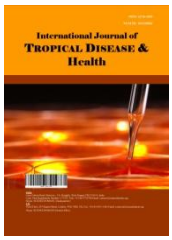

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Transmitted HIV-1 Drug-resistance Mutations among Newly Diagnosed Patients of the North Western Region of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LEA, JNT, AKN, PO and FC conceived and designed the experiments. Author LEA enrolled the patients. Authors LEA, LAT, YC and TD performed the experiments. Authors LEA, JNT, AKN and FC data management, analysis, and interpretation. Authors LEA, JNT, AKN, LAT, YC, TD, PO and FC prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: First line antiretroviral treatment (ART) regimens in Cameroon comprises of reverse transcriptase inhibitors (RTIs). As ART continues to expand, the emergence of HIV drug resistance

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mutations (DRMs) is a challenging problem. The ongoing scale up of ART in Cameroon has prompted the interest in surveillance of transmitted drug resistance (TDR). The aim of this study was to evaluate the prevalence of TDR among drug naïve individuals residing in the North West Region (NWR) of Cameroon.

Methods: Ethics approval was obtained from the National Ethics Committee of Cameroon and patients informed consents were obtained. A total of 81 HIV-1 infected and drug naïve patients were recruited from randomly selected clinics located in the NWR of Cameroon from February 2016 and April 2016. HIV-1 protease-reverse transcriptase region was sequenced using an in-house protocol. HIV drug resistance mutations were identified and analyzed using Stanford HIVDR database and the Calibrated Population Resistance (CPR) tools. Data was analyzed using SPSS version 23.

Results: Of the 81 samples analyzed, the prevalence of TDR was 11.1% (9/81). Of these 9.9% (8/81) had drug resistance mutations to nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) and a single individual (1.2%) with protease inhibitors. A total of 8.6 % (7/81), 4.9(4/81), and 3.7 % (3/81) individuals had a major mutation to NRTIs, NNRTIs, and both NRTIs and NNRTIs respectively. Thymidine analogue mutations [TAM] (M41ML, D67N, K70T/R, T215TA/F, and K219Q) were detected in five individuals. The most frequent NRTI and NNRTI DRMs were K219Q (n=2) and E138A (n=2) respectively. Mutations associated with NRTIs were TAM and M184MV; NNRTIs were A98G, K103N, V108I, V179E, and Y181C and I54IFV for PI. CPR tool revealed a 4.9% (4/81) prevalence with the following single mutations K103N D67N, K70R, M184V, T215F, K219Q.The difference between two methods was statistically significant p=0.0001. These mutations confer resistance to all NRTIs and NNRTIs drugs.

Conclusions: Although moderate transmitted drug resistance (11.1%) levels were detected in this study, this calls for routine drug resistance surveillance among patients on ART. This will ensure relative maintenance of low viral suppression amongst HIV patients.

Keywords: HIV-1, Antiretroviral therapy, transmitted Drug resistance, Cameroon.

ABBREVIATIONS

3TC: Lamivudine
ABC: Abacavir
APOBEC3G: Apolipoprotein B MRNA Editing Enzyme Catalytic Subunit 3G
ATV/r: Boosted Atazanavir
AZT: Zidovudine
CPR: Calibrated Population Resistance
D4T: Stavudine
DDI: Didanosine
DNA: DEOXYRIBONUCLEIC ACID
DRM: DRUG-RESISTANT MUTATION
EFV: Efavirenz
ETR: etravirine
FTC: emtricitabine
HIV: HUMAN Immunodeficiency Virus
LPV/r : Boosted Lopinavir
NNRTI: Non-nucleoside Reverse Transcriptase Inhibitor
NRTI: Nucleoside Rverse Tanscriptase Inhbitor
NVP: Nevirapine
PCR: Polymerase Chain Reaction
PI: Protease Inhibitor
PRRT: Protease-Reverse Transcriptase
PMTCT: Prevention of mother-to-child transmission
PEP: post-exposure prophylaxis;
PrEP: pre-exposure prophylaxis
TDF: Tenofovir

