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Drug Resistance Mutations Among South African Children Living With HIV on WHO-recommended ART Regimens

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Abstract

Background.—Children living with human immunodeficiency virus (HIV) (CLHIV) receiving antiretroviral therapy (ART) in resource-limited settings are susceptible to high rates of acquired HIV drug resistance (HIVDR), but few studies include children initiating age-appropriate World Health Organization (WHO)-recommended first-line regimens. We report data from a cohort of ART-naive South African children who initiated first-line ART.

Methods.—ART-eligible CLHIV aged 0–12 years were enrolled from 2012 to 2014 at 5 public South African facilities and were followed for up to 24 months. Enrolled CLHIV received standard-of-care WHO-recommended first-line ART. At the final study visit, a dried blood spot sample was obtained for viral load and genotypic resistance testing.

Results.—Among 72 successfully genotyped CLHIV, 49 (68.1%) received ABC/3TC/LPV/r, and 23 (31.9%) received ABC/3TC/EFV. All but 2 children on ABC/3TC/LPV/r were <3 years, and all CLHIV on ABC/3TC/EFV were ≥3 years. Overall, 80.6% (58/72) had at least one drug resistance mutation (DRM). DRMs to nonnucleoside reverse transcriptase inhibitors (NNRTIs)

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and nucleoside reverse transcriptase inhibitors (NRTIs) were found among 65% and 51% of all CLHIV, respectively, with no statistical difference by ART regimen. More CLHIV on ABC/3TC/EFV, 47.8% (11/23), were found to have 0 or only 1 effective antiretroviral drug remaining in their current regimen compared to 8.2% (4/49) on ABC/3TC/LPV/r.

Conclusions.—High levels of NNRTI and NRTI DRMs among CLHIV receiving ABC/3TC/LPV/r suggests a lasting impact of failed mother-to-child transmission interventions on DRMs. However, drug susceptibility analysis reveals that CLHIV with detectable viremia on ABC/3TC/LPV/r are more likely to have maintained at least 2 effective agents on their current HIV regimen than those on ABC/3TC/EFV.

Keywords

HIV drug resistance; pediatric HIV; children; resource-limited settings

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In 2018, 1.7 million children under the age of 15 years were living with human immunodeficiency virus (HIV) (CLHIV), and only 54% were receiving antiretroviral therapy (ART) [1, 2]. Among children on treatment, data suggests many are not achieving viral suppression (VS). A pooled analysis of CLHIV on ART in resource-limited settings showed only 73% achieved a viral load (VL) <1000 copies/mL by 12 months on ART [3]. Population-based HIV Impact Assessments conducted in 6 sub-Saharan African countries revealed a low pooled estimate of viral suppression (VS) of 53% among CLHIV on ART, ranging from 30.4% in Tanzania to 73.9% in Eswatini [4, 5].

One critical driver of poor VS in children is the continued use of suboptimal ART regimens [3]. The World Health Organization (WHO) has recommended the use of lopinavir/ritonavir (LPV/r)-based first-line ART for CLHIV <3 years since 2010; however, use of nonnucleoside reverse transcriptase inhibitors (NNRTIs), such as nevirapine (NVP) and efavirenz (EFV), remains common [6]. Reasons for poor uptake of optimal pediatric ART include lack of palatable pediatric formulations, higher cost of protease inhibitors, and a scarcity of pediatric-trained clinicians which contributes to prolonged use of failing regimens in children [6–8].

High levels of pretreatment drug resistance (PDR) among CLHIV due to exposure to prevention of mother-to-child transmission (PMTCT) interventions underscores the importance of initiating an effective first-line ART regimen [7, 9]. A 2017 systematic literature review found 42.7% and 12.7% PDR among PMTCT-exposed and unexposed children, respectively, with most harboring NNRTI drug resistance mutations (DRMs) [7]. Two studies among South African children found similarly high rates of PDR, 53.0% and 52.3%, with NNRTI DRMs being most common [10, 11]. Prior studies examining acquired drug resistance (ADR) in South African CLHIV revealed DRM in over 90% [12–15]. However, these studies included few ART-naive children initiating WHO-recommended first-line ART regimens. These findings emphasize the need to better understand the programmatic impact of optimal ART regimens on HIVDR among ART-naive CLHIV.

