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Original Article

High performance of integrase genotyping on diverse HIV-1 clades circulating in Cameroon: toward a successful transition to dolutegravir-based regimens in low and middle-income countries

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ABSTRACT

A successful transition to dolutegravir-based regimens in low and middle-income countries (LMICs) requires an integrase genotyping assay effective on diverse HIV-1 clades. We herein developed and validated an in-house integrase genotyping protocol on plasma samples from 195 HIV-infected patients in Cameroon. Median [IQR] viremia was 23,574 (518-109,235) copies/mL; 128/195 participants had ≥1000copies/mL (i.e., WHO-threshold for genotypic resistance testing in LMICs). A total of 18 viral clades were detected: 72(51.1%) CRF02_AG, 38(26.9%) pure subtypes and 31(22.0%) other recombinants. Following WHO-threshold (≥1000copies/ml), sequencing performance was 82.81%(106/128). Regarding viremia, performance was 85.00%(68/80) with ≥100,000copies/mL versus 76.67%(23/30) with 10,000 to 99,999copies/mL ($P = 0.22$); 83.33%(15/18) with 1,000 to 99,999copies/mL ($P = 0.55$); 73.68%(14/19) with 500 to 999copies/mL ($P = 0.19$); 50%(13/26) for 200 to 499copies/mL ($P = 0.0005$) and 36.36%(8/22) for <200copies/mL ($P < 0.0001$). The developed in-house integrase-genotyping is highly effective on both pure and recombinant viral clades, even at low-level viremia. This performance underscores its usefulness in monitoring integrase-resistance mutations and supporting the scale-up of dolutegravir-based regimens in LMICs.

1. Introduction

Despite considerable progress in terms of antiretroviral-coverage worldwide (which led to a decrease in HIV-related death), there is an increasing burden of HIV drug resistance (HIVDR) in low and middle income countries (LMICs) (Phillips et al., 2017, Inzaule et al., 2016, Bessong and Nwobegahay, Mar. 2013, World Health Organization, 2017), with an overall rate of pretreatment drug resistance above the threshold of 10% for non−nucleoside reverse transcriptase inhibitors (NNRTIs) (World Health Organization, 2017, Gupta et al., 2018, Organization, 2018). This critical threshold suggests a substantial reduction in the efficacy of first-line ART, which prompts the need for new, and more potent drug combinations (Gupta et al., 2018, Organization, 2018). To address this public health threat, the World Health Organization (WHO) recommended transitioning from first-generation NNRTI-based to dolutegravir (DTG)-based regimens as preferred first-line antiretroviral therapy

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(ART) and as a possible alternative in managing cases of multi-resistance in LMICs (World Health Organization, 2017, Organization, 2018, World Health Organization 2016, Wainberg et al., 2016, Inzaule et al., 2019, Hauser et al., 2020, Mikasi et al., 2020). According to WHO updated recommendations, a regimen with 2 nucleoside reverse-transcriptase inhibitors (NRTIs) paired with DTG is more effective, with a higher viral suppression, a better CD4 cell count recovery and a lower risk of treatment discontinuation compared with efavirenz-based regimens among treatment-naive adults (Organization, 2018). DTG also has better efficacy at suppressing viral loads than other integrase strand transfer inhibitors (INSTI), greater potency, high genetic barrier to resistance (Organization, 2018, Vitoria et al., 2018) and is currently the most affordable regimen (Organization, 2018, World Health Organization 2017, Phillips et al., 2018b, Phillips et al., 2018a, Dorward and Hamers, Feb. 2019). In addition to its high genetic barrier, the prevalence of DTG resistance-associated mutations among INSTI-naïve patients is low in LMICs, and especially within sub-Saharan Africa (SSA) where several viral clades have been found (Bessong and Nwobegahay, Mar. 2013, Ndashimye et al., 2018, Ndashimye, 2017, Mboumba Bouassa et al., 2019, Inzaule et al., 2018, Digban et al., 2019, Brado et al., 2018, El Bouzidi et al., 2020, Mabeya et al., Jan. 2020, Semengue et al., 2021). In this context, using optimal strategies for preventing, monitoring and responding to HIVDR is of paramount importance to maintaining current achievements, improving treatment outcomes for people living with HIV, protecting investments, and guaranteeing the long-term sustainability of care, and treatment programs (World Health Organization, 2017). An essential component in meeting the aforementioned goals is the use of genotyping to guide on the selection of appropriate ART both for policy-making and for individualized clinical management. Of note, genotypic resistance testing (GRT) appears as a suitable tool for HIVDR (World Health Organization, 2017) as it involves sequencing the relevant parts of the virus genome and interpreting the sequence with respect to the resistance phenotype of the virus (Santoro and Perno, 2016, Collier et al., 2019, World Health Organization (WHO) 2018).

