ORIGINAL RESEARCH

Alarming rates of virological failure and HIV-1 drug resistance amongst adolescents living with perinatal HIV in both urban and rural settings: evidence from the EDCTP READY-study in Cameroon

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Objectives

Adolescents living with perinatal HIV infection (ALPHI) experience persistently high mortality rates, particularly in resource-limited settings. It is therefore clinically important for us to understand the therapeutic response, acquired HIV drug resistance (HIVDR) and associated factors among ALPHI, according to geographical location.

Methods

A study was conducted among consenting ALPHI in two urban and two rural health facilities in the Centre Region of Cameroon. World Health Organization (WHO) clinical staging, self-reported adherence, HIVDR early warning indicators (EWIs), immunological status (CD4 count) and plasma viral load (VL) were assessed. For those experiencing virological failure (VF, VL ≥ 1000 copies/mL), HIVDR testing was performed and interpreted using the Stanford HIV Drug Resistance Database v.8.9–1.

Results

Of the 270 participants, most were on nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens (61.7% urban *vs.* 82.2% rural), and about one-third were poorly adherent (30.1% *vs.* 35.1%). Clinical failure rates (WHO-stage III/IV) in both settings were < 15%. In urban settings, the immunological failure (IF) rate ($CD_4 < 250$ cells/μL) was 15.8%, statistically associated with late adolescence, female gender and poor adherence. The VF rate was 34.2%, statistically associated with poor adherence and NNRTI-based antiretroviral therapy. In the rural context, the IF rate was 26.9% and the VF rate was 52.7%, both statistically associated with advanced clinical stages. HIVDR rate was over 90% in both settings. EWIs were delayed drug pick-up, drug stock-outs and suboptimal viral suppression.

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Conclusions

Poor adherence, late adolescent age, female gender and advanced clinical staging worsen IF. The VF rate is high and consistent with the presence of HIVDR in both settings, driven by poor adherence, NNRTI-based regimen and advanced clinical staging.

Introduction

Since its discovery in 1983, over 70 million people have been infected with HIV and over 35 million deaths have been reported. By the end of 2017, over 36.9 million people were found to be living with HIV, and there were about 940 000 deaths during that same year [1–4]. The greatest burden of disease is borne by sub-Saharan Africa (SSA), accounting for *c.* 70% of the global infection [5]. It is notable that West and Central Africa account for 21% of the world's new HIV infections and 30% of global deaths from AIDS-related illness, and this includes Cameroon [6]. In 2019, the prevalence of HIV in Cameroon was 2.7%, with a high rate of new infections among adolescents and young people.

Antiretoviral therapy (ART) coverage for treating HIV infection was estimated at 21.7 million worldwide by the end of 2017. ART is a key element of the ambitious 90–90–90% strategy put in place by the Joint United Nations Program on HIV/AIDS (UNAIDS) with the goal of ending the AIDS epidemic by 2030, indicating that 90% of people living with HIV (PLHIV) should know their status, 90% of those diagnosed should be on ART and 90% of these should be on virological suppression (VS) [6]. By the end of 2018 in Cameroon, among 520 069 PLHIV nationwide, 72.2% (337 862) were tested for HIV infection, 66.7% (281 083) of those tested were receiving ART, 57.2% (119 708) of those receiving ART were tested for viral load (VL) and 77.8% (93 139) of those tested for VL had viral suppression (< 1000 RNA copies/mL) [7–10]. In spite of this progress, there are challenges in meeting the desirbale target performance, of which HIV drug resistance (HIVDR) is part of the bottleneck [10].

HIV drug resistance emergence and transmission are of greater concern in SSA due to other programmatic factors, such as inappropriate prescribing practices, drug supply, poor retention in care and limited access to reference laboratory monitoring assays (genotyping, VL, CD4, etc.) [11].

Although the number of AIDS-related deaths has decreased by 30% for all ages, the exception is the adolescent age group (10–19 years old), who experienced a 50% increase in the same period [12–18]. Notably, current uptakes in Prevention of Mother-to-Child Transmission (PMTCT) and universal ART for children suggest that any infected child stands at high risk of initial HIVDR, related to varying PMTCT practices over time, use of drugs with low genetic barrier to resistance, frequent drug stock-outs and poor adherence. Coupled with high viraemia in children and limited laboratory monitoring, detection of treatment failure is often delayed, leading to accumulation of HIVDR at rates as high as 80%, which jeopardizes treatment outcomes in adolescence [16–17]. In such a context, strategies for optimal sequencing of ART regimens would maximize long-term treatment success and reduce AIDS-related morbidity and mortality for adolescents living with HIV (ALHIV) as they grow towards adulthood [18–19]. When evaluating the efficacy of first- and second-line paediatric ART regimens in resource-limited settings (RLSs), Boerma *et al.* [2] reported the necessity to set up salvage paediatric therapies that could be affordable in RLSs, which entails an eventual need for newer second-line or third-line ART. In the Central African Republic, an assessment of virological response and drug resistance mutations (DRMs) revealed 34% of children in need of a second-line ART after first-line failure, while 47% of those under second/third-line ART were experiencing virological failure (VF) with at least one major DRM, thus calling for improved paediatric ART and monitoring strategies. Among Cameroonian children (aged up to 12 years), a high rate of HIVDR (*c.* 90%) was reported at first-line failure, after a median time on ART of 2 years only; second-line ART guided by genotyping showed *c.* 70% with undetectable viraemia and *c.* 90% with viraemia < 1000 RNA copies/mL; and CRF02_AG was the main viral clade [20]. As these children grow older in Cameroon, evidence-based strategies are needed to limit VF and HIVDR at adolescence, thus ensuring long-term ART success and an effective transitioning to adult care.

Our goal was to generate information towards improved recommendations for a successful long-term management of adolescents living with perinatal HIV infection (ALPHI) in SSA in general, and Cameroon in

particular, with adequate and affordable transition policies from paediatric to adult regimens. To achieve this, we assessed the therapeutic response to first- and second-line regimens, drug resistance profiles and genotypes in HIV-1 in the urban and rural settings of the Centre Region of Cameroon. Specifically, we sought to determine the proportion of these patients with clinical and immunovirological failure; evaluated DRMs and circulating subtypes among those experiencing VF; determined potentially active antiretroviral drugs for those with multiple DRMs having multi-drug resistance (MDR); and evaluated HIVDR early warning indicators (EWIs) in the study population.

Methods

Study design and target population

An analytical and cross-sectional study was carried out from December 2018 to May 2019 among ALPHI aged 10–19 years and receiving ART in one of the selected health facilities within the Resistance Evolution among Adolescents in Yaoundé and its surroundings (READY-study) in the Centre Region of Cameroon. Participants were recruited following exhaustive sampling.

Description of study sites

The study sites were from both urban and rural settings of the Centre region of Cameroon, classified as follows:

- two health facilities from the referral treatment centres specialized in paediatric care, the Mother-Child Centre of the Chantal BIYA foundation, (MCC-FCB), and the National Social Welfare Hospital (NSWH), both in Yaoundé (Fig. 1);
- two health facilities from the management units located 30–50 km (Fig. 1) from the urban settings, the Mbalmayo District Hospital, (HDMB), Mbalmayo, and the Mfou District hospital, (HDMF), Mfou, with the Nkomo Integrated Health Centre (NIHC) and the Bikop Catholic Health Centre (BCHC) as satellite sites (Fig. 1) in the Center Region of Cameroon.

