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Pretreatment HIV drug resistance predicts accumulation of new mutations in ART-naïve Ugandan children

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Abstract

Aim: To assess the prevalence of pretreatment drug resistance (PDR) and its association with virologic outcomes after 24 weeks of antiretroviral therapy (ART), within an urban cohort of Ugandan children.

Methods: Prospective observational study. Baseline and 24-week assessments of viral load (VL) and genotypic drug resistance to nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) were performed.

Results: Ninety-nine ART-naïve children (3–12 years) initiated efavirenz-based ART 2015–2016 and 18/90 (20%) had baseline NRTI/NNRTI associated drug resistance mutations (DRMs). By 24 weeks, 72/93 (77%) children had VL < 40 copies/mL and a total of 23 children had DRMs. Children with PDR accumulated new DRMs with a mean number (SD) of 1.4 (2.35) new mutations compared to 0.26 (0.98) in 67 children with wild-type virus ($P = .003$). High pretreatment VL and PDR (number of baseline DRMs) predicted viremia ($P = .003$; $P = .023$) as well as acquired drug resistance ($P = .02$; $P = .04$).

Conclusion: Pretreatment drug resistance to NNRTI/NRTI was common among ART-naïve Ugandan children and predicted viremia and new resistance mutations after only 24 weeks of efavirenz-based therapy. PDR may compromise long-term ART outcomes—especially when access to resistance testing and VL monitoring is poor. The long-term importance of PDR for non-NNRTI-based regimens needs further evaluation.

Abbreviations: ADR, Acquired drug resistance; ART, Antiretroviral therapy; ARV, Antiretroviral drug; DRM(s), Drug resistance mutation(s); DTG, Dolutegravir; EFV, Efavirenz; HIVDR, HIV drug resistance; LMIC, Low- and middle-income country/Low- and middle-income countries; NNRTI(s), Non-nucleoside reverse transcriptase inhibitor(s); NRTI(s), Nucleoside reverse transcriptase inhibitor(s); NVP, Nevirapine; PDR, Pretreatment drug resistance; PMTCT, Prevention of mother-to-child transmission; SSA, Sub-Saharan Africa; VL, Viral load; WHO, World Health Organization.

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KEYWORDS

child, drug resistance, viral, efavirenz, HIV, Uganda

1 | INTRODUCTION

About half of children living with HIV globally had access to ART in 2017 compared to 22% in 2010.¹ Still, service delivery of HIV care remains challenging in many countries, with suboptimal monitoring of viral load (VL), drug stock-outs and poor retention in care potentially leading to the development of HIV drug resistance (HIVDR).² Drug resistance mutations (DRMs) can be acquired during treatment but may also be present prior to ART initiation. The prevalence of pretreatment HIV drug resistance (PDR) is increasing in low- and middle-income countries (LMIC) and is associated with poor viral suppression and accumulation of new DRMs.^{3,4} Children are particularly at risk, due to exposure in utero to antiretroviral drugs (ARVs) within prevention of mother-to-child transmission (PMTCT) programs. A recent meta-analysis estimated PDR prevalence at more than 40% in PMTCT-experienced children in sub-Saharan Africa (SSA).⁵ Other studies indicate an increased prevalence of PDR even in children without PMTCT exposure.⁶⁻⁸

Efavirenz-based treatment has been the most commonly used ART in adults and children from 3 years of age. Due to the rising HIVDR prevalence, WHO recently changed the recommendations for first line ART to include dolutegravir (DTG) combined with two nucleoside reverse transcriptase inhibitors (NRTIs) from six years of age.⁹ Uganda among other LMIC is already implementing this shift in therapy.¹⁰ Yet, many children still are or have been exposed to the non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (NVP) and efavirenz (EFV) which can affect future therapeutic options. Viral susceptibility to newer NNRTIs may be reduced through cross-resistance.¹¹⁻¹³ Persistent viremia due to NNRTI resistance also predisposes emergence of new DRMs¹⁴⁻¹⁶ and may compromise the NRTI backbone in future DTG-based ART regimens.

The occurrence of PDR as well as development of resistance to antiretroviral drugs (ARV) during treatment among children is not sufficiently studied. Neither are the characteristics of HIV-1 drug resistance profiles before and during ART in children living with HIV in SSA. As HIVDR is critical for future treatment results, we studied the occurrence and characteristics of HIVDR before and after 24 weeks of efavirenz-based therapy in previously ART-naïve Ugandan children living with HIV.

2 | PATIENTS AND METHODS

2.1 | Ethics

The study was approved by the Ethical Institutional Review Boards of School of Biomedical Sciences and Higher Degrees, Makerere University College of Health Sciences (SBS-HDREC 174), Uganda

Key notes

- Pretreatment HIV drug resistance (PDR) towards efavirenz based antiretroviral therapy (ART) is increasing among children with HIV and WHO now recommends non-efavirenz based therapy.
- PDR was present in 20% of ART-naïve Ugandan children initiating efavirenz based therapy 2015-2016 and predicted viremia and new resistance mutations after 6 months of therapy, despite no detectable viremia in 77% of the patients.
- The long-term importance of PDR for non-efavirenz-based regimens needs further evaluation.

