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Original Article

Evaluation of two bioinformatic algorithms for the interpretation of HIV-1 drug resistance and subtyping in Cameroon: Translational application for ART optimization in low-and middle-income countries

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List of abbreviations: AB1, Applied Biosystems files; AIDS, Acquired Immuno Deficiency Syndrome; ART, Antiretroviral Therapy; ARV, Antiretroviral; CD4, Cluster differentiation 4; CD8, Cluster differentiation 8; CDC, Centre for Disease Control and Prevention; cDNA, Complementary Deoxyribonucleic acid; CIRCB, *Centre International de Référence "Chantal BIYA" pour la recherche sur la prévention et la prise en charge sur le VIH/SIDA*; CTRL, Control key; CRF, Circulating Recombinant Forms; DNA, Deoxyribonucleic Acid; DRMs, Drug Resistance Mutations; FASTA, Fast Alignment Sequence Test for Application; HIV, Human Immunodeficiency Virus; HIVdb, HIV database; HIVDR, HIV drug resistance; INSTI, Integrase Strand Transfer Inhibitor; IUPAC, International Union of Pure and Applied Chemistry Nomenclature; IQR, Interquartile range; LMICs, Low- and middle-income countries; NGS, Next-Generation Sequencing; NNRTI, Non-Nucleoside Reverse Transcriptase Inhibitor; NRTI, Nucleoside Reverse Transcriptase Inhibitor; PCR, Polymerase Chain Reaction; PDR, Pre-treatment Drug Resistance; PLHIV, People Leaving with HIV; PMTCT, Prevention of Mother -To-Child Transmission; PI, Protease Inhibitor; PI/r, Protease Inhibitor Ritonavir boosted; Pol, Polymerase; PR, Protease; RNA, Ribonucleic Acid; RT, Reverse Transcriptase; RTI, Reverse Transcriptase Inhibitors; TDR, Transmitted Drug Resistance; VL, Viral Load; QASI, Quality Assessment and Standardization of Indicators.

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ABSTRACT

Background: Efficient monitoring of HIV drug resistance (HIVDR) relies on standardized bioinformatics tools for accurate identification of drug resistance mutations (DRMs). Thus, we sought to compare the concordance of HIV-1 genotypic profiling from sequences analyzed with two commonly-used editing algorithms in low- and middle-income countries (LMICs).
Methods: A laboratory-based comparative study was conducted among treatment-experienced people living with HIV attending the Chantal BIYA International Reference Centre in Yaoundé-Cameroon from October-2022 through July-2023. For each individual, raw data of HIV-1 sequences were analyzed simultaneously using RECall (semi-automated) vs. Exatype (automated) algorithms. Outputs were compared for DRMs, polymorphisms and subtyping between the two algorithms, with significance at $p<0.05$.
Results: Overall, 221 participants were included (mean-age 32±15 years, 52.5% female). Validation of sequence quality was 70.1% (155/221) by RECall vs. 60.2% (133/221) by Exatype, Ka=0.78 ($p<0.0001$), indicating a good agreement between both algorithms. Importantly, a perfect concordance (100%) was found in HIV-1 clade inference (CRF02_AG [82/82], A1 [29/29], G [5/5], F2 [5/5] and others [12/12]). Similarly, high concordances were found for the identification of DRMs to protease-inhibitors (99.0%), nucleoside reverse-transcriptase inhibitors (98.0%), non-nucleoside reverse-transcriptase inhibitors (98.6%) and integrase-inhibitors (100.0%). The average turn-around-time was two-folds longer with RECall (5.5±1.7 min) vs. Exatype (2.5±1.1 min); giving a lower efficiency (i.e. validation rate/time) with RECall (12.7) vs. Exatype (24.1).
Conclusions: Semi-automated (RECall) and automated (Exatype) tools showed excellent agreement in detecting HIV-1 clades and DRMs, supporting their interoperability in clinical practice. Following efficiency, Exatype can be considered preferential, while RECall remains a quite suitable alternative for LMICs.

