HIV-1 drug resistance testing is essential for heavily-treated patients switching from first- to second-line regimens in resource-limited settings: evidence from routine clinical practice in Cameroon

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Abstract

Background: With the phase-out of stavudine (d4T), change to first-line regimens with zidovudine (AZT) or tenofovir (TDF) in resource-limited settings (RLS) might increase risks of cross-resistance to nucleos(t)ide reverse transcriptase inhibitors (NRTI). This would restrict the scope of switching to the World Health Organisation (WHO)-recommended standard second-line combinations (SLC) without HIV drug resistance (HIVDR)-testing in routine clinical practice.

Methods: An observational study was conducted among 101 Cameroonian patients (55.4% male, median [IQR] age 34 [10–41] years) failing first-line antiretroviral therapy (ART) in 2016, and stratified into three groups according to NRTIs exposure: exposure to both thymidine analogues AZT “and” D4T (group-A, n = 55); exposure to both TDF and AZT “or” D4T (group-B, n = 22); exposure solely to D4T (group-C, n = 24). Protease-reverse transcriptase HIVDR was interpreted using the HIVdb penalty scores (≥60: high-resistance; 20–59: intermediate-resistance; < 20: susceptible). The acceptable threshold for potential-efficacy was set at 80%.

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Background

By the end of 2017, about 70% of HIV-infected people knew their status, 77% of these were receiving antiretroviral therapy (ART) and 82% of treated patients achieved viral suppression [1, 2]. This progress led to 54% coverage of ART (14 million people) in Africa, with a coverage that is expected to double by 2020 as per the 90–90–90 targets (90% of all people living with HIV have been diagnosed; 90% of all people with diagnosed HIV infection are receiving ART; 90% of all people receiving ART have suppressed viral load) [2].

A major setback in achieving viral suppression is the emergence of HIV drug resistance (HIVDR), which is driven by delayed detection of virological failure and accumulation of drug resistant mutations (DRMs) in resource-limited settings (RLS). This later increases cases of new HIV-infections with resistant viruses, as well as AIDS-associated-morbidity and mortality [2, 3]. While HIVDR is gradually under control in high-income countries, the resistant patterns are rising in RLS and especially within sub-Saharan Africa (SSA) [3]. In these settings, one in every 10 adults starting ART harbour resistant virus; three in every 10 adults restarting first-line ART (i.e. women exposed to antiretrovirals for the prevention of mother-to-child transmission (PMTCT) or individuals re-initiating first-line ART after a period beyond 3 months of treatment interruption) harbour resistant virus; and five in every 10 young children diagnosed with HIV harbour resistant virus [3]. Regarding adults living in different SSA regions, resistance rates to first-generation non-nucleoside reverse-transcriptase inhibitors (NNRTIs), i.e. nevirapine and efavirenz, are already beyond 10% in East and Southern Africa regions [4], thus requiring treatment initiation with NNRTI-sparing regimens for optimal ART response in these settings, compared to West and Central Africa regions where the burden may still be bearable [4, 5]. However, the estimated incremental annual increase of 17% pre-treatment drug resistance (PDR) in West and Central Africa settings over time (p = 0.0017), the implementation of the “treat-all” strategy and the continuous use of NNRTI-based first-line ART [2], virological failure (VF) and acquired HIVDR to current first-line ART would be cumulative [5, 6], especially for countries like Cameroon where the national estimates of NNRTI-PDR are near the 10% critical threshold: 7.8% by Fokam et al. in 2017 and 8.3% by WHO in 2017 [7, 8].

With the complete phased-out of stavudine (D4T) from current first-line ART in RLS, tenofovir (TDF)-containing regimens are now widely used while patients with contraindication to TDF are generally being prescribed zidovudine (AZT)-containing regimens as an alternative [9, 10].