RAM: Resistance-associated Mutation
RPV: Rilpivirine
SRDM: Surveillance Drug Resistance Mutation
TAMs: Thymidine Analogue Mutations

1. INTRODUCTION

The use of combined antiretroviral therapy (cART) has shown to be the most potent intervention for both treatment and prevention of HIV [1-3]. Cameroon like most of the countries adopted the use of first and second-line drug regimens in its national treatment guidelines. As of 2016, the first-line drugs consist of two nucleoside reverse transcriptase inhibitors (NRTI) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) while second line regimen constitute the use of one ritonavir-boosted protease inhibitor [PI/r] plus two NRTIs) regimens for those failing firsts line [1,4,5].

ART was introduced in Cameroon in 2002 where patients had to pay the US $23–$100 for their drugs monthly [6]. By May 2007, All eligible individuals received ARV drugs free of charge through a national distribution program [4, 6]. Cameroon, like many other countries, is increasing its HIV prevention and treatment efforts through the current WHO guidelines that recommend the “test and treat” approach to ART for everyone who tests HIV positive and the use of pre/post-exposure prophylactic antiretroviral drugs to subjects at increased risks of infection [7,8]. As such ART has also been extensively used among HIV pregnant and lactating women and individuals at risk of contracting HIV infection [9]. Furthermore, the use of pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP) to protect HIV negative individuals has shown to be very effective and reduces the chances of HIV infection to nearly zero [2]. As such, there has been a rapid scale-up of ART with an increasing national coverage from 0% in 2001, 2% in 2003, 22% in 2014 and 32% in 2016 of the eligible patients [4, 7, 10, 11].

With increased treatment coverage, HIV drug resistance (HIVDR) and onward transmission of drug resistance mutations (DRMs) are expected to increase. Based on the simplified early warning indicators in Cameroon which include; On-time pill pick-up of drugs, retention in care, pharmacy stock-outs, pharmacy dispensing practice and virological suppression are major factors favoring HIVDR emergence [12]. This has limited ARV treatment responses to first-line ART among HIV infected persons and increases the risk of treatment failure. Consequently, the development and transmission of drug-resistant viruses have become a major concern with the scaling up of ART [13, 14].

Although TDR mutations have been studied extensively in high-income countries such as France, United States, and Denmark [13, 14], there is still a paucity of data in Cameroon especially in the NWR.

In Cameroon, studies have shown that there is increasing prevalence of TDR over time and ranging from 1 to 10.4% [15-17]. Nevertheless, these studies were designed only for a particular locality like Centre, and Littoral regions. As such with the rapid expansion of the ART program in Cameroon, the success of ART programs will require drug resistance genotyping before drug administration. Therefore, with limited resources and facilities to implement proper care and monitoring of HIV patients, Cameroon may face increasing rates of HIV-1 TDR in the near future [11]. It is, therefore, necessary to understand the rate of resistant HIV-1 variants to ARV regimens in drug naïve patients prior to drug administration.

This study was set up to evaluate the prevalence of TDR mutations, among drug-naïve individuals seeking HIV care in the North West Region of Cameroon. Data from this study is expected to guide the policy makers on the current status of first line drugs being used for treatment in the studied region.

2. MATERIALS AND METHODS

2.1 Study Population

A total of one hundred individuals were consented and enrolled into this study from HIV comprehensive clinics in the northern region of Cameroon. The sampled participants were recruited from both urban and rural settings from AIDS Treatment Centres of; Bamenda, Bali, Bafut, Santa and Ndop Health districts.

2.2 Study Design

A cross-sectional study design was used and five ART clinics; Bafut, Bali, Ndop, and Santa District

Hospitals and Bamenda Regional hospital of the Northwest Region of Cameroon were randomly sampled. Pretested structured questionnaire was use to collect demographic and clinical information from February 2016 to April 2016. The sampled sites better represent the different backgrounds of individuals from the entire NWR of Cameroon. Bafut, Santa, Bali, and Ndop are in rural areas while Bamenda is from urban settings.