National Council for Science and Technology UNCST (HS1659), Baylor College of Medicine Children's Foundation IRB Texas (H35046) and the Regional Ethical Review Board in Stockholm, Sweden (2016/1026-31). Written informed consent was obtained from caretakers. Treatment and services offered followed standard clinical routines of Baylor Uganda.

2.2 | Study design

The study protocol is part of a prospective observational cohort study called GENEFA ('The importance of pharmacogenetic variation on Efavirenz levels and treatment effects in ART-naïve HIV-infected Ugandan children aged 3-12 years'). GENEFA is a collaboration between Baylor College of Medicine Children's Foundation-Uganda (Baylor Uganda), Makerere College of Health Sciences (MakCHS), Uganda and Karolinska Institutet (KI), Sweden.

2.3 | Study participants and setting

Enrolment was performed during February 2015 to February 2016. Children were eligible if they were HIV-positive, aged 3-12 years, had a body weight ≥ 10 kg, were previously ART naïve with no treatment with any drug/substance potentially interacting with efavirenz (St Johns Worth, carbamazepine, phenytoin, phenobarbitone and rifampicin) within 12 weeks prior to study start. Out of 120 children screened, 99 were enrolled (Figure 1).

Prescription followed Ugandan national HIV treatment guidelines; efavirenz and two NRTIs (abacavir and lamivudine) dosed according to weight bands. Follow-up visits were scheduled at 2, 6, 12 and 24 weeks post-ART initiation.