After VF on a standard first-line ART in RLS, an optimal selection of SLC is challenging due to limited access to HIVDR testing [2, 5, 6]. In such situations, 3TC could be recycled as part of the NRTI-backbone in SLC because it selects for the M184 V mutation, which decreases viral replication and increases susceptibility to AZT and TDF for potential use in SLC with PI/r [10, 11]. In this frame, the WHO guidelines also recommend the use of AZT + 3TC as NRTI backbone for SLC for those patients failing on TDF-containing first-line regimen; likewise, TDF + 3TC is recommended as NRTI backbone in SLC for patients failing on AZT-containing first-line regimen [9, 11]. Of note, failure on TDF leads to viral hyper-susceptibility to AZT (due to K65R mutation) while failure on AZT leads to possible cross-resistance to TDF. Moreover, in

Keywords: HIV drug resistance, Antiretroviral therapy, First-line, Protease, Reverse transcriptase, Cameroon
routine clinical practice, patients may experience several NRTI substitutions on first-line, which renders difficult drug recycling for use alongside PI/r in SLC. In an attempt to predict acquired HIVDR, Rutstein et al. reported a very poor sensitivity (14.7–28.0%) by using a risk-score approach [12]. Another report from South-India revealed that only half of the patients could be eligible for recycling NRTIs in the SLC [13], which somehow indicate an increasing need of implementing HIVDR testing in routine clinical practice of RLS [14]. However, Phillips et al. reported that HIVDR testing, as part of the decision to switch to SLC, was not cost-effective [15], thereby calling for the need of affordable HIVDR testing assays to the better management of ART in RLS [13–15]. A study in 9 countries revealed varying resistance patterns at first-line failure and consistent dual-class resistance [16], which raise concerns on using HIVDR testing in deciding to switch to SLC in RLS in the frame of increasing assess to reference laboratory platforms [15, 16]. Therefore, for a switch to SLC, assessing the potential effectiveness of the current WHO recommendation in real-life would be of great benefit for ART management in RLS.

Considering the peculiarity of the national ART program in Cameroon (scale-up of ART since 2003, generalised HIV epidemic, broad viral genetic diversity) [17, 18], we sought to evaluate the patterns of DRMs according to treatment history of patients failing first-line ART in routine clinical practice and the potential susceptibility of NRTIs for selecting active SLC.

**Methods**

**Study design and settings**

A cross-sectional and analytical study was conducted in 2016 among 101 HIV-infected patients failing first-line ART (defined as viral load: ≥ 1000 HIV-1 RNA copies/ml) and referred for HIVDR testing at the Chantal BIYA International Reference Centre (CIRCB) for research on HIV/AIDS prevention and management (CIRCB), located in Yaoundé, the capital city of Cameroon.

The CIRCB is a government institution of the Ministry of Public Health dedicated to HIV research and patient monitoring in several aspects, among which: (a) HIV early infant diagnosis in the frame of the national PMTCT program; (b) diagnosis of co-infections with HIV; (c) viral load measurement; (d) CD4 and CD8 T lymphocytes counts; (e) biochemical and haematological tests for drug safety; (f) HIV-1 genotypic drug resistance testing at subsidised costs; with a quality control program conducted in partnership with QASI (http://www.circb.cm/btc_circb/web/).

**RNA extraction, reverse transcription and polymerase chain reaction**

Viral RNA was extracted from plasma samples using the QIAmp viral RNA Mini kit (Qiagen Hilden, Germany) according to the manufacturer’s protocol; extracted viral RNA was then reverse transcribed, amplified and sequenced as previously described [19]. Briefly, RNA was retro-transcribed and amplified using the kit One-Step Invitrogen SuperScript for long templates RT-PCR (Foster City, CA) and 2 sequence-specific primers for 40 cycles; for insufficiently amplified samples after the first round PCR, a second round PCR (semi-nested PCR) was performed; direct sequencing reaction was then carried out using 7 overlapping primers [19]; Capillary electrophoresis was performed using an Applied Biosystems 3130 XL genetic analyzer (Applied Biosystems, Tokyo, Japan) and sequences were assembled using SeqScape Version 2.7 to generate contigs [20].

**Nucleotide sequences were aligned with subtype/circulating recombinant form (CRF) reference sequences from the Los Alamos National Laboratory database using the CLUSTALW integrated into Bioedit 7.2.5 [21, 22]. Following comparison of each sequence with reference sequences (database accessed on 8/01/2018) [23], gaps were then removed from the final alignment, and the phylogenetic tree was constructed by using Splitstree [24]. Recombination among HIV-1 subtypes were confirmed by SCUЕAL [25], COMET [26], SimPlot [27], and Rega subtyping tool v.3 [28].**

**Interpretation of drug resistance mutations**

DRMs of the protease-reverse transcriptase regions were analyzed using the Stanford HIVDR database algorithm version 8.1 [29]. PI/r, NRTIs and NNRTIs effectiveness were interpreted using the genotypic scoring system for drug susceptibility with the following penalty: ≥60 high-resistance; 20–59: intermediate-resistance; < 20: susceptible. Resistance profile was then compared according to first-line ART-regimens received by each patient, and the potential drug efficacy was evaluated for SLC.