We report HIVDR in a cohort of ART-naive infants and children with detectable VL receiving first-line, WHO-recommended ART regimens in South Africa. We describe the

proportion of children with DRMs by antiretroviral (ARV) class and analyze current and alternate ARV susceptibility.

METHODS

Study Design and Procedures

Data came from a previously described observational cohort of CLHIV receiving routine HIV services at 5 health facilities in South Africa [16]. ART-naive CLHIV from birth to 12 years were enrolled at ART eligibility (identified by healthcare providers). South Africa ART guidelines changed during the study. 2010 guidelines called for ART for (1) all children <12 months; (2) children 1–5 years with WHO clinical stage 3 or 4, $CD4^+$ cell count ($CD4^+$) <25%, or absolute $CD4^+$ ≤750 cells/mm^3; and (3) children >5 years with WHO stage 3 or 4 or $CD4^+$ ≤350 cells/mm^3. 2013 guidelines recommended ART for all children <5 years and those 5–15 years with WHO clinical stage 3 or 4 or $CD4^+$ ≤350 cells/mm^3 [17, 18]. The first-line regimen for children <3 years was abacavir (ABC), lamivudine (3TC), and LPV/r and for children ≥3 years, ABC, 3TC, and EFV. Viral load monitoring guidelines also changed from ART initiation, 6 and 12 months, and then annually (2010) to every 6-month VL testing for children <5 years (2013). After study enrollment, CLHIV were followed for up to 24 months. Caregivers provided informed consent and children ≥8 years provided assent.

During study follow-up, children received routine HIV standard of care following South African guidelines, including ART, opportunistic infection management, and laboratory monitoring (the study did not provide medical care). Additionally, enrolled children attended quarterly study visits, which included caregiver questionnaires, additional physical exams, and blood specimen collection. Children who missed study visits were traced through phone calls and home visits. Ethical review was received from Columbia University, University of Cape Town, East London Hospital Complex Research Ethics Committee, Walter Sisulu University Health Research Ethics Committee, and Eastern Cape Department of Health. The protocol was reviewed in accordance with the Centers for Disease Control and Prevention (CDC) human research protection procedures and was determined to be research, but CDC investigators did not interact with human subjects or have access to identifiable data or specimens for research purposes.

Study data were abstracted from medical records of enrolled children. Enrollment characteristics included age at diagnosis as reported by caregivers and recorded in clinic charts, hospitalization at enrollment, tuberculosis (TB) at enrollment (up to 90 days prior), history of TB (diagnosis in chart >90 days prior), weight-for-age z-score, $CD4^+$ and VL (up to 1 year prior or 1 month after enrollment), maternal age, and history of PMTCT interventions. The study also collected information on routinely conducted laboratory tests, including $CD4^+$ (FC500 Cytomics MPL 1, Beckman Coulter) and VL (Cobas© 6800/8800, Roche Molecular Systems), conducted at the National Health Laboratory Services (NHLS) at Livingstone and Dora Nginza Hospitals in Port Elizabeth and at Frere and Cecilia Makiwane Hospitals in East London. At the final study visit 12–24 months postenrollment, two dried blood spot (DBS) cards for VL and HIVDR testing were collected.

HIV-1 Viral Load Testing and Drug Resistance Genotyping

DBS cards were shipped and stored at −80°C at the Molecular Haematology and Virology Laboratory at Charlotte Maxeke Johannesburg Academic Hospital under the University of the Witwatersrand and NHLS. In 2016, specimens were shipped to the International Laboratory Branch (ILB), Division of Global HIV and TB at CDC, Atlanta, GA, USA. Viral load testing was performed on the DBS samples at the ILB using the Abbott RealTi*m*e HIV-1 VL optimized one spot assay on the fully automated Abbott m2000 platform (Abbott Molecular Inc.) [19]. Data on VS during study follow-up have been previously reported [16].