For more than a decade, HIV personalized medicine in high-income settings has paved the way for GRT in all drug targets including the integrase gene (Armenia et al., 2014). This approach has been proven to treat each HIV-patient according to the viral strain found in the plasma, in order to adjust drug dosage according to genotypic profile, and to prompt a rapid/sustained viral control (Santoro and Perno, 2016, Mu et al., 2018, Lengauer et al., Mar. 2014). In the frame of the ongoing transition to integrase inhibitor-based regimens in LMICs, integrase-genotyping is becoming more, and more essential for appropriate management of HIV-infection (Inzaule et al., 2016, Mikasi et al., 2020). Of note, the reduced efficacy of INSTI in patients treated with NNRTI-based regimens, underscores the need for close monitoring of DTG-efficacy among those with long therapeutic history in SSA where multiple viral clades are circulating (Siedner et al., 2020, Seatla et al., 2019). Unfortunately, no integrase-genotyping assays issued up to date have been recommended in LMICs for the monitoring of INSTI-based ART in clinical settings. Furthermore, the few developed assays cover a limited number of viral clades, which henceforth weakens their broader utility (Seatla et al., 2019, Nyamache et al., 2012).

With the goal to improve the response to ART while scaling-up DTG-based regimens in LMICs, we sought to develop, and validate an in-house integrase-genotyping assay that could be highly sensitive on a wide range of circulating HIV-1 clades for the routine surveillance of integrase resistance-associated mutations. For a real-life clinical application, we considered a wide range of HIV-1 plasma viral loads to define a reasonable threshold for ensuring an acceptable INSTI genotyping strategy in LMICs as a whole (Fig 1, Fig 2).

2. Materials and methods

2.1. Study design

A cross-sectional study was carried out among HIV-infected patients seen at the Virology Laboratory of the Chantal BIYA International Reference Centre for research on HIV/AIDS prevention and management (CIRCB) in Yaoundé-Cameroon, from February 2020 to January 2021. These patients were visiting the facility for their routine monitoring of HIV-1 protease/reverse transcriptase drug resistance mutations, regardless of WHO clinical staging.

2.2. Ethical considerations

Administrative authorization was received from the Directorate General of CIRCB (N°0191/019L/CIRCB/DG/SAA/BRH); ethics approval was obtained from the Cameroon National Ethics Committee for research on human health (2019/06/34/CE/CNERSH/SP). Written informed consent (proxy-consent by the parent/legal guardian for children/adolescents) was obtained from each participant; data were protected using specific identifiers for the purpose of confidentiality and stored in a password encrypted computer; all laboratory results were freely returned to participants for possible benefit in their personal clinical management.

2.3. Study population and procedures

Following informed consent, participants were enrolled among those who came for routine monitoring of protease/reverse transcriptase drug resistance mutations. Age, gender, and ART history data (treatment initiation date, treatment regimens, ART duration, and reasons for switch) were extracted from medical records.

2.4. HIV-1 RNA viral load

Plasma viremia was determined using the Abbott Real Time HIV-1 (Chicago, Illinois); this assay quantifies HIV-1 RNA over the range of 40 to 10,000,000 copies/mL.

