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ORIGINAL ARTICLE

Compromise of Second-Line Antiretroviral Therapy Due to High Rates of Human Immunodeficiency Virus Drug Resistance in Mozambican Treatment-Experienced Children With Virologic Failure

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Background. Virologic failure (VF) is highly prevalent in sub-Saharan African children on antiretroviral therapy (ART) and is often associated with human immunodeficiency virus drug resistance (DR). Most children still lack access to routine viral load (VL) monitoring for early identification of treatment failure, with implications for the efficacy of second-line ART.

Methods. Children aged 1 to 14 years on ART for ≥12 months at 6 public facilities in Maputo, Mozambique were consecutively enrolled after informed consent. Chart review and caregiver interviews were conducted. VL testing was performed, and specimens with ≥1000 copies/mL were genotyped.

Results. Of the 715 children included, the mean age was 103 months, 85.8% had no immunosuppression, 73.1% were taking stavudine/lamivudine/nevirapine, and 20.1% had a history prevention of mother-to-child transmission exposure. The mean time on ART was 60.0 months. VF was present in 259 patients (36.3%); 248 (95.8%) specimens were genotyped, and DR mutations were found in 238 (96.0%). Severe immunosuppression and nutritional decline were associated with DR. M184V and Y181C were the most common mutations. In the 238 patients with DR, standard second-line ART would have 0, 1, 2, and 3 effective antiretrovirals in 1 (0.4%), 74 (31.1%), 150 (63.0%), and 13 (5.5%) patients, respectively.

Conclusion. This cohort had high rates of VF and DR with frequent compromise of second-line ART. There is urgent need to scale-up VL monitoring and heat-stable protease inhibitor formulations or integrase inhibitorsfor a more a durable first-line regimen that can feasibly be implemented in developing settings.

Key words: drug resistance; HIV; pediatric; virologic failure.

At the end of 2015, an estimated 1.8 million children younger than 15 years were infected with human immunodeficiency virus (HIV) globally, and only 49% of them were on antiretroviral therapy (ART). Despite the low ART coverage rate, there has been a massive global scale-up of pediatric treatment, including an increase from 354 000 children on ART at the end of 2009 to 872 524 at the end of 2015 [1]. An estimated 82.7% of children on ART live in sub-Saharan Africa (SSA), where most countries have not yet been able to pivot from clinical and immunologic monitoring to virologic monitoring of ART response because of financial, technical, and logistic constraints [2].

This situation is problematic in light of the large body of evidence for the superiority of virologic over clinical and immunologic monitoring in diagnosing ART failure in children and adults [3, 4]. Virologic failure (VF) invariably precedes clinical and immunologic failure, and delayed detection of VF has been shown to be associated with the development of drug resistance (DR), which progressively worsens with accumulating DR mutations (DRMs) over time without an appropriate switch in ART regimens [5, 6]. The issue is even more problematic in children who are less likely to achieve virologic suppression after ART initiation, have unique barriers to ART adherence, and might also have been exposed to antiretroviral drugs (ARVs) as part of prevention of mother-to-child transmission (PMTCT) in HIV programs [7–10]. Many countries with a high HIV burden, including Mozambique, have not been able to implement a protease inhibitor (PI)-based first-line regimen for children younger than 3 years, which is recommended by the World Health Organization (WHO) on the basis of evidence

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of its increased efficacy and durability compared to those of non–nucleoside reverse transcriptase inhibitor (NNRTI)-based therapy [11, 12].

Pediatric HIV treatment outcomes in SSA have indicated that VF is alarmingly prevalent. One systematic review found VF rates in pediatric cohort studies in Africa that ranged from 13% to 61% [13]. Other regional studies have also reported high rates of VF (19.3%–38%) in children on first-line ART [14–16]. The factors associated with VF have varied among studies, but exposure to PMTCT and poor adherence have been cited frequently [17]. A study from Mozambique and Uganda found advanced clinical stage with tuberculosis and an age between 3 and 5 years to be risk factors for VF [18].

VF is highly associated with DR in children on ART in resource-limited settings. A review of data from Africa, Latin America, and Asia found that 90% of children with first-line ART failure harbored viruses with DRMs [13], and a retrospective cohort study in South Africa found DR in 81% of children with VF [19]. Also, a pediatric cohort study in Mozambique found that after 12 months on ART, 23% of children had VF and 10% harbored viruses with DRMs and that exposure to PMTCT, baseline DR, and missed medication are risk factors for DR [20].