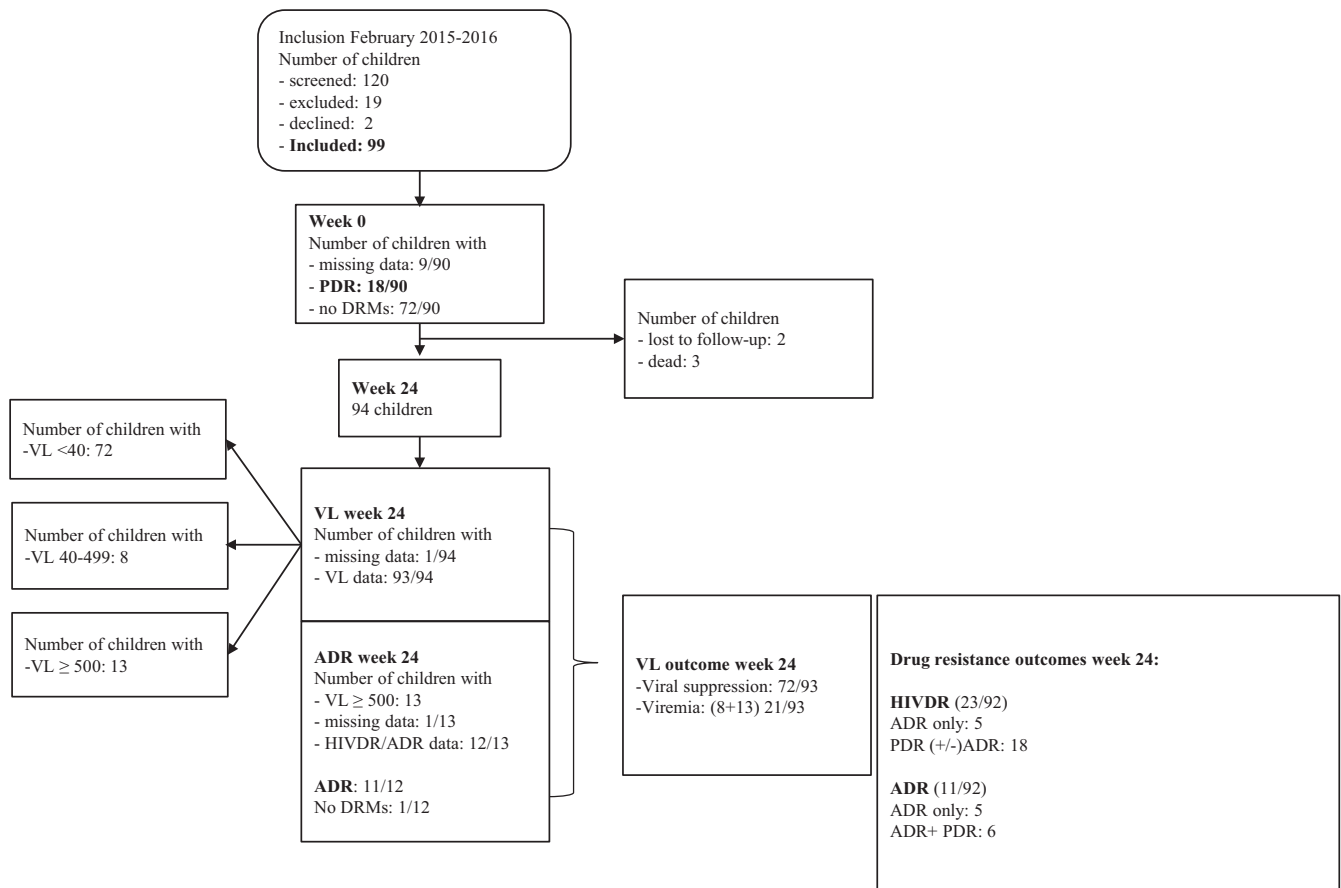


FIGURE 1 GENEFA study flow chart. Enrolment, follow-up and virologic outcomes before and after 24 wk of efavirenz-based therapy in 99 Ugandan children aged 3-12 y. Ten children were excluded due to co-infection with tuberculosis and nine for various other reasons. At week 24, 72/93 children had viral load (VL) < 40. Twelve children had VL ≥ 500 and available data from HIV drug resistance assay (HIVDR). Eleven of 92 children had acquired HIV drug resistance (ADR), defined as acquisition of at least one new drug resistance mutation (DRM) compared to baseline, while 81 children with VL < 500 and missing HIV drug resistance (HIVDR) assay at week 24 were assumed not to have ADR. All 18 children with pretreatment HIV drug resistance (PDR) were considered to still harbour baseline DRMs and were accordingly classified as HIVDR cases at week 24. The prevalence of HIVDR at 24 wk was 23/92

The study was conducted at the clinic of Baylor Uganda, which is a donor-funded Non-Governmental Organization located within the Mulago National Referral Hospital, Kampala and offers outpatient HIV services to HIV-infected children and their families, living in or within a 50 km radius from urban Kampala.

2.4 | Clinical assessments and variables monitored

The outcomes measured were prevalence of PDR, acquired HIVDR (ADR) (acquisition of ≥ one new DRM) and viremia (VL ≥ 40 copies/mL) after 24 weeks. Baseline factors potentially affecting VL and ADR were investigated. DRMs were identified by the 2015 IAS-USA mutation list,¹⁷ and HIVDR was classified according to Stanford HIV Drug Resistance Database (HIVdR), scoring predicted viral resistance levels as susceptible, potentially low, low, intermediate or high.¹⁸ HIVDR was defined as the presence of baseline DRMs known to confer any level of impaired susceptibility to any NRTI/NNRTI. HIVDR testing for other drug classes was not performed.

At baseline, demographic and clinical characteristics of the cohort were assessed and are listed in Table 1. Status of PMTCT exposure was collected through self-reported history. Immunodeficiency was classified according to WHO criteria¹⁹ (Table 1). Adherence was measured by pill count at all visits. A mean adherence was calculated for each individual and considered poor if <90%.

2.5 | Laboratory methods

CD4-cells were assayed by routine flow cytometry at the routine laboratory at Baylor Uganda. VL and HIVDR were analysed in CFAR Molecular Virology Lab/Resistance Lab of the Joint Clinical Research Centre Kampala. The laboratory is accredited by the College of American Pathologists' Laboratory Accreditation Program.²⁰

Viral load was detected and quantified with the Abbott m2000sp/rt platform (Abbott Laboratories) using Abbott RealTime HIV-1 assay. The lower limit of detection was 40 copies/mL.

TABLE 1 Baseline characteristics of a cohort of 99 ART-naïve Ugandan children aged 3-12 y, followed for 24 wk after initiating efavirenz-based ART at Baylor Uganda during 2015-2016

Variable	Variable number or median (IQR)	Percentage
Sex (Females)	59	60
Age (years)	6.2 (4.2-8.3)	
WHO Clinical stage 3/4	18	18
CD4 ⁺ absolute count (cells/ μ L)	545 (249-880)	
CD4 ⁺ (%)	17 (8-24)	
Immunodeficiency [†]	- None	38
	- Mild	16
	- Advanced	32
	- Severe	13
Primary caregiver	- Mother/Father	59
	- Other	40
Education, primary caregiver	- None	10
	- Primary	50
	- Secondary or higher	39
Maternal PMTCT [‡]	Yes	1
	No	84
	Unknown	14
Infant PMTCT	Yes	0
	No	83
	Unknown	16
Viral load (copies/mL) (Log ₁₀)	108,164 (29,666-423 365) 5.04 (4.42-5.63)	
[§] PDR (n = 90)	NNRTI and/or NRTI ^{††}	18
	NNRTI drug resistance	16
	NRTI drug resistance	4
	EVF/NVP drug resistance ^{‡‡}	12
HIV subtype (n = 90)	A	57
	D	25
	C	6
	B	1
	CRF02_AG [¶]	1

[†]According to WHO classification of immunodeficiency, based on CD4 percentage (% CD4⁺) and CD4⁺ absolute count of cells/ μ L (CD4⁺): None: % CD4⁺ >25% in children <5 y; CD4⁺ $\geq$ 500 in children $\geq$ 5 y; Mild: % CD4⁺ = 20%-35% in children <5 y; CD4⁺ = 350-499 in children $\geq$ 5 y; Advanced: % CD4 = 15%-19% in children <5 y; CD4⁺ = 200-349 in children $\geq$ 5 y; and Severe: % CD4 < 15% in children < 5 y; CD4⁺ < 200 or % CD4⁺ <15% in children $\geq$ 5 years.