2.5. Genotyping method

Briefly, our in-house assay was as follows; Viral RNA was first extracted from 1 mL of plasma aliquots. These aliquots were centrifuged at 20000 x g at 4°C for 120 minutes to harvest an adequate viral pellet. Viral RNA extraction was performed using the QIAamp Viral RNA mini kit (Qiagen, Milan, Italy), as per the manufacturer's protocol. Viral RNA was then retro-transcribed and amplified using the kit One-Step Invitrogen (SuperScript® One-Step for long templates RT/PCR; Foster City, CA) and 2 different sequence-specific primers «GGACATATCAAATTTATCAAGA» as the forward, located at 3559 to 3580 (pol), and «CTAGTGGGATGTGTACTTCT» as the reverse, located at 5199 to 5218 (vif) (the given position refers to the HXB2 strain of HIV-1). The RT-PCR reaction contained for each sample 25 μL reaction mix, 8 μL MgSO4 (5 mM), 3 μL DNAse- and RNAse-free water, 0.75 μL of each primer (10μM stock), 1 μL RNAse-OUT (5 U/ μL Invitrogen), 1.5 μL RT-Taq (Superscript III RT/Platinum high fidelity enzyme) and 10 μL of extracted RNA. The RT-PCR conditions consisted of an initial step of 1 cycle at 50° for 30 minutes, 1 cycle at 94°C for 2 minutes; 40 cycles (95°C, 30 seconds; 51°C, 30 seconds; 72°C, 2 minutes and 30 seconds); 1 cycle at 72°C for 10 minutes; 1 cycle at 4°C for 30 minutes and 1 cycle at 10°C forever. Amplification results were revealed after agarose-gel electrophoresis and positive results were kept for the sequencing process. In case of negative results, a second round PCR (nested PCR) was performed with another set of 2 primers «TTGGAGGAAATGAACAAGTAGA», as the forward, located at 4174 to 4195 (pol), and «GTGGGATGTGTACTTCTGAAC» as the reverse,