Based on classification of healthcare facilities, the two urban sites were secondary and the three rural sites were tertiary structures. All sites were affiliated to the public sector except for the NSWH which was para-statal. First- and second-line ART regimens were provided at all sites. ART registers and patient medical files were available at all sites.
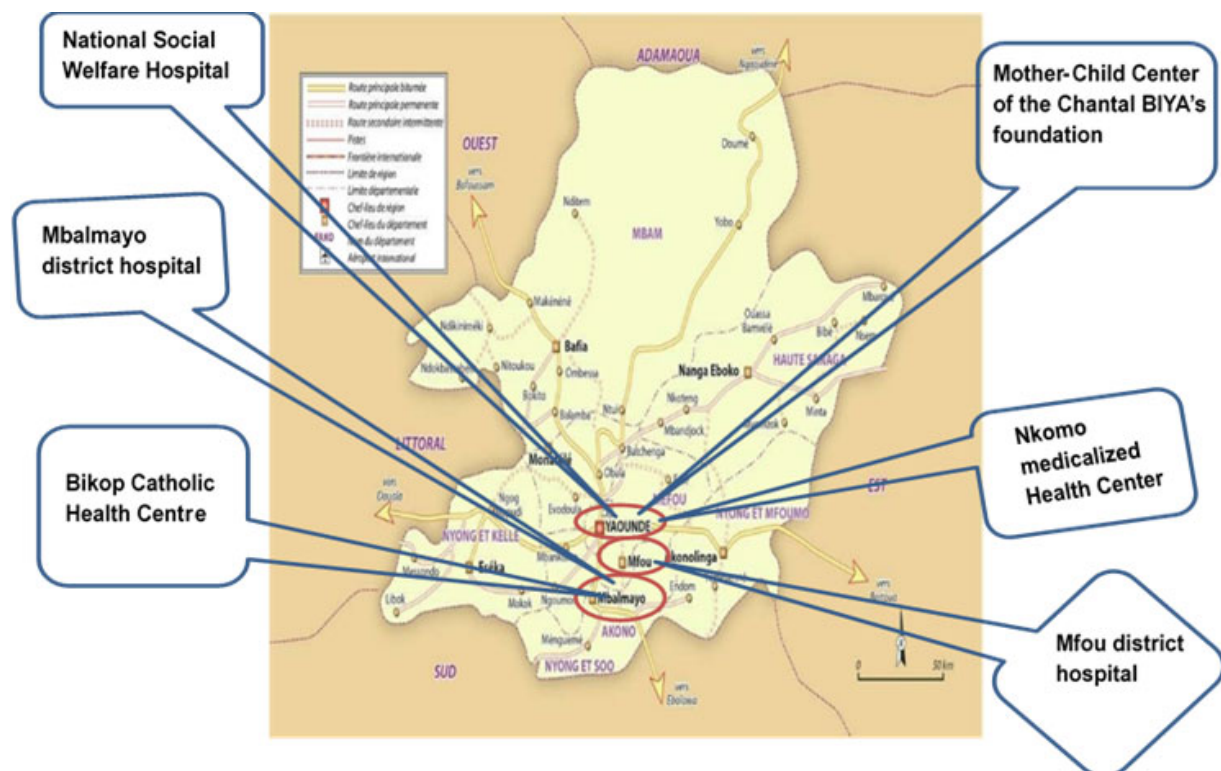

Fig. 1 Distribution of study sites. [Colour figure can be viewed at wileyonlinelibrary.com]

Sampling method and eligibility criteria

Site selection was on the basis of: (1) years of experience in paediatric ART management ($\geq$ 3 years); (2) availability of first- and second-line ART regimens at the clinic pharmacy; (3) conformity to national ART guidelines; (4) availability of ART registers, patient medical files and/or a database; (5) the number of adolescents on ART per site ($n \geq 15$), and geographical location (urban *vs.* rural).

An exhaustive and non-randomized sampling method was used for participant enrolment, following eligibility criteria.

Eligibility criteria

Inclusion criteria
The study included every ALHIV who was aged 10–19 years, who had been receiving a standard first- or second-line ART regimen for at least 6 months at one of the study sites, and who provided written assent and written proxy-informed consent from their legal guardian(s). ALHIV were enrolled irrespective of PMTCT antiretroviral history (exposed, not exposed or unknown exposure).

Non-inclusion criteria
The following were not considered for enrolment: ALHIV not found (i.e. not formally registered) in the ART monitoring system of a study site, as well as ALHIV reported to be ART-naïve, to be on a drug regimen not included in the national guidelines, or to be on a structured treatment interruption.

Exclusion criteria
Any participant who decided deliberately/freely to withdraw during the course of the study, or who transferred away from a study site before the mid- or endpoint was excluded from the study.

Consecutive enrolment of adolescents on ART was performed in every study site, until full sampling was achieved. The minimal sample size required to carry out this study was established following statistical calculations [21].

Sample size

Assuming the rate of VF at 40%, a 95% confidence interval (CI) and 80% statistical power, the sample size was 174 patients. Adding 10% potential long-term follow-up (LTFU) during the 1-year study period and 20% sequencing failure rate, the minimal sample size was 243 ALHIV, rounded-up to 250, and then further stratified into 150 ALHIV in the referral centres (75 per site) and 100 in the rural HIV management unit settings (50 per site), as per coverage in ART in these two geographical locations. Effectively 270 participants were enrolled, 196 from referral centres and 74 from management units.

Data collection, quality assurance and data validation

Participants who were on ART for $\geq$ 6 months, attended one of the study sites for their follow-up visits and agreed to take part in this study were invited to sign an informed assent and provide written proxy-informed consent from their legal guardian(s). We administered a standardized questionnaire to all ALHIV, covering sociodemographic data, treatment history, adherence level, as well as basic clinical and biological parameters. In addition, data were collected from ART registers, patient medical files, and pharmacy records. Incoherent data were resolved by retrieving additional source documents available at the clinic. Making use of the self-reported method for assessing adherence, poor adherence was defined as the number of missing doses during the last 30 days.

To ensure reliability in collected data, a final-year medical student and an MSc student were trained in HIVDR, program quality indicators (PQIs) and ART programme, as well as in the procedures for PQI data abstraction following the WHO strategy [22–25]. Collected data were proofread for consistency, and then data validation was done by a supervisor (senior virologist) with field experience of the collection, monitoring, evaluation and reporting of data in the ART programme.

Clinical and laboratory procedures

From each participant, we collected 10 mL of whole blood in the study sites and transported these within 6 h to the Chantal Biya International Reference Centre for research on HIV/AIDS prevention and management (CIRCB), Yaoundé, Cameroon. From these, plasma aliquots were obtained after centrifugation at 1600 rpm for 10 min and stored at −80°C for further analysis.

CD4 cell count was performed using the Pima CD4 (Abbott/Pantech (Pty) Ltd, Westville, South-Africa) automatic test as per the manufacturer's instructions. Plasma VL measurement was performed using the Abbott Applied Biosystem platform (Real Time PCR AB m2000RT) as-per the manufacturer's instructions (Abbott Laboratories, Des Plaines, IL, USA), with a detection threshold of 40 copies/mL (lower) and 10 000 000 copies/mL (upper).

To increase sensitivity of the polymerase chain reaction (PCR) amplification, HIV-1 RNA extraction was performed from 1000 μL of plasma, and an initial 2-h

refrigerated centrifugation at 14 000 rpm in order to concentrate viral RNA was also performed.