A review of pooled data from pediatric DR in low-resource countries found 88%, 80%, and 54% of children with genotypic resistance to NNRTIs, nucleoside reverse transcriptase inhibitors (NRTIs), and PIs, respectively [13]. Studies in Africa have revealed high rates of NNRTI and NRTI resistance and lesser degrees of PI resistance; 1 study from Mozambique found resistance rates of 92%, 88%, and 0.0%, respectively [6, 21–24].

In Mozambique, as the pediatric ART program continues to grow and mature, the need to better understand trends in VF and emerging DR patterns is critical. Our study aimed to describe the prevalence of VF and HIV DR and the impact of HIV DRMs on susceptibility to the national recommended second-line regimens for children in a cohort of HIV-infected Mozambican children on first-line ART.

METHODS

Between August 2013 and March 2014, a cross-sectional study was conducted at 6 urban and periurban facilities (1 central hospital, 3 general hospitals, and 2 primary care health centers) located in Maputo City and Maputo Province. HIV-infected children, aged 12 months to 14 years, actively on ART for at least 12 months were eligible. Children were recruited consecutively from outpatient clinics. After the caregiver(s) provided written consent, each child at each site was enrolled according to site-specific targets relative to the overall pediatric ART patient volume. Each patient was receiving routine care per national guidelines with a standard first-line ART regimen of a fixed-dose combination tablet containing stavudine (d4T), lamivudine (3TC), and nevirapine (NVP) or a fixed-dose combination tablet containing zidovudine, 3TC, and NVP.

Demographic data (including age and sex) and clinical data (including clinic name, duration on ART, ART regimen, CD4 count, hemoglobin level, weight-for-age z score [WAZ], and exposure to PMTCT at the time of data collection) were collected through chart reviews and standardized caregiver interviews. Blood from each patient was collected in ethylenediaminetetraacetic acid (EDTA) tubes and used for viral load (VL) and DR testing. All study events occurred within a single patient encounter.

Plasma was prepared from venous blood and tested for VL using a COBAS AmpliPrep/COBAS TaqMan HIV type 1 (HIV-1) v2.0 test (Roche Diagnostics, Basel, Switzerland) at Instituto Nacional de Saude laboratory in Mozambique. The lower limit of detection for the assay was 20 copies/mL. VF was defined as a plasma HIV-1 RNA VL of ≥1000 copies/mL according to 2013 WHO guidelines [2]. All specimens that indicated VF were genotyped for DR by using dried blood spots. Genotyping of the protease and reverse transcriptase regions of the HIV-1 *pol* gene was performed using a broadly sensitive in-house genotyping assay at the US Centers for Disease Control and Prevention (CDC) laboratory in Atlanta, Georgia [25].

Statistical analyses were performed using SAS 9.2 software (SAS Institute, Inc, Cary, North Carolina) and Stata 14 (StataCorp, College Station, Texas). The cohort's demographic and clinical characteristics are summarized using means for continuous variables, frequencies for categorical variables, and survey-adjusted 95% confidence intervals (CIs) to account for survey design. For some covariates, principally current hemoglobin level and immune status, up to 17% of the data were missing (see Table 1); by exploring patterns of missing data and the association between missing data and the outcomes of interest, the missing-at-random (MAR) assumption was considered plausible [26]. To best manage missing covariate data in regression models, both complete case analyses and multiple-imputation (MI) approaches were used to better assess sensitivity of the regression models to missing data. Results of analyses using complete case analyses and those using an MI approach were similar. Because MI is generally preferred to complete case analysis when missing data are considered missing at random [27, 28] because this approach is less likely to yield biased parameter estimates, results of the MI approach are presented [28]. In the MI approach, the ice [29] procedure in Stata was used to create 20 imputed data sets for each outcome (VF and DR) separately [30]. For all analyses using imputed data, estimates were combined across imputed data sets according to Rubin's rules [31]. Multivariate logistic regression, in which we accounted for survey design, was used to assess predictors of VF and DR.