**Statistical analysis**

Levels of DRMs, defined as any mutation with a genotypic penalty score of either a high or intermediate threshold, were used to classify patients failing ART with HIVDR. Per group (A, B and C), adequacy for using AZT versus TDF in SLC was defined as ≥80% of patients reporting drug efficacy based on a genotypic susceptibility profile within each group. Comparison of DRMs was performed by group (A, B and C) and following the local HIV-1 molecular epidemiology (CRF02_AG versus non-CRF02_AG). Chi-squared or Fisher’s exact test, where appropriate, was used for statistical analysis of categorical variables to determine the statistical significance of bivariate analysis, with p < 0.05 considered statistically significant.
Ethical considerations
Ethical clearance was obtained from the Ethics Review and Consultancy Committee of the Cameroon Bioethics Initiative (Reference number: CBI/2013/0139/ERCC/ CAMBIN), and written informed consent was provided. For those participants under 16 years of age, a written proxy-informed consent was provided to the study coordinators by the respective parent(s) or legal guardian(s), followed by an assent provided by the corresponding participant. Data were de-identified for purpose of confidentiality and privacy in data management.

Results
Characteristics of the study population
All study participants were failing treatment on a first-line ART available in the national guidelines, after a median time-on-ART of IQR 4 [2–5] years.

All the participants were exposed to lamivudine plus efavirenz or nevirapine, plus at least one other NRTIs used in the first-line ART of Cameroon. According to NRTI-exposure on first-line ART, patients were then classified into three groups (group-A: patients with prior but not concomitant exposure to both thymidine analogues AZT “and” D4T; group-B: exposed to TDF + a thymidine analogue D4T “or” AZT, and group-C: exposed solely to D4T), as shown in Table 1.

HIV drug resistance according to first line ART exposure
Globally, the rate of HIVDR among these patients failing first-line ART was 89.1% (90/101). Interestingly, up to 83.2% of patients harboured the M184V mutation, associated with high-level resistance to 3TC and FTC and serving as adherence marker.

In all the three groups of ART-exposure, the overall prevalence of DRMs (both high and intermediate levels combined) to AZT was higher compared to TDF, with respectively: 56.4% (31/55) vs. 29.1% (16/55) in group A, p = 0.0038; 36.4% (8/22) vs. 31.8% (7/22) in group B, p = 0.0765, leading to higher rates of HIVDR to AZT compared to TDF (Table 2 Group A, B and C). TDF-mutation K65R was significantly associated with TDF-exposure: 0% in group-A, 22.7% (5/22) in group-B, 4.2% (1/24) in group-C (p = 0.0013). Only 1.98% (2/101) of patients harboured PI/r major DRMs, in accordance with the history of non-exposure to PI/r.

Table 2 Resistance to each NRTI among patients failing first-line antiretroviral therapy

| HIVDR       | Group-A: AZT and D4T n = 55 |  | Group-B: TDF + D4T or AZT n = 22 |  | Group-C: D4T (i.e. Triomune) n = 24 |  |
|-------------|----------------------------|----------------|---------------------------------|----------------|-----------------------------------|----------------|
| HIVDR       | High (%) | Intermediate (%) | Susceptible (%) | High (%) | Intermediate (%) | Susceptible (%) | High (%) | Intermediate (%) | Susceptible (%) |
| 3TC         | 45 (81.8%) | 0 (0.0%) | 10 (18.2%) | 19 (86.4%) | 0 (0.0%) | 3 (13.6%) | 20 (83.3%) | 0 (0.0%) | 4 (16.7%) |
| ABC         | 11 (20.0%) | 19 (34.5%) | 25 (45.5%) | 10 (45.5%) | 6 (27.3%) | 6 (27.3%) | 7 (28.3%) | 8 (33.3%) | 9 (37.5%) |
| AZT         | 23 (41.8%) | 8 (14.6%) | 24 (43.6%) | 3 (13.6%) | 0 (0.0%) | 14 (63.6%) | 10 (41.7%) | 0 (0.0%) | 9 (37.5%) |
| D4T         | 23 (41.8%) | 7 (12.7%) | 25 (45.5%) | 4 (18.2%) | 9 (40.9%) | 9 (40.9%) | 23 (95.2%) | 1 (4.5%) | 13 (54.2%) |
| DDI         | 14 (25.4%) | 11 (20.0%) | 30 (54.6%) | 10 (45.5%) | 2 (9.1%) | 5 (45.5%) | 7 (28.3%) | 1 (4.5%) | 6 (25.0%) |
| FTC         | 45 (81.8%) | 0 (0.0%) | 10 (18.2%) | 19 (86.4%) | 0 (0.0%) | 3 (13.6%) | 20 (83.3%) | 0 (0.0%) | 4 (16.7%) |
| TDF         | 3 (5.5%) | 13 (23.6%) | 39 (70.9%) | 6 (27.3%) | 1 (4.5%) | 15 (68.2%) | 2 (8.3%) | 2 (8.3%) | 20 (83.3%) |