2.3 Ethical Statement

This study commenced after obtaining ethics approval from National Ethics Committee of Cameroon with reference number N°2016/01/689/CE/CNERSH/SP. Written informed consent was provided by each study participant prior to sample collection.

2.4 Sample Collection

Five-millilitres of venous blood samples and demographic information data were collected from consenting participants and analysed at Centre International de Reference Chantal Biya (CIRCB) Yaoundé.

2.4.1 $CD4^+$ T cell counts

The CD4+ T lymphocyte count was determined by flow cytometry using Fluorescence Activated Cell Sorting (FACS) Count Analyzer with the BD FACS Count tri CD4/CD8/CD3 reagent kit (Becton Dickson, Beiersdorf, Germany) equipped with automated acquisition and analysis software according to the manufacturer's instructions [18].

2.4.2 Viral RNA extraction cDNA synthesis

HIV-1 Viral RNA was extracted from 1 ml plasma using the QIAamp Viral RNA Mini kit (Qiagen, Valencia, California). The protease-reverse transcriptase region was then reverse transcribed and amplified using the One-Step RT-PCR system with Platinum® Taq High Fidelity enzyme, and super mix high fidelity enzyme for the second PCR (Invitrogen: Carlsbad, USA*).*

The RT_PCR (cDNA synthesis) was performed using the 10 µM BS primer [5'GAC AGG CTA ATT TTT TAG GG 3' located at 2075-2094 *gag* of HXB2] as the forward primer and 10 µM GIO_2 [5' TTT CCC CAT ATT ACT ATG CTT3'located at 3683-3703 bp of HXB2] as the reverse primer under the following thermo cycling conditions: Initial denaturation: (1 cycle, 50°C for 30mins), denaturation: (1 cycle, 94 °C for 2mins), amplification: 40 cycles (consisting of denaturation: 95 °C for 30s, annealing: 52 °C for 30s, extension: 72 °C for 1mins), final elongation: 1 cycle 72 °C for 10mins using an AB 2720 thermocycler (Applied Biosystems, California, USA). The RT-PCR was regularly launched with a positive and a negative PCR control [19].

2.4.3 Nested PCR

HIV-*pol* region corresponding to 2075-3583 nucleotides in HIV-1 HXB2 was amplified in a nested-PCR. with 3 µl amplicons from the RT-PCR in a reaction containing; 45 µl Platinum™ PCR supermix high fidelity (H_2O, Buffer TAQ [10X], $MgCl_2$ [25 mM], dNTPs [12.5 mM], TAQ Gold) (Invitrogen, USA), 1.5 µl sense primer (10 µM stock; BS primer, 1.5 µl antisense primer (10 µM stock; TAK 3{5' GGC TCT TGA TAA ATT TGA TAT GT 3'} located at 3561-3583 pol) as a reverse primer.

The Nested PCR was run under the following conditions; Initial denaturation and consisted of the following cycles: denaturation: (1 cycle, 93°C for 12 mins), amplification: 40 cycles (denaturation: 94°C for 30s, annealing: 53°C for 45s, extension: 72°C for 2 mins), final elongation: 1 cycle 72°C for 10mins, 1 cycle 4^0C for 30 mins and 10^0C for infinity. The expected cDNA is about 1510 bp (position 2075 [gag] to 3583 [pol]) in length. For each reaction, positive and negative controls were used to ensure the effectiveness of the reaction and the absence of contamination respectively. The reaction was run in the AB 2720 thermocycler (Applied Biosystems, California, USA).

