785 nucleotides long) of the pol gene of 231 samples with a sufficient viral genome copy number were amplified via nested PCR and analyzed by Sanger sequencing.

Treatment failure (TE) was defined as PVC ≥ 1000 HIV-1 RNA copies/ml; plasma samples from study participants who underwent VF were designated for HIV-1 sequencing for DRM detection and for viral subtyping.

Drug Resistance Testing

RNA extraction and amplification: Viral RNA is extracted from 140 µl of plasma or serum using the QIAamp Viral RNA Mini Kit (AM-MO-136). For viral loads below 2000 copies/ml, up to 2 ml of material is centrifuged at 25,000 × g for 60 minutes at 4°C. The supernatant is discarded except for 140 µl, and the pellet is resuspended in the remaining plasma. RNA extraction then follows the manufacturer's protocol. Reverse transcription is performed using the SuperScript III One-Step RT-PCR System with Platinum Taq Polymerase.

Sequencing of the samples: The ANRS AC11 protocol was used for the amplification and sequencing of the protease (PR) and reverse transcriptase (RT) regions of HIV-1, using the GeneAmp PCR System 9700 thermal cycler [17]. Briefly, amplification was performed using the Titan One version 13 RT-PCR kit (Boehringer Mannheim, Manneheim, Germany), with first-round PCR using primers amplifying 941 bp of RT (MJ3/MJ4) and 653 bp which encompasses the entire RA region (5'Prot1/3'Prot1). Second-round (nested) PCR was performed with A35/NE(1)35 primers spanning 731 bp

of RT and 5′prot2/3′prot2 primers spanning 507 bp which encompasses the entire PR region. The other outer primers used for RT were RT18/RT21 and for PR 5′eprB/3′eprB, while the other nested primers were RT1/RT4 for RT and 5′prB/3′prB for regions of PR. Primer sequences are provided in Supplementary File 1.

Revelation of PCR products was performed by 4% ethidium bromide agarose gel electrophoresis, with a predicted size of 731 bp for RT and 507 for PR, including positive and negative controls along a molecular scale (Tacklt™ ΦX174 RF DNA/Hae III Fragment). The amplicons were purified by PCR using the NucleoFast® 96 PCR (Macherey-Nagel).

In accordance with the ANRS AC11 protocol, the HIV-1 PR-RT was sequenced using deoxyterminator overlapping primers [17]. The sequences were purified using Sephadex G-50 resin, and identified after capillary electrophoresis on an Applied Biosystem (ABI) "3130" genetic analyzer.

Interpretation of HIV-1 drug resistance: According to the sequencing protocol used [17], the HIV-1 DRMs were interpreted according to the FastSeq50_Pop7_1 algorithm (http://www.hivfrenchresistance.org/). Viruses with a mutant, or a mixture of wild type and mutant, at the one amino acid position were considered to have the resistant variant. Patients were considered carriers of wild-type virus if their viral load was < 1000 RNA copies/ml (virological success) or with a non-amplifiable sample.

Drug resistance analysis: We evaluated clinically relevant resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs) using the Stanford University HIV Drug Resistance Database Genotypic Resistance Interpretation Algorithm (version 8.8) and the International Antiviral Society Drug Resistance Mutation list [16]. The degree of drug resistance to each antiretroviral drug was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance.

Statistical analysis

Data were processed using the version of R. The chi-square test was used to analyze categorical data on TE by ART regimens, including 95% CI, with a $P < 0.05$ considered statistically significant. Spearman's correlation was used for quantitative data on treatment failure outcomes obtained with the two different instruments, with $R \geq 0.8$ considered a strong positive correlation.

Ethical Considerations

We conducted this study in accordance with the ethical principles outlined in the Declaration of Helsinki and received ethical approval from the Cameroon National Ethics Committee for Research on Human Health (Ref. N° 2016/03/727/CE/CNERSH/SP), along with administrative authorization from the Regional Delegate of Public Health of the Center Region. All participants provided written informed consent or assent (depending on age) prior to their enrolment in the study. Confidentiality of participant data was maintained throughout the study.

RESULTS

1. Socio-demographic and virological parameters

Table 1: Basic characteristics of study participants

Parameters		Effective (n=231)	Percentage (%)
Sex	Female	156	67.53
	Male	75	32.47
Age group	[21-35]	116	50.21
	[36-51]	91	39.39
	[52-66]	23	9.95
	≥67	1	0.45
Marital status	married	113	48.91
	single	79	34.19
	Widower	39	16.9
Mode of contamination	Sharp object	13	5.62
	unknown	99	42.85
	sexual	119	51.51
Therapeutic problem	Adherence	8	3.46%
	Education	94	40.69
	Financial constraint	17	7.35
	None	35	15.15
	Social stigma	17	7.35
	Supply	60	29.46
Treatment	none	96	41.55
	AZT-3TC-EFV	52	22.51
	AZT-3TC-NVP	42	18.18

	TDF-3TC-EFV	27	11.68
	TDF-3TC-NVP	14	6.08
Mean duration on ART (month)	6		
Subtypes	A1	27	11.68
	CRF02_AG	149	64.50
	CRF09_cpx	3	1.29
	CRF11_cpx	3	1.29
	CRF13_cpx	4	1.73
	CRF45_cpx	1	0.43
	CRF49_cpx	1	0.43
	D	9	3.89
	F2	9	3.89
	G	16	6.92
	J	6	2.66
	O	3	1.29
Median viral load (copies/mm^3)	536262,2913		

Demographic Profile

The cohort of 231 participants was predominantly female (67.53%), aligning with global trends where women in sub-Saharan Africa are disproportionately affected by HIV due to biological, social, and economic vulnerabilities. This imbalance may also reflect healthcare-seeking behavior, as women are more likely to access testing and treatment services. The majority of participants were aged 21–51 years (89.6%), highlighting HIV's burden on the economically productive population. This underscores the need for workplace-based interventions and youth-focused prevention programs. Nearly half (48.91%) were married, suggesting potential transmission risks within marital partnerships and the importance of couple-centered testing and counseling.