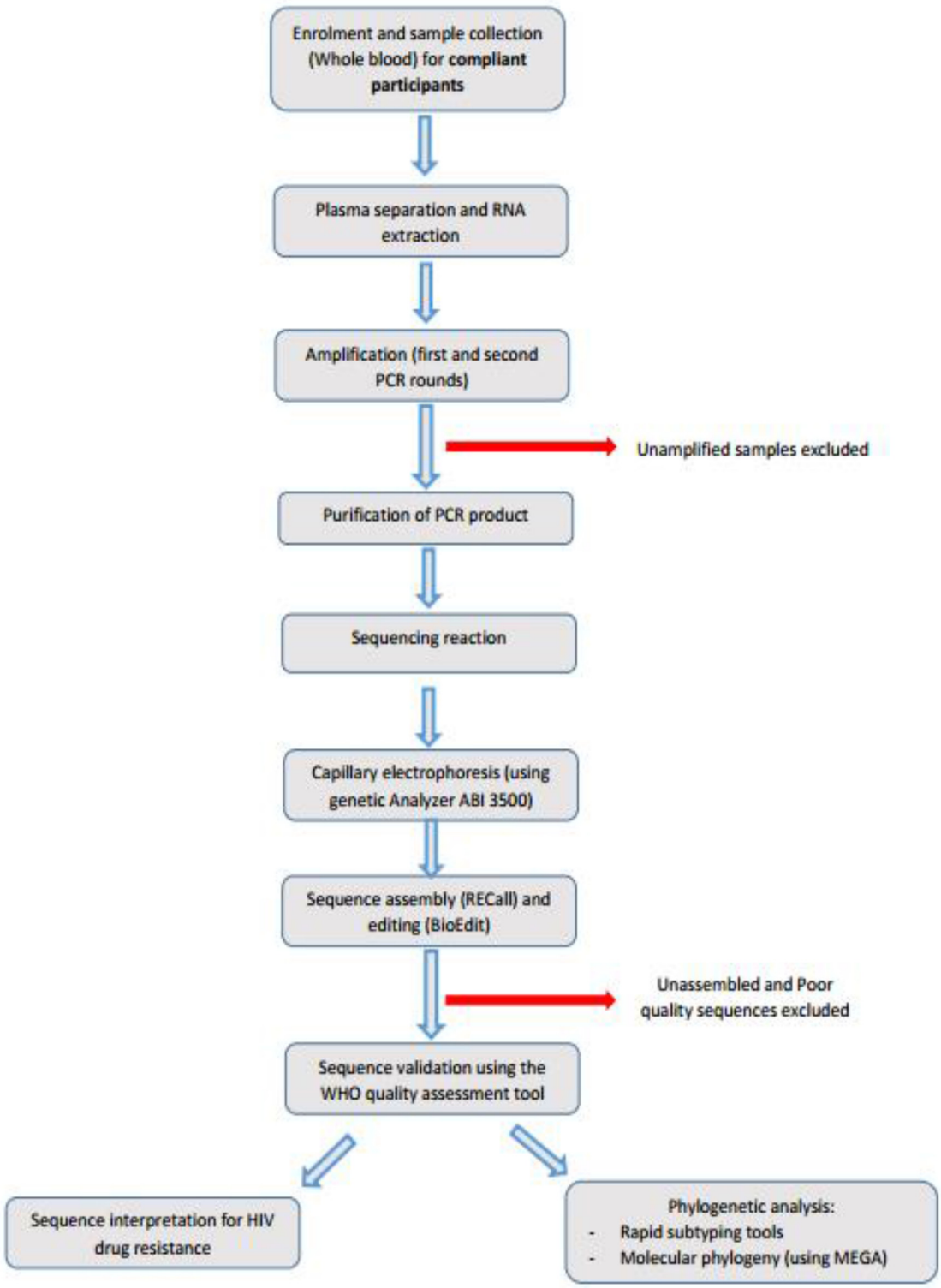

Fig. 1. General description of the genotyping pipeline for HIV-Integrase at the CIRCBs virology laboratory – Yaoundé, Cameroon.

located at 5195 to 5215 (vif). The nested PCR contained for each sample 32.5 μL DNAse- and RNAse-free water, 5 μL 10x PCR buffer (Applied Biosystems^{TM}), 4 μL MgCl2 (25 mM), 1 μL dNTPs (10 mM), 0.9 μL of each primer (10μM stock), 0.7 μL AmpliTaq Gold (5 U/ μL) (Applied Biosystems) and 5 μL of cDNA. Reaction conditions consisted of 1 cycle at 93°C for 12 minutes; 40 cycles (94°C, 30 seconds; 56°C, 30 seconds; 72°C, 1 minutes, and 20 seconds); 1 cycle at 72°C for 10 minutes; and 1 cycle at 4°C forever. Again, after agarose-gel electrophoresis, sequencing process was initiated in the case of positive results, whereas negative results, were considered as unamplified samples. For each PCR reaction, positive and negative controls were used to guarantee the efficiency of the reaction and the absence of contamination, respectively. PCR products were purified through the ExoSAP-IT^{TM} kit (Applied Biosystems^{TM}, Lithuania) and then sequenced through direct Sanger-sequencing reaction, which involved all previous forward, and reverse primers:

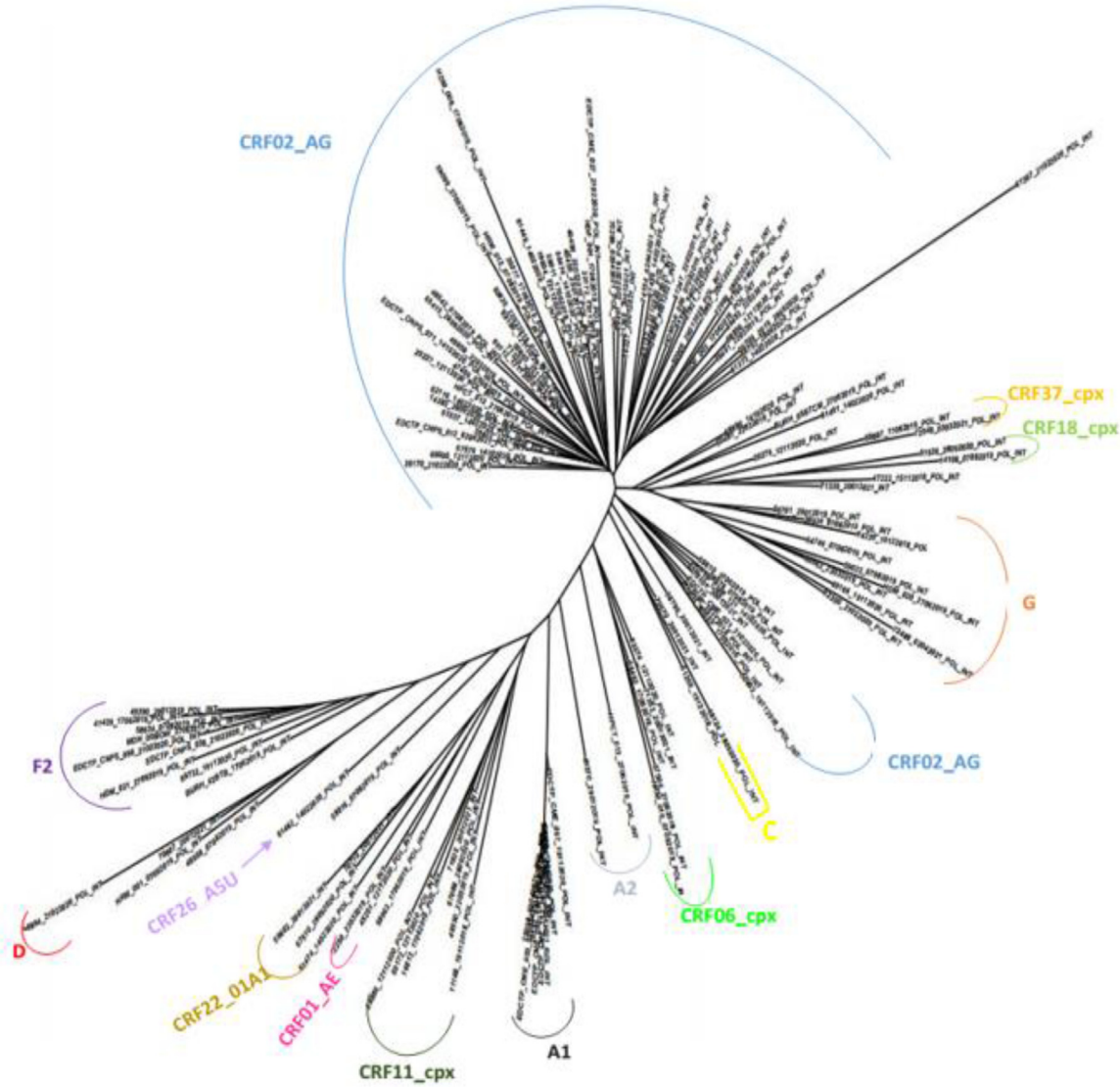

Fig. 2. HIV-1 phylogenetic Tree of the 141 sequences generated at the CIRCBs virology laboratory – Yaoundé, Cameroon. Evolutionary relationship of HIV strains was inferred by means of maximum likelihood method. Reference sequences have been omitted to enable better visualization; the scale bar represents 5% of genetic distance. CRF, circulating recombinant form.

«GGACATATCAAATTTATCAAGA», «CTAGTGGGATGTGTACTTCT», «TTGGAGGAAATGAACAAGTAGA», «GTGGGATGTGTACTTCTGAAC»; the reaction contained for each sample 1.5 μLbig dye terminator v 3.1 cycle sequencing kit (Applied Biosystems^{TM}), 3.2 μLof each primer (1 μM stock), 6.5 μLbig dye diluent (from the kit), 4.8 μLDNAse- and RNAse-free water. Conditions for the reaction were: 1 cycle at 96°C for 1 minute, 35 cycles (96°C, 10 seconds, 55°C, 10 seconds and 60°C 4 minutes) and 1 cycle at 4°C forever. The final product was purified using Sephadex G-50 fine powder. Sequences were obtained after capillary electrophoresis on the 3500 Genetic Analyzer (Applied Biosystems^{TM}, USA) and sequences of at least 864 nucleotides of the integrase region were assembled and manually edited using RECall (CDC, Atlanta, GA) (Woods et al., 2012).

2.6. Sequence analysis

All HIV-1 integrase sequences generated were aligned through BioEdit software version 7.2.6 (Tom Hall, Raleigh, NC) with CLUSTAL W algorithm, and compared with reference sequences for the major HIV-1 subtypes and circulating recombinant forms (CRFs), available in the Los Alamos database (http://www.hiv.lanl.gov), gaps were then removed from the final alignment. Sequences were then compared using the WHO quality control tool following the operational framework recommendations (World Health Organization (WHO) 2020) to ensure good quality sequences were generated. The phylogenetic tree was inferred using maximum likelihood method on the MEGA software v7 for both subtyping and to ensure that there was no cross-contamination of samples. The statistical robustness and reliability of the branching order within the phylogenetic tree were confirmed through bootstrap analysis using 1000 replicates on a maximum likelihood tree obtained by molecular phylogeny. Sequences were then analyzed for interpreting drug resistance mutations (DRMs) using both Stanford HIVdb v9.0 (https://hivdb.stanford.edu/hivdb/by-sequences/; last updated on February 22, 2021 and the IAS-USA 2019 drug resistance mutations list (Wensing et al., 2019) using consensus B (HXB2 from LANL HIV db; Genbank accession n° K03455) as the reference strain for defining DRMs.

2.7. Assay assessment conditions

For assay efficiency, we set the desirable success rate of this integrase-genotyping assay at ≥80% considering the broad HIV-1 genetic

diversity found locally for samples with at least 1.000 copies/mL. This threshold represented our expected efficiency for this assay as we proceeded with a wide range of viral loads and diverse HIV-1 subtype specimens. In case this desirable success rate was not achieved, we set the acceptable genotyping performance for this assay at 60% to 79%, which represents the minimum threshold for acceptable efficiency.