The PCR amplifications were confirmed by visualization with ethidium bromide staining of the gel. The positive generated amplicons were directly sequenced using primers in nested PCR by labeling the amplicons using Big Dye technology on ABI 3130 genetic analyzer (Applied Biosystems, Foster City, USA). 5' AGC AGA CCA GAG CCA ACA GC 3' (2140-2159 *gag*), 5' CCA TCC ATT CCT GGC TTT AAT 3' (2582-2602 pol), 5' CAG GAA TGG ATG GCC CAA AA3' (2590-2609 pol), 5' AGC AGA CCA GAG CCA ACA GC 3' (2140-2159 gag), 5' TTG TAC AGA AAT GGA AAA GGA AGG 3' (2660-2683 pol), 5' CCC TGT GGA AAG CAC ATT GTA 3' (2985-3004*pol* with an insertion) 5' GCT TCC ACA GGG ATG GAA A 3' (2993-3011 pol), 5' CTA TTA AGT CTT TTG ATG GGT CA 3' (3506-3528 pol), and 5' GGC TCT TGA TAA ATT

TGA TAT GT 3'(3561-3583 pol). These positions are given referring to the HXB2 strain from Los Alamos National Laboratory database [20].

2.4.4 Genotypic drug resistance analysis

The generated sequences were analyzed for HIV-1 drug associated resistance mutations using a calibrated Population Resistance (CPR) Tool and Stanford University HIV database genotypic resistance interpretation algorithms [21] and confirmed by consensus mutation figures of the International AIDS Society-USA. In addition, the reliability of each TDR mutation (or surveillance drug resistance mutation - SDRM) was also examined to rule out APOBEC-mediated hypermutation artifact.

2.4.5 Phylogenetic analysis

Generated sequences were aligned using CLUSTAl W version 1.8.3 with subsequent inspection and manual modification with pair wise evolutionary distances estimated by Kimura's two-parameter method with bootstrap analysis of 1000 replicates. Bootstrap resampling (1000 data sets) of multiple alignments was performed to test the statistical robustness of the trees. Viral recombinants were confirmed using recombinant identification program (RIP) subtyping tool available at [22].

2.5 Data Analysis

Statistical analysis was done using SPSS version 23 SPSS Inc. Chicago, IL, USA). Baseline characteristics of study patients were described using standard descriptive statistics (frequency and percentages). Associations between the CPR tool and the Stanford University HIV database was assessed using the Chi- square test for categorical variables and a p-value <0.05 was considered significant.

3. RESULTS

3.1 Study Populations

Of the 100 patients enrolled into the study, a total of 81(81%) samples were successfully sequenced. Of the 81 study participants, 45(55.6%) were female and 36 (44.4%) male with their ages ranging between 18 and 61 years old and an average age of 36.9 (±1.03) years. The $CD4^+$ T cell count was ranged between 8–498 cells/ mm^3 with mean (±SEM) $CD4^+$ T cell count of 194.3 (±15.01) cells/ mm^3 with slight average of them 59.3 % (48) having CD4 count of <200 cells/ mm^3. Most of the participants resided in urban settings 54.3% (47) and 48.1% (39) was classified as WHO stage 2 of AIDS (Table 1).

3.2 Drug Resistance Mutations

Overall the prevalence of TDR was 11.1 % (9/81). Drug associated mutations conferring resistance to NRTI, NNRTI and PI were detected in 8.6% (7/81), 4.9% (4/81) and 1(1.2%) patients respectively. The most commonly occurring mutations were K219Q (2.5%; 2/81) and E138A (2.5%; 2/81) which confers resistance to NRTIs and NNRTIs respectively.

The most commonly occurring mutations were K219Q (2.5%; 2/81) and E138A (2.5%; 2/81) which confers resistance to NRTIs and NNRTIs respectively. The NRTI DRMs included K65E, M184MVand the following thymidine analogue mutations (TAMs): M41ML, D67N, K70T/R, T215TA/F and K219Q.