[‡]PMTCT: prevention of mother-to-child transmission.

[§]PDR pretreatment drug resistance. CRF02_AG: Subtype A/G recombinant form.

^{††}NNRTI: non-nucleoside reverse transcriptase inhibitors, NRTI: nucleoside reverse transcriptase inhibitors.

^{‡‡}EVF/NVP: efavirenz and/or nevirapine. N = 99 unless otherwise stated.

Genotypic resistance was assayed by sequencing of the reverse transcriptase region using 3730xl Applied Bio-systems platform (Life Technologies). If a sequencing failed, one second assay was performed. Sequences were edited in REcall (beta V3.01), and

a web-based HIV drug resistance sequence analysis software²¹ (BC Centre for Excellence in HIV/AIDS) freely available at <http://pssm.cfenet.ubc.ca/> and then entered into HIVdB to obtain drug resistance profiles and HIV subtypes.

2.6 | Data analysis and statistical methods

Data were managed with REDCap.²² STATA 14.2 (StataCorp LLC) was used for statistical analysis.

Association between baseline characteristics and 24-week outcomes of virologic suppression and ADR was summarised in a cross-table using Fisher's exact test or chi-squared test and investigated with odds ratio (OR) using logistic regression. Statistically significant variables in univariable logistic regression were included in a multivariable model. Means were compared with *t* tests, medians with Mann-Whitney *U* test. All *P*-values were 2-sided and considered significant if <0.05 .

All baseline samples underwent PDR assay. When sequencing failed, results were classified as missing. At week 24, only samples with VL ≥ 500 copies/mL were sequenced. Children with VL < 500 copies/mL and no PDR were classified as not having HIVDR in week 24 data analysis. Conversely, all children with PDR were assumed to still harbour baseline DRMs, even if virally suppressed by week 24.

3 | RESULTS

3.1 | Baseline characteristics

Baseline characteristics of the 99 study participants are summarised in Table 1. The NRTI backbone was lamivudine and abacavir in all but one child who received zidovudine instead of abacavir. Only one child had a self-reported history of PMTCT. No/unknown PMTCT history was reported by 84/99 (85%) and 14/99 (14%) of children, respectively. In 52/99 (53%), the mother accompanied the child at the first visit, and in 59/99 (60%), the mother (53%) or the father (7%) was reported as the primary caregiver.

3.2 | Pretreatment HIV drug resistance (PDR)

Genotypic HIV drug resistance sequences were available in 90/99 cases, and mutations conferring resistance to any NNRTI/NRTI were present in 18/90 (20%) children (Table 1). The prevalence of NNRTI,