1. Introduction

Human immunodeficiency virus (HIV) infection remains a global public health concern and particularly in sub-Saharan Africa that harbours 70% of the global burden [1]. The scale-up of antiretroviral therapy (ART) has significantly reduced HIV-related mortality and morbidity in low- and middle-income countries (LMICs) [2,3]. Interestingly, early ART initiation is associated with rapid viral suppression, an improved quality of life, as well as prevention of HIV drug resistance (HIVDR) emergence. Of note, HIVDR is characterized by a change in the genetic structure of the viral protein referred to as a mutation (i.e. nucleic acid substitution that leads to a change in amino acid). Such mutations are associated with a decreased viral susceptibility of antiretroviral drugs, thus leading to ART failure in clinical practice, due to the inability of antiretrovirals (ARV) to block the viral replication [4]. There exist three main types of drug resistance; firstly, we have pretreatment drug resistance (PDR) which is observed in individuals starting ART for the first time or in people reinitiating first-line ART [4,5]. Secondly, transmitted drug resistance (TDR) is detected in recently infected individuals with no history of ARV drug exposure. It occurs when previously uninfected individuals are infected with a virus harbouring drug resistance mutation (DRMs). Thirdly, acquired drug resistance (ADR) develops when mutations emerge due to viral replication under suboptimal regimens or in non-adherent individuals receiving ART [2].

Effective management of ART at individual and population levels requires optimal monitoring and surveillance of HIVDR [6]. In this frame, genotypic resistant testing (GRT) remains the gold standard to detect DRMs and to tailor optimized ART regimens, in order to reduce events of virological failure [6–10]. Such ART optimization strategy, currently applicable in both high and LMICs, can be successfully implemented through rigorous and well-conducted genotyping. Of note, genotyping entails several steps from the viral nucleic acid extraction of the genetic material to HIV sequence interpretation for the identification of DRMs and viral genetic diversity in a geographical setting.

It is noteworthy that one of the key steps in sequence interpretation is the editing procedure; which is crucial for the reliability of results. Of relevance, some discrepancies observed following genotyping are partly attributed to sequence editing/analysis through diverse bioinformatics pipelines [11]. This in turns will affect the genotypic profiling and subsequent interpretation of HIVDR on sequences generated routinely by trained staffs with expertise in the use of editing tools. There are several editing tools in the market which include either automated, semi-automated or manual algorithms. These algorithms are based either on customized, standardized, in-house or commercially developed bioinformatics pipelines. These tools could either work as stand-alone or web-based, with diverse field realities depending on various settings. Nonetheless, it is postulated that discrepancies between these editing algorithms could result from the rate of mixtures identified by each of the tools, the degree of forgiveness in DRMs selection and also the fluctuations in hands-on time [12]. Given the workload of sequence editing on the quality of HIVDR interpretation, comparing the outcome from editing tools used routinely in LMICs is of paramount importance to: [1] delineate the operational characteristics of each tool, [2] select the most efficient tool for preferential use, [3] set-up a pragmatic algorithm to secure reliability in HIVDR and subtyping results which could be generalisable in several LMICs.

We therefore engaged in a laboratory-based comparative study with the goal to ascertain the level of interoperability between commonly used bioinformatics platforms for HIV sequence analysis/editing in LMICs. Such evidence could help minimize discrepancies in the interpretation of HIV-1 sequences generated routinely in Cameroon and similar LMICs [13]. To achieve this goal, we sought as objective to study the rate of concordance in detecting DRMs, polymorphisms and viral clades between two different algorithms, RECall (which is semi-automated) and Exatype (which is automated).

[1] contributed equally as second authors
[2] Contributed equally as senior authors.

2. Results

2.1. Sociodemographic and clinical characteristics

From a total of 244 virally unsuppressed clients (i.e. Viral Load (VL)>1000 copies/mL) received at the virology laboratory of the Chantal BIYA International Reference Centre in Yaoundé-Cameroon during the study reporting period, 221 samples were successfully sequenced and thus enrolled for the purpose of the study analysis.

Participants had a mean of 32±15 years; female participants were 116/221 (52.5%). Median VL was 13,508 [IQR: 3,810–290,876] copies/mL. Majority of the participants (163/221; i.e. 73.76%) were failing efavirenz/nevirapine-based first-line ART while 20.36% (45/221) were failing protease inhibitor-based second-line (atazanavir- or lopinavir-based) and 5.88% (13/221) were failing darunavir based third-line ART at the moment of the study. None was failing integrase inhibitors-containing regimens. Median ART duration was 10 [4-13] years and about 85.07% (188/221) were at WHO clinical stage I (Fig. 1).

2.2. Overall sequence validation with both algorithms

Following each pipeline for sequence quality control, 155/221 (70.14%) sequences were validated and 66/221 (29.86%) were failed with RECall while 133/221 (60.18%) were validated and 88/221 (39.82%) failed with Exatype. The sequences rescued by RECall and failed by Exatype were 66/221 (29.86%). Table 1 shows the overall concordance and discordance between both editing algorithms. The Kappa=0.78 [95%CI: 0.69-0.83] suggests a good concordance between RECall and Exatype.