Table 1. Demographic and Clinical Descriptive Statistics for Cohort

Characteristic	Age Group			Total (N = 715)
	<5 years (n = 88)	5–9 years (n = 394)	10–14 years (n = 233)	
Sex, female (n [%]) (95% CI)	41 (46.6) (27.1–67.2)	187 (47.5) (40.3–54.8)	101 (43.3) (33.0–54.3)	329 (46.0) (39.3–52.8)
Length of time on ART (n [%]) (95% CI)				
12–36 mo	56 (63.6) (45.8–78.4)	53 (13.5) (4.7–32.8)	27 (11.6) (2.9–36.6)	136 (19.0) (6.7–43.3)
>36 mo	32 (36.4) (21.6–54.2)	341 (86.5) (67.2–95.3)	206 (88.4) (63.4–97.1)	579 (81.0) (56.7–93.3)
Current ART regimen				
d4T/3TC/NVP (n [%]) (95% CI)	80 (90.9) (35.4–99.5)	302 (76.6) (46.4–92.6)	140 (60.3) (30.6–84.0)	522 (73.1) (43.4–90.6)
AZT/3TC/NVP (n [%]) (95% CI)	5 (5.7) (0–63.1)	75 (19.0) (4.9–51.8)	72 (31.0) (11.6–60.6)	152 (21.3) (6.4–51.5)
Other (n [%]) (95% CI)	3 (3.4) (0–18.7)	17 (4.3) (2.1–8.7)	20 (8.6) (4.2–16.7)	40 (5.6) (3.0–10.0)
Missing data (n)	0	0	1	1
Current immunosuppression				
None (n [%]) (95% CI)	38 (88.4) (48.7–98.4)	302 (90.1) (86.7–92.8)	168 (78.5) (53.0–92.2)	508 (85.8) (75.2–92.3)
Mild (n [%]) (95% CI)	2 (4.7) (1.0–19.2)	19 (5.7) (3.6–8.8)	20 (9.3) (5.1–16.4)	41 (6.9) (4.6–10.3)
Advanced (n [%]) (95% CI)	1 (2.3) (0.2–21.5)	5 (1.5) (0.7–3.3)	12 (5.6) (1.1–23.5)	18 (3.0) (1.0–8.8)
Severe (n [%]) (95% CI)	2 (4.7) (0.4–37.3)	9 (2.7) (1.2–5.9)	14 (6.5) (2.0–19.1)	25 (4.2) (1.8–9.6)
Missing data (n)	45	59	19	123
Current WAZ				
Higher than –2 (n [%]) (95% CI)	71 (87.7) (74.8–94.4)	339 (88.5) (80.8–93.4)	166 (71.2) (58.3–81.4)	576 (82.6) (77.4–86.9)
–2 to –3 (n [%]) (95% CI)	9 (11.1) (5.4–21.6)	31 (8.1) (5.4–12.0)	46 (19.7) (13.1–28.7)	86 (12.3) (9.6–15.8)
Less than –3 (n [%]) (95% CI)	1 (1.2) (0.2–6.1)	13 (3.4) (1.3–8.8)	21 (9.0) (4.2–18.2)	35 (5.0) (2.8–8.8)
Missing data (n)	7	11	0	18
≥1 SD decrease from peak WAZ				
No (n [%]) (95% CI)	58 (71.6); 65.6, 76.9	276 (72.1); 63.6, 79.2	166 (71.2); 65.9, 76.1	500 (71.7) (67.5–75.6)
Yes (n [%]) (95% CI)	23 (28.4); 23.1, 34.4	107 (27.9); 20.1, 36.4	67 (28.8); 23.9, 34.1	197 (28.3) (24.4–32.5)
Missing data (n)	7	11	0	18
Current hemoglobin level				
≥8 g/dL (n [%]) (95% CI)	65 (97.0) (83.5–99.5)	310 (99.7) (96.4–100.0)	187 (98.9) (96.8–99.7)	562 (99.1) (97.5–99.7)
<8 g/dL (n [%]) (95% CI)	2 (3.0) (0.5–16.5)	1 (0.3) (0–3.6)	2 (1.1) (0.3–3.2)	5 (0.9) (0.3–2.5)
Missing data (n)	21	83	44	148
VL suppressed (<1000 copies/mL) (n [%]) (95% CI)				
Yes	54 (61.4) (42.0–77.7)	255 (64.7) (55.1–73.3)	147 (63.1) (44.8–78.2)	456 (63.8) (53.5–72.9)
No	34 (38.6) (22.3–58.0)	139 (35.3) (26.7–44.9)	86 (36.9) (21.8–55.2)	259 (36.2) (27.1–46.5)
Median (IQR) VL (of those with VF) (copies/ml)	42 296 (21 148–287 614)	22 829 (8875–67 617)	28 807 (14 207–74 262)	27 734 (10 755–71 906)
PMTCT history				
No (n [%]) (95% CI)	29 (33.3) (24.9–43.0)	164 (42.1) (20.5–67.2)	117 (52.2) (30.2–73.4)	310 (44.2) (25.4–64.9)
Yes (n [%]) (95% CI)	45 (51.7) (37.0–66.2)	80 (20.5) (17.2–24.2)	16 (7.1) (1.2–32.5)	141 (20.1) (15.0–26.5)
Unknown (n [%]) (95% CI)	13 (14.9) (8.1–25.9)	146 (37.4) (16.5–64.4)	91 (40.6) (23.6–60.2)	250 (35.7) (17.6–59.0)
Missing data (n)	1	4	9	14