Legend. HIVDR HIV drug resistance, 3TC Lamivudine, EFV Efavirenz, NVP Nevirapine, ART antiretroviral therapy, AZT Zidovudine, D4T Stavudine, TDF Tenofovir, Triomune D4T + 3TC + NVP. All patients had received 3TC plus EFV or NVP. Footnote: Prior exposure to D4T and AZT was not concomitant.
AZT and TDF potential efficacy according to treatment history after failing first-line ART

In group-A (i.e. exposed prior and not concomitantly to regimens containing both thymidine analogues AZT “and” D4T), the potential efficacy of AZT was significantly lower (43.64%) compared to that of TDF (70.91%); \( p = 0.0038 \).

In group-B (i.e. exposed prior and not concomitantly to TDF and a thymidine analogue AZT “or” D4T), the potential efficacy of AZT (63.64%) was similar to that of TDF (68.28%); \( p = 1.0000 \).

In group-C (i.e. exposure to D4T-containing regimen only), the potential efficacy of AZT was significantly lower (37.50%) compared to that of TDF (83.33%); \( p = 0.0032 \).

As shown in Table 2 Group A, B and C, the very high prevalence of resistance to 3TC and FTC were due to the high rate of M184 V mutation. Though not exposed to TDF, Groups A + C reported low prevalence of TDF-resistance, due to cross-resistance induced by the accumulation of other NRTI-mutations, especially TAMs. Of note, TAMs-1 were predominant (T215F/Y: 46.5%; M41 L: 22.8%; L210 W: 8.9%) and associated with higher levels of resistance to both AZT and TDF; as compared to TAMs-2 that had relatively lower prevalence (D67N: 21.8%; K70R: 19.8%; K219Q/E: 18.8%) and were associated preferentially with AZT/D4T-resistance.

Genetic diversity of HIV-1 protease-reverse transcriptase

The genetic analysis revealed ten subtypes: 63.4% CRF02_AG (64/101), 8.9% CRF11.cpx (9/101), 7.9% A1 (8/101), 5.9% G (6/101), with only one case of subtype C known as an uncommon strain in west-central Africa (Fig. 1).

According to the local HIV-1 molecular epidemiology, rate of DRMs was slightly higher in CRF02-AG (92.2%) compared to non-02_AG clades (83.8%), without any statistically significant difference \( (p = 0.204) \). Of note, L74I (following exposure to TDF-containing regimens) and L210 W (following exposure to regimens containing a thymidine analogue) mutations were found only in the group

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**Fig. 1** HIV-1 genetic diversity among patients failing first-line antiretroviral therapy in Cameroon. Legend. The reference sequences were from the Los Alamos Database (https://www.hiv.lanl.gov/components/sequence/HIV/search/search.html); Some references have been omitted to enable better visualization. The scale bar represents 1% genetic distance. CRF: circulating recombinant form
of patients infected with CRF02_AG viruses (Fig. 2). HIVDR by drug-class also revealed similar rates between CRF02_AG and non-CRF02_AG infected populations (Tables 3 and 4).

**Discussion**

With the limited access to HIVDR testing in RLS, successful switch to SLC remains a major clinical challenge, especially for patients heavily treated on first-line ART (i.e. substitution of several NRTIs) [2, 5, 6]. Thus, implementing local strategies to ensure a successful switch to SLC is warranted [10].