The TAMs were identified in 6.2% (5/81) patients. Singleton mutations associated with NNRTI mutations were A98G, K103N, V108I, V179E, and Y181C. Among the DRM; T215F, M184V, K65E, and K103N, Y181C resistant mutations that confer high resistance to NRTI and NNRTI respectively were also recorded. Also, dual-class DRM involving NRTI and NNRTI was observed in three patients (3.7%; 3/81). One participant (1.2%) presented with a PI mutation which includes the I54IFV mutation (Table 2).

Assessing transmitted drug resistance (TDR) using the CPR tool showed that four of the 81 HIV-1 RT sequences (4.9%) had TDR mutations. These were K219Q, M184V, D67N, K70R, T215F, M41L of NNRTI, and K103N and Y181C of NRTI, and none in the protease region (Table 2).

3.3 Prevalence of TDR among Study Participants

The prevalence of TRD was high among individual from Urban area 5(11.4%), males 5(13.9%), participant of age <30years 3(17.6%), those with CD4 count <200 cells/ µL 6(12.5%) and those who presented with WHO stage 2, 5(12.8%). However, these differences were not significant (p>0.05) Table 3.

Table 1. Descriptive characteristics of study participants (n=81)

Indicator	Variable (n)	Female (%) n=45	Male (%) n=36
Site	Rural (37)	24(53.3)	13 (36.1)
	Urban (44)	21(46.7)	23 (63.9)
Age in years	Mean ± SEM	36.31±1.41	37.39±1.51
	Range	18-56	20-61
	<30 (17)	12 (70.6)	5 (29.4)
	30-40 (37)	18 (48.8)	19(51.2)
	>40 (27)	15 (55.6)	12(44.4)
CD4 Classification cells/ µL	Mean ± SEM	207.36±21.27	177.97±20.84
	Range	8-489	31-498
	<200 (48)	23 (49.9)	25 (52.1)
	200-350 (19)	13 (68.4)	6 (31.6)
	350-500 (14)	9 (64.3)	5 (35.7)
WHO stage	1 (10)	5 (50.0)	5 (50.0)
	2 (39)	18 (46.2)	21 (53.8)
	3 (28)	19 (67.9)	9 (32.1)
	4 (4)	3 (75)	1 (25)

3.4 HIV Subtypes

Phylogenetic analysis of the 81 sequences revealed a high HIV-1 group M isolates genetic diversity with four subtypes and five CRFs. The subtypes were F2 (6: 7.4%), D (6:7.4%), G (4: 4.9%), A1 (1: 1.2%) and the CRF were CRF02_AG (59: 72.8%), CRF22_01A1 (2: 2.4%), CRF06_cpx (1: 1.2%), CRF09_cpx (1: 1.2%), and CRF11_cpx (1: 1.2%). From this study CRF02_AG remains the most predominant circulating HIV-1 strains in Cameroon. TDR was insignificantly (x^2= 1.004, p=0.316) high in patient harboring CRF02_AG (13.1%) than those harboring Non-CRF02_AG 1(5.1%).

4. DISCUSSION

The prevalence of TDR was 11.1% and a total of 1.2%, 8.6 %, 4.9, and 3.7 % individuals had a major mutation to protease inhibitors, NRTIs, NNRTIs, and both NRTIs and NNRTIs respectively.

The present study detected moderated levels (11.1 %) of TDR which was higher than the 7.3% and 7.55% recorded in the Centre region of Cameroon [7, 11] and10.4 % from rural and urban towns in Cameroon [23]. However, it was lower than the 13.9% from the South west region [24], and 18.8% from rural villages in Cameroon [17]. In addition TDR rates ranging from 4.9%-8% has also been recorded in children [15, 23] in pregnant women [25] from Cameroon. These observations could be associated with the continuous scale-up of ART, pre-treatment drug resistance, poor treatment adherence and low rate of patient retention in care that led to the transmission of resistant HIV strains [7, 8].