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; d4T, stavudine; IQR, interquartile range; NVP, nevirapine; PMTCT, prevention of mother-to-child transmission; SD, standard deviation; VF, virologic failure; VL, viral load; WAZ, weight-for-age z score.

Most recent CD4 counts, measured no more than 12 months before study enrollment, were used to classify the degree of current immunosuppression according to standard WHO age-based tables, preferentially using CD4 percentage values for children aged <5 years and absolute CD4 count values for children aged ≥5 years [32]. DRM interpretation and drug susceptibility were assessed using the Stanford University Drug-Resistance Database (HIVDB 7.0) (Palo Alto, California).

This study was approved by the institutional review board of the Centers for Disease Control and Prevention (protocol 5448) and the Mozambique National Bioethics Committee (19/CNBS/13).

RESULTS

In total, 723 children were enrolled, and data were analyzed for 715 (98.9%) of them. Eight patients were excluded from analysis because of missing data or having not had a blood sample collected. The mean age was 103.0 months (95% CI, 81.2–124.8 months), the mean age at ART initiation was 35.4 months (95% CI, 25.0–45.8 months), and the mean time on ART was 60.0 months (95% CI, 18.5–102.5 months). Of the patients with a recent CD4 count measurement, 85.8% (95% CI, 75.2%–92.3%) had no immunosuppression on the basis of WHO classifications, and only 4.2% (95% CI, 1.8%–9.6%) had severe immunosuppression. The current WAZ was between –2 and –3 in 86

children (12.3% [95% CI, 9.6%–15.8%]) and less than −3 in 35 children (5.0% [95% CI, 2.8%–8.8%]), and 197 children (X% [95% CI, Y%–Z%]) had a ≥1 standard deviation decrease from their peak WAZ. The majority of the patients, 73.1% (95% CI, 43.4%–90.6%), were on d4T/3TC/NVP. PMTCT exposure was documented in 20.1% (95% CI, 15.0%–26.5%) of the patients. Other age-stratified clinical and demographic characteristics of the study cohort are detailed in Table 1.

Among all 715 children included in the analysis, 342 (47.8% [95% CI, 32.4%–63.5%]) had a VL of <50 copies/mL, 114 (16.0% [95% CI, 9.3%–26.0%]) had a VL of 50 to <1000 copies/ml, and 259 (36.3% [95% CI, 27.1%–46.6%]) had VF (VL ≥ 1000 copies/ml). Among the 259 patients with VF, samples from 248 (95.8%) were successfully genotyped, and DRMs were present in 238 (96.0%) of them. In both univariate and multivariate analyses, all forms of immunosuppression had a statistically significant association with VF and DR, whereas a decrease from the peak WAZ was significant for only DR. None of the other variables included in the model showed statistically significant associations with VF or DR. Full details of the regression analysis are provided in Table 2.

In the 238 patients with DRMs, high levels of NRTI and NNRTI mutations were observed with M184V (n = 220 [92.4%]) and Y181C (n = 69 [29.0%]), which were most common in each class, respectively. K65R was found in only 2.9% of the patients with DR. Thymidine analog mutations (TAMs) and major PI DRMs occurred in 83 (33.5%) and 4 (1.6%) patients, respectively. TAM2 was the main pathway observed (69 [83.1%]), and a mixture of both pathways was found in 12 patients (14.5%). The most prevalent NRTI and NNRTI mutations observed according to age group are detailed in Figure 1.