2.3. Concordance in detecting HIV-1 drug resistance mutations and polymorphisms between the two algorithms

From the 133 samples validated by both algorithms, we found an excellent concordance (>98%) in detecting DRMs and polymorphisms in the PR, the RT and the IN regions. The Table 2 provides details on the concordance obtained for each portion.

Table 1
Overall concordance of both algorithms.

Algorithm	RECall (+)	RECall (-)	Total
Exatype (+)	133	0	133
Exatype (-)	22	66	88
Total	155	66	221

2.4. Comparing subtyping and sequence homology of HIV-1 strains

Importantly, RECall and Exatype algorithms had 100% concordance in identifying HIV clades. Among the clades identified in this study we had: A1 (29/133), F2 (5/133), G (5/133), D (3/133), and F1 (1/33) as concerns the pure subtypes while the circulating recombinant forms were CRF02_AG (82/133), CRF18_cpx (3/133), CRF01_AE (2/133), CRF13_cpx (2/133) and CRF37_cpx (1/133). The phylogenetic tree constructed with a 1000 bootstrap and confirming the homology between these sequences can be seen in Fig. 2.

2.5. Turn-around time for sequence editing

Regarding the turn-around time, the average time spent for sequence editing with RECall was 5.5 ±1.7 min whereas the operators spent approximately 2.5 ±1.1 min while editing HIV-1 sequences with Exatype.

2.6. Evidence-based approach for HIV-1 sequence editing in routine practice

With a validation rate of 70.1% and a turn-around time of 5.5 min, RECall yielded an efficiency of 12.75% of validation/min whereas Exatype had an efficiency of 24.08% of validation/min (validation rate of 60.2% and a turn-around time of 2.5 min).

This higher efficiency observed with the automated editing algorithm led to the stepwise and pragmatic approach for sequence editing illustrated in Fig. 3. From the proposed approach, after editing 221 sequences with Exatype, 133 were passed with Exatype and from the 88 rejected by Exatype, 66 were passed with RECall using RECall. The total

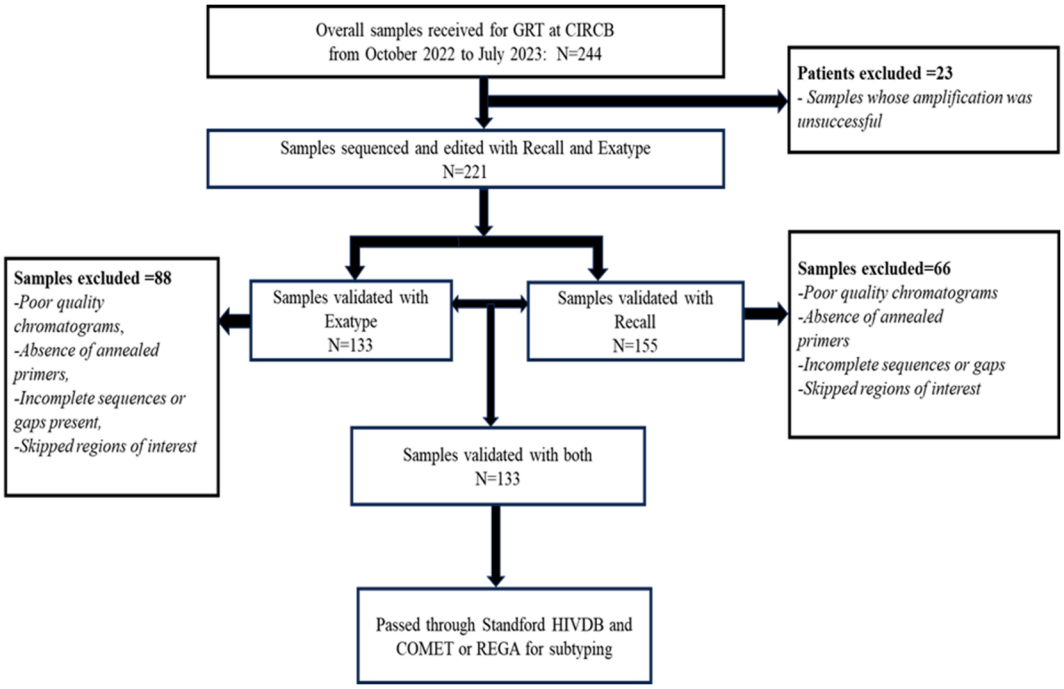

Fig. 1. Algorithm for enrolling of participants and sample screening.