We reported a higher prevalence of NRTIs which concurs with previous studies conducted elsewhere [14, 26]. However, these findings are contrarily to those previous obtained in Cameroon especially in urban settings of Cameroon where NNRTIs has been highly reported with high frequency [8, 16, 23]. The high prevalence of NRTIs seen in this study is most probably due to the change in treatment guideline from ARV monotherapy to HAART since 2012 [5].

The NRTIs mutation K219Q that confers resistance to AZT, D4T, AZT, D4T, DDI [26, 27] was the most predominant NRTI mutation was detected. The detected high frequency of K219Q as well as M184V mutation could be assocciated with transmission of a resistant viral strain from individual failing AZT, D4T, and DDI regimens [7, 28, 29]. Similarly, E138A (2.5%) was the most prevalent NNRTI mutation conferring resistance to etravirine and rilpivirine which are drugs not currently being used in Cameroon [5].

Contrary to several reports from Cameroon we found no K65R mutation which explain the high frequency of TAMs observed in our study [7, 8, 23]. The high prevalence of TAMs (6.2%) may be explained by the extensive use of thymidine analogues (AZT and d4T) since 2002 in our settings. TAMs are associated with inducing resistance to almost all NRTIs but the degree to

which the resistance is observed depends on the specific mutations and the number of mutations involved in the particular individual viral strain. The detected TAMs mutations seen in this study can lead to cross-resistance across NRTI analogues hence compromising first-line therapy [7, 27, 28].

The detected 4.9% (4/81) prevalence of NNRTIs mutations were found to be below 10% similar to what has been reported in other studies in Cameroon [16, 23, 29]. The K103N mutation which is known to confer cross resistance to the entire class of NNRTIs was also detected in the current study [26, 27, 29]. This implies that, treatment of the infected individual with this viral strain could pose a challenge. We recorded E138A (2.5%; 2/81) as the most common NNRTI mutations. E138A mutation is weakly selected in patients receiving second-generation NNRTIs (ETR and RPV) which are drugs not being used in Cameroon as such use of NVP and EVP remains efficient [5,30]. However, this high prevalence of E138A may limit the role of RPV or ETR based ART in patients who develop resistance to first-generation NNRTIs [27, 30]. As such with increasing frequency of E138A TDR mutations, RPV or ETR antiretroviral drugs may become less suitable as salvage therapy.

The low PI-mutation (1.2%) recorded in this study is consistent with reports from other countries in Central and West Africa [16, 31, 32, 33]. The low prevalence of PI mutations is because first-line ART in Cameroon is a combination of two NRTIs plus one NNRTI as such only a few patients are being treated with PIs [32, 33]. Secondly, studies have shown that PI mutations do not exist as natural polymorphisms or are not easily transmitted as compared to NNRTI and NRTI [26, 34]. Even though PI-containing regimens of HAART are recommended as first-line treatment in rare cases, the use of PI containing PrEP/PEP and PI-based regimens as first-line protocol in HIV-infected infants [5] requires close follow up.

Although the prevalence of TDR resistance is moderate (5-15%) in this study, the use of ARV in PMTCT and the prevention of new HIV-1 infections depicts a public health problem. In line with the WHO 2012 PMTCT guidelines, which recommend the initiation of lifelong combination ART (cART) for all pregnant and breastfeeding women living with HIV-1 regardless of CD4 count or WHO clinical stage, the mutations seen in this study were found to confer possible resistance to pediatric HAART options (AZT, 3TC, ABC/ EFV, NVP /LPV/r) [5]. As such optimal policies need to be put in place to prevent the spread of these DR mutant viruses so as to curb the rate of treatment failure and mortality in children.

Taken into consideration that the mutations **(**D67N, K70T, K219Q, T215F, M184V/I) seen in this study can also affect the current PrEP/PEP protocol (AZT + 3TC + LPV/r or TDF + 3TC or FTC + LPV/r) [2, 5] the use of these drugs on a larger scale, will decrease the effectiveness of these regimens at the level of prevention and treatment. Thus DRM genotyping is necessary before drug initiation.