HIV-1 RNA was extracted manually from 140 μL of plasma using the Qiagen protocol (QIAamp® DNA Mini-kit; Qiagen, Courtaboeuf, France). Genotypic resistance testing (GRT) was done when the VL was > 1000 copies/mL using a homemade protocol [26], which amplifies the protease and reverse-transcriptase regions in the pol gene fragment (~1600 bp). PCR products were sequenced using the Applied Biosystems 3500 genetic analyser; the sequences thus obtained were edited using Bio-edit v.5.0.26 software and DRMs were interpreted using the Stanford HIV drug resistance database v.8.9-1. Subtyping was done using MEGA v.10 for molecular phylogeny.

Data interpretation

The major outcomes of the study were the rate of immunovirological failure among ALHIV, antiretroviral (ARV) drug resistance profile including ARV DRMs with ARV drug resistance defined as the presence of a genotypic resistance to at least one ARV drug, and the viral genetic diversity among these adolescents. A normal immune status was defined as absolute CD4 count ≥ 250 cells/μL and immunological failure (IF) as < 250 CD4 cells/μL, virological suppression (VS) as VL < 40 HIV-1 RNA copies/mL and VF as VL ≥ 1000 RNA copies/mL. Adherence was evaluated by self-reporting, and poor adherence was defined as missing more than one dose of ART during the last 30 days.

HIV drug resistance EWIs were interpreted following the WHO recommendations as defined by the respective target performance from EWI 1 to EWI 7, shown in Table 1.

Statistical analysis

Dependent variables were IF, VF and HIVDR, while independent variables were age range, duration on ART, ART regimen line, adherence level, gender, clinical stages and viral subtypes. χ^2 and Fisher's exact tests were used to determine associations between qualitative variables. Multivariate logistic regression models were used to assess the relationship between the dependent and independent variables (which had a P-value ≤ 0.2 in univariate analyses), and a linear regression model was used to evaluate the predictive value of CD4 count in cases of VF. Data were analysed using Epi Info v.7.2.2.16 (CDC, www.cdc.gov) with P < 0.05 considered statistically significant.

Ethical considerations

Ethical clearance no. 2018/01/981/CE/CNERSH/SP for the study was obtained from the national ethics committee for research on human subjects in Yaoundé, Cameroon. A research authorization was obtained from the CIRCB directorate and administrative authorizations from the study sites. Confidentiality and core ethical values were respected. Laboratory results of viraemia CD4 cell count and GRT were freely delivered to each participant for their clinical benefits, data management was kept strictly confidential through the use of unique identifiers and access to data was protected by a password.

Results

Sociodemographic characteristics of study population

After exhaustive sampling, a total of 196 (112 from MCC-FCB and 84 from NSWH) adolescents and 74 (51 from HDMF and 23 from HDMB) adolescents were enrolled in the two urban study sites and in the two rural study sites respectively. With respect to geographical location, females were more represented (56.1%) in the urban sites while both males and females were equally distributed in rural sites; the median (IQR) age of participants was 16 (14–18) years in urban and 14 (12–17) years

Table 1 Early warning indicators (EWIs) and their target performances

EWIs	EWI target (%)	Green (%)	Amber (%)	Red (%)
1. On-time pill pick-up (% of patients who picked up drug before or on the planned date at the hospital pharmacy during the past 12 months of ART)	> 90	> 90	80–90	<80
2. Retention on ART (% of patients retained during the past 12 months)	> 85	> 85	75–85	<75
3. No pharmacy drug stock-out (% of months with no stock-out of any ARV drug during the past 12 months)	100	100	NA	<100
4. Dispensing practices (% of adolescents receiving a triple ART as per the national/WHO guidelines), without monotherapy or dual therapy	100	100	NA	<100
5 Viral load coverage (% of patients on ART receiving at least one routine viral load test in a year)	≥ 90	> 70	NA	<70
6. Viral load suppression (% viral load suppression among people on ART with a viral load test available).	> 90	> 90	75–90	<75
7. Proportion of switch to second-line ART (at least 5% of people receiving second-line ART)	≥ 5	100	NA	<100

ART, antiretroviral therapy; ARV, antiretroviral; NA, not applicable; WHO, World Health Organization.

in rural sites; with respect to age ranges, late-aged adolescents (15–19 years) accounted for 64.8% in urban sites whereas early aged adolescents (10–14 years) represented (52.7%) in rural sites.

Clinical and biological characteristics of study population

The median [interquartile range (IQR)] duration on ART was 9 (4–11) years in urban sites *vs.* 5 (3–9) years in rural sites; 61.7% (121/196) of participants were on nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens in urban sites [with 67.9% (57/84) and 71.4% (80/112) good self-reported adherence in NSWH and MCC-FCB, respectively] *vs.* 82% (61/74) in rural sites [with 64.86% (48/74) good self-reported adherence to ART]. The majority of the adolescents in both urban [91.0% (178/196)] and rural sites [87.8% (65/74)] were in clinically asymptomatic stages (I and II).

The median (IQR) CD4 count was 541 (330.5–772) cells/μL with a 15.8% (31/196) prevalence of IF in urban sites *vs.* 482 (195–808) cells/μL with a 26.9% (18/67) prevalence of IF in rural sites (Table 2). The median VL was 64 RNA copies/mL (with values ranging from < 40 to 2 331 109 RNA copies/mL) in urban sites *vs.* 2649 RNA copies/mL (with values ranging from < 40 to 3 512 122 RNA copies/mL) in rural sites. Viral load values were distributed as shown in Table 3. The prevalence of VF was 34.2% (67/196) in urban *vs.* 52.7% (39/74) in rural sites [with odds ratio (OR) (95% CI) = 2.15 (1.25–3.69) and *P*-value of 0.005 between the two sites].

In the urban setting, bivariate analysis revealed that IF was significantly higher in older adolescents (19.9%) than in younger ones (8.7%) [*P* = 0.04, OR (95% CI) = 2.57 (1.0–6.6)]. Patients with poor adherence (23.7%) were more likely to experience IF as opposed to those with good adherence (12.4%) [OR (95% CI) = 2.2 (1.1–4.9), *P* = 0.04]. However, there was no significant association between IF and study site, gender, duration on ART and ART regimen line. Multivariate analysis (Table 4) revealed that the factors that could serve as predictors of IF were older age [OR (95% CI) = 1.24 (1.03–1.5), *P* = 0.02], female sex [0.4 (0.2–0.9), *P* = 0.04] and poor adherence to ART [0.4 (0.2–0.9), *P* = 0.04].

In the rural setting, bivariate analysis between IF and gender, age range, study sites, ART regimen line and adherence level revealed that there was no significant association; however, there was a statistically significant association between IF and clinical stages [OR (95% CI) = 0.128 (0.03–0.59), *P* = 0.003]. Multivariate analysis (Table 4) with the variables regimen line, WHO clinical stages, self-reported adherence, site group and age ranges revealed that WHO clinical stage was the only variable that was independently associated with IF [OR (95% CI) = 0.10 (0.02–0.58), *P* = 0.009].