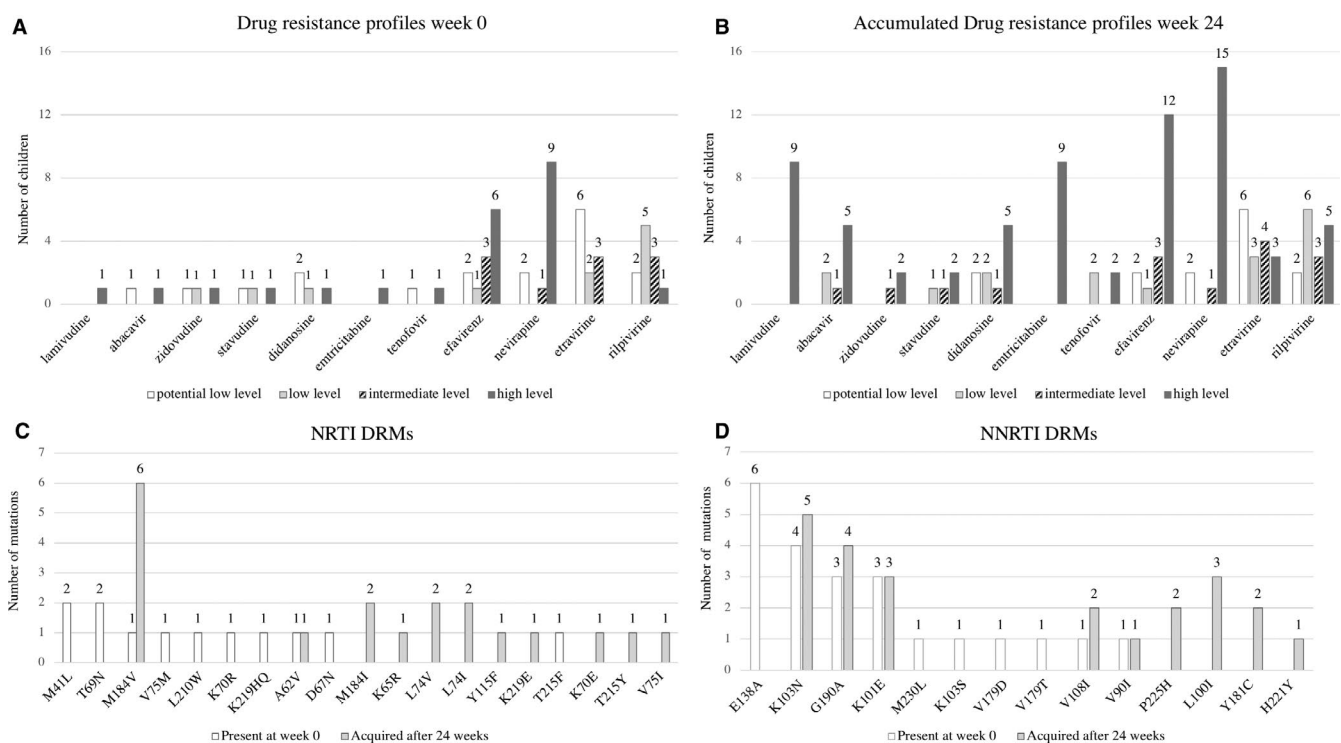


FIGURE 2 Distribution of individual drug resistance profiles and NNRTI and NRTI drug resistance mutations in ART-naïve Ugandan children at baseline and after 24 wk of efavirenz-based therapy. A, Drug resistance profiles among eighteen children with pretreatment drug resistance. Ninety out of 99 children had a successful HIVDR assay. The number of children with virus susceptible to the individual drugs is not shown. B, Accumulated drug resistance profiles at week 24 (ie pretreatment and acquired drug resistance). At week 24, 92 children were sampled for VL. Out of them, successful HIVDR assay was performed in 12/13 children with (VL) ≥ 500 copies/mL. Newly acquired DRMs were detected in 5 children without pretreatment drug resistance at baseline. Children with baseline pretreatment drug resistance ($n = 18$) were considered to still harbour drug resistant virus, irrespective of VL level at week 24. In summary, 23 children were considered to have HIVDR at week 24. Children with VL < 500 copies/mL and no pretreatment drug resistance were classified as having virus susceptible to all drugs. The number of children with virus susceptible to the individual drugs is not shown. C-D. NRTI and NNRTI DRMs detected in HIVDR assay at week 0 and at week 24. DRMs, drug resistance mutations; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor

NRTI and dual-class resistance was 16/90 (18%), 4/90 (4%) and 2/90 (2%), respectively. The one child reporting PMTCT experience had no DRMs detected. Predicted resistance pattern for each drug is shown in Figure 2.

In total, 13 (14%) children had reduced viral susceptibility to at least one of the drugs in the current regimen. One child had high-level resistance to all drugs tested, except etravirine.

Twelve (13%) children had reduced viral susceptibility to both EFV/NVP, of which nine (10%) displayed intermediate- and/or high-level resistance to both drugs. Four (4%) had intermediate/high resistance to rilpivirine and/or etravirine.

In total, 33 DRMs were detected in the 18 children with PDR (Figure 2). The median number of DRMs per patient in the PDR group was 1 (range 1-10). NNRTI mutations represented 64% of the DRMs at baseline, and the predominating DRM was E138A (Figure 2). Details of the children with DRMs are displayed in Table S1.

3.3 | Follow-up

The median (IQR) follow-up time was 24.2 (24.0-24.9) weeks. At week 24, 94 children remained in the study, two were lost to follow-up and three had died (Figure 1). Two deaths occurred within four weeks from diagnosis due to severe acute malnutrition and pneumonia, respectively, while one child died after eleven weeks, due to suspected HIV-associated nephropathy and severe acute malnutrition. Among the children that completed 24 weeks, nine received in-patient care during the study, the most common cause being malaria, followed by septicæmia and pneumonia.

3.4 | Viral Load and predictors of detectable viremia at week 24

VL results were available in 93 children of whom 72 (77%) had VL < 40 copies/mL and 80 (86%) had VL < 400 copies/mL. Out of 21 children with viremia, thirteen had VL $\geq$ 500 copies/mL (1531-1 258 054 copies/mL) and eight had viremia between 55 and 322 copies/mL (Figure 1).

Eighty-five children had available results from both baseline PDR assay and 24-week assays of VL and drug resistance. Among them, 55 out of 67 (82%) children without baseline DRMs were virally suppressed while only 10/18 (56%) of children with PDR achieved VL < 40 ($P = .02$). In children with missing results from PDR assay, 7/9 achieved suppression.

Pretreatment drug resistance and other baseline characteristics are shown in Table 2 and were further evaluated as potential risk factors for VL $\geq$ 40 copies/mL at 24 weeks (Table 3). The odds for VL $\geq$ 40 copies/mL increased 9.6 times for every $\log_{10}$ increase in VL ($P = .003$) and by 3.6 for every additional DRM at baseline ($P = .023$). Immunodeficiency predicted VL $\geq$ 40 copies/mL in the univariable model, but not when adjusted for baseline viral load and PDR. Sex,

age and mean adherence were also not associated with VL $\geq$ 40 copies/mL.