In this study, most of the TDR mutations were associated with predicted resistance level to DDI followed by D4T. This accounts for the reason why these drugs are no longer recommended for use in our setting [5]. The likely explanation for the high spread of D4T and DDI mutants is due to the suboptimal use of D4T and DDI based therapy at the beginning of ART scale up. Although mutations that causes high-Level resistance (M184MV/M184V associated with 3TC and FTC; Y181C associated with NVP and K103N associated with NVP and EFV) were identified in the study, the use of first line treatment regimen is still very effective in the NWR.

Therefore, HIV resistance test before initiation of HAART will ensure maximum efficiency of HAART that will avoid extra costs by maximizes the opportunity for successful second-line with reduced morbidity and mortality. Secondly, it will also limit the transmission of drug-resistant viruses. These studies suggest that the price of resistance testing be subsides and made affordable such that resistance testing should become part of routine HIV diagnostics.

The associations of TDR distribution with other variables were not statistically significant similar to what was reported by Onywera et al., [34]. The absence of association seen in this study may be a function of sample size rather than lack of association.

Several bioinformatics tools are available for interpretation of HIV drug resistance levels which show significant differences in the output data and results.

Table 2. Prediction of patient-level HIV-1 drug resistance versus population-level transmitted drug resistance

The serial number of patient	Study Site	WHO Clinical stage	HIV-1 Subtype	ARV Drug Class	Type of mutation		*Resistance-associated Mutation predicted ARV drug resistance
					Stanford HIV Drug Resistance db mutations	(CPR) mutations	
1	Urban	4	CRF02_AG	NRTI	K219Q	K219Q	AZT[a] , D4T[a]
2	Rural	2	CRF02_AG	NRTI	T215TA	None	AZT [b], D4T[b], DDI[a]
3	Urban	2	CRF02_AG	NRTI	M184MV	M184V	ABC [b], DDI[b], FTC[d], 3TC[d]
				NNRTI	K103N	K103N	EFV[d], NVP[d]
4	Urban	2	CRF02_AG	NNRTI	E138A	None	ETR[a], RPV [b]
5	Urban	2	CRF02_AG	NRTI	K70T	None	ABC[b], D4T[b], TDF[b], DDI [b], FTC[a], 3TC[a]
				NNRTI	E138A		ETR[a], RPV [b]
6	Rural	3	CRF22_01A1	PI	I54IFV	None	ATV/r[b], LPV/r[b]
7	Urban	4	CRF02_AG	NRTI	D67N, K70R, M184V, T215F, K219Q	D67N, K70R, M184V, T215F, K219Q	AZT[d]ABC[d], D4T[d], TDF[c],FTC[d] 3TC[d]DDI[d]
				NNRTI	A98G, V108I, V179E, Y181C	Y181C	
8	Rural	3	CRF02_AG	NRTI	M41ML	None	AZT[b], D4T[b],DDI[c]
9	Rural	2	CRF02_AG	NRTI	K65E	None	D4T[a],TDF[a],DDI[a]

**Stanford HIV DR Db analysis* $x^2 = 33.66$, $p=0.0001$

Legend

NRTI: Nucleoside reverse transcriptase inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; ABC: abacavir; FTC: emtricitabine; 3TC: lamivudine; TDF: tenofovir; AZT: zidovudine; D4T: stavudine; DDI: didanosine; EFV: efavirenz; ETR: etravirine; NVP: Nevirapine; RPV: rilpivirine; ATV/r: Boosted atazanavir; LPV/r: lopinavir/ritonavir

Predicted Drug Response (Stanford HIV Drug Resistance db Tool) a: Potential low-level resistance (mutation net drug score (10–14), b: Low-level resistance (mutation net drug score of 15–30), c: Intermediate resistance (mutation net drug score of 31–59), d: High-level resistance (mutation net drug score of ≥60)