With respect to VF within the urban context, bivariate analysis revealed that VF was significantly associated with poor adherence [OR (95% CI) = 2.1 (1.1–3.9), *P* = 0.02]. In addition, participants on first-line ART were twice as likely to experience VF [OR (95% CI) = 2.1 (1.1–4.1), *P* = 0.02]. Moreover, participants in clinical failure were three times more likely to experience VF (95% CI: 1.3–9.3, *P* = 0.01), and being in immunological failure increased the probability of experiencing VF [OR (95% CI) = 3.9 (1.7–8.6), *P* < 0.001]. However, factors such as age ranges, study site, gender and duration on ART had no significant association with VF. Multivariate analysis (Table 5) between VF and its associated factors, age, ART line, adherence, gender and study site, revealed that the predictive factors of VF were: being on first-line ART [OR (95% CI) = 2.58 (1.29–5.17), *P* = 0.01] and poor adherence to ART [0.4 (0.24–0.90), *P* = 0.02].

In the rural setting, bivariate analysis revealed that there was no significant association between VF and its associated factors (age range, gender, ART regimen line, adherence) except with advanced clinical stages [OR (95%

Table 2 Distribution of CD4 count ranges at urban study sites (a) and rural study sites (b)

CD4 count ranges	Frequency	Percentage
(a) Rural sites		
< 100	6	8.96%
100–250	12	17.91%
251–499	17	25.37%
≥ 500	32	47.76%
Total	67	100.00%
(b) Urban sites		
< 100	8	4.08%
100–250	23	11.73%
251–499	61	31.12%
≥ 500	104	53.06%
Total	196	100.00%

CD4, cluster of differentiation 4

Table 3 Distribution of viral load ranges at rural study sites (a) and urban study sites (b)

Viral load range	Frequency	Percentage
(a) Rural sites		
< 40	29	39.19%
40–999	6	8.11%
≥ 1000	39	52.70%
(b) Urban sites		
< 40	86	43.88%
40–999	43	21.94%
≥ 1000	67	34.18%

Table 4 Predictors of immunological failure (IF) at urban study sites (a) and rural study sites (b)

	Odds of immunological failure	95% CI	P-value	Interpretation
(a) Urban sites				
Age (10–14/15–19 years)	1.24	1.03–1.50	0.02	Being an older adolescent increases the risk of immunological failure 1.24-fold
Gender (male/female)	0.40	0.20–0.90	0.04	Males have less risk of IF than do females
Adherence (good/poor)	0.40	0.20–0.90	0.04	Being poorly adherent to ART increases the risk of IF
ART line (first/second)*	0.60	0.20–1.32	0.20	
Site (CME/CNPS)	1.63	0.70–3.90	0.25	
(b) Rural sites				
ART line (first/second)*	0.46	0.10–2.13	0.324	
WHO clinical stages (I/II; III/IV)	0.10	0.02–0.58	0.009	The likelihood of participants in clinical stages I/II experiencing IF is lower than that for those in stages III/IV
Adherence (good/poor)	1.86	0.45–7.76	0.396	
Site group (HDMF/CMAN, HDMB/CSCB)	0.72	0.18–2.82	0.637	
Age ranges (10–14/15–19 years)	1.97	0.56–6.88	0.289	
Gender (female/male)	0.74	0.22–2.51	0.634	

ART, antiretroviral therapy; CI, confidence interval; CMAN, Nkomo Integrated Health Centre; CME, Mother-Child Centre of the Chantal BIYA foundation; CNPS, National Social Welfare Hospital; CSCB, Bikop Catholic Health Centre; HDMB, Mbalmayo District Hospital; HDMF, Mfou District Hospital.
*First-line ART consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) + one nonnucleoside reverse transcriptase inhibitor (NNRTI); second-line ART line consisted of two NRTIs + one ritonavir-boosted protease inhibitors (PI/r).

Table 5 Predictors of virological failure (VF) at urban study sites (a) and rural study sites (b)

Variables	Odds of VF	95% CI	P-value	Interpretation
(a) Urban sites				
Age (10–14/15/19 years)	1.1	0.98–1.27	0.09	
ART line (first/second)*	2.58	1.29–5.17	0.01	Being on first-line ART increases the chance of being in VF two-fold
Adherence (Good/Poor)	0.47	0.24–0.90	0.02	Being poorly adherent to ART increases the chance of being in VF
Gender (female/male)	0.75	0.40–1.42	0.38	
Site (CME/CNPS)	1.74	0.92 −3.30	0.08	
(b) Rural sites				
Age range (10–14/15–19 years)	2.356	0.842–6.593	0.103	
ART line (first/second)*	0.314	0.069–1.409	0.130	
Adherence (good/poor)	0.635	0.216–1.866	0.409	
Clinical stages (I/II, III/IV)	0.099	0.011–0.929	0.043	Participants with clinical stages III/IV have greater odds of VF than do those with clinical stages I/II.

ART, antiretroviral therapy; CI, confidence interval; CMAN, Nkomo Integrated Health Centre; CME, Mother-Child Centre of the Chantal BIYA foundation; CNPS, National Social Welfare Hospital; CSCB, Bikop Catholic Health Centre; HDMB, Mbalmayo District Hospital; HDMF, Mfou District Hospital.
*First-line ART consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) + one nonnucleoside reverse transcriptase inhibitor (NNRTI); second-line ART line consisted of two NRTIs + one ritonavir-boosted protease inhibitor (PI/r).

CI) = 0.11 (0.01–0.96), P = 0.02]. Multivariate analysis between VF and its associated factors, age range, ART regimen line, adherence and clinical stages (Table 5), showed that the only variable that was independently associated with VF was clinical stage [OR (95% CI) = 0.099 (0.01–0.93) and P = 0.043].
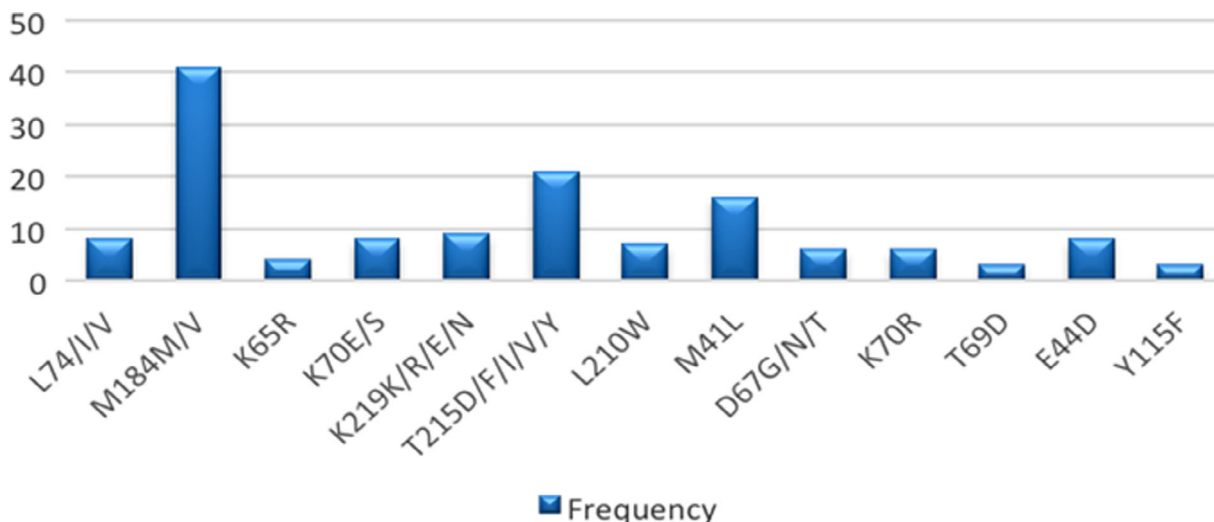
HIV-1 genotypic resistance profile

Among the 67 patients in VF (34.2%) in the urban study sites, 64 sequences were obtained. Of the 64 sequences, 49 (76.6%) belonged to participants on first-line ART and 15 (23.4%) on second-line with a 92.2% (59/64) prevalence of acquired HIVDR. In the rural setting, of the 74

participants enrolled, 39 (52.7%) experienced VF. From the latter, 31 sequences were successfully obtained and, of these, 28 (90.32%) were shown to experience HIVDR to the ART regimen they were exposed to with at least one DRM.