Among 86 individuals with a mean adherence >90%, 18 had viremia and a higher median VL at baseline ($\log_{10} = 5.72$; $\log_{10} = 4.89$, $P < .001$) than 68 children with virologic suppression. Baseline advanced/severe immunodeficiency was also more common in the subgroup with viremia (83% vs 53%, $P = .02$), while there was no statistically significant difference in the distribution of age, sex or PDR between these two subgroups.

3.5 | HIV drug resistance and predictors of ADR at week 24

HIVDR could be analysed in 12/13 children with VL $\geq$ 500 copies/mL at 24 weeks. Eleven children had ADR, including five with wild-type virus at baseline. As we considered all 18 children with PDR to still harbour baseline DRMs at week 24 (detectable or not), the number of children with any HIVDR was 23/92 (25%) (Figure 1). Corresponding counts for NNRTI, NRTI and dual-class resistance were 22/92 (24%), 11/92 (12%) and 10/92 (11%), respectively. Where baseline data were available ($n = 84$), comparisons showed an increase of NNRTI, NRTI and dual-class resistance from 19% to 26% ($P = .014$); from 2% to 11% ($P = .008$); and from 2% to 11% ($P = .008$), respectively.

Figure 2 displays the week 24 resistance profiles for the individual drugs tested. Thirteen per cent (12/92) had high-level resistance against EFV compared to 6.7% (6/90) at baseline, and 6.5% (6/92) harboured intermediate-/high-level resistance to all three antiretrovirals prescribed. In addition, three children had intermediate-/high-level resistance to zidovudine and/or tenofovir, both recommended as second line NRTI backbone.²³

The children with ADR had an average of 3.8 new DRMs (range 1-8) compared to baseline ($P = .0001$). The accumulation of new DRMs was significantly higher among 18 children with baseline DRMs, with a mean (SD) of 1.4 (2.35) compared to 0.26 (0.98) in 66 children with baseline wild-type virus ($P = .0029$). Associations between baseline characteristics and ADR were evaluated (Tables 2 and 3). Odds for acquiring at least one new DRM at week 24, increased 3.2 times for every added baseline DRM. High VL at baseline and poor mean adherence also predicted ADR. No child with missing results from HIVDR baseline assay had developed DRMs by week 24. The most frequent NNRTI and NRTI DRMs were K103N and M184V (Figure 2).

3.6 | CD4 and immunological status at week 24

The proportion of children without immunodeficiency increased from 35% at baseline to 62% ($P = .0003$) after 24 weeks. Corresponding figures for children with PDR/wild-type virus were 33%-72% ($P = .019$) and 33%-60% ($P = .0015$). There was no difference between children with and without PDR regarding mean

TABLE 2 Baseline characteristics and treatment adherence of children with/without viremia and with/without acquired HIV drug resistance at 24 wk

	Viremia n (%)	No viremia n (%)	Total N	P- value	ADR n (%)	No ADR n (%)	Total N	p-value
Sex				.803				0.340
Female	12 (57)	44 (61)			5 (45)	50 (62)		
Male	9 (43)	28 (39)			6 (55)	31 (38)		
	21(100)	72(100)	93		11 (100)	81 (100)	92	
Age				.628				0.752
<6 y	11 (52)	33 (46)			6 (55)	38 (46)		
6-12 y	10 (48)	39 (54)			5 (45)	43 (54)		
	21 (100)	72 (100)	93		11 (100)	81 (100)	92	
Immune deficiency [†]				.002				0.066
No	4 (19)	33 (46)			2 (18)	35 (43)		
Mild	2 (9)	11 (18)			2 (18)	13 (16)		
Advanced	1 (5)	13 (15)			0 (0)	12 (15)		
Severe	14 (67)	15 (21)			7 (64)	21 (26)		
	21 (100)	72 (100)	93		11(100)	81(100)	92	
Caregiver				.024				0.116
Mother/father	17 (81)	38 (53)			9 (82)	45 (56)		
Other	4 (19)	34 (47)			2 (18)	36 (44)		
	21(100)	72 (100)	93		11(100)	81(100)	92	
Viral load baseline, copies/ml				.000				0.006
<100 000	2 (9)	42 (58)			1 (9)	43 (53)		
100 000-500 000	9 (43)	25 (35)			6 (55)	28 (35)		
>500 000	10 (48)	5 (7)			4 (36)	10 (12)		
	21 (100)	72 (100)	93		11 (100)	81 (100)	92	
PDR (all NNRTI/NRTI) [‡]				.018				0.003
No	12 (60)	55 (85)			5 (45)	61 (84)		
Yes	8 (40)	10 (15)			6 (55)	12 (16)		
	20 (100)	65 (100)	85		11 (100)	73 (100)	84	
[§] PDR (EFV/NVP)				.006				0.007
No	13 (65)	60 (92)			6 (55)	66 (90)		
Yes	7 (35)	5 (8)			5 (45)	7 (10)		
	20 (100)	65 (100)	85		11 (100)	73 (100)	84	
Mean adherence				.188				0.035
<90%	3 (14)	4 (6)			3 (27)	4 (5)		
≥90%	18 (86)	68 (94)			8 (73)	77 (95)		
	21(100)	72(100)	93		11(100)	81(100)	92	

Note: Viremia defined as viral load (VL) >40 copies/mL after 24 wk of efavirenz-based therapy. Acquired HIV drug resistance (ADR) is defined as acquisition of at least one new DRM at week 24 compared to baseline.