Table 3. Prevalence of TDR among study participants

Indicator	Variable (n)	Transmitted drug resistance		Statistical analyses	
		No (%)	Yes (%)	x^2	P value
Site	Rural (37)	33(89.2)	4(10.8)	0.006	0.937
	Urban (44)	39 (88.6)	5 (11.4)		
Sex	Female (45)	41(91.1)	4 (8.9)	0.506	0.477
	Male(36)	31(86.1)	5 (13.9)		
Age in years	Mean ± SEM	37.0±1.10	36.33±2.9	*0.401	0.84
	Range	18-61	26-52		
	<30 (17)	14 (82.4)	3(17.6)	1.114	0.573
	30-40 (37)	33(89.2)	4(10.8)		
	>40 (27)	25 (92.6)	2(7.4)		
CD4 Classification cells/µL	Mean ± SEM	207.36±21.27	177.97±20.84	*0.462	0.499
	Range	8-489	8-498		
	<200 (48)	42 (87.5)	6 (12.5)	0.324	0.85
	200-350 (19)	17 (89.5)	2 (10.5)		
	350-500 (14)	13(92.9)	1 (7.1)		
WHO stage	1 (10)	9(90.0)	1(10.0)	1.356	0.716
	2 (39)	34 (87.2)	5 (12.8)		
	3 (28)	26 (92.9)	2 (7.1)		
	4 (4)	3 (75)	1 (25)		

** represent F test*

Analysis of our results throws more light on two very important definitions that is CRP (epidemiologic) and clinical relevance (individual level) of RAMs. From this study, a TDR rate of 4.9% (4/81) was reported using CRP and 11.1% (9/81) using Stanford. Similar discrepancies have been recorded in Cameroon [12] and in USA [31]. This differences might jeopardize future treatments and thus explains why caution should be taken in interpreting genotypic HIV DR results and to seek expert opinion in selecting optimal ARV combinations of the available drugs. Thus it is required that thorough virological monitoring be carried out during treatment.

Though drug resistance patterns did not vary significantly across CD4 class and WHO stage, higher TDR prevalence was seen in participants with CD4 count<200 cells/ µl (12.5%) and in those presenting with WHO stage 4. It may be associated to the fact that mutant variants decline more frequently in relation to more fit wild type viruses over time [35]. Similar results has been reported among patients failing treatment [36, 37]. However these finding requires further investigation.

It is likely that the majority of the subjects in this study had been infected for years as subjects had to have a CD4 counts below 350 cells/mm3 to receive ART through the national China CARES program during this time period. Given that these subjects were likely chronically infected (the majority had CD4 counts<200 cells/mm^3) prior to presenting for ART initiation.

Molecular phylogenetic analysis confirmed CRF02_AG has consistently been found to be the most predominant in the cities and rural villages of Cameroon [7, 19, 33]. This high suggests that this viral strain may be well adapted in the Cameroonian population due to a founder effect from the parent strains subtype A and G and as such may be used as a vaccine candidate to design of an effective vaccine. The presence of recombinant forms requires full-length genome sequencing to assess the extent of HIV-1 diversity in our study settings.

5. CONCLUSIONS

The prevalence of HIV-1 TDR in Northern region of Cameroon was at moderate level (11.1%) of transmitted drug resistance despite the provision of HIV-related services. Further, surveillance of TDR is required in this region. Therefore, there is a need to emphasize continuous HIVDR surveillance, treatment adherence, and monitoring of viral transmission in Cameroon.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request. The DNA sequences of the HIV-1 protease-reverse

transcriptase sequences that were determined in this study were submitted to Gen Bank under the following accession numbers: MK061035-MK061115.

CONSENT

Participant's consent to participate in the study also included consent for the data to be published without the identification of personal names or contacts.

ETHICS APPROVAL AND CONSENT

This study protocol was reviewed and approved by all Health Institute Review Boards and the National Ethics Committee of Cameroon (N^{O}2016/01/689/CF/CNERSH/SP), and all patients gave their written informed consent for participation in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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