Table 2. Risk Factors for Virologic Failure and HIV Drug Resistance

	Virologic Failure				Drug Resistance			
	Univariate Regression		Multivariate Regression		Univariate Regression		Multivariate Regression	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age group								
<5 years	Reference		Reference		Reference		Reference	
5–9 years	0.86 (0.38–1.98)	.65	1.10 (0.35–3.42)	.82	0.87 (0.43–1.77)	.62	1.05 (0.38–2.94)	.89
10–14 years	0.93 (0.23–3.83)	.89	0.91 (0.20–4.08)	.86	0.94 (0.28–3.13)	.90	0.86 (0.25–2.93)	.75
Sex								
Male	Reference		Reference		Reference		Reference	
Female	0.86 (0.60–1.23)	.30	0.89 (0.62–1.28)	.40	0.93 (0.64–1.33)	.58	0.98 (0.69–1.39)	.89
Time on ART								
12–36 mo	Reference		Reference		Reference		Reference	
>36 mo	0.74 (0.39–1.41)	.26	0.85 (0.51–1.41)	.40	0.79 (0.39–1.59)	.39	0.91 (0.49–1.68)	.67
Current ART regimen								
d4T/3TC/NVP	Reference		Reference		Reference		Reference	
AZT/3TC/NVP	1.17 (0.57–2.42)	.57	1.07 (0.64–1.80)	.71	1.12 (0.50–2.52)	.71	1.02 (0.55–1.90)	.93
Other	0.77 (0.52–1.15)	.14	0.48 (0.21–1.10)	.07	0.77 (0.42–1.41)	.30	0.46 (0.20–1.08)	.06
Current immunosuppression								
None	Reference		Reference		Reference		Reference	
Mild	4.05 (2.03–8.09)	.01[a]	4.72 (2.28–9.73)	.01[a]	3.51 (1.50–8.23)	.02[a]	4.17 (1.79–9.68)	.01[a]
Advanced	5.44 (1.86–15.94)	.02[a]	6.16 (2.05–18.50)	.02[a]	6.06 (2.08–17.62)	.01[a]	6.96 (2.54–19.11)	.01[a]
Severe	8.64 (2.53–29.51)	.01[a]	11.30 (2.71–47.13)	.02[a]	9.36 (2.54–34.46)	.02[a]	12.78 (2.88–56.77)	.02[a]
Current WAZ								
Greater than –2	Reference		Reference		Reference		Reference	
–2 to –3	0.68 (0.37–1.26)	.15	1.09 (0.32–3.73)	.85	0.66 (0.40–1.09)	.08	1.13 (0.44–2.95)	.72
Less than –3	1.16 (0.43–3.10)	.69	2.06 (0.41–10.28)	.27	1.13 (0.51–2.51)	.68	2.10 (0.57–7.76)	.18
Decrease from peak WAZ								
No	Reference		Reference		Reference		Reference	
Yes	1.26 (0.91–1.75)	.11	1.25 (0.84–1.86)	.19	1.45 (1.14–1.84)	.02[a]	1.48 (1.07–2.04)	.03[a]
Current hemoglobin level								
≥8 g/dL	Reference		Reference		Reference		Reference	
<8 g/dL	1.93 (0.08–49.25)	.53	2.35 (0.08–66.03)	.44	1.80 (0.72–45.04)	.58	2.22 (0.08–59.93)	.47
PMTCT exposure								
No	Reference		Reference		Reference		Reference	
Yes	1.18 (0.66–2.11)	.46	1.37 (0.83–2.26)	.16	1.17 (0.61–2.24)	.53	1.34 (0.79–2.27)	.19
Unknown	1.17 (0.76–1.79)	.37	1.16 (0.75–1.80)	.38	1.20 (0.72–1.99)	.38	1.20 (0.72–2.03)	.37

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; d4T, stavudine; HIV, human immunodeficiency virus; IQR, interquartile range; NVP, nevirapine; OR, odds ratio; PMTCT, prevention of mother-to-child transmission; WAZ, weight-for-age z score.

[a]Statistically significant result.