The prevalence of HIV DRMs was higher in participants on a first-line reverse transcriptase inhibitor-based (RTI-based) ART regimen than in those on second-line ritonavir-boosted protease inhibitor (PI/r)-based ART, with proportions of 78.1% (50/64) *vs.* 80.7% (25/31) to nucleoside reverse transcriptase inhibitors (NRTIs), 89.1% (57/64) *vs.* 90.3% (28/31) to NNRTIs, and 4.7% (3/64) *vs.* 3.2% (1/31) to PI/r, in urban *vs.* rural sites, respectively. The major HIV DRMs to all three ARV drug classes are shown in Figs 2–4. With respect to the class of NNRTIs, the prevalence of DRMs to first-generation NNRTIs was 89.1% (57/64) *vs.* 90.3% (28/31), and to second- generation NNRTIs was 81.3% (52/64) *vs.* 38.7% (12/31) in urban *vs.* rural sites, respectively, in spite of the absence of second-generation NNRTIs in the Cameroonian national ART programme. In addition, the prevalence of dual class resistance (NRTIs + NNRTIs) was 76.6% (49/64) *vs.* 77.4% (24/28) and that of triple class resistance (NRTIs + NNRTIs + PI/r) was 4.7% (3/64) *vs.* 3.2% (1/28) in urban *vs.* rural sites, respectively.

Association analysis revealed that there was no significant association between HIVDR and factors such as gender, age range, regimen line, viral subtypes (CRF02_AG *vs.* non CRF02_AG) and adherence in both urban and rural study sites. Details are provided in Figures 2, 3 and 4.

Genetic diversity of HIV-1 subtypes

The most common genetic variant was circulating recombinant form, CRF02_AG (60.9% *vs.* 54.8% in urban *vs.* rural sites, respectively). The presence of two unclassified unique recombinant forms (URFs), URF A1/G and URF G/A1, in the urban sites is noteworthy. Moreover, the proportion of pure subtypes *vs.* recombinants was 23.5% *vs.* 76.5% and 32.3% *vs.* 67.7% in urban and rural sites, respectively. Subtype/genetic variant distribution is shown in Table 6.

Assessing early warning indicators (EWIs)

Seven HIVDR EWIs were assessed using WHO standard values shown in Table 1. This revealed that the major factors driving HIVDR were delayed drug pick-up, drug stock-outs and poor VL suppression rate in both urban and rural study sites, as shown in Table 7.

Discussion

Increased access to ART for HIV-infected children in SSA has resulted in reductions in HIV-related morbidity and mortality in children, but these benefits are threatened as they mature into adolescents, with increasing mortality rates [27]. This study addressed therapeutic outcomes and HIV-1 drug resistance patterns among ALPHI in urban and rural settings in Cameroon, as there are limited findings in the target population in our setting. A total of 270 adolescents were enrolled who met the inclusion
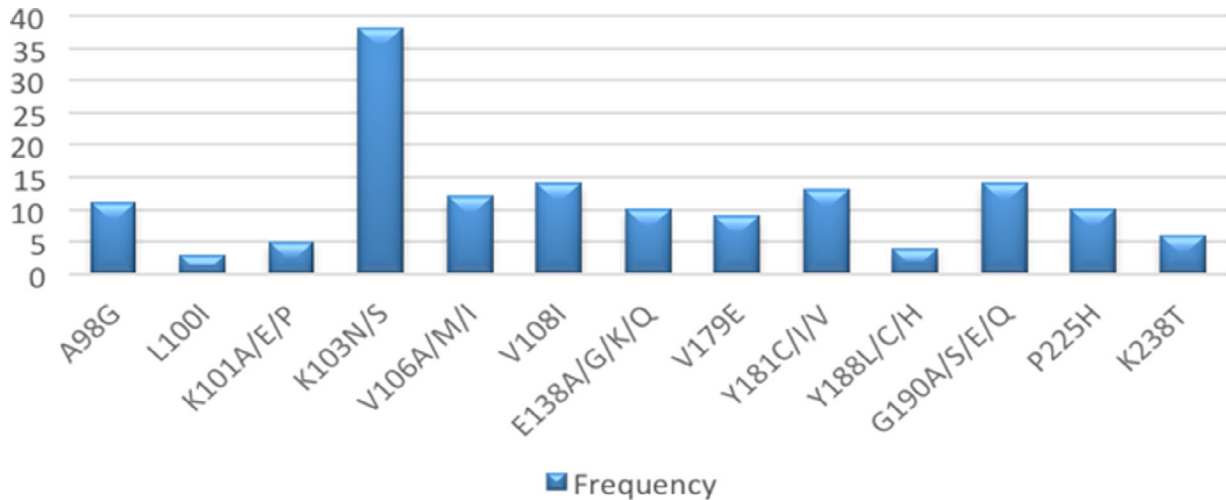

Fig. 2 Frequency of drug resistance mutations (DRMs) to nucleoside reverse transcriptase inhibitors (NRTIs) at urban sites. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig. 3 Frequency of major drug resistance mutations (DRMs) to nonnucleoside reverse transcriptase inhibitors (NNRTIs) at urban sites. [Colour figure can be viewed at wileyonlinelibrary.com]
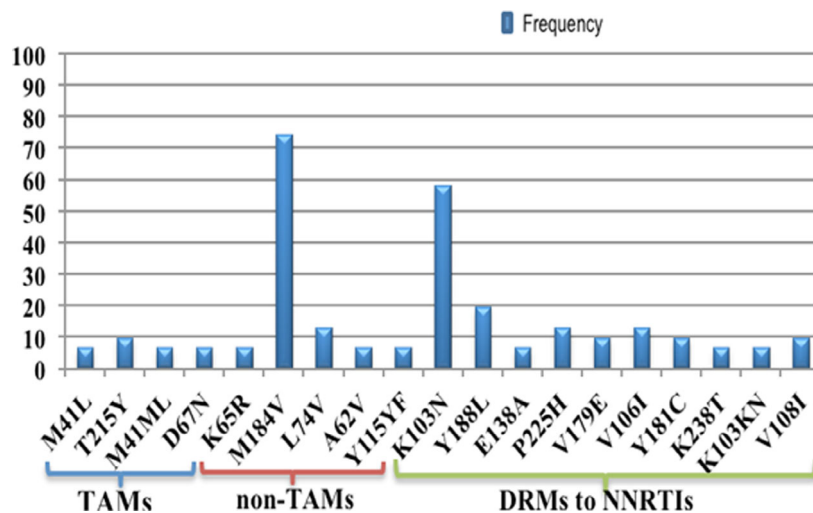

Fig. 4 Major drug resistance mutations (DRMs) to nucleoside reverse transcriptase inhibitors (NRTIs) [with proportions of thymidine analogue mutations (TAMs) and non-TAMs] and non-NRTIs (NNRTIs). [Colour figure can be viewed at wileyonlinelibrary.com]

criteria, with a sampling rate beyond minimal size, which supports the representativeness of our findings and external validation of our data with regard to the target population of ALPHI in similar settings in SSA.