Table 2
Overall concordance table for drug resistance mutations and polymorphisms.

Region		Number of analysed sequences	Overall DRMs concordance	Observed DRMs discordances		Overall concordance with Polymorphisms
				With Exatype	With RECall	
Protease		105	99%	None **L90M**	**L89T** **L89T**	99%
Reverse transcriptase	NRTI		98%	K219**KQ** None D67**DH** K70**KR** D67**DN**, T215**TFIS** None M184**MV**	S68**SN** T215**TI** None None None **M41L, M184V, T215F** **M41L**, M184**V, T215F**	99%
	NNRTI		98.6%	None P236**PL** M230**MI** **V179E** **G190A**	G190**GA** None None **K103N** None	
Integrase		28	99.25%	None	E157**EQ**	99.22%

Legend. INSTI: Integrase Strand Transfer Inhibitor; NRTI: Nucleoside Reverse-Transcriptase Inhibitor; NNRTI: Non-Nucleoside Reverse-Transcriptase Inhibitor

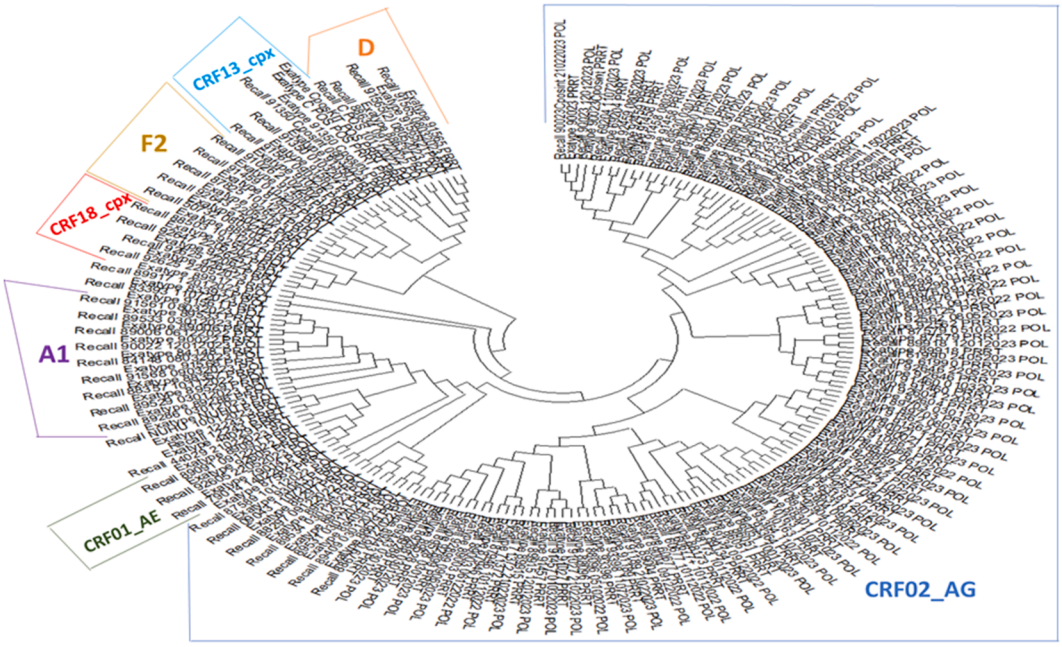

Fig. 2. Phylogenetic tree.
Legend. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 266 mucleotide sequences. Codon positions included were ***1st+2nd+3rd+Noncoding***. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1036 positions in the final dataset. Evolutionary analyses were conducted in MEGAll. RECall: sequences generated with RECall softwave; Exatype sequences generated with Exatype software.

reportable sequences was 155 with 22 sequences rejected by RECall. The total validation rate becomes 90.04% with a total turn-around time of 395.5min, thereby giving an overall efficiency (validation/min) of 22.77%.

3. Discussion

Optimization of ART especially in LMICs is key to achieving UNAIDS' third 95 target for HIV elimination by 2030. This requires selecting for the most optimal regimens as supported by the principle of personalized medicine currently applied in the western world. This calls for scaling-up genetic sequencing in LMICs, leveraging from platforms acquired during the COVID-19 outbreak.

With the goal to ensure the reliability of results generated routinely during the interpretation of DRMs, the present study is unique due to its focus in HIV-1 sequence analysis based on user-friendly systems that are generalisable in several LMICs to spin-off molecular virology applied to the clinical management of HIV/AIDS and pathogens. By comparing the performance of two editing algorithms in analyzing HIV-1 sequences, samples were mainly from routine practice, which therefore gives room for a wider translational application of our study findings.