All DBS samples with VL results above the Abbott DBS VL lower limit of detection (839 copies/mL) were HIV-1 genotyped. One DBS spot per sample was used for nucleic acid extraction using the NucliSENS on easyMAG platform (Biomerieux), following the manufacturer's instructions [20]. Genotyping of the protease and reverse transcriptase regions of the HIV-1 *pol* gene was performed using the Thermo Fisher (TF) HIV-1 Genotyping Kit (Life Technologies). The TF kit was developed based on a broadly sensitive CDC in-house genotyping assay [21].

In brief, a 1084 base-pair segment of the 5' region of the *pol* gene covering the protease and 5' segment of the reverse transcriptase (*RT*) region was generated by reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR using the kit Amplification Module. The purified PCR fragment was then sequenced using the kit Cycle Sequencing Module and analyzed on the ABI Prism™ 3730 Genetic Analyzer (Applied Biosystems). The customized ReCALL (version 2.27) software program was used to edit the raw sequences and generate consensus sequences, while sequence quality assurance was performed on each sequence using MEGA [22, 23]. HIV DRMs and drug susceptibility profiles were generated using Stanford University's HIVdb algorithm (version 8.4) [24]. HIV-1 subtypes were determined by REGA HIV-1 Subtyping Tool version 3 [25].

Data Analyses

Children were included in this analysis if they had an end-of-study DBS specimen with a detectable VL and a successfully amplified genotyping product. Enrollment characteristics were compared based on first-line regimen type using Pearson Chi-square tests for categorical variables (or Fisher's exact tests) and Wilcoxon signed rank tests for continuous variables. HIVDR by DRM and drug class were described based on first-line ART regimen. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc.). We also present data on drug susceptibility according to the HIVDR mutations identified. As facilities were not randomly selected and may not be representative of all facilities treating children in South Africa, inferences were limited to the included facilities.

RESULTS

Study Population and Participant Characteristics

The study enrolled 397 children <12 years, among whom 307 (77.3%) completed study follow-up; 35 (8.8%) children died, 49 (12.3%) withdrew due to changing care locations, and 6 (1.5%) were lost to follow-up. A total of 301 DBS specimens were collected, stored,

and shipped to the CDC laboratory. Four specimens had duplicate study IDs (only one of each was analyzed) and four specimens had >99% similarity in genotype results (all were excluded). VL testing was performed on 293 samples; among these, 95 (32.4%; 95% confidence interval [CI] = 27.1, 38.1) had a detectable VL, of which 72 (75.8%) were successfully sequenced and 23 (24.2%) failed to sequence. Among 293 unique children with specimen collection, 128 (43.7%) initiated ABC/3TC/EFV and 165 (56.3%) initiated ABC/3TC/LPV.

Among the 72 children with successful amplification, 49 (68.1%) initiated ABC/3TC/LPV/r and 23 (31.9%) ABC/3TC/EFV (Table 1). Among children who initiated ABC/3TC/LPV/r, 95.9% (47/49) were <3 years, and all children who initiated ABC/3TC/EFV were 3–12 years. At enrollment, 31.9% (23/72) of children were hospitalized, 22.2% (16/72) had TB, and 54.2% (39/72) had any maternal PMTCT exposure. Mothers of children on ABC/3TC/LPV/r were more likely to have received ART for PMTCT versus other or no PMTCT regimen compared to mothers of children on ABC/3TC/EFV (30.6% vs 4.4%; P = .0142) and to be the primary caregivers rather than grandmother or other caregiver (91.8% vs 65.2%; P = .0083). The median log VL at enrollment was significantly higher among children on ABC/3TC/LPV/r, 6.2 (interquartile range (IQR) 5.5–6.7), compared to 5.4 (IQR 5.1–5.8) among children on ABC/3TC/EFV (P = .0037). The median time on ART at DBS sampling was 20 months (IQR 14–24) for children on ABC/3TC/LPV/r and 24 months (IQR 14–24 months) for those on ABC/3TC/EFV (Table 1).