Transmission Dynamics

Sexual contact was the primary reported route (51.51%), emphasizing the urgency of scaling up condom distribution, pre-exposure prophylaxis (PrEP), and sexual health education. The high proportion of "unknown" transmission (42.85%) signals gaps in patient education or stigma-related underreporting.

Therapeutic Challenges

Only 0.02% reported adherence issues, which likely underestimates the true burden given the high viral load (median: 536,262 copies/mm^3). The dominant therapeutic problems—lack of education (40.69%) and drug supply shortages (29.46%)—suggest systemic healthcare barriers, including poor patient counseling and stockouts of antiretrovirals (ARVs). 41.55% were not on ART, reflecting gaps in treatment access or linkage to care. This is alarming given Cameroon's "Treat All" policy and risks perpetuating transmission.

Treatment Regimens

First-Line ART including AZT-3TC-EFV (22.51%) and AZT-3TC-NVP (18.18%) were most common, consistent with Cameroon's national guidelines. However, the low uptake of TDF-based regimens (17.76% combined) may reflect regional prescribing preferences or cost barriers. The median ART duration of 6 months suggests many participants were recently initiated, potentially impacting long-term adherence and resistance patterns.

Virological and Genetic Insights

HIV-1 Subtypes CRF02_AG (64.50%) dominated, as seen in West/Central Africa. Subtypes A1 (11.68%) and G (6.92%) indicate genetic diversity, which may influence drug resistance pathways and vaccine development efforts. The median viral load of 536,262 copies/mm^3 reflects poor virological control, likely due to delayed ART initiation, suboptimal adherence, or pre-existing resistance.

2. Detected HIV Drug Resistance Mutations

2.1. Mutation and drug resistance frequencies

Table 2: Mutation and drug resistance frequencies

Number (n=231)	NAIVE		TREATMENT-EXPERIENCED		TOTAL
Classes of ARV drugs	frequency	P value	Frequency	P value	Frequency
PI/r	26(11.25%)	0,002	19(8.23%)	0.05	45(19.48%)
NRTI	0(0%)	/	1(0.43%)	0.45	1(0.43%)
NNRTI	3(1.30%)	0,001	14(6.06%)	0.42	17(7.36%)
PI/r+ NNRTI	1(0.43%)	0,001	0(0%)	0.001	1(0.43%)
NNRTI+NRTI	1(0.43%)	0,001	4(1.73%)	0.47	5(2.16%)
PI/r+ NNRTI+NRTI	0(0%)	/	4(1.73%)	0.28	4(1.73%)
TOTAL	31(13.41%)		42(18.18%)		73(31.59%)

Table 2 underscores a dual challenge in Cameroon: transmitted resistance in untreated populations and acquired resistance under suboptimal treatment adherence. The overall resistance burden was 31.59% (73/231) of participants harbored resistance mutations (Table 2), highlighting a significant public health challenge in Cameroon. Resistance was higher in treated patients (18.18%) than naive individuals (13.41%), reflecting accumulated resistance under treatment pressure. However, the substantial rate in naive patients signals transmitted drug resistance (TDR), likely due to incomplete viral suppression in the community.

Protease inhibitors (PI/r) resistance showed highest prevalence: 19.48% (45/231), with 11.25% in naive patients (P=0.002) and 8.23% in treated patients (P=0.05) (Table 2). The high PI/r resistance in naive patients may stem from natural polymorphisms in HIV-1 subtypes (CRF02_AG) or undiagnosed prior ARV exposure such as PMTCT. The significant (P=0.002) resistance suggests either transmitted resistance or subtype-associated polymorphisms such as CRF02_AG that may harbor PI-associated mutations without prior PI exposure.

NNRTIs resistance was found in 7.36% (17/231), with 6.06% in treated patients and 1.30% in naive (P=0.001) (Table 2). NNRTI resistance is widespread due to historical reliance on efavirenz/nevirapine. Highly significant (P=0.001) resistance in naive patients aligns with global TDR trends, driven by prolonged NNRTI use in first-line regimens. The significant TDR underscores the need for baseline resistance testing before initiating NNRTI-based regimens. Higher NNRTI (6.06%) and PI/r (8.23%) resistance in treated patients reflect adherence challenges and regimen-specific selective pressure

NRTIs was the rare resistance (0.43%, 1/231), limited to treated patients (P=0.45) (Table 2). Lamivudine/ tenofovir retain efficacy, but vigilance is needed to prevent emerging resistance.

Multi-Class Resistance was also found with NNRTI+NRTI: 2.16% (5/231), predominantly in treated patients (1.73%, P=0.47); PI/r+NNRTI+NRTI: 1.73% (4/231), exclusively in treated patients (P=0.28) (Table 2). The multi-class resistance complicates treatment options, necessitating costly second-line regimens (boosted PIs or integrase inhibitors).