Drug susceptibility analysis for patients with DRMs revealed high levels of resistance to NNRTIs across the age groups; 96.2% had high-level resistance to NVP, and 68.9% had high-level resistance and 27.3% intermediate-level resistance to efavirenz. In addition, 2 (0.4%) patients harbored intermediate- and high-level resistance to both lopinavir/ritonavir (LPV-r) and atazanavir/ritonavir in the 10- to 14-year-old group. The results of susceptibility analysis for the NRTI class according to age group are shown in Figure 2.

ARV susceptibility results were then used to assess the efficacy of the current standard Mozambique pediatric second-line ART regimens of abacavir (ABC)/3TC/LPV-r (for children <35 kg) and tenofovir (TDF)/3TC/LPV-r (for children ≥35 kg), assuming that patients with susceptibility and those with low-level resistance would still experience drug efficacy. Of the patients with DRMs, 5.5%, 63.0%, and 31.1% would have 3, 2, or 1 efficacious ARV drug in the second-line regimen, respectively. For 1 patient in the 10- to 14-year-old age group, no drugs in the standard second-line regimen were effective. Age-stratified results are presented in Figure 3.

DISCUSSION

To our knowledge, this is the first report to describe the prevalence of VF and DR and patterns in DR in a pediatric population enrolled in the Mozambique National ART program. VF was present in 36.3% of the children, and that rate could be even higher if some of the 114 (16%) children who had a VL between 50 and 1000 copies/mL were considered to have VF according to a more stringent threshold than that recommended by the WHO [2]. Our data are consistent with results of studies from South Africa and Botswana in which the majority of sequences in patients with VF (96.0%) carried a DRM [23, 33]. The resistance patterns observed in our study have critical implications for currently available second-line regimens in Mozambique that rely on reuse of NRTIs.

Any form of immunosuppression was found to be a significant risk factor for VF and DR in both univariate and multivariate analyses. This result differs from those of other pediatric studies in which immunosuppression was found to be poorly predictive of VF and DR [4, 14]. Interesting to note is that current nutritional status as defined by the WAZ was not significantly associated with VF or DR, but a >1 standard deviation decrease from previous peak WAZ score was significantly associated with DR, and we found a trend toward significance for VF. This finding highlights the importance of following not only current nutritional status but also nutritional trends over time as part of routine clinical monitoring of ART effectiveness.

Moreover, age was not found to be a factor associated with VF or DR, but differences in trends in mutation frequencies and complexity patterns between age categories were observed, as was increased complexity in older patients. K101E, K103N, Y181C, and G190A were the most frequent NNRTI mutations found, and they were more prevalent in children younger than 5 years and older than 10 years. Younger patients were far more likely to have been exposed to PMTCT regimens (including NVP), which is a well-documented risk factor for NNRTI class resistance [34]. A history of known PMTCT exposure had a non–statistically significant association with VF and DR across all age groups in this study, but many of the older patients had an unknown PMTCT history. Also, with respect to age, studies in SSA have found lower rates of ART adherence in younger children and adolescents [6, 35, 36]. We were not able to include retrospective ART adherence data in this cross-sectional study,

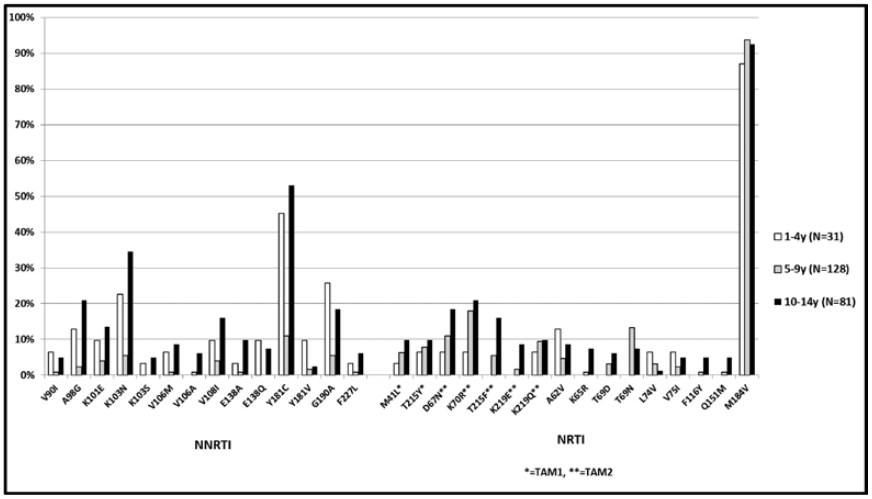

Figure 1. Nucleoside reverse transcriptase inhibitor (NRTI) and non–nucleoside reverse transcriptase inhibitor (NNRTI) mutation frequencies in children with any drug resistance mutation according to age group (includes only mutations with at least a 5% frequency in at least 1 age group). Abbreviations: TAM1 and TAM2, thymidine analog mutations 1 and 2, respectively.