Gender was equally distributed in this study and all participants were virally unsuppressed, which gives room for comparability with similar assessments conducted following the WHO framework for HIV drug resistance laboratory landscape. The median viremia was fairly high (~4log), which represents the main range of people with a confirmed

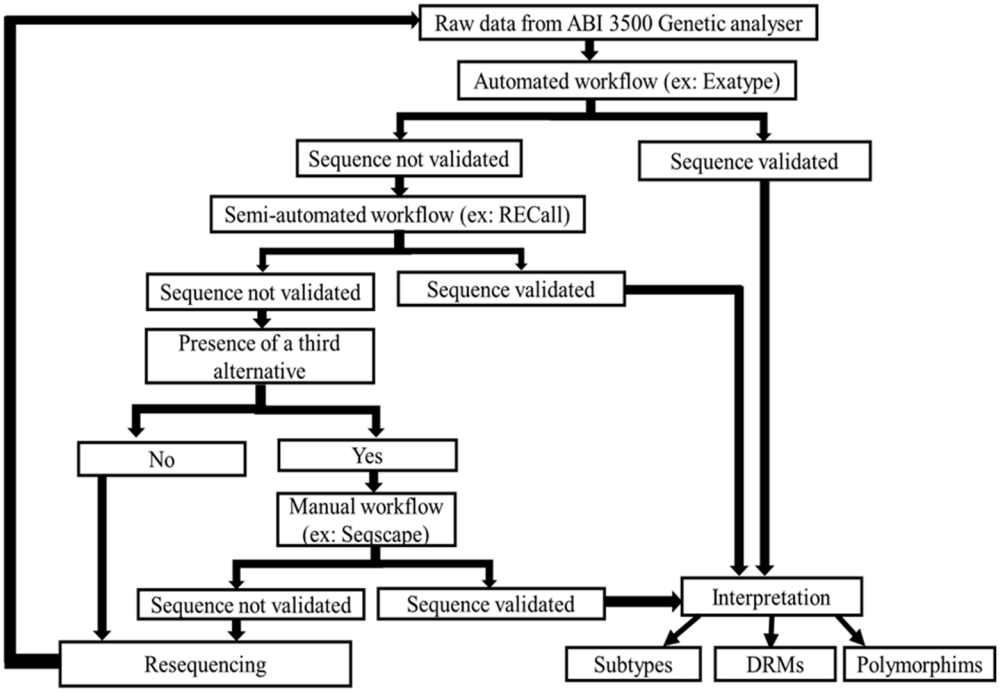

Fig. 3. A pragmatic sequence editing approach in routine clinical practice.

treatment failure in several LMICs. About half of the study participants have been on ART for a decade or more; the majority of whom failed efavirenz/nevirapine-based first-line ART at the time of study enrolment. Similar observations were made in previous studies wherein heavily treated patients were more likely to fail ART due to decreased treatment adherence favoured by possible drug holidays, forgetfulness and absentmindedness on the first-hand [14], and the use of very low genetic barrier drugs (i.e. efavirenz and nevirapine) especially in LMICs on the other hand [15–20]. Such situation is expected to change with the wide transition/use of dolutegravir-based regimens in the present era.

Our data highlight a good agreement in HIV-1 sequence validation with these two bioinformatic tools, suggesting an interoperability of these editing algorithms for sequence analysis in routine clinical applications. Furthermore, the comparisons of sequences validated by both algorithms yielded an excellent concordance in detecting DRMs and polymorphisms and a perfect concordance in identifying HIV-1 clades, which confirms the reliability of results obtained by each of the methods. These findings are in line with those of a Canadian study conducted in 2012 in which there was 98.1% and 98.7% concordance between manual and semi-automated editing tools for the detection of PI and RTI DRMs respectively [11]. As expected, the few differences observed could be justified by the presence or absence of human intervention in the process which is mostly translated by the high forgiveness for single primer coverage and variable stringency in the application of the rules [11,12]. After a detailed analysis and manual proofreading, we noticed that the rescued sequences by RECall (29.86%; 66/221) is explained by the fact that, RECall being a semi-automated algorithm suggest a higher tolerance leaving the possibility for even low-quality primers to be rescued by the operator since it recognized all primers, pending manual review and editing, it is therefore possible to adjust low-quality chromatograms and borderline calls compared to the automated algorithm which did not recognize some primers (succeeded in aligning only one primer), has strict quality checks and fixed mixture calling thresholds, thereby showing poor tolerance for potentially corrupted primers rejecting automatically some sequences [11].