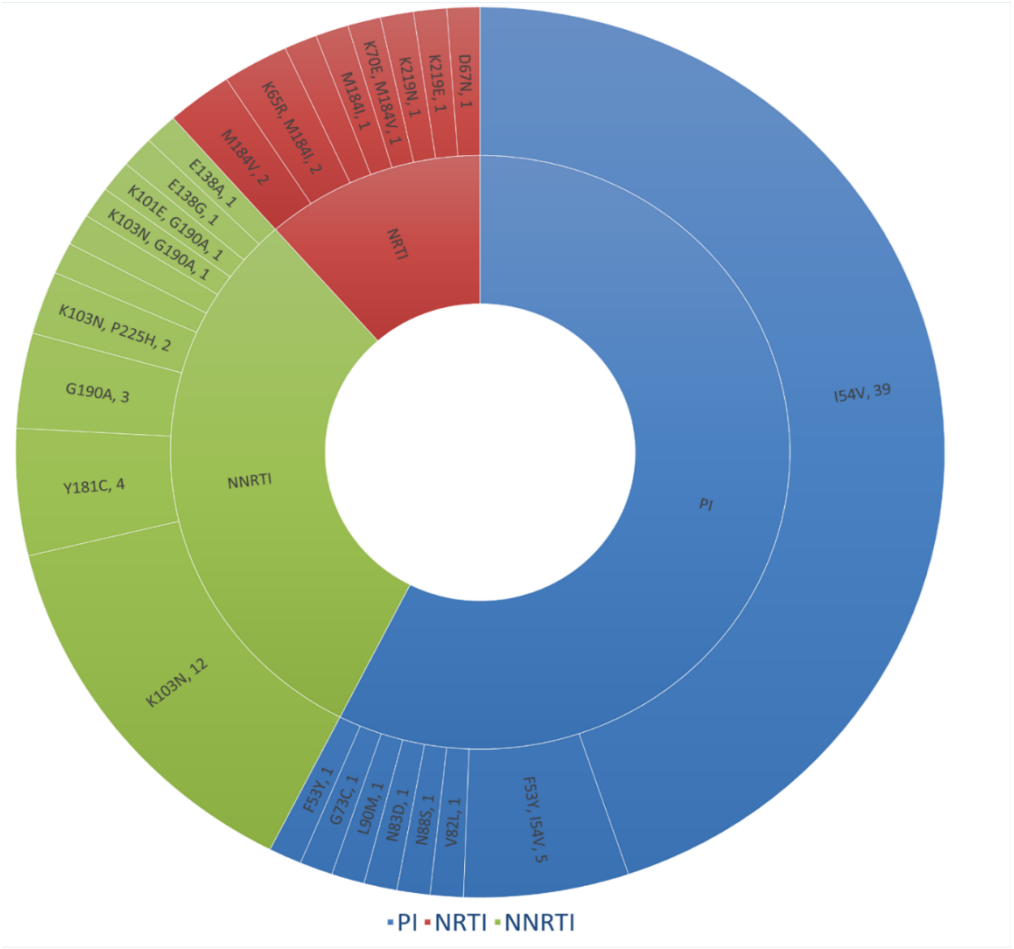

Figure 1: Prevalence and Distribution of HIV-1 Drug Resistance Mutations in Yaoundé, Cameroon. Frequency of drug resistance mutations across codon positions in the HIV-1 reverse transcriptase (RT) and protease (PR) genes among ART-experienced and treatment-naïve patients in Yaoundé, Cameroon. Mutations are annotated by codon position (M184V, K103N, I54V), with bar heights indicating their prevalence in the cohort. Key resistance-associated mutations to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) are highlighted. Data derived from sequencing of plasma viral RNA (2017–2021) in 231 participants.

Protease inhibitor mutation frequencies

The data presented in Figure 1 strongly suggest that the I54V mutation is the primary driver of resistance to the protease inhibitor(s) in this population. The high frequency of this mutation indicates that it is likely to confer a significant advantage to the virus in the presence of the inhibitor, leading to its selection and dominance. The presence of the F53Y/I54V combination at a notable frequency (10%) also highlights its importance in resistance. It is possible that the F53Y mutation, when occurring in combination with I54V, further enhances resistance or arises through a specific evolutionary pathway. The very low frequencies of the other individual mutations (F53Y, G73C, L90M, N83D, N88S, V82L) imply that they are either less effective in conferring resistance to this particular protease inhibitor(s) or are less likely to emerge in this specific viral context.

Nucleotide reverse transcriptase inhibitor mutation frequency

Figure 1 illustrates the frequencies of different nucleotide reverse transcriptase inhibitor (NRTI) mutations. The data in Figure 2 suggests that multiple NRTI mutations are prevalent, indicating that resistance to these inhibitors can arise through various genetic pathways. The high frequency of K65R/M184I, K70E/M184V, and M184V (all at 20%) suggests that these mutations are particularly effective in conferring resistance to the specific NRTI(s) being studied. The M184V mutation is frequently observed in the context of resistance to lamivudine (3TC) and emtricitabine (FTC), which are NRTIs. The presence of combinations like K65R/M184I and K70E/M184V further emphasizes the complexity of resistance development. The mutations occurring at a frequency of 10% (D67N, K219E, K219N, M184I, M41L/M184V/T215F) also contribute to NRTI resistance, although they appear to be less dominant than the 20% frequency group in this specific dataset. The M41L, M184V, and T215F combination is a well-known thymidine analog mutation (TAM) pattern associated with resistance to drugs like zidovudine (AZT) and stavudine (d4T).

Non-nucleotide reverse transcriptase inhibitory mutations

From this figure 1, it appears that the highest in NNRTIs was K103N, followed by Y181C. We have two triple mutations, and three double mutations. The data presented in Figure 3 highlights that the K103N mutation is the most dominant NNRTI resistance mutation in this study population, occurring in a substantial proportion of cases. This finding is consistent with previous research that has identified K103N as a key mutation conferring resistance to commonly used NNRTIs like efavirenz and nevirapine. The significant frequency of the K103N/Y181C/G190A combination (14.82%) suggests that the accumulation of multiple resistance mutations can lead to higher levels of drug resistance or may arise under specific treatment pressures. The G190A mutation also appears to be an important single mutation contributing to NNRTI resistance. The lower frequencies of other mutations indicate that while they can contribute to resistance, they are less prevalent in this specific context. The presence of various mutation combinations suggests the complex evolutionary pathways the virus can take to evade the effects of NNRTI drugs.

2.6. Correlation between ARV regimens and the emergence of drug resistance

Table 3 presents the correlation between specific resistance mutations within different classes of antiretroviral (ARV) drugs (Protease Inhibitors boosted with ritonavir - PI/r, Nucleotide Reverse Transcriptase Inhibitors - NRTI, and Non-Nucleotide Reverse Transcriptase Inhibitors - NNRTI) and the first-line treatment regimen received by individuals.