All participant ages ranged from 10 to 19 years, with a median age of 16 years in urban settings and 14 years in rural settings. These findings are different from findings in a study carried out by Fokam *et al.* [3] in a similar urban context in 2017, where the median age was 13 years, thus indicating progressive growth to adulthood. With regard to classification of adolescents into age groups, the early-age adolescents were a larger proportion compared with the late-age adolescents in the rural context. This could be explained by the fact that older adolescents have consistently been susceptible to challenges such as sexual development (puberty), decreased adherence, disclosure of HIV status, adverse social stigma with regard to their condition in both resource-rich and -limited countries, all of which are factors that favour decreased adherence to treatment, loss to follow-up and hence high mortality rate in adolescents aged ≥ 15 years [28]. It is worth noting that some of these patients transition to adult care in urban settings with the aim of furthering their studies or learning a

Table 6 Distribution of HIV-1 subtypes/genetic variants at urban sites (a) and rural sites (b)

Subtypes	Frequency	Percentage
(a) Urban sites		
A1	6	9.38%
URF A1/G	2	3.13%
URF G/A1	1	1.56%
CRF01_AE	1	1.56%
CRF02_AG	39	60.94%
CRF13_cpx	2	3.13%
CRF11_cpx	1	1.56%
CRF18_cpx	1	1.56%
CRF37_cpx	1	1.56%
D	1	1.56%
F2	5	7.81%
G	4	6.25%
Total	64	100%
(b) Rural sites		
A	2	6.45%
CRF11_cpx	1	3.23%
CRF13_cpx	1	3.23%
CRF18_cpx	1	3.23%
CRF37_cpx	1	3.23%
CRF02_AG	17	54.84%
D	1	3.23%
F2	4	12.9%
H	1	3.23%
G	2	6.45%
Total	31	100%

Cpx, complex; CRF, circulating recombinant form; URF, unknown recombinant form.

trade. This may explain a predominance of late-age adolescents in the urban context (65%). There was a slight female predominance in the urban context (female/male ratio of 6/5), which is in line with the national data from the birth registry in the country. This distribution pattern was also similar to studies by Fokam *et al.* [3], Koech *et al.* [29] and Sithole *et al.* [30] in Cameroon, Uganda and Zimbabwe respectively, all studies showing a female predominance of 52% and 82%, respectively. This shows that females are probably more at risk of being infected with HIV than males even in the urban context, as stated in recent WHO findings [31–33], but this is not the case in rural settings with a female/male ratio of 1:1. With regard to duration of treatment in urban and rural contexts, the overall median durations on ARV were 9 and 5 years, respectively. This indicates longer exposure to ARVs at urban sites than at rural sites, due to relative differences in accessibility to healthcare, standards of living and literacy, leading to earlier diagnosis and commencement of ART. Over 61.7% *vs.* 82% of these adolescents were on first-line (NNRTI-based regimens), in urban *vs.* rural settings, respectively. Findings by Fokam et al. [3] in 2017 gave a proportion on first-line therapy of > 92%, implying a relative decrease in NNRTI use, following increased detection of VF, which is concordant

with the national ART programme [34]. With regard to self-reported adherence to ART, we observed that up to 30% and 35% of our participants in urban and rural settings, respectively, had poor levels of adherence. These findings are dissimilar to those obtained by Fokam *et al.* in 2017, with up to 64% of poor adherence; findings by Natukunda *et al.* [35] in Uganda rather showed a poor adherence level of 20%. The observed difference between these studies is due to varying behaviours throughout adolescence and possible information bias attributed to the self-reported approach used [3,35]. Moreover, other researchers recommend use of multiple methods such as caregiver and youth self-reports, pill count and pharmacy records [36].

The overall rate of clinical failure was < 15% (9.2% urban *vs.* 12.2% rural). Our findings are relatively different from a study carried out by Muri *et al.* [37] in 2016 in Tanzania where they had clinical failure rates as high as 45% (done only in rural setting). This discrepancy can be explained by the fact that the children enrolled in the latter study had a longer follow-up period, combined with suboptimal adherence [OR (95% CI) =3.90 (1.11–13.68)] and pre-ART data suggesting that > 85% had acquired key mutations during treatment that resulted in therapeutic failure translating into clinical failure.

We observed IF rates (CD4 < 250 cells/μL) of 15.8% and 26.9% in urban and rural settings, respectively. This is slightly different from the 21.4% figure in a study by Fokam *et al.* in the same urban setting in 2017, a discrepancy that can be attributed to a lower cut-off for immune failure in our study. Recent guidelines from WHO explain the relatively lower rate in the urban context [38]. In the rural setting, the rate of IF was higher in males, 28.13%, within the early adolescence age range. This was not in agreement with the study carried out by Bailey *et al.* [39] within a cohort of 903 ALPHI in South America. This discrepancy could be attributed to adherence issues, disclosure of HIV status or rigorous follow-up of the young adolescent by parents or counsellors. The overall relatively low prevalence of IF in adolescents could be due to their better immune recovery as compared with older adults, related to high thymic scores and immune restoration, giving them greater capacity to recruit and repopulate CD4 cells. Considering multivariate analysis IF in urban settings, it was noted that a poorer immune status remained significantly associated with older age, female gender and poor adherence. This can be explained by a decline in CD4 as adolescents grow towards adulthood, which in turn indicates a higher vulnerability to disease and increased risk of mortality [40]. These findings are in agreement with those of Makadzange *et al.* [27], where immunological

Table 7 Program quality indicators for urban study sites (a) and rural study sites (b)

EWIs per site	EWI_1	EWI_2	EWI_3	EWI_4	EWI_5	EWI_6	EWI_7
Target	> 90%	≥ 85%	100%	100%	≥ 90%	≥ 90%	≥ 5%
(a)							
Mother and child centre	81.7% (98/120)	92.6% (112/121)	75.0% (9/12)	100% (120/120)	100% (121/121)	63.1% (70/111)	24.0% (23/96)
National social welfare hospital	73.9% (68/92)	91.3% (84/92)	50.0% (6/12)	100% (92/92)	100% (92/92)	71.1% (54/76)	8.3% (5/60)
(b)							
Mfou District Hospital	60.0% (9/15)	83.3% (15/18)	75.0% (9/12)	100% (15/15)	100% (11/11)	36.4% (4/11)	27.3% (3/11)
Nkomo Medical Centre	60.0% (12/20)	93.0% (40/43)	58.3% (7/12)	100% (20/20)	100% (40/40)	55.0% (22/40)	17.1% (7/40)
Mbalmayo District Hospital	30.6% (11/36)	65.0% (13/20)	50.0% (6/12)	100% (36/36)	100% (13/13)	38.5% (5/13)	7.7% (1/13)

EWI, early warning indicator(s).
Performance: green, desirable; amber, fair; red, poor. NNRTIs. [Colour table can be viewed at wileyonlinelibrary.com]

failure was more frequent in older adolescents, which proves that advanced age leads to significant immune remodelling and decline [41]. In rural settings, however, the risk of IF in participants in clinical stages III/IV was higher than for those in stages I/II ($P = 0.03$). This could be explained by the fact that severe clinical symptoms have severe immunodepression as an underlying cause; therefore, patients in clinical stage class III/IV are generally immunodepressed, resulting in the emergence of opportunistic infections.