In effect, fully automated procedures will tolerate few ambiguous amino acids called, less mixtures and less uncovered regions as it will strictly apply all the cut-offs and thresholds set for the analysis. Thus, the manual step involved in the semi-automated algorithm, yet helps in yielding a greater validation rate but may lead to potential misclassification. This calls for high-level trained staff and a second sequence reviewer prior to confirm the quality of the sequence output [11]. This point is of major relevance in a continent, like Africa, where the routine use of sequencing is still to come, while it is highly desirable. Availability of reliable tools, together with the skill of the technicians and biologists, is of paramount importance to achieve valid results applicable in clinics.

As regarding the turn-around time, the automated editing algorithm was unsurprisingly faster, which encouraged its prioritization over the semi-automated tool to prompt decision-making for treatment optimization of patients already failing ART (Fig. 3). However, even though human intervention increases the turn-around time, it is worth noting that the semi-automated tool led to a higher validation of sequences. According to the proposed workflow, the overall efficiency is higher than RECall's efficiency but less than Exatype's efficiency by little, since Exatype's time which is faster is influenced by RECall's turn-around time. This is obviously because RECall still has a higher validation rate since all the sequences validated by Exatype are also validated by RECall, but the turn-around time for RECall is still more. In effect, human intervention enables a better appraisal of the quality of sequence reads and could be crucial for the analysis of complex and recombinant HIV-strains; in which for example the variation of breaking points may not allow proper annealing of primers, resulting in ambiguous/uncovered regions [21–23]. As a result, the primary limitation of the semi-automated algorithm is the need for highly trained and qualified operators which will help compensate the higher stringency of the automated editing algorithm [11].

Importantly, all HIV-1 clades (both pure and circulating recombinant forms) observed in this study and confirmed by both algorithms, clearly depicting the HIV molecular epidemiology found in Cameroon [18, 24–26]. This underscores the reliability of the generated outputs, the good performance of staffs involved in the analytical pipeline, and the user-friendly features of these editing tools for generalisability in LMICs participating in HIVDR clinical monitoring and population-based surveillance following the WHO target product profile (TPP) applicable in these settings [27].

4. Conclusion

The analysis of HIV-1 sequences using automated (Exatype) and semi-automated (RECall) bioinformatic tools highlights an excellent agreement in detecting HIV-1 clades, DRMs and polymorphisms, thus underscoring the interoperability of these tools in routine clinical practice. Taken together, the efficiency observed during utilization, due to the importance of rapid decision-making for treatment optimisation of patients failing their ART, suggests a slight preference for Exatype as the most suitable editing pipeline (owing to its fully automation with shorter turn-around-time), while RECall remains a quite valid tool to be used in LMICs.

5. Materials and methods

5.1. Study design

A laboratory-based comparative study was carried out from October 2022 through July 2023 among ART-experienced people living with HIV received for HIVDR testing at the Virology laboratory of the Chantal Biya International Reference Centre (CIRCB) for research on HIV/AIDS prevention and management, located in Yaoundé-Cameroon [28].

The CIRCB is a government institution of the ministry of Public Health dedicated to HIV research and person monitoring in several aspects. Among these aspects are (a) HIV early infant diagnosis in the frame of the national PMTCT program, (b) diagnosis of coinfections with HIV, (c) viral load measurement, (d) CD4 and CD8 T lymphocyte counts, (e) biochemical and hematological tests for drug safety, and (f) genotypic HIVDR testing (GRT) at subsidized costs, with quality-control programs conducted in partnership with the Quality Assessment and Standardization of Indicators (QASI) and other international agencies [26,28]. Furthermore, the expertise of the virology laboratory of CIRCB on the interpretation of protease-, reverse transcriptase- and integrase resistance-associated mutations has led to the implication of the institution (CIRCB) in national health policies implementation and the positioning at the international level as a final stage candidate for the WHO national HIV drug resistance laboratory [29].

5.2. HIV-1 Genotypic resistance testing procedure

Following the WHO laboratory operational framework for HIV drug resistance [30], samples eligible were collected from clients with a confirmed viral non-suppression (VL>1000 copies/ml).