Table 3: Correlation between resistance type and treatment class

Classes of ARV drugs		TREATMENT					
		AZT-3TC-EFV	AZT-3TC-NVP	TDF-3TC-EFV	TDF-3TC-NVP	R	P
PI/r	I54V	12	1	4	1		
	L90M	1	0	0	0		
	N88S	1	0	0	0		
	G73C	0	1	0	0		
	V82L	0	1	0	0		
	N83D	0	0	1	0	0.03	0.12
NRTI	M184I	1	0	0	0		
	M184V	2	0	0	0		
	K65R, M184I	1	0	1	0		
	K70E, M184V	1	0	0	0		
	D67N	0	0	1	0	0.05	0.04

	K219E	0	0	1	0		
	M41L, M184V, T215F	0	0	1	0		
NNRTI	E138G	1	1	0	0		
	K103N	2	5	1	1		
	K103N, G190A	1	0	0	0		
	K103N, P225H	2	0	0	0		
	K103N, Y188C, M230L	1	0	0	0		
	G190A	0	2	0	0		
	Y181C	0	1	1	0		
	K103N, Y181C, G190A	0	0	1	0		
	K101E, G190A	0	0	1	0		
	TOTAL	26	12	13	2	0.04	0.08

The columns labeled "R" and "P" likely represent the total count of individuals with that mutation across all treatment groups and the p-value associated with the correlation between the mutation and the treatment group, respectively.

The analysis of Table 3 reveals that the most significant finding is the strong correlation between NRTI resistance mutations and the first-line treatment regimen (p=0.04). This suggests that the specific NRTI drugs used in the regimen (AZT-3TC vs. TDF-3TC) significantly influence the type of NRTI resistance mutations that emerge. While the NNRTI class as a whole might not have a statistically significant correlation as strong as NRTIs, specific mutations like G190A (p=0.04) and Y181C (p=0.08) show a statistically significant or borderline significant association with the treatment regimens. G190A appears to be more prevalent in the AZT-3TC-NVP group, while Y181C is seen in both AZT-3TC-NVP and TDF-3TC-EFV groups. The individual PI mutations listed do not show strong statistically significant correlations with the treatment regimens based on their p-values. The most frequent PI mutation, I54V, has a p-value of 0.12, indicating a lack of strong association with any specific treatment group in this dataset. The total number of resistance mutations observed is highest in the AZT-3TC-EFV group (26), which could be due to various factors such as the duration of treatment, virological failure rates, or the specific resistance profiles associated with this regimen.

2.7. Correlation of viral load with resistance patterns in different drug classes

Table 4: Correlation of viral load with resistance type and subtypes

Parameters		VL copies/mL					
		40-1000	1001-10000	>10001	TOTAL	R	P. value
subtypes	A1	1	1	6	8	0.001	0.71
	D	1	0	2	3		
	F2	0	0	2	2		
	G	1	2	4	7		
	J	0	1	2	3		
	O	0	0	1	1		
	CRF02_AG	4	6	36	46		
	CRF09_cpx	0	0	1	1		
	CRF11_cpx	0	0	1	1		
	CRF13_cpx	0	0	1	1		
PI/r	F53Y, I54V	0	2	3	5	0.23	0,0001
	I54V	0	2	38	40		

	G73C	0	0	1	1		
	L90M	1	0	0	1		
	N83D	0	0	1	1		
	N88S	0	0	1	1		
	V82L	0	0	1	1		
	NONE	4	2	7	13		
NRTI	D67N	0	1	0	1	0.001	0.71
	M184I	1	0	0	1		
	M184V	0	0	2	2		
	K219E	0	0	1	1		
	K219N	0	0	1	1		
	K70E, M184V	0	0	1	1		
	K65R, M184I	0	0	2	2		
	M41L, M184V, T215F	0	0	1	1		
	NONE	6	9	48	63		
NNRTI	E138G	1	0	0	1	0.24	0,009
	G190A	1	0	2	3		
	K103N	3	4	5	12		
	E138A	0	1	0	1		
	Y181C	0	0	4	4		
	K103N, P225H	1	0	1	2		
	K101E, G190A	0	0	1	1		
	K103N, G190A	0	0	1	1		
	K103N, Y181C, G190A	0	0	1	1		
	K103N, Y188C, M230L	0	0	1	1		
	NONE	1	5	40	46		

Table 4 reveals significant correlations between viral load and resistance to protease inhibitors and non-nucleotide reverse transcriptase inhibitors. The highly significant p-value (0.0001) for the PI mutation combination F53Y, I54V suggests a strong association with higher viral loads. This implies that individuals with this specific PI resistance pattern tend to have poorer viral control. The overall PI resistance also shows a significant correlation with viral load. The significant p-value (0.009) for the NNRTI mutation E138G suggests an association with lower viral loads, as it is only found in the 40-1000 copies/mL range. The overall NNRTI resistance also shows a significant correlation with viral load. The presence of Y181C exclusively in the highest viral load category also points towards a potential association. There is no statistically significant correlation between the HIV-1 subtypes and viral load in this dataset. Similarly, individual NRTI mutations do not show strong correlations with viral load, and the overall NRTI resistance is not mentioned as significantly correlated.

3. Resistance mutation distribution in treated and untreated individuals

3.1 Summary of Resistance Profiles in Treated and Untreated Individuals.

This table summarizes the distribution of resistance to each drug class and the extent of multi-drug resistance in the studied population, broken down by whether individuals were on HIV drug treatment or not.

Table 5: Summary of Resistance Profiles in Treated and Untreated Individuals

Drug Class	HIVDT = Yes (n=42)	HIVDT = No (n=31)	Overall (n=73)
PI	22 (52.4%)	25 (80.6%)	47 (64.4%)
NRTI	7 (16.7%)	2 (6.5%)	9 (12.3%)
NNRTI	14 (33.3%)	7 (22.6%)	21 (28.8%)
Resistance to 2+ Classes	7 (16.7%)	3 (9.7%)	10 (13.7%)
Resistance to 3 Classes	3 (7.1%)	1 (3.2%)	4 (5.5%)
No Resistance	0 (0.0%)	5 (16.1%)	5 (6.8%)

In this study, the distribution of antiretroviral resistance revealed that a higher proportion of treatment-naive individuals exhibited resistance to protease inhibitors (80.6%) compared to those on treatment (52.4%), a notable finding that warrants further investigation within this context. As anticipated, resistance to nucleotide and non-nucleotide reverse transcriptase inhibitors was more prevalent in the treated group (16.7% and 33.3% respectively) than in the untreated group (6.5% and 22.6%), though the presence of NNRTI resistance in some untreated individuals suggests potential transmission of resistant strains in Cameroon. Multi-drug resistance was also more common among treated individuals, and importantly, no individual on treatment showed complete susceptibility to all tested drug classes, indicating that in this Cameroonian cohort, treatment exposure was invariably associated with the development of resistance.