HIV Drug Resistance Results

Overall 80.6% (58/72; 95% CI = 69.5, 88.9) of children successfully genotyped had at least one DRM; 83.7% (41/49; 95% CI = 73.3, 94.0) on ABC/3TC/LPV/r and 73.9% (17/23; 95% CI = 51.6, 89.8) on ABC/3TC/EFV (Table 2). Nucleoside reverse transcriptase inhibitors (NRTI) mutations were found in 51.4% (37/72; 95% CI = 39.3, 63.4) of all children; 51.0% (24/49; 95% CI = 34.4, 63.7) on ABC/3TC/LPV/r and 52.2% (12/23; 95% CI = 30.6, 73.2) on ABC/3TC/EFV. Among children with any NRTI resistance on EFV-based regimens, 91.7% (11/12) had any resistance to 3TC and ABC compared to 88.0% (22/25) of children on LPV/r-based regimens. DRMs to NNRTIs were most common and occurred in 65.3% of all children (47/72; 95% CI = 53.1, 76.1), with almost identical results by regimen (65.3%, 32/49 vs 65.2%, 15/23, 95% CI = 50.4, 78.3 and 42.7, 83.6, respectively) (Table 2). Among children with any NNRTI resistance on EFV-based regimens, 80% (12/15) had any resistance to NVP and EFV compared to 87.5% (28/32) on LPV/r-based regimens (Figure 1). Dual-class resistance to NNRTIs and NRTIs was 32.6% (16/49; 95% CI = 20.0, 47.5) among those on ABC/3TC/LPV/r compared to 43.5% (10/23; 95% CI = 23.2, 65.5) on ABC/3TC/EFV. Among all children with DRMs, 15.5% (9/58) had 2 or more NRTI mutations, 39.7% (23/58) had 2 or more NNRTI mutations, and 1.7% (1/58) had multiple PI mutations (not shown).

The M184V mutation was the most common mutation among all children (45.8%, 33/72). The most common NNRTI mutation was K103N (33.3%, 24/72) and was more common among children on ABC/3TC/LPV/r (38.8%, 19/49) than on ABC/3TC/EFV (21.7%, 5/23). Among the 19 children on ABC/3TC/LPV/r with a K103N mutation, 78.9% (15/19)

reported exposure to maternal PMTCT compared to 21.1% (4/19) who reported no known PMTCT exposure. Among 5 children with a K103N mutation on ABC/3TC/EFV, 80% (4/5) reported no known PMTCT exposure, and 1 child had missing PMTCT data. Thymidine analog mutations were found in 8.3% (6/72) of children. Only 2.8% (2/72) of children, both on ABC/3TC/LPV/r, had PI mutations, and only one of these children had resistance to LPV/r. One of these children reported concurrent TB treatment, and the other stopped treatment within a month of starting and did not restart. The prevalence of each DRM is shown in Figure 2.

Drug susceptibility analysis among children with DRMs revealed high- and intermediate-level NNRTI resistance in most children across both ART regimens; 69.0% (40/58) had high-level resistance to NVP, while 58.6% (34/58) had high-level and 10.3% (6/58) intermediate-level resistance to EFV. High-level resistance to 3TC/FTC was present in 56.9% (33/58) of children, while only 12.1% (7/58) showed high- or intermediate-level resistance to ABC. High- or intermediate-level resistance to ABC was lower among children on ABC/3TC/LPV/r (7.3%, 3/41) than those on ABC/3TC/EFV (23.5%, 4/17) (Figure 1). Figure 1 also demonstrates the variable patterns of drug susceptibility based on individual children's DRM combinations and reveals that no children on either EFV- or LPV/r-based therapy selected mutations consistent with zidovudine resistance, and only one child had intermediate- or high-level resistance to tenofovir.