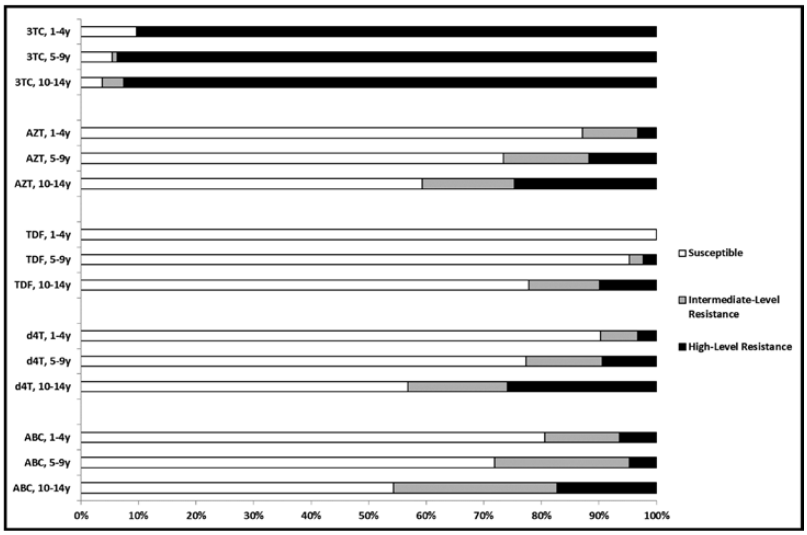

Figure 2. Nucleoside reverse transcriptase inhibitor susceptibility/drug resistance according to age group. Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; TDF, tenofovir.

but if the same trends were present in our cohort, they also could account for some of the age-based differences in NNRTI DR that we observed.

The M184V mutation was observed with high frequencies in all age groups of children with DR and TAMs. These findings were expected, given the standard first-line pediatric ART regimens that contain 3TC. TAMs were found in 33.5% of the patients with DR, which is within the range of TAM prevalence in other studies of children from the region with DR (19%–64%) [19, 22, 23]. The TDF- and d4T-associated mutation K65R was found in only 2.9% of the patients with DR in our study, occurring mostly in adolescents. This mutation frequency is lower than those reported from pediatric cohorts in Botswana (4.4%) and South Africa (14%) and is likely explained by the phase-out of d4T from regular use in 2012 and the very limited use of TDF in adults and children in Mozambique up to the time of this study [23, 33].

Drug-susceptibility results were used to predict the efficacy of the current standard second-line ART regimens available in Mozambique for children who were initiated on an NNRTI-based first-line regimen (ABC/3TC/LPV-r for children <35 kg and TDF/3TC/LPV-r for children >35 kg). Very few patients

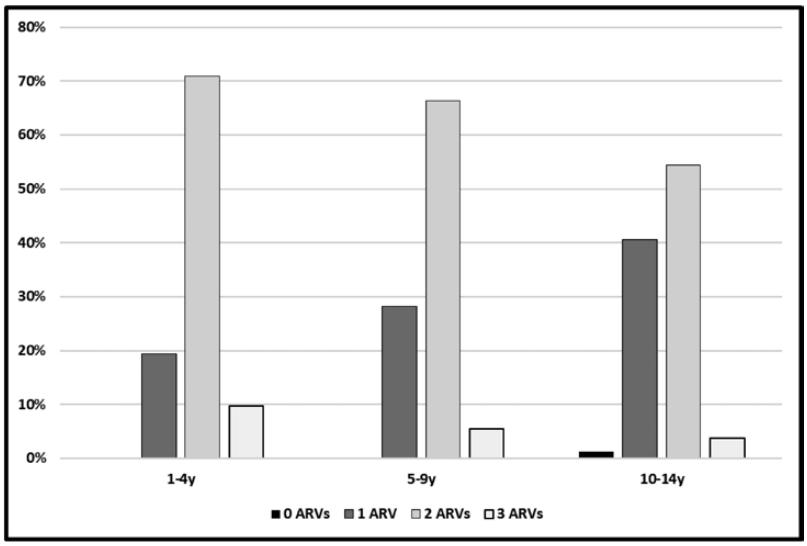

Figure 3. Effectiveness of standard second-line antiretroviral therapy in patients with human immunodeficiency virus drug resistance according to age group. Regimens were abacavir/lamivudine/lopinavir/ritonavir in children <35 kg and tenofovir/lamivudine/lopinavir/ritonavir in children ≥35 kg. Intermediate- and high-level resistance was used to define the efficacy of antiretrovirals (ARVs).