[†]Immune deficiency: According to WHO classification of immunodeficiency, based on CD4 percentage (% CD4⁺) and CD4⁺ absolute count of cells/μL (CD4⁺):None: % CD4⁺ >25% in children <5 y; CD4⁺ ≥ 500 in children ≥5 y; Mild: % CD4⁺ = 20%-35% in children < 5 y; CD4⁺ = 350-499 in children ≥5 y; Advanced: % CD4 = 15%-19% in children <5 y; CD4⁺ = 200-349 in children ≥5 y; and Severe: % CD4 < 15% in children <5 y; CD4⁺ < 200 or % CD4⁺ < 15% in children ≥5 y.

[‡]PDR (all NNRTI/NRTI): pretreatment drug resistance to any non-nucleoside reverse transcriptase inhibitor/ nucleoside reverse transcriptase inhibitor.

[§]PDR (EFV/NVP): pretreatment drug resistance to efavirenz and/or nevirapine.

TABLE 3 Risk factors and odds ratio (OR) for viremia and acquired drug resistance after 24 wk of efavirenz-based therapy

Logistic Regression	Viremia		Acquired drug resistance (ADR)					
	Univariable N = 93 [†]		Multivariable N = 85			Univariable N = 92 [†]		
Baseline characteristics (Referent for categorical variable, unit for continuous variable)	Viremia [‡] n/N ₁		OR	95% CI	P-value	ADR [‡] n/N ₁	OR	95% CI
Sex	9/37	0.85	(0.32-2.27)	0.74	-	6/37	0.52	(0.15-0.18)
(male)	12/56					5/55		
female	21/93					11/92		
Total								
Age (years) [§]	-	0.97	(0.81 - 1.17)	0.76	-	-	0.95	(0.75-1.22)
Mean Adherence (%)	-	0.94	(0.85-1.04)	0.20	-	-	0.85	(0.76-0.96)
Immunodeficiency [§]	6/52	4.40	(1.53-12.79)	0.006	0.13	4/52	2.55	(0.69-9.40)
(None-mild)	15/41					7/40		
Advanced-Severe	21/93					11/92		
Total								
Viral load baseline [§] (Log ₁₀)	-	7.81	(2.51-24.27)	0.000	0.003	-	5.00	(1.42-17.72)
PDR [§] (no. of baseline mutations)	-	2.75	(1.23-6.16)	0.014	0.023	-	4.10	(1.60-10.23)
							8.90	(1.39-57.03)
							3.20	(1.04-9.70)

Note: Association between baseline characteristics, treatment adherence and viremia/ADR estimated with univariable and multivariable logistic regression. Variables with statistically significant odds ratio (OR) estimates in univariable analysis were included in a multivariable logistic regression model.

[†]N = 93 or 92 for all variables in univariable analyses except PDR where data were available for 85 and 84 patients.

[‡]Viremia n/N₁: number (n) of individuals with viral load ≥40 copies/mL/number (N₁) of children in each category (for categorical variables: sex, immunodeficiency).

[‡]ADRn/N₁ number (n) of individuals with viral ADR/number (N₁) of children in each category (for categorical variables: sex, immunodeficiency).

[§]Investigated as continuous variable.

CD4% (26.2 vs 26.8, $P = .83$) or CD4 absolute count (882.6 vs 906.3, $P = .87$) at 24 weeks.

4 | DISCUSSION

In this prospective study in ART-naïve Ugandan children, we found a 20% PDR prevalence with mutations conferring potential low-level to high-level resistance to NRTI/NNRTI. After 24 weeks of efavirenz-based therapy, the burden of resistance increased and 25% of children had experienced HIVDR. Yet, 77% achieved full viral suppression (VL < 40 copies/mL).

Most children reported no or unknown exposure to PMTCT, suggesting that other sources contributed to the PDR detected. Vertical transmission of resistant virus is plausible as WHO estimates that 19% of Ugandan women initiating ART have DRMs.² Possibly, we underestimated the PMTCT rate, as the history of PMTCT exposure relied solely on self-reporting. In a West-African cohort of ART-naïve children where few had reported PMTCT experience, 38% of children with PDR had antiretroviral drugs detected in plasma, suggesting unreported exposure to maternal ART.⁷ However, high PDR prevalence within cohorts of ART-naïve children characterised by poor PMTCT coverage is previously reported.^{7,8,14}

The overall PDR prevalence of 20% is somewhat higher than observed in the MARCH study, where 16.9% of Ugandan children initiating ART 2010-2011 had PDR (and 5% reported PMTCT exposure).¹⁴ This likely reflects methodological differences, as our definition of HIVDR was broad and included DRMs conferring *any* level of resistance, although an increase in PDR prevalence cannot be ruled out.