With a median duration of 4 years on ART, the severe immunodeficiency (CD4 < 200 cells/mm$^3$) and the high viral load (HIV-RNA > 10,000 copies/ml), there is a late detection of treatment failure and a substantial accumulation of DRMs in about nine out of ten patients in routine care [12–14, 17]. This observation therefore urges the need for early viral load monitoring for timely detection of ART failure and adequate switch to SLC with limited risk of HIVDR emergence [30–32]. Our findings are similar to several reports in Cameroon [31, 32], but with higher HIVDR prevalence compared to a study conducted at 36-months ART [33]. This is due to differences in study design (virologically suppressed and unsuppressed patients) and durations [33].

Most importantly, with only ~2% PI/r resistance, the use of PI/r as back bone for SLC remains standard for patients failing first-line regimens in settings with similar ART programs [2, 4, 9, 11], pending the selection of potentially active NRTIs [10–14, 16].

**Table 3** Prevalence of HIV-1 drug resistance among CRF02_AG

| Resistance Category | No. sequences | Percentage with DRM |
|---------------------|---------------|---------------------|
| PI/r                | 64            | 1.6%                |
| NRTI                | 64            | 87.5%               |
| NNRTI               | 64            | 92.2%               |

Legend. PI/r ritonavir boosted protease inhibitor, NRTI nucleos(t) ide reverse transcriptase inhibitor; NNRTI non-nucleoside reverse transcriptase inhibitor, DRM drug resistance mutations

In group-A (both AZT + D4T-exposure), level of HIVDR to AZT was almost two times higher as compared to TDF. This could be explained by the fact that these patients were previously exposed to D4T-containing regimens (i.e. Trionyune) and were subsequently moved to AZT, most likely due to D4T-adverse events or the phased-out of D4T [34]. In the frame of treatment failure, the accumulation of TAMs would further jeopardise the efficacy of TDF due to cross-resistance mainly driven by TAMs-1 [34, 35]. Therefore, among patients exposed to both thymidine analogues, TDF still stands as the preferable option despite risks of TAMs-induced cross-resistance (~30%). Thus, in routine clinical practice, patients failing ART with such treatment history should either: (a) be referred for HIVDR testing or (b) be switched blindly to SLC with close viral load monitoring to detect those at risk of failure due to TDF cross-resistance [30, 34].

In group-B (exposure to TDF + a thymidine analogue D4T “or” AZT), about one-third of patients harboured HIVDR to AZT, also similar for TDF ($p = 1.0000$). This is due to the selection of DRMs (TAMs and/or K65R) following previous ART with a thymidine analogue and

![Fig. 2 HIV-1 drug resistance mutations according to major subtypes distribution. Legend. CRF02_AG: circulating recombinant form; non-CRF02_AG: other subtypes other than CRF02_AG](image-url)
subsequently with TDF-containing regimens [35]. Thus, because one-third of patients managed on first-line with such NRTIs substitution has lost both TDF- and AZT-efficacy [30–34], HIVDR testing would be of great clinical relevance for selecting SLC NRTIs [14, 36].

In group-C (exposed only to a single regimen D4T-3TC-NVP), level of HIVDR was significantly higher to AZT as compared to TDF (about 4-folds), with minimal effects of TAMs inducing cross-resistance to TDF [20]. Thus, in case of exposure to a single first-line regimen, the use of TDF in SLC would have a high predictive efficacy in the majority of patients (≥80%). Thus, for such patients living in RLS, using TDF in SLC without referring to HIVDR testing might be acceptable in clinical practice [15].

As expected, K65R was only found from the group of TDF-exposed patients (group-B). Of note, K65R is a mutation known to reverse the excision phenotype of AZT resistance mutations, to increase viral susceptibility to AZT, which in turns improves the clinical efficacy of AZT [10, 37]. Thus, a wider use of TDF in current first-line regimen might improve the efficacy of AZT for subsequent use in SLC. However, the poor rate of AZT-efficacy in this group is attributed to TAMs derived from previous exposure to thymidine analogues (AZT or D4T). This implies that without HIVDR testing, AZT could be used in SLC solely for those failing on TDF-containing regimens without any previous substitution of NRTIs [8–11].

The very high frequency of M184V (>80%) underscores the utility of this mutation as an indicator of therapeutic compliance in clinical practice for patients receiving any regimen containing 3TC or FTC [9]. Though FTC has an enhanced incorporation efficiency (~10-fold) compared to 3TC during HIV-1 RT-catalyzed RNA-dependent DNA synthesis [38], these two cytidine analogues appear as suitable alternatives without the need for programme-wide substitution of FTC for 3TC in current clinical practice [39, 40].