would have 3 effective drugs (defined as no or low-level resistance) in their second-line regimen, given the very high prevalence of the M184V mutation and the inclusion of 3TC in both the first- and second-line standard ART regimens. Alarming is that 19.3%, 28.1%, and 40.5% of patients aged <5 years, 5 to 9 years, and 10–14 years, respectively, would be on a second-line regimen with only 1 effective drug, LPV-r, given frequent dual-class NRTI and NNRTI mutations. It should be mentioned that adult data from the Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) study suggest that genotypic NRTI DR might not accurately predict which patients have actual NRTI activity in PI-based second-line ART, but similar studies in children are lacking [37].

These drug-susceptibility findings, along with the results on specific DRMs and TAMs, suggest that many children were on a failing first-line regimen for some time, which has implications for the durability of second-line ART [6]. Our findings of immunosuppression and falling WAZs being associated with VF and DR show that immunologic and clinical failure diagnoses were being missed. Virologic monitoring of ART has been shown to be superior for timely diagnosis of treatment failure, and Mozambique is currently implementing a staged roll-out of VL testing, in which children aged 2 to 5 years and all patients suspected of treatment failure on the basis of CD4 and clinical criteria are being prioritized in the early phases [2].

Our findings also highlight a major operational challenge that Mozambique has faced in terms of implementing the WHO-recommended PI-based first-line regimen for children aged <3 years [2]. The requirements for cold-chain distribution and refrigerated storage of LPV-r syrup have been a major obstacle, and the vast majority of infants in the country have been, and continue to be, initiated on first-line ART that contains both NVP and 3TC, 2 drugs with very low genetic barriers to resistance. There are plans to introduce the new heat-stable LPV-r granules in the country in 2018, which represents a major opportunity for improved treatment of HIV-infected infants and young children. In addition, new 2018 guidance from the WHO calls for the expanded use of integrase inhibitors (IIs), specifically dolutegravir (DTG), as part of standard first- and second-line ART regimens and raltegravir as an option for neonatal treatment [38, 39]. Unanswered questions regarding DTG use in women who conceive while on treatment, TB cotreatment, and dosing/safety in younger, lower-weight children remain [40, 41]. Mozambique has not yet adopted a policy for transition, but II-containing regimens represent another future option for more efficacious and durable pediatric ART.

This study had limitations that need to be addressed. First, the cross-sectional design with retrospective data collection of clinical variables precluded an accurate assessment of the impact of ART adherence on VF and DR, and it also was not possible to know the duration of VF, because VL testing was not performed routinely. Also, the study was only conducted at sites within the capital city of Maputo, which might not be representative of the rest of the country. The use of dried blood spots for DR testing has been validated in children and was not considered a limitation in study methodology [42]. However, a range of study sites representing different levels of care was selected, and this cohort was large and represented approximately 16% of all children on ART in Mozambique at the time of the study [43].

Additional studies in SSA are needed to assess treatment outcomes for children on second-line ART. Furthermore, national and international policy makers need to use the results from this evaluation and other studies of pediatric DR to guide the development of new pediatric fixed-dose combination regimens and treatment guidelines that will enable resource-constrained countries such as Mozambique to offer HIV-infected children more effective and durable first- and second-line ART.

Notes

Author contributions. J. H., P. V., N. B., and I. J. conceived and designed the study; L. C., A. S., C. A., and E. M. led study implementation; J. D. and C. Y. coordinated the laboratory analyses and supervised VL and DR testing in accordance with good laboratory practice; A. A. and J. S. coordinated data management and performed the statistical analysis; C. B., D. B., N. B., C. A., K. J., and P. V. contributed to the interpretation of results; C. B. and D. B. had primary responsibility for manuscript writing; and all the authors reviewed and approved the final version of the manuscript.

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