The predominating baseline DRM, E138A, is a polymorphic mutation reducing viral susceptibility towards the second generation NNRTIs etravirine/rilpivirine.^{24,25} It is not selected by efavirenz treatment and five out of six children harbouring E138A at baseline achieved VL < 40 copies/mL by 24 weeks. E138A has been detected in up to 8% of ART-naïve individuals and was reported as more common in HIV1-subtype C than B.⁶ We only detected E138A in children with subtype A and D, that were the predominating subtypes, as seen in other Ugandan cohorts.^{6,26}

Other dominating NNRTI baseline mutations were K103N, G190A and K101E, which are non-polymorphic DRMs typically selected during nevirapine and efavirenz therapy.²⁷ Y181C which is frequently seen in nevirapine exposed children (due to PMTCT) was not detected.⁸ This implies a low PMTCT rate, as reported by participants, but could also reflect that mutations commonly selected under NNRTI pressure, may be archived after sufficient time has passed from exposure and are present at frequencies too low for detection with standard HIVDR assay, as used in this study.²⁸

A meta-analysis of paediatric studies in LMIC, estimated that the rate of achieving VL < 1000 copies/mL within 6 months from ART start, increased from 62% to 82% between 2000 and 2010.²⁹ Our results were encouraging as 72/93 (77%) children achieved VL < 40 and 86% VL < 400 copies/mL after 24 weeks. Similar results

were reported from the ARROW trial, where 80% of a Ugandan/Zimbabwean paediatric cohort had achieved VL < 80 and 90.5% < 400 copies/mL after 36 weeks of efavirenz-based therapy.³⁰

We found that PDR and high baseline VL predicted ADR after 6 months of NNRTI-based treatment, which is in line with previous findings in ART-naïve Ugandan children.¹⁴

In other short- and long-term follow-ups, both young children^{16,30,31} and adolescents³⁰ experienced higher risk of poor viral suppression, but age did not predict virologic suppression in our study. Neither did sex nor CD4 levels which is in line with the 2-year follow-up from the MARCH cohort.¹⁴ Furthermore, poor mean adherence seemed predictive of ADR but not viremia. While high VL and poor immunostatus at baseline could impede viral decay, adherence assessments may also have been inaccurate.

Eleven children had detectable NRTI/NNRTI mutations at 24 weeks including ADR, but HIVDR prevalence was 23/92 (25%) as all 18 children with PDR were considered to still harbour baseline DRMs. Comparisons between baseline and week 24 ($n = 84$), showed that NRTI resistance increased markedly from 2% to 11%, mainly due to the emergence of M184V. This DRM is commonly selected under lamivudine pressure but is known to reduce viral fitness and may benefit net antiviral activity, even during lamivudine monotherapy.^{32,33}

The presence of NRTI DRMs could affect future DTG regimens. Although DTG has a high genetic barrier to drug resistance, viral failure and emergence of DTG-related DRMs have been observed in individuals on DTG monotherapy.³⁴ Current paediatric guidelines include DTG in combination with two NRTIs,⁹ but these regimens could be reduced to *functional* DTG monotherapy in individuals where the NRTI backbone has become compromised by HIVDR. This is of potential concern for settings like Uganda where PDR prevalence is high, but access to HIVDR assaying and regular VL testing is limited. The implications of DTG rollout for long-term trends of PDR and viral outcomes in Ugandan children need further evaluation with monitoring of the drug resistance patterns in both children and adults.

The strengths of our study include the prospective design and that almost 95% of the participants completed the study. The follow-up time is a limitation, as 24 weeks are short when evaluating viral suppression and the dynamics of HIVDR. The HIVDR assay could not detect minority variants, and we are likely to have underestimated the prevalence of PDR. Thus, we may have misclassified DRMs as acquired by week 24 even though they were already present at baseline. HIVDR could only be assayed when VL $\geq$ 500 copies/mL and children with low-level viremia at week 24 were classified as not having HIVDR in statistical analysis. As their VL ranged between 53 and 322 copies/mL and the duration of therapy short, we hypothesised that their residual viremia reflected slow viral decay, rather than viral non-suppression due to newly emerged drug mutations. Consequently, we may have underestimated the frequency of significant DRMs. Our definition of poor viral suppression did not include study participants who died or were lost to follow-up, possibly underestimating the rate of poor viral response.

5 | CONCLUSIONS

Pretreatment NNRTI/NRTI drug resistance was present in 20% of ART-naïve children from this urban Ugandan cohort. Yet, virologic suppression was achieved in 77% of the participants after 24 weeks of efavirenz-based therapy. The occurrence of PDR was predictive of viremia and ADR. The burden of resistance increased with time, even though the short-term outcome could be considered successful. Resistance may compromise the long-term outcome of both NNRTI and non-NNRTI regimens. If efavirenz-based therapy is used in Ugandan children, it should ideally be preceded by HIVDR testing, irrespective of PMTCT exposure. The long-term importance of PDR for non-NNRTI-based regimens needs further evaluation.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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