With regard to VF, we observed VF rates as high as 34.2% and 52.7% in urban and rural settings, respectively. This implies that up to a third of ALPHI in urban settings, and up to half of those in rural settings, fail to suppress and sustain their VL to the < 1000 RNA copies/mL threshold. This is below the 90–90–90 targets recommended by UNAIDS [1]. These results are different from findings by Fokam *et al.* with a VF rate of 20.7% [3]. This discrepancy can be attributed to the fact that ART effectiveness is increasingly challenged by the remoteness in Cameroon, especially in rural settings, and could be due to both patient-related factors, such as longer distance and time to reach the hospital, lower incomes, lower literacy, and poorer living conditions in these RLSs, and programmatic factors such as poor knowledge and experience of healthcare workers in HIV/AIDS management and ART use, staff shortage (especially physicians and pharmacists), poor laboratory infrastructure and high frequency of drug stock-outs [42]. Multivariate analysis of VF confirms poor adherence and NNRTI-based regimen use as strong predictors in urban settings, showing that good adherence and use of second-line ART produce better virological outcomes, due to the higher genetic barrier of ritonavir-boosted protease inhibitors (PI/r). This is in agreement with findings by Natukanda *et al.* [35], Muri

et al. [37] and Salou *et al.* [43], but disagrees with findings by Fokam *et al.* [26] and Zanoni *et al.* [44]. In rural settings, younger adolescence and advanced clinical stage were found to be predictors of VF. This disagreed with a previous study by Adejumo *et al.* [36], which suggested that VF was poorer among older adolescents due to poorer ART adherence patterns.

Among 64/67 patients and 31/39 who benefited from GRT in urban and rural samples, respectively, prevalence of HIV DRMs was very high (92.2% *vs.* 90.3%), particularly in those on a NNRTI-based regimen, due to its weaker genetic barrier and prolonged use as opposed to the high genetic barrier and higher potency of PI/r. With respect to ARV drug class, the prevalence rates of HIVDR in urban *vs.* rural setting were 78.1% *vs.* 80.7% to NRTIs, 89.1% *vs.* 90.3% to NNRTIs, and 4.7% *vs.* 3.2% to PI/r [31, 37, 43–46]. It is also worth noting that DRM rate to second-generation NNRTIs is high (81.3% *vs* 38.7% in urban and rural settings despite an absence of these drugs in the Cameroonian ART programme. This is due to cross-resistance from first-generation NNRTIs in those who present with the highest DRM rate. Thus, VF is effectively a result of DRMs, which in turn greatly renders ART ineffective [47].

With regard to EWIs, factors that favoured HIVDR were delayed ARV pick-up, consistent drug stock-outs and poor VS in both settings. These could be as a result of suboptimal adherence and heavy workload at pharmacies to dispense these drugs. Another reason for the stock-out could be the relative absence of a concise plan for adolescents who have to be managed with either paediatric or adult regimens, without a specific dedicated facility. These findings are partly in agreement with findings by Fokam et *al.* [48] in 2016 in the Centre Region of Cameroon with respect to drug stock-outs. However, the factor

regarding VS was not assessed in similar studies within the same country [48,49].

Limitations included the cross-sectional design of the study and the inability to take into account psychosocial effects that could impact therapeutic outcomes.

Conclusions

In summary, ALPHI receiving ART at both urban and rural study sites generally appeared asymptomatic, with a low rate of IF. Nonetheless, IF increases with older age (15–19 years), female gender and poor adherence. VF at both urban and rural study sites is alarming, especially with first-line NNRTI-based ART urban settings and advanced clinical stage in rural settings. This high rate of VF is consistent with acquired drug resistance in both urban and rural settings, driven by resistance to the NNRTI class. This is also alarming, considering that these adolescents need lifelong treatment (i.e. presumably for many decades), while the MDR-based restriction of therapeutic options might hamper their life expectancy considerably in the coming years. Although CRF02_AG remains the most predominant variant for both the urban and rural study sites, there is a growing genetic diversity with potential novel unique recombinants within the urban context. Programmatic factors favouring HIVDR are delayed ARV drug pick-up, pharmacy stock-outs and poor VS.

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References

1 UNAIDS. Fact sheet 2016 Available at http://www.unaids.org/en/resources/factsheet (Geneva, Switzerland) (accessed 12 august 2020).

2 Boerma RS, Bunupuradah T, Dow D *et al.* Multicentre analysis of second-line antiretroviral treatment in HIV-infected children: adolescents at high risk of failure. *J Int AIDS Soc* 2017; **20**: 21930

3 Fokam J, Billong SC, Jogue F *et al.* Immuno-virological response and associated factors amongst HIV-1 vertically infected adolescents in Yaoundé-Cameroon. *PLoS One* 2017; **12**: e0187566.

4 WHO | HIV/AIDS / WHO. Available at http://www.who.int/gho/hiv/en/ (accessed 6 December 2018).

5 Mbanya D, Sama M, Tchounwou P. Current status of HIV/AIDS in Cameroon: how effective are control strategies? *Int J Environ Res Public Health* 2008; **5**: 378–383.

6 unaids-data-2018_en.pdf.

7 Coetzee J, Hunt G, Jaffer M *et al.* HIV-1 viraemia and drug resistance amongst female sex workers in Soweto, South Africa: a cross sectional study. *PLoS One* 2017; **12**: e0188606.

8 Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis* 2016; **46**: 292–307.

9 Bertagnolio S, De Luca A, Vitoria M *et al.* Determinants of HIV drug resistance and public health implications in low- and middle-income countries. *Antivir Ther* 2012; **17**: 941–953.

10 Zazzi M, Hu H, Prosperi M. The global burden of HIV-1 drug resistance in the past 20 years. *Peer J* 2018; **6**: e4848. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5971836/.

11 Kaleebu P, Kirungi W, Watera C *et al.* Virological response and antiretroviral drug resistance emerging during antiretroviral therapy at three treatment centers in Uganda. *PLoS One* 2015; **10**: e0145536.

12 Fitzgerald F, Penazzato M, Gibb D. Development of antiretroviral resistance in children with HIV in low- and middle-income countries. *J Infect Dis* 2013; **207**: S85–S92.

13 Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK. Panel on clinical practices for treatment of HIV. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Ann Intern Med* 2002; **137**: 381–433.

14 Rusine J, Asiimwe-Kateera B, van de Wijgert J *et al.* Low primary and secondary HIV drug-resistance after 12 months of antiretroviral therapy in human immune-deficiency virus type 1 (HIV-1)-infected individuals from Kigali, Rwanda. *PLoS One* 2013; **8**: e64345.

15 Bhatti AB, Usman M, Kandi V. Current scenario of HIV/AIDS, treatment options, and major challenges with compliance to

antiretroviral therapy. *Cureus* 2016; **8**: e515. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4818110/

16 Cameroon_art_2015.pdf. Available at https://aidsfree.usaid. gov/sites/default/files/cameroon_art_2015.pdf (accessed 20 May 2019).