Phylogeny confirms CRF02_AG as the major circulating clade [41–45]. The single case of subtype C (uncommon in Cameroon) may be due to phylodynamics from Southern/Eastern Africa [46, 47]. Even though the analysis of CRF02_AG versus non-AG showed no major effect of the local subtype distribution on emerging DRMs, molecular epidemiology surveillance merits further investigations, which include the preferential pattern of L74I as a potential signature in CRF02_AG-infected patients [48, 49]. Findings with clinical responses after switch to SLC would provide greater insights for translational application [50].

Conclusion
In a nutshell, first-line ART-failure exhibits variable levels of NRTI-resistance (from 17 to 62.5%) in a routine clinical setting of Cameroon, with a remarkable higher level of AZT-resistance as compared to TDF-resistance (recently introduced drug). Thus, regarding efficacy, first-line ART-failure without NRTI-substitution (i.e. exposed to only AZT or TDF) on first-line could switch to SLC following the WHO-approach. However, failure after substitution only between thymidine analogues may receive TDF as part of SLC pending a close viral load or HIVDR-testing whenever possible. Most importantly, failure after multiple NRTI-substitutions on first-line (exposed to both “thymidine-analogue and TDF”) should be referred for HIVDR-testing for selecting active NRTIs for an optimal SLC in clinical practice.

Additional files

Table 4 Prevalence of HIV-1 drug resistance among non-CRF02_AG

| Resistance Category | No. sequences | Percentage with DRM | 1 DRM | 2 DRMs | 3 DRMs | ≥4 DRMs |
|---------------------|--------------|---------------------|-------|--------|--------|--------|
| PI/RT               | 37           | 2.7%                | 0     | 0      | 0      | 1      |
| NRTI                | 37           | 75.7%               | 7     | 6      | 5      | 10     |
| NNRTI               | 37           | 83.8%               | 19    | 10     | 2      | 0      |

Abbreviations
ART: Antiretroviral therapy; CIRCB: Chantal BIYA International Reference Centre for research on HIV/AIDS prevention and management; DNA: Deoxyribonucleic acid; DRM: Drug resistance mutations; HIV: Human immunodeficiency virus; HIVDR: HIV drug resistance; IQR: Interquartile range; NRTI: Non-Nucleoside reverse transcriptase inhibitor; NNRTI: Non-Nucleoside reverse transcriptase inhibitor; PLHIV: People living with HIV; RLS: Resource-limited settings; RNA: Ribonucleic acid; SLC: Second-line combination; SSA: Sub-Saharan Africa; TAM: Thymidine analogue mutation; UNAIDS: United Nations program on AIDS; WHO: World health organisation.

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Availability of data and materials
All datasets on which the conclusions of the manuscript are drawn are duly presented in the main paper and related tables and figures. Sequence data are submitted to Genbank with the following accession numbers: JF273951, JF273953, JF273955, JF273957, JQ796149, JQ796150, JF273961, JF273962, JF273965, JQ796152, JQ796153, JQ796154, JQ796155, JQ796156, JQ796157, JQ796158, JQ796159, JQ796160, KR229828, JQ796163, JF273935, JF273936, JQ796168, JQ796169 and GenBank Submissions group 6,957,604. All sequences are also provided as Additional file 1 (SMF1 - accession numbers) and Additional file 2 (SMF2 - GenBank Submissions grp 6,957,604).
Authors’ contributions
Designed the study: DT, JF, MMS, SCB, RS, FCS, CFP, VC, AN. Plan and perform the experiments: JF, DT, MMS, GT, AJN, SMS, CG. Analyse and interpret the data: GC, CNF, BD GT, DT, JF, MMS, FCS. Initiate the manuscript: JF, GT, DT, MMS. Revise the manuscript: All the authors Approved the final version of the manuscript: All the authors.

Ethics approval and consent to participate
Ethical clearance was obtained from the Ethics Review and Consultancy Committee of the Cameroon Bioethics Initiative (Reference number: CBI/2013/0139/ERC/CA/MBIN), and written informed consent was provided. For those participants under 16 years of age, a written proxy-informed consent was provided to the study coordinators by the respective parent(s) or legal guardian(s), followed by an assent provided by the corresponding participant. Data were de-identified for purpose of confidentiality and privacy in data management.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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