3.2. Analysis of cross-mutation patterns and art implications in HIV-1 patients

Table 6: Distribution of resistance in population study

ID	SUBTYPE	HIVDT	PI Major	NRTI	NNRTI
K10560	D	Yes	I54V	None	None
K10601	CRF02_AG	Yes	I54V	M184V	K103N, P225H
K10630	CRF02_AG	Yes	I54V	None	None
K10646	CRF02_AG	Yes	None	M184I	K103N, P225H
K10674	G	Yes	I54V	None	None
K10683	CRF02_AG	Yes	None	None	K103N
K10687	D	Yes	G73C	None	None
K10698	CRF02_AG	Yes	None	None	G190A
K10707	G	Yes	I54V	None	None
K10708	G	Yes	None	None	K103N
K10709	CRF09_cpx	Yes	None	K70E, M184V	K103N, G190A
K10721	CRF02_AG	Yes	I54V	K65R, M184I	K103N, Y181C, G190A
K10728	CRF02_AG	Yes	None	None	K103N
K10737	CRF02_AG	Yes	None	None	G190A
K10740	G	Yes	None	None	K103N
K10744	CRF02_AG	Yes	I54V	M184V	K103N
K10761	CRF02_AG	Yes	I54V	None	None
K10610	D	Yes	None	None	E138G
K10624	CRF02_AG	Yes	None	D67N	None
K10628	J	Yes	None	None	K103N
K10635	CRF02_AG	Yes	I54V	None	None
K10638	CRF13_cpx	Yes	I54V	None	None

K10642	CRF02_AG	Yes	I54V	None	None
K10643	CRF02_AG	Yes	None	None	E138A
K10652	CRF02_AG	Yes	I54V	None	None
K10657	CRF02_AG	Yes	I54V	None	None
K10675	CRF11_cpx	Yes	V82L	None	None
K10681	CRF02_AG	Yes	None	K65R, M184I	K103N, Y188C, M230L
K10689	A1	Yes	I54V	None	None
K10696	CRF02_AG	Yes	I54V	None	None
K10801	CRF02_AG	Yes	N88S	None	None
K10847	A1	Yes	None	None	K103N
K10906	O	Yes	None	None	Y181C
K10907	CRF02_AG	Yes	None	None	Y181C
K10916	CRF02_AG	NO	None	None	K103N
K10917	A1	NO	I54V	None	None
K10922	A1	Yes	N83D	M41L, M184V, T215F	K101E, G190A
K10928	CRF02_AG	Yes	I54V	None	None
K10929	A1	Yes	None	K219E	Y181C
K10949	CRF02_AG	Yes	L90M	None	None
K10950	G	Yes	I54V	None	None
K11087	J	Yes	None	None	K103N
K10895	CRF02_AG	NO	F53Y	None	None
K10908	CRF02_AG	NO	None	None	K103N
K10836	CRF02_AG	Yes	I54V	None	None
K10837	A1	Yes	None	None	K103N
K10126	J	NO	I54V	None	None
K10150	F2	NO	I54V	None	None
K10170	CRF02_AG	NO	F53Y, I54V	None	None
K10199	CRF02_AG	NO	I54V	None	None
K10203	CRF02_AG	NO	I54V	None	None
K10204	CRF02_AG	NO	F53Y, I54V	None	None
K10206	G	NO	I54V	None	None
K10207	CRF02_AG	NO	F53Y, I54V	None	None
K10213	CRF02_AG	NO	I54V	None	None
K10214	CRF02_AG	NO	I54V	None	None
K10216	CRF02_AG	NO	I54V	None	None
K10225	A1	NO	I54V	None	None
K10227	CRF02_AG	NO	F53Y, I54V	None	None
K10228	CRF02_AG	NO	I54V	None	None
K10229	CRF02_AG	NO	I54V	None	None
K10236	CRF02_AG	NO	I54V	None	None
K10244	CRF02_AG	NO	None	K219N	Y181C

K10247	CRF02_AG	NO	None	None	G190A
K10248	CRF02_AG	NO	I54V	None	None
K10250	CRF02_AG	NO	I54V	None	None
K10251	CRF02_AG	NO	I54V	None	None
K10259	CRF02_AG	NO	I54V	None	None
K10264	G	NO	I54V	None	None
K10267	CRF02_AG	NO	I54V	None	None
K10271	CRF02_AG	NO	I54V	None	None
K10284	A1	NO	I54V	None	None
K10288	CRF02_AG	NO	F53Y, I54V	None	K103N

This study highlights critical trends in HIV-1 drug resistance among patients in Yaoundé, Cameroon, emphasizing the interplay between protease inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), and non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations. Table 5 reveals a significant prevalence of antiretroviral resistance in the studied population, with 42 individuals on treatment and 31 not on treatment exhibiting resistance mutations.

The most prevalent PI mutation, I54V, was identified in 37% of patients, often co-occurring with F53Y, suggesting a propensity for PI resistance in this population. Among NRTIs, M184V/I (11.3% prevalence) dominated, linked to lamivudine/emtricitabine resistance, while NNRTI resistance was driven by K103N (24.2%) and G190A, which compromise first-line regimens like efavirenz and nevirapine.

Notably, cross-class resistance was observed in several patients, reflecting complex treatment challenges. For example, patient K10601 harbored I54V (PI) + M184V (NRTI) + K103N/P225H (NNRTI), a combination that undermines both boosted PIs and first-line NRTI/NNRTI regimens. Similarly, K10721 exhibited a multi-drug-resistant profile (I54V + K65R/M184I + K103N/Y181C/G190A), indicative of advanced virological failure. These patterns underscore the risk of accumulating resistance mutations in settings with limited regimen options.