Drug susceptibility results among all children with detectable VL were used to assess the number of potentially effective drugs in a child's current ART regimen, with ARV drugs without any related DRMs or DRMs conferring potential or low-level resistance classified as effective. Among children on ABC/3TC/LPV/r, 8.2% (4/49) had one effective ARV (3 had LPV/r and 1 had ABC), 36.7% (18/49) had 2 effective drugs (all LPV/r and ABC), and 55.1% (27/49) had 3 effective ARVs in their current regimen (Figure 3). Among children on ABC/3TC/EFV, 13.0% (3/23) had no effective ARVs, 34.8% (8/23) had one effective ARV, 8.7% (2/23) had 2 effective ARVs (both had ABC and 3TC), and 43.5% (10/23) had 3 effective ARVs in their current regimen (Figure 3).

CONCLUSIONS

This report describes HIVDR among South African children recently initiated on age-appropriate WHO-recommended first-line ART. Overall, we found high rates of DRMs, with 80.6% of children with detectable viremia having at least one DRM and 38.9% with dual-class HIVDR. As previously described, we found low rates of PI resistance, but high rates of NRTI and NNRTI mutations among children on both regimens, likely representing both ADR and PDR selected by prior exposure to PMTCT interventions. We also found that the vast majority (91.8%) of children with detectable VL on ABC/3TC/LPV/r retained at least 2 active ARVs. These novel data are meaningful as countries develop optimized pediatric ART strategies utilizing the limited pediatric ARVs currently available.

As previously reported, we found high rates of NNRTI and NRTI resistance among children with detectable viremia, with K103N and M184V the most common mutations in each class, respectively [7, 12, 26–29]. Novel to our study is the finding that there was no

difference in the proportion of children with NNRTI DRMs by regimen. The high proportion of children on LPV/r-based ART in our analysis with NNRTI resistance (65.3%, 32/49) is almost double that reported from prior studies [12, 13, 30, 31]. One possible cause is the high rate of ARV exposure from PMTCT (73.5% of mothers received ARVs for PMTCT including single dose NVP, zidovudine and NVP, or ART), which are associated with high PDR rates in infants [7]. While our findings are limited by a lack of pretreatment DRM information and limited PMTCT history, 2 prior studies of PDR in newly-diagnosed HIV-infected infants in South Africa between 2010 and 2013 found NNRTI DRMs in 52% and 56.8% of infants, comparable to levels seen in our cohort [10, 11]. We hypothesize that NNRTI PDR mutations reemerged in the children on nonsuppressive PI-based ART.

PIs have been previously shown to have a high genetic barrier to resistance among children [12, 13, 30–32]; however, most existing data have included relatively small numbers of children on LPV/r with the WHO-recommended backbone of ABC+3TC. Our data, showing only 2 children with PI DRMs, further support the finding that LPV/r retains its high genetic barrier to resistance when paired with an ABC+3TC backbone. Of note, both children had a history of TB treatment. While we lack information on TB treatment regimens for these children, national guidelines called for super-boosting of LPV/r and no change for children on EFV-based ART regimens. It is possible that additional pill burden contributed to suboptimal adherence and drug–drug interactions between ARVs and TB medications could explain the resistance we identified [30, 33].

Our study includes DRM data on the largest cohort of children on WHO-recommended first-line ABC/3TC/LPV/r regimen to date. Drug susceptibility results showed that over 90% of children with detectable viremia on ABC/3TC/LPV/r, retained at least 2 effective ARVs, including LPV/r and ABC (+/− 3TC) compared to less than half of those on ABC/3TC/EFV (47.8%) (Figure 3), suggesting that children on ABC/3TC/LPV/r could achieve VS with improved adherence to their current regimen. This finding is encouraging, as data show that few CLHIV with viral failure are being switched to second-line ART [34]. In comparison, our findings of high rates of EFV resistance suggest that children failing EFV-based ART would be less likely to suppress if maintained on the same regimen.