2.8. Statistical analysis

Descriptive statistics were performed for socio-demographic data and biological parameters wherever available. Median and interquartile range (IQR) were reported for continuous variables. The χ^2 test and the Fisher's exact test were used to compare variables where appropriate. *P*-values ≤0.05 were considered statistically significant. All analyses were performed using Epi Info version 7.

3. Results

3.1. Participants' overview

Globally, 195 HIV-infected Cameroonians were successfully enrolled in this study. Participants were aged 4 to 80 years with few children/adolescents enrolled (general median age: 37; IQR: [(Mabeya et al., Jan. 2020, Semengue et al., 2021, Santoro and Perno, 2016, Collier et al., 2019, World Health Organization (WHO) 2018, Armenia et al., 2014, Mu et al., 2018, Lengauer et al., Mar. 2014, Siedner et al., 2020, Seatla et al., 2019, Nyamache et al., 2012, Woods et al., 2012, World Health Organization (WHO) 2020, Wensing et al., 2019, Van Laethem et al., 2015, Tekola-Ayele and Rotimi, 2015, Van Laethem et al., 2008, Fokam et al., 2020aa, Santoro and Perno, 2013, Daar, Jan. 2005, Fokam et al., 2020bb, Low et al., 2009, Garijo et al., 2015)] years) and we counted 58. 9% (115/195) females. Median (IQR) viremia was 23,574 (518-109,235) copies/mL, median (IQR) CD4 cell count was 121 [(Daar, Jan. 2005 −353] cells/mm^3.

3.2. Clinical and biological parameters

As regarding treatment exposure, we counted 14.36% (28/195) ART-naïve participants (viremia >100.000 copies/mL), 34.87% (68/195) failing NNRTI-based regimens, 44.10% (86/195) failing protease inhibitors-based regimens and 6.67% (13/195) participants previously exposed to INSTI. By stratifying for viremia levels, 11.28% (22/195) of participants had viremia <200 copies/mL; 13.33% (26/195) had viremia 200 to 499 copies/mL; 9.74% (19/195) had viremia 500 to 999 copies/mL; 9.23% (18/195) had viremia 1000 to 9999 copies/mL; 15.38% (30/195) had viremia 10,000 to 99,999 copies/mL; 41.03% (80/195) had viremia ≥100,000 copies/mL.

3.3. Assay performance

Out of the 195 plasma samples processed, 35.90% (70/195) were successfully amplified after first round PCR and 63.20% (79/125) after second round PCR; giving an overall 76.41% (149/195) amplification rate. Regarding sequencing, 141 HIV-1 integrase-sequences were successfully generated out of the 149 processed (94.63%), giving an overall genotyping performance 72.31% (141/195). According to viral loads, assay performance was 85.00%(68/80) with ≥100,000copies/mL versus 76.67%(23/30) with 10,000 to 99,999copies/mL ($P = 0.22$); 83.33%(15/18) with 1000 to 99,999copies/mL ($P = 0.55$); 73.68%(14/19) with 500 to 999copies/mL ($P = 0.19$); 50%(13/26) for 200 to 499copies/mL ($P = 0.0005$), and 36.36%(8/22) for <200copies/mL ($P < 0.0001$). Considering the threshold of ≥1,000 copies/mL for clinical application in real-life, the sequencing performance was 82.81% (106/128).

3.4. Sequence quality and HIV-1 genetic diversity

Overall, good quality sequences were generated and we followed WHO operational framework for HIVDR (World Health Organization (WHO) 2020) to confirm there was no cross-contamination during the whole process. As expected, we found a much-diversified molecular epidemiology with 18 different HIV-1 subtypes including both pure subtypes and circulating recombinant forms (CRF). These comprised: CRF02_AG(72), G(11), A1(10), F2(9), CRF11_cpx(8), CRF06_cpx(5), D(4), CRF22_01A1(4), CRF13_cpx(3), A2(2), C(2), CRF01_AE(2), CRF18_cpx(2), CRF37_cpx(2), CRF02_AG/A3(2), CRF26_A5U(1), CRF36_cpx(1), and CRF01_AE/CRF02_AG(1) (Table 1).