Subtype analysis revealed CRF02_AG as the dominant strain, associated with the highest mutation diversity. This subtype accounted for most cases of I54V and K103N, including in treatment-naïve individuals, suggesting potential subtype-specific resistance transmission. For instance, six untreated patients (43% of naïve cases) carried I54V, signaling transmitted PI resistance—a rare but alarming finding with implications for pre-ART genotyping.

The comparison between treatment-experienced (HIVDT=Yes) and naïve (HIVDT=No) patients further emphasized public health concerns. While NNRTI mutations like K103N and G190A were prevalent in both groups, PI resistance in naïve patients (e.g., I54V in untreated individuals) highlights gaps in resistance surveillance. This underscores the need for baseline genotype testing to guide regimen selection, particularly as Cameroon transitions to dolutegravir-based regimens.

Clinical and public health priorities include phasing out NNRTIs, adopting integrase inhibitors, and enhancing resistance monitoring. The high prevalence of K103N (24.2%) aligns with WHO recommendations to prioritize dolutegravir, which has a higher genetic barrier to resistance. Additionally, the detection of multi-class resistance in CRF02_AG underscores the urgency of subtype-tailored strategies to mitigate treatment failure.

In conclusion, these findings reveal a pressing need for updated guidelines, robust surveillance, and targeted interventions to address cross-class resistance and transmitted mutations. Strengthening laboratory capacity for genotyping and prioritizing newer antiretrovirals will be vital to sustaining ART efficacy in Cameroon's HIV response.

DISCUSSION

In this study, we reported on a large cohort of 231 patients infected with HIV-1 followed in health facilities in the central region, including 135 patients on first-line antiretroviral treatment and 96 who were not on any antiretroviral treatment. This study provides a comprehensive analysis of HIV-1 drug resistance patterns in Cameroon's Centre Region, contextualizing genetic diversity, treatment outcomes, and public health challenges in a resource-limited setting. Below, we expand on the implications of our findings, integrating regional and global perspectives to inform future interventions.

The dominance of the CRF02_AG subtype (64.5%) aligns with its established prevalence in West and Central Africa, where recombination events between subtypes A and G have driven its dissemination. However, the secondary predominance of subtypes A1 (11.68%) and G (6.92%) contrasts with studies from Cameroon's Northwest and Southwest regions, where subtypes G and F are more common [16, 17]. This geographical heterogeneity may reflect differences in viral introduction events, population mobility, or founder effects. For instance, urban centers like Yaoundé, with high population density and migration, could act as hubs for CRF02_AG transmission. Such variability underscores the need for decentralized surveillance systems to track

subtype-specific trends, as viral diversity can influence disease progression, vaccine efficacy, and drug resistance pathways.

The overall prevalence of resistance mutations (31.59%) exceeds rates reported in Kenya (23.1% [18]) and mirrors rising resistance trends across sub-Saharan Africa. This escalation is likely multifactorial such as irregular treatment follow-up (reported by 40.69% of participants) and limited health literacy delay the detection of virological failure, allowing resistance to accumulate. As well, the high prevalence of NNRTI resistance (7.36%) reflects Cameroon's historical reliance on nevirapine/efavirenz-based first-line regimens, which have low genetic barriers to resistance. Similarly, the M184V mutation (NRTI) is strongly associated with lamivudine, a backbone drug in national protocols. Surprisingly, 13.41% of treatment-naive individuals harbored major mutations, particularly PI-associated I54V (11.25%). Natural polymorphisms, amplified by HIV-1's error-prone replication [22], may seed these mutations. This poses a critical challenge: initiating ART in naive patients with pre-existing resistance risks rapid regimen failure, especially if PI-based therapies are introduced without baseline resistance testing.

The high PI resistance rate (19.48%)—far exceeding earlier Cameroonian reports (1.3%)[19]—signals a shifting landscape. While PIs are not yet widely used in first-line regimens in Cameroon, their increasing adoption in second-line therapy may explain this trend. Notably, the predominance of I54V—a major PI resistance mutation—in untreated patients (39/50 sequences) suggests transmission of resistant strains or de novo mutagenesis. This mirrors global concerns about "hidden" resistance in ART-naive populations, documented in regions like East Africa and Southeast Asia. The persistence of such mutations, even at low frequencies, could undermine future PI-based regimens, as suboptimal adherence may allow resistant quasispecies to dominate.

The strong association between NRTI use and resistance (p=0.04) highlights the impact of long-term lamivudine/tenofovir exposure in driving M184V and K65R mutations. Similarly, the link between high viral loads and PI/NNRTI resistance (p<0.01) underscores the role of uncontrolled replication in accelerating mutagenesis. These findings align with studies demonstrating that viral loads >100,000 copies/mL correlate with accelerated resistance development. In resource-limited settings, where viral load monitoring is often delayed or unavailable, this creates a vicious cycle: poor adherence → viral rebound → resistance → treatment failure.

The high NNRTI resistance rates (7.36%) support Cameroon's ongoing shift to dolutegravir, an integrase inhibitor with a higher genetic barrier. However, baseline resistance testing for PIs and NRTIs remains critical to pre-empt cross-resistance. Scaling up point-of-care viral load testing and training healthcare workers to interpret results could reduce delays in detecting virological failure. Partnering with community organizations to address stigma, improve health literacy, and provide socioeconomic support such as transportation subsidies may enhance adherence. Establishing a national HIV drug resistance database would enable real-time tracking of emerging mutations and guide regimen updates.

While this study offers critical insights, its cross-sectional design limits causal inferences. Longitudinal cohorts are needed to evaluate how pre-treatment resistance impacts clinical outcomes. Overrepresentation of women (67.53%) and urban populations may limit generalizability. Precludes causal inferences about factors driving low adherence or high viral loads. Risk of recall bias, especially for transmission routes and adherence. Additionally, the focus on *pol* gene mutations overlooks resistance mechanisms in *integrase* or *envelope* regions, which may gain relevance as dolutegravir becomes widespread. Future work should also explore socio-cultural barriers to adherence through mixed-methods research.