Critical to optimizing pediatric ART is the selection of the NRTI backbone. While ABC+3TC has been one of several first-line pediatric ART backbones since 2013, its inclusion as the only WHO-recommended first-line pediatric backbone in 2018 prompted scrutiny in light of ABC resistance in children [6]. In this cohort, only 7.3% (3/41) of children on ABC/3TC/LPV/r with any DRMs developed intermediate- or high-level ABC resistance compared to 23.5% (4/17) of children on ABC/3TC/EFV. The limited ABC resistance suggests it may remain an effective ARV for use in second-line pediatric ART regimens, however it also highlights the importance of routine VL monitoring and rapid transition to potent regimens when treatment failure is identified.

A key strength of our study is presentation of new data on DRMs in a large cohort of children initiating first-line in routine care settings in South Africa. Unlike findings from clinical trials, children in our study experienced a standard of care similar to those in other resource-limited settings. A limitation of the study is the lack of PDR and standardized VL

testing that would have allowed for DRM evaluation at the same time point after treatment initiation for all children. This constrains our ability to understand whether DRMs were transmitted or acquired and whether they occurred among children meeting WHO definition of virological failure (VL above 1000 copies/mL from 2 consecutive VL measurements in a 3-month interval) [35]. Additionally, the small sample size of children with DRMs limited our ability to make statistical comparisons between the regimen groups.

In this cohort of South African children on WHO-recommended first-line ART regimens, the high levels of NNRTI and NRTI DRMs suggest a lasting impact of failed PMTCT interventions on DRMs, even among those on LPV/r-based regimens. Our data demonstrate superior susceptibility to the current treatment regimen of children with detectable viremia on ABC/3TC/LPV/r compared to ABC/3TC/EFV. Our findings also underscore the durability of ABC as part of the backbone of an LPV/r-based regimen and add to the growing body of literature demonstrating the urgent need for better ARV formulations and more robust, potent agents such as dolutegravir and ritonavir-boosted darunavir for the treatment of CLHIV.

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ART Regimen	Atazanavir	Darunavir	Lopinavir	Lamivudine	Abacavir	Zidovudine	Tenofovir Disoproxil Fumarate	Efavirenz	Etravirine	Nevirapine	Rilpivirine
ABC+3TC+EFV	0	0	0	4	4	0	4	4	0	4	0
ABC+3TC+EFV	0	0	0	4	4	0	0	4	3	4	4
ABC+3TC+EFV	0	0	0	4	4	0	0	4	2	4	2
ABC+3TC+EFV	0	0	0	4	2	0	0	4	1	4	2
ABC+3TC+EFV	0	0	0	4	2	0	0	4	3	4	3
ABC+3TC+EFV	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+EFV	0	0	0	4	2	0	0	4	1	4	1
ABC+3TC+EFV	0	0	0	4	2	0	0	4	1	4	2
ABC+3TC+EFV	0	0	0	4	2	0	0	4	3	4	3
ABC+3TC+EFV	0	0	0	4	2	0	0	4	2	4	4
ABC+3TC+EFV	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+EFV	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+EFV	0	0	0	4	4	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+EFV	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+EFV	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+EFV	0	0	0	0	0	1	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+EFV	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	3	0	2	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	3	0	2	4	1	4	2
ABC+3TC+LPV/r	0	0	0	4	3	0	2	4	4	4	4
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	3	4	3
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	3	4	4
ABC+3TC+LPV/r	0	0	0	4	2	0	0	4	1	4	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	1	4	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	1	4	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	0	4	0
ABC+3TC+LPV/r	0	0	0	0	0	1	0	4	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	4	3	4	4
ABC+3TC+LPV/r	0	0	0	4	2	0	0	3	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	3	1	4	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	3	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	3	3	4	3
ABC+3TC+LPV/r	0	0	0	0	0	0	0	3	3	4	4
ABC+3TC+LPV/r	0	0	0	0	0	0	0	3	3	4	3
ABC+3TC+LPV/r	3	0	3	4	2	0	0	0	1	0	2
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	4	2	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	1	0	2
ABC+3TC+LPV/r	0	0	0	0	0	2	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0
ABC+3TC+LPV/r	0	0	0	0	0	0	0	0	0	0	0

Figure 1.