3.5. Integrase DRMs

Regarding INSTI-resistance, we found only 1 patient with therapeutic failure after exposure to raltegravir (for 7years) and DTG (for 2 years). At the time of suspicion of DTG-failure, viremia was >100,000 copies/mL and major integrase-DRMs found wereE138KQ, G140A, Q148R and S147G. Notably, 7 other INSTI-unexposed participants were found with accessory DRMs (T97A, E157Q, V151VA) (Table 1). These accessory mutations weren't associated neither to pure subtypes (versus all CRFs; $P = 0.38$) nor to CRF02_AG (versus non−CRF02_AG; $P = 0.22$). Finally, according to viremia, we found a statistical significance between viremia >1000 copies/mL, and the occurrence of accessory mutations ($P = 0.047$).

4. Discussion

As LMICs in general are progressively transitioning to DTG, setting up an accurate, reproducible, highly effective but also affordable assay for monitoring the emergence of integrase-DRMs among patients exposed to INSTI is becoming more and more essential (Siedner et al., 2020). With this context, next generation sequencing (NGS) technology would have been a great asset in LMICs. To the best of our knowledge, there is need to establish a clear consensus for the use of NGS in clinical practice below the threshold of 20% (i.e., current threshold of the Sanger system) due to existing discrepancies in detecting minority variants between different NGS platforms (Mu et al., 2018, Van Laethem et al., 2015). In this frame, routine clinical monitoring through a well-implemented Sanger-sequencing (wherever available or possible) could help in closing the gap and maintaining the strides achieved so far in the prevention and surveillance of DRMs emergence in LMICs (Van Laethem et al., 2015). The current approach is particularly true for Cameroon and several LMICs that encounter challenges in implementing NGS, mainly due to limited bioinformatics pipelines, inadequately equipped research and clinical laboratories, shortage of scientifically skilled personnel, lack of awareness on the importance of genomics to guide public health, and lagging public health policy frameworks (Van Laethem et al., 2015, Tekola-Ayele and Rotimi, 2015).

In this study we developed and validated an in-house assay for integrase genotyping using Sanger-sequencing approach in a context of high HIV-1 genetic diversity, taking into account a wide variety of viral loads. Our in-house integrase genotyping assay was able to amplify 76.41% (149/195) of patients' plasma samples and this rate was achieved due to the use of a sensitive nested PCR. These rates are lower than what reported in Belgium by Laethem K et al. (Van Laethem et al., 2008) and recently in Botswana by Seatla KK et al. (Seatla et al., 2019). These discrepancies may be explained by low sample size and genetic diversity in the study by Laethem K et al. and the other study by Seatla KK et al. was done essentially on HIV-1 subtype C samples, while our assay involved about 18 different HIV-1 subtypes. The clade diversity in this study reflects the real-life HIV-molecular epidemiology in Cameroon (Semengue et al., 2021,

Table 1
Distribution of the genotyping performance according to the viral load, subtype covered, and presence of accessory DRMs.

Viral load (HIV-RNA copies/mL)	Genotyping performance *n/N*, % (95%CI)	Subtypes covered			Presence of accessory drug resistance mutations[a]
		CRF02_AG, *n* (%)	Non CRF02_AG, *n* (%)	Specific subtypes	
≥100,000	68/80, 85.00% (74.67 - 91.79)	37 (54.41%)	31 (45.59%)	A1, A2, C, D, F2, G, CRF01_AE, CRF02_AG, CRF06_cpx, CRF11_cpx, CRF13_cpx, CRF18_cpx, CRF22_01A1, CRF36_cpx, CRF01_AE/CRF02_AG, CRF02_AG/A3.	T97A (1), E157Q (1)
10,000 to 99,999	23/30, 76.67% (57.72 - 90.07)	8 (34.78%)	15 (65.22%)	A2, D, F2, G, CRF02_AG, CRF06_cpx, CRF11_cpx, CRF22_01A1, CRF26_A5U, CRF37_cpx	T97A (2), V151VA (1)
1000 to 9,999	15/18, 83.33% (58.58 - 96.42)	7 (46.67%)	8 (53.33%)	D, F2, CRF01_AE, CRF02_AG, CRF11_cpx, CRF22_01A1, CRF37_cpx	T97A (1), E157Q (2)
500 to 999	14/19, 73.68% (48.80 - 90.85)	9 (64.29%)	5 (35.71%)	A1, CRF02_AG	None
200 to 499	13/26, 50% (29.93 - 70.07)	7 (53.85%)	6 (46.15%)	A1, G, CRF02_AG, CRF11_cpx	None
<200	8/22, 36.36% (17.20 - 59.34)	4 (50%)	4 (50%)	A1, D, CRF02_AG, CRF18_cpx	None

CI = confidence interval
[a] accessory DRMs were found only among INSTI-unexposed with viremia >1000 HIV-RNA copies/mL. The only case of major DRMs has already been documented (Fokam et al., 2020bb) as earlier mentioned.