CONCLUSION

In summary, CRF02 was the most common HIV subtype (64.5%). Drug resistance mutations were detected in 18.18% of ART-experienced and 13.41% of treatment-naïve patients. NRTI mutations were infrequent (0.43%), while NNRTI mutations were more prevalent (7.36%), with M184V and K103N being the most frequent. Protease inhibitor mutations, predominantly I54V, were found in 19.48% of sequences. ART and treatment failure increase the risk of resistance. Viral diversity, pre-treatment resistance, and adherence challenges in Cameroon necessitate precision public health strategies including resistance testing, optimized regimens, and addressing gaps in care to curb rising HIV drug resistance and improve progress towards UNAIDS 95-95-95 targets. Global partnerships and funding are crucial to support these efforts. The data highlight the need for: (1) baseline resistance testing to guide treatment for newly diagnosed patients due to high transmitted NNRTI and PI resistance; (2) accelerated adoption of dolutegravir-based regimens given the observed NNRTI resistance and dolutegravir's higher resistance barrier; (3) addressing educational gaps to improve adherence and reduce virological failure; and (4) continuous surveillance to monitor circulating subtypes like CRF02_AG and detect subtype-specific resistance patterns.

Author Contributions

Conceptualization: Bello Djoda, Jerome Fru-Cho, Patrick Achiangia Njukeng, Alain Nantchouang, Stefanie Van Cleaf, Josef Eberle, Oliver T. Keppler, Oliver Bosnjak

Data curation: Bello Djoda, Ezechiel Ngoufack Jagni Semengue, Patrick Valere Tsouh Fokou, Evariste Molimbou, Naomi-Karell Etame

Formal analysis: Bello Djoda, Jerome Fru-Cho, Ezechiel Ngoufack Jagni Semengue, Evariste Molimbou, Joseph Fokam, Patrick Valere Tsouh Fokou

Funding acquisition: Oliver Bosnjak.

Investigation: Bello Djoda, Jerome Fru-Cho, Alain Nantchouang, Stefanie Van Cleaf.

Methodology: Bello Djoda, Jerome Fru-Cho, Patrick Achiangia Njukeng, Alain Nantchouang, Patrick Valere Tsouh Fokou, Stefanie Van Cleaf, Josef Eberle, Oliver Till Keppler, Oliver Bosnjak

Project administration: Bello Djoda, Jerome Fru-Cho, Patrick Achiangia Njukeng, Alain Nantchouang, Stefanie Van Cleaf, Josef Eberle, Oliver Till Keppler, Oliver Bosnjak.

Resources: Bello Djoda, Jerome Fru-Cho, Alain Nantchouang, Oliver Till Keppler, Oliver Bosnjak.

Supervision: Jerome Fru-Cho, Patrick Achiangia Njukeng, Christopher Dächert, Josef Eberle,

Validation: Jerome Fru-Cho, Patrick Achiangia Njukeng, Christopher Dächert, Josef Eberle.

Writing, original draft: Bello Djoda.

Writing, review & editing: Bello Djoda , Jerome Fru-Cho, Patrick Achiangia Njukeng, Ezechiel Ngoufack, Jagni Semengue, Evariste Molimbou, Naomi-Karell Etame, Rodrigue Kamga, Patrick Valere Tsouh Fokou, Bauer Wilhelm, Christopher Dächert, Alain Nantchouang , Maximilian Muenchhoff, Abdel Wade, Stefanie Van Cleaf, Josef Eberle, Charles Fokounang, Joseph Fokam, Oliver T. Keppler, Oliver Bosnjak

Competing interests: The authors declare that they have no competing interests.

Availability of data and material: All data generated or analyzed during this study are included in this manuscript.

Consent to publication: All authors have given their consent for publication.

Acknowledgement: We extend our sincere gratitude to all individuals and institutions who contributed to this study. In memoriam, we honor the late Prof. Lazare Kaptue, whose visionary leadership and dedication to HIV research in Cameroon laid the foundation for this work. His pioneering contributions to understanding antiretroviral resistance in resource-limited settings continue to inspire our team, and we humbly dedicate this study to his lasting legacy. We acknowledge the BIOMEX GmbH for their financial, technical and logistical support. We are deeply indebted to the healthcare staff at the five treatment centers in Cameroon's Centre Region and to the study participants for their invaluable cooperation. Our appreciation extends to the National Reference Centres for Retroviruses in Germany (LMU Munich and FAU Erlangen-Nürnberg) for their technical collaboration. Finally, we acknowledge our families and mentors for their unwavering support.

REFERENCES

1. World Health Organization. Prevalence of HIV in the world. Available at: https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/data-use/hiv-data-and-statistics

2. Johnston V, Fielding KL, Charalambous S, Churchyard G, Phillips A, Grant AD. Outcomes following virological failure and predictors of switching to second-line antiretroviral therapy in a South African treatment program. J Acquir Immune Defic Syndr. 2012;61(3):370-80. https://doi.org/10.1097/QAI.0b013e318266ee3f PMid:22820803 PMCid:PMC3840925

3. Kuritzkes DR. Preventing and managing antiretroviral drug resistance. AIDS Patient Care STDS. 2004;18(4):259-73. https://doi.org/10.1089/108729104323076007 PMid:15186710

4. DeGruttola V, Dix L, D'Aquila R, Holder D, Phillips A, Ait-Khaled M, Baxter J, Clevenbergh P, Hammer S, Harrigan R, Katzenstein D, Lanier R, Miller M, Para M, Yerly S, Zolopa A, Murray J, Patick A, Miller V, Castillo S, Pedneault L, Mellors J. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. Antivir Ther. 2000;5:41-8. https://doi.org/10.1177/135965350000500112 PMid:10846592