Heat map of HIV drug resistance and drug susceptibility among South African children 0–12 years with detectable viremia (viral load >832 copies/mL) and successful amplification of dried blood spot samples by ART regimen (n = 72). Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; HIV, human immunodeficiency virus; LPV/r, lopinavir/ritonavir.

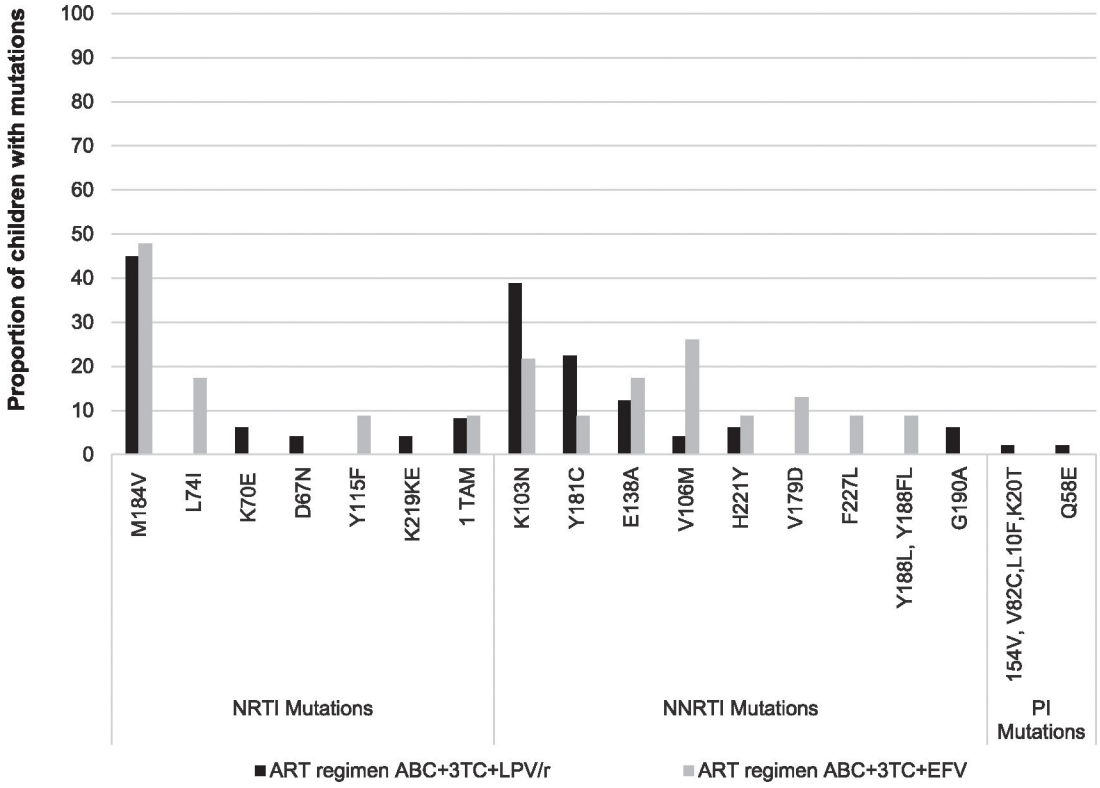

Figure 2.

Major drug resistance mutations by ART regimen type among South African children on LPV/r- and EFV-based therapy in South Africa from 2012–2015. Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors..