HIV-1 GRT was performed on HIV-1 plasma RNA following in-house PR, RT and IN genotyping assays as described and published elsewhere [26,31,32]. Briefly, viral nucleic acids were extracted using the Qiagen protocol with a viral RNA extraction kit. Amplification, gel electrophoresis, purification of amplified products, and sequencing reactions were realized accordingly. By the end of the capillary electrophoresis, the raw “ab1 files” (ie. Applied Biosystems files) generated by the genetic analyzer (ABI 3500) were later edited using two editing algorithms; one semi-automated (RECall) and the other automated (Exatype) to comparability.

5.3. Sequence editing using RECall (semi-automated pipeline)

The software required a specific file format to group multiple sequence reads from the same sample into a single consensus sequence automatically [11]. The step-by-step procedure is described elsewhere [30]. Briefly, the procedure goes as follows; to get started with RECall we need to launch the software and log into your account to access the sample manager. After adding the samples (generally a single file containing all sequence reads from different patients and various primers) to the sample manager, choose the reference sequence ("Project") with which to align your sequence reads (here "CDC_HIVDR" for PR-RT and "CDC_INTEGRASE-288" for Integrase). The software automatically puts together the reads from the same sample and starts the analysis. Once it is over, samples will be listed at the bottom of the left-right side and highlighted as green (if passed or approved), orange (if manual review is required), red (if failed), or black (in case the raw data is incomplete, corrupted or does not seem very good; no amount of editing will make it pass).

The editing properly starts with sample viewing on the "Sequence finisher" window, which allows to navigate and edit your sample sequence. Base editing is done simply by navigating through the window and typing/correcting ambiguous nucleotides according to the International Union of Pure and Applied Chemistry Nomenclature (IUPAC) mixtures list present on the toolbar. Once editing was completed, only passed/Approved sample sequences could be exported.

5.4. Sequence editing using Exatype (automated pipeline)

The software requires a good internet connectivity to begin the editing process. The step-by-step procedure is described elsewhere [12]. Briefly, the procedure goes as follows; firstly, you have to create an account online and login to launch the software. You will be directed to a dashboard where you have to click on “create a job”. After creating the task you will have to choose the type of assay used for running the samples (“thermofisher with integrase kit” or “thermofisher without integrase kit”) and then upload your sequence reads. The identifiers in the filenames are colored according to the specifications provided so, a verification is needed to be sure that the sample name, primer, gene region, and plate name/number (if applicable) identifiers are shown correctly. Samples will then be uploaded and the analysis will start if everything has been correctly inserted. Duration of the analysis will depend on the quality of the internet connexion and once completed, the operator will have an overview of the total number of samples analysed; the ones “coloured in green” (i.e. the task does not require further editing), the ones “coloured in orange” (i.e. human action is required to either validate or reject the sequence) and those “coloured in red” (i.e. the sequence is of poor quality and has been rejected).

Base editing (for samples requiring additional intervention) was done by navigating through the window and typing/correcting ambiguous nucleotides according to the IUPAC mixtures list present on the toolbar. Once editing was completed, only passed/Approved sample sequences could be exported. Importantly, this software automatically generated a Stanford datasheet for DRMs interpretation directly on the report page.

5.5. Sequence validation criteria at laboratory level

All generated sequences whose primers cover minimal regions in the protease (PR) from codons 10-93; reverse transcriptase (RT) from codons 41-238; and integrase (IN) from codons 51-263 were finally validated(30). Incomplete sequences after genotyping and those with too much background noise and poor-quality chromatograms were all excluded from the analysis. Quality check was realised on every sample file to ensure that the sequence was acceptable. Table 3 and Table 4 show the sequence rejection criteria using either of the editing algorithms.

Of note, the editing process was realised by only one operator to avoid operator bias, but overall process was supervised by two other senior staffs who were responsible to ensure reliability of the results from primary operator.

5.6. Interpretation of HIV-1 drug-resistance mutations and subtyping

After assembling and editing the sequences by using RECall (https://recall.bccfe.ca/) and Exatype (https://hyraxbio.com/exatype-platform/solutions/) algorithms, they were then analysed for interpreting DRMs by using Stanford HIVdb v9 as described elsewhere [33]. Samples with resistant mutant or a mixture of wild type and mutant at an amino

Table 3
Configuration variables for nucleotide mixture calling and base "marking" for clinical drug resistance genotyping(12).

Parameter	Value	Interpretation
Quality censoring cutoff	<10	Quality scores cut off for excluding bases during assembly.
Mixture area (%)	≥20	The area of the uncalled peak must be at least 20% of the called peak area. If 50% of the reads pass this threshold, a mixture is called.
Mark area (%)	≥15	The area of the uncalled peak must have at least 17.5% of the called peak area. If ≥50% of the reads pass this threshold, then a mark is made.
Mark average quality cut-off pared score Additional marks	<20	If the average quality of the base across all reads is below the cutoff, then a mark is made. Insertions, deletions, and single primer coverage are also marked.