5. Wainberg MA, Zaharatos GA, Brenner BG. Mechanisms of disease development of antiretroviral drug resistance. N Engl J Med. 2011;365(1):37-46. https://doi.org/10.1056/NEJMra1004180 PMid:21848464

6. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, Botes ME, Mandaliya K, Wellington M, Osibogun A, Stevens WS, van Vugt M, de Wit TF. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr. 2011;58(1):23-31. https://doi.org/10.1097/QAI.0b013e318227fc34 PMid:21694603

7. Boulle A, Van Cutsem G, Cohen K, Hilderbrand K, Mathee S, Abrahams M, Goemaere E, Coetzee D, Maartens G. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. JAMA. 2008;300(5):530-9. https://doi.org/10.1001/jama.300.5.530 PMid:18677025

8. Pasquet A, Messou E, Gabillard D, Minga A, Depoulosky A, Deuffic-Burban S, Losina E, Freedberg KA, Danel C, Anglaret X, Yazdanpanah Y. Impact of drug stock-outs on death and retention to care among HIV-infected patients on combination antiretroviral therapy in Abidjan, Côte d'Ivoire. PLoS One. 2010;5(10):e13414. https://doi.org/10.1371/journal.pone.0013414 PMid:20976211 PMCid:PMC2955519

9. Wainberg MA, Zaharatos GJ, Brenner BG. Development of antiretroviral drug resistance. N Engl J Med. 2011;365(7):637-46. https://doi.org/10.1056/NEJMra1004180 PMid:21848464

10. Wainberg MA. HIV-1 Subtype Distribution and the Problem of Drug Resistance. AIDS. 2004;18 Suppl 3:S63-8. https://doi.org/10.1097/00002030-200406003-00012 PMid:15322487

11. Kantor R, Katzenstein D. Drug Resistance in Non-Subtype B HIV-1. J Clin Virol. 2004;29(3):152-9. https://doi.org/10.1016/S1386-6532(03)00115-X PMid:14962783

12. Ojesina AI, Sankalé JL, Odaibo G, Langevin S, Meloni ST, Sarr AD, Olaleye D, Kanki PJ. Subtype-Specific Patterns in HIV Type 1 Reverse Transcriptase and Protease in Oyo State, Nigeria: Implications for Drug Resistance and Host Response. AIDS Res Hum Retroviruses. 2006;22(8):770-9. https://doi.org/10.1089/aid.2006.22.770 PMid:16910833

13. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010. Available at: http://www.who.int/hiv/pub/arv/adult2010/en/index.html

14. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. 2013. Available at: http://www.who.int/hiv/pub/guidelines/arv2013/en/

15. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating HIV infection: recommendations for a public health approach. 2nd ed. 2016. Available at: http://www.who.int/hiv/pub/arv/arv-2016/en/

16. Agyingi L, Barengolts D, Mayr L, Kinge T, MBida M, Nyambi P. Genetic variability and drug resistance mutations in HIV-1 infected individuals on HAART or drug naïve in Limbe, Cameroon. Retrovirology. 2012;9 Suppl 2:P158.

https://doi.org/10.1186/1742-4690-9-S2-P158 PMCid:PMC3441775

17. Ceccarelli L, Salpini R, Moudourou S, Cento V, Santoro MM, Fokam J, Takou D, Nanfack A, Dori L, Torimiro J, Sarmati L, Andreoni M, Perno CF, Colizzi V, Cappelli G. Characterization of drug resistance mutations in naive and ART-treated patients infected with HIV-1 in Yaounde, Cameroon. J Med Virol. 2012;84(5):721-7. https://doi.org/10.1002/jmv.23244 PMid:22431019

18. Kinyua JG, Lihana RW, Kiptoo M, Muasya T, Odera I, Muiruri P, Songok EM. Antiretroviral resistance among HIV-1 patients on first-line therapy attending a comprehensive care clinic in Kenyatta National Hospital, Kenya: a retrospective analysis. Pan Afr Med J. 2018;29:186. https://doi.org/10.11604/pamj.2018.29.186.10796 PMid:30061964 PMCid:PMC6061825

19. Meriki HD, Tufon KA, Anong DN, Atanga PN, Anyangwe IA, Cho-Ngwa F, Nkuo-Akenji T. Genetic diversity and antiretroviral resistance-associated mutation profile of treated and naive HIV-1 infected patients from the Northwest and Southwest regions of Cameroon. PLoS One. 2019;14(11):e0225575. https://doi.org/10.1371/journal.pone.0225575 PMid:31751428 PMCid:PMC6874083

20. Kurt Yilmaz N, Swanstrom R, Schiffer CA. Improving Viral Protease Inhibitors to Counter Drug Resistance. Trends Microbiol. 2016;24(7):547-57. https://doi.org/10.1016/j.tim.2016.03.010 PMid:27090931 PMCid:PMC4912444

21. Mata-Munguía C, Escoto-Delgadillo M, Torres-Mendoza B, Flores-Soto M, Vázquez-Torres M, Gálvez-Gastelum F, Viniegra-Osorio A, Castillero-Manzano M, Vázquez-Valls E. Natural polymorphisms and unusual mutations in HIV-1 protease with potential antiretroviral resistance: a bioinformatic analysis. BMC Bioinformatics. 2014;15:72. https://doi.org/10.1186/1471-2105-15-72 PMid:24629078 PMCid:PMC4003850

22. Coffin J, Swanstrom R. HIV Pathogenesis: Dynamics and Genetics of Viral Populations and Infected Cells. Cold Spring Harb Perspect Med. 2013;3(1):a012526. https://doi.org/10.1101/cshperspect.a012526 PMid:23284080 PMCid:PMC3530041

23. Rhee SY, Sankaran K, Varghese V, Winters MA, Hurt CB, Eron JJ, Parkin N, Holmes SP, Holodniy M, Shafer RW. HIV-1 Protease, Reverse Transcriptase, and Integrase Variation. J Virol. 2016;90(13):6058-70. https://doi.org/10.1128/JVI.00495-16 PMid:27099321 PMCid:PMC4907232