Fokametal.,2020aa),thereforesupportingtheuseofthisassayforanaccuratemonitoringofINSTI-basedtherapyinLMICsandbeyond,evenincases of patients experiencing early treatment failure with low-level viremia. Also, we successfully sequenced 94.63% (141/149) of amplified samples which is similar to what obtained by both previous authors (Seatla et al., 2019, Van Laethem et al., 2008). Furthermore, performance of this in-house integrase-genotyping was significantly higher as viremia increased, translating increasing primers' affinity with high RNA concentration. Again, Seatla et al. had similar observations with their homebrew protocol as regarding viral loads >1000copies/mL. Armenia et al. (Armeniaetal.,2014)publishedevengreatersuccessratesatlowlevelviremia among Italian patients genotyped over the years 2006 to 2013. However, they reported in their study that genotyping platforms had been upgraded (equipment, kits, and reagents) within that same period of time; but this wasn't taken into account while evaluating overall genotyping success (Armenia et al., 2014). Besides, failure during amplification/sequencing in the present study was most probably due to low viral load or poor quality of RNA (SantoroandPerno,2013,Daar,Jan.2005).

As for the unique case of INSTI-resistance observed, it was previously reported as a complete 4-class multidrug resistance to all antiretrovirals available locally with complete loss of boosted darunavir and DTG efficacy (Fokam et al., 2020bb). In effect, recurrent poor adherence to ART even in the presence of INSTI (raltegravir and then DTG) was the leading factor associated to the emergence of drug resistance (Fokam et al., 2020bb). Conversely in the present study, the statistically significant presence of accessory DRMs among INSTI-unexposed participants with viremia >1000copies/mL ($P = 0.047$) was probably due to error-prone replication leading to various polymorphisms, adaptation to immune activity or intrinsically less fit viruses (Inzaule et al., 2016, Daar, Jan. 2005, Low et al., 2009, Garijo et al., 2015, Ceccherini-Silberstein et al., 2009). This finding strongly advocates for the close surveillance of conserved regions in the integrase gene of HIV as DTG is being scaled-up in order to ensure long-term effectiveness of these drug-regimens (Semengue et al., 2021, Ceccherini-Silberstein et al., 2009). On the other side, no association was found between accessory DRMs, and subtype distribution, probably because of the limited sample size. Nevertheless, absence of integrase DRMs (major or accessory) in the presence of INSTI strongly supports the continuation of these very potent drug-regimens (Mikasi et al., 2020, Mboumba Bouassa et al., 2019, Inzaule et al., 2018, Brado et al., 2018, Mabeya et al., Jan. 2020), thus framing the way for the achievement of UNAIDS third 95-goal by 2025 in LMICs.

A limitation in this study was the small proportion of samples from patients with low viremia as compared to those with high levels of viremia given the delayed detection of virological failure in our country (Fokam et al., 2011, Njom Nlend et al., 2017). Nonetheless, the acceptable success rate of this in-house genotyping assay beyond 500 copies/mL may call for a new definition of viral suppression for optimal monitoring of ART-strategies in LMICs, pending confirmatory studies. Further investigations with larger sample size will help evaluate accuracy and reproducibility of this in-house integrase genotyping assay; assessments on subtypes not found in this study would strengthen the usefulness of tis assay toward its global endorsement beyond LMICs.

5. Conclusions

In summary, this in-house HIV-1 integrase-genotyping assay can serve as a very effective tool for the surveillance of INSTI-DRMs in LMICs, especially with the high genetic diversity of HIV found locally. Indeed, this assay is accessible, easily reproducible, effective even at low-level viremia (500-999copies/mL) and covers a wide range of HIV-1 pure and recombinant clades. This assay performance underscores its usefulness to support the successful scale-up of DTG-based regimens in Cameroon, in other LMICs, and beyond.

Data availability

Integrase sequences generated in this study are available in GenBank under the accession numbers MW328641 - MW328713, MN520219, MZ044346-MZ044408, and OK086757-OK086758.

Authors' contributions

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Declaration of competing interests

Authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2021.115574.

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