17 Bekker LG, Hosek S. HIV and adolescents: focus on young key populations. *J Int AIDS Soc* 2015; **18**: 20076. Available at https://doi.org/10.7448/IAS.18.2.20027

18 Kisesa A, Chamla D. Getting to 90–90–90 targets for children and adolescents HIV in low and concentrated epidemics: bottlenecks, opportunities, and solutions. *Curr Opin HIV AIDS* 2016; **11**: S1–S5.

19 Rapport_annuel_cnls_2016.pdf.

20 (Enquête) [Internet]. Journal du Cameroun. 2019. Available at https://www.journalducameroun.com/le-taux-de-prevale nce-du-vihsida-est-de-2,7-au-cameroun-enquete/ (accessed 20 august 2020).

21 Chernick MR, Friis RH. *Introductory biostatistics for the health sciences: modern applications including bootstrap.* Hoboken, NJ, Wiley series in probability and statistics. Wiley-Interscience, 2003.

22 World Health Organisation. *Global action plan on HIV drug resistance. 2018 progress report.* Geneva, World Health Organisation, 2018.

23 Fokam J, Billong SC, Bissek ACZK *et al.* Declining trends in early warning indicators for HIV drug resistance in Cameroon from 2008-2010: lessons and challenges for low-resource settings. *BMC Public Health*, 2013; **13**: 308.

24 Fokam J, Elat J-BN, Billong SC *et al.* Monitoring HIV drug resistance early warning indicators in Cameroon: a study following the revised world health organization recommendations. *PLoS One* 2015; **10**: e0129210.

25 World Health Organization. *Consolidated guidelines on person-centred HIV patient monitoring and case surveillance.* Guidelines. Geneva, World Health Organization, 2017: 1–224.

26 Fokam J, Salpini R, Santoro MM *et al.* Performance evaluation of an in-house human immunodeficiency virus type-1 protease-reverse transcriptase genotyping assay in Cameroon. *Archive of Virology* 2011; **156**: 1235–1243.

27 Makadzange AT, Higgins-Biddle M, Chimukangara B *et al.* Clinical, virologic, immunologic outcomes and emerging HIV drug resistance patterns in children and adolescents in public ART care in Zimbabwe. *PLoS One* 2015; **10**: e0144057.

28 Judd A, Davies M-A. Adolescent transition among young people with perinatal HIV in high-income and low-income settings. *Curr Opin HIV AIDS* 2018; **13**: 236–248.

29 Koech E, Teasdale CA, Wang C *et al.* Characteristics and outcomes of HIV-infected youth and young adolescents enrolled in HIV care in Kenya. *AIDS Lond Engl* 2014; **28**: 2729–2738.

30 Sithole Z, Mbizvo E, Chonzi P *et al.* Virological failure among adolescents on ART. Harare City, 2017- a case-control study. *BMC Infect Dis* 2018; **18**: 469.

31 WHO | Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection [Internet]. WHO. Available at http://www.who.int/hiv/pub/arv/arv-2016/en/ (accessed 18 December 2018).

32 World Health Organization. Global action plan on HIV drug resistance 2017-2021: 2018 progress report, July 2018: executive summary. 2018; Available at https://apps.who.int/ iris/handle/10665/273049 (accessed 22 May 2019).

33 WHO_Consolidated_Guidelines_Annexes_2.4.6.pdf [Internet]. Available at https://www.who.int/hiv/pub/guidelines/WHO_ Consolidated_Guidelines_Annexes_2.4.6.pdf?ua=1 (accessed 22 May 2019).

34 Cameroon-report. National Guidelines to the Prevention and Management of HIV/AIDS in Cameroon [Internet]. Cameroon-report.com. 2018. Available at https://cameroon-report.com/sante/national-guidelines-to-the-prevention-and-management-of-hiv-aids-in-cameroon/ (accessed 20 May 2019).

35 Natukunda J, Kirabira P, Ong KIC, Shibanuma A, Jimba M. Virologic failure in HIV-positive adolescents with perfect adherence in Uganda: a cross-sectional study. *Trop Med Health* 2019; **47**: 8.

36 Adejumo OA, Malee KM, Ryscavage P, Hunter SJ, Taiwo BO. Contemporary issues on the epidemiology and antiretroviral adherence of HIV-infected adolescents in sub-Saharan Africa: a narrative review. *J Int AIDS Soc* 2015; **18**: 20049.

37 Muri L, Gamell A, Ntamatungiro AJ *et al.* Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. *AIDS* 2017; **31**: 61–70.

38 WHO | What's new in treatment monitoring: viral load and CD4 testing [Internet]. WHO. Available at http://www.who. int/hiv/pub/arv/treatment-monitoring-info-2017/en/ (accessed 22 May 2019).

39 Bailey H, Cruz MLS, Songtaweesin WN, Puthanakit T. Adolescents with HIV and transition to adult care in the Caribbean, Central America and South America, Eastern Europe and Asia and Pacific regions. *J Int AIDS Soc* 2017; **20**: 21475.

40 Ford N, Meintjes G, Vitoria M, Greene G, Chiller T. The evolving role of CD4 cell counts in HIV care. *Curr Opin HIV AIDS* 2017; **12**: 123–128.

41 Evolution of the immune system in humans from infancy to old age [Internet]. Available at https://www.ncbi.nlm.nih.gov/ pmc/articles/PMC4707740/ (accessed 29 May 2019).

42 Boullé C, Guichet E, Kouanfack C *et al.* Virologic failure and human immunodeficiency virus drug resistance in rural Cameroon with regard to the UNAIDS 90–90-90 treatment targets. *Open Forum Infect Dis* 2016; **3**: ofw233.

43 Salou M, Dagnra AY, Butel C *et al.* High rates of virological failure and drug resistance in perinatally HIV-1-infected children and adolescents receiving lifelong antiretroviral therapy in routine clinics in Togo. *J Int AIDS Soc* 2016; **19**: 20683.

44 Zanoni BC, Archary M, Buchan S, Katz IT, Haberer JE. Systematic review and meta-analysis of the adolescent HIV continuum of care in South Africa: the cresting wave. *BMJ Glob Health* 2016; **1**: e000004.

45 Guichet E, Aghokeng A, Serrano L *et al.* Short communication: High viral load and multidrug resistance due to late switch to second-line regimens could be a major obstacle to reach the 90-90-90 UNAIDS objectives in Sub-Saharan Africa. *AIDS Res Hum Retroviruses* 2016; **32**: 1159–1162.

46 Szubert AJ, Prendergast AJ, Spyer MJ *et al.* Virological response and resistance among HIV-infected children receiving long-term antiretroviral therapy without virological monitoring in Uganda and Zimbabwe: observational analyses within the randomised ARROW trial. *PLoS Med* 2017; **14**: e1002432.

47 Clutter DS, Fessel WJ, Rhee SY *et al.* Response to therapy in antiretroviral therapy-naive patients with isolated non-nucleoside reverse-transcriptase inhibitor-associated transmitted drug resistance. *J Acquir Immune Defic Syndr* 2016; **72**: 171–176.

48 Fokam J, Elat J-BN, Billong SC *et al.* Monitoring HIV drug resistance early warning indicators in cameroon: a study following the revised World Health Organization Recommendations. Paraskevis D, editor. *PLoS One* 2015; **10**: e0129210.

49 Billong SC, Fokam J, Penda CI *et al.* Predictors of poor retention on antiretroviral therapy as a major HIV drug resistance early warning indicator in Cameroon: results from a nationwide systematic random sampling. *BMC Infect Dis* 2016; **16**: 678.