Table 4
Criteria used by RECall and Exatype for rejecting a sequence.

Failure category	Description
Stop codons	Any unambiguous stop codon (TGA, TAA, or TAG)
Bad inserts	An insertion relative to the reference sequence that is not a multiple of three bases, resulting in a frameshift
Bad deletion	A deletion relative to the reference sequence that is not a multiple of three bases, resulting in a frameshift
Too many mixtures	>3.5% of nucleotides sequences called as mixtures
N count	≥5 Ns (any base) in the sequence
Mark count	≥100% positions marked as been potentially problematic
Single coverage	>3 consecutive bases of single-read coverage with phred scores of 40
Low quality	Any section where the quality of all coverage is too low to make a call

acid position were considered resistant.

For subtyping, sequences were aligned in BioEdit version 7.2.6 (Tom Hall, Raleigh, NC, USA) by using CLUSTAL W, 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. The phylogenetic tree was inferred by using both Neighbor-Joining and maximum likelihood method on the MEGA software v7.0.26 primarily for subtyping and to ensure that there was no cross-contamination of samples [26].

Sequence quality assessment

Quality assessment of duplicated sequences (edited with each algorithm) was done following the WHO framework quality control tool available online (https://sequenceqc.bccfe.ca/who_qc).

Statistical analysis

Primary outcomes for the study were the level of agreement between the two bioinformatics tools using kappa; secondary outcomes were the rates of concordance in the detection of DRMs, viral polymorphisms and HIV-1 clade assignation. P-value<0.05 was considered statistically significant.

Ethics Statement

Administrative authorizations were issued by the CIRCB directorate (reference N°0191/019L/CIRCB/DG/SAA/BRH); ethical clearance for the study was obtained from the Research Ethics Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I (Authorization Number: N°0450/UY1/FMSB/VDRC/DAASR/CSD).

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Data availability

Part of the data described in this article to support the findings of this study are included within this article, the ones concerning the WHOQC framework are openly available in the link: h ttps://sequenceqc.bccfe.ca/who_qc/upload_results/1713179716.

CRediT authorship contribution statement

Joseph Fokam: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Naomi-Karell Etame:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Ezechiel Ngoufack Jagni Semengue:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Collins Ambe Chenwi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Seth C. Inzaule:** Writing – review & editing, Validation, Supervision. **Désiré Takou:** Writing – review & editing, Conceptualization. **Evariste Molimbou:** Writing – review & editing, Validation, Investigation. **Alex Durand Nka:** Writing – review & editing, Validation, Methodology. **Derrick Tambe Ayuk Ngwese:** Writing – review & editing, Investigation. **Davy-Hyacinthe Gouissi Anguechia:** Writing – review & editing, Investigation. **Aude Christelle Ka'e:** Writing – review & editing, Investigation. **Grâce Beloumou Angong:** Writing – review & editing, Methodology, Investigation. **Sandrine Claire Djupsa Ndjeyep:** Writing – review & editing, Methodology, Investigation. **Aurelie Minelle Kengni Ngueko:** Writing – review & editing, Methodology, Investigation. **Rachel Audrey Nayang Mundo:** Writing – review & editing, Investigation. **Larissa Gaelle Moko Fotso:** Writing – review & editing, Investigation. **Pamela Patricia Tueguem:** Writing – review & editing, Investigation. **Vincent Kamaël Mekel:** Writing – review & editing, Investigation. **Michel Carlos Tommo Tchouaket:** Writing – review & editing, Investigation. **Samuel Martin Sosso:** Writing – review & editing, Investigation. **Rogers Ajeh Awoh:** Writing – review & editing, Validation. **Maria-Mercedes Santoro:** Writing – review & editing, Validation. **Francesca Ceccherini-Silberstein:** Writing – review & editing, Validation. **Anne-Cecile Bissek Zoung-Kanyi:** Writing – review & editing, Validation. **Giulia Cappelli:** Writing – review & editing, Validation. **Vittorio Colizzi:** Writing – review & editing, Validation. **Carlo-Federico Perno:** Writing – review & editing, Validation. **Nicaise Ndembi:** Writing – review & editing, Validation. **Francois-Xavier Mbopi-Keou:** Writing – review & editing, Validation. **Alexis Ndjolo:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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