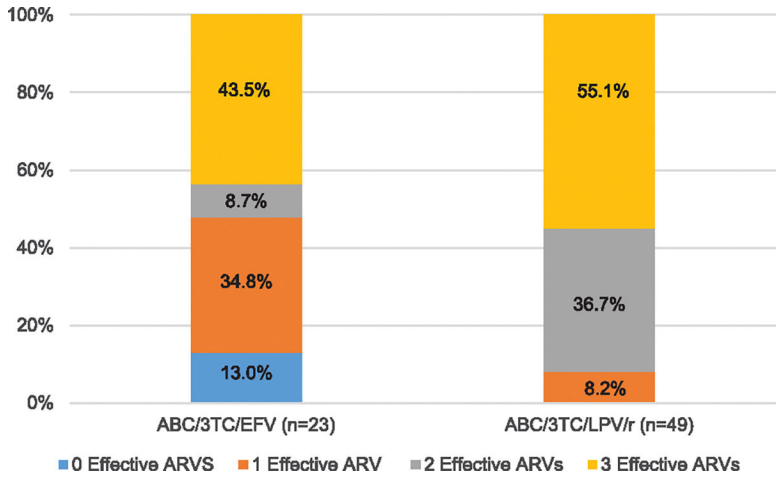

Figure 3.

Effectiveness of current ART regimen among South African children 0–12 years living with HIV with detectable viremia (viral load >832 copies/mL) and successful amplification of dried blood spot samples (n = 72). Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ARVs, antiretrovirals; EFV, efavirenz; HIV, human immunodeficiency virus; LPV/r, lopinavir/ritonavir.

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Table 1.

Characteristics at Study Enrollment (2012–2014) Among South African Children 0–12 Years Living With HIV With Detectable Viremia (Viral Load >832 Copies/mL) and Successful Amplification of Dried Blood Spot Samples (n = 72)

	All children		ART regimen				
			ABC+3TC+LPV/r		ABC+3TC+EFV		
	n	%	n	%	n	%	*P* value
	72	100.0	49	68.1	23	31.9	
Age at ART initiation							
median in months (IQR)	17 (6–78)		10 (4–18)		108 (69–132)		<.0001
<12 m	27	37.5	27	55.1	0	0.0	<.0001
1–2 y	20	27.8	20	40.8	0	0.0	
3–5 y	6	8.3	1	2.0	5	21.7	
6–12 y	19	26.4	1	2.0	18	78.3	
Age at HIV diagnosis							
median in months (IQR)	12 (2–52)		3 (1–4)		88 (52–124)		<.0001
<12 m	36	50.0	35	71.4	1	4.4	<.0001
1–3 y	12	16.7	12	24.5	0	0.0	
4–5 y	10	13.9	1	2.0	9	39.1	
6–12 y	14	19.4	1	2.0	13	56.5	
Sex							
Female	32	44.4	22	44.9	10	43.5	1.00
Male	40	55.6	27	55.1	13	56.5	
Child hospitalized at enrollment	23	31.9	18	36.7	5	21.7	.2806
Child hospitalized ever	42	58.3	29	59.2	13	56.5	1.00
Child TB at enrollment	16	22.2	9	18.4	7	30.4	.3618
Child TB history	30	41.7	15	30.6	15	65.2	.0096
Child knows HIV status (among children ≥8 y)	4	5.6	0	0.0	4	17.4	.0086
Mother PMTCT, reported or chart							
sdNVP	2	2.8	2	4.1	0	0.0	<.0001
AZT/NVP	21	29.2	19	38.8	2	8.7	
ART	16	22.2	15	30.6	1	4.4	

	All children		ART regimen				
			ABC+3TC+LPV/r		ABC+3TC+EFV		
	n	%	n	%	n	%	*P* value
None	29	40.3	13	26.5	16	69.6	
Missing	4	5.6	0	0.0	4	17.4	
Any PMTCT (among 68 with data)	39	54.2	36	73.5	3	15.8	<.0001
Mother PMTCT ART vs. other/none	16	22.2	15	30.6	1	4.4	.0142
Mother alive at enrollment	67	93.1	49	100.0	18	78.3	.0024
Primary caregiver (n = 348)							
mother	60	83.3	45	91.8	15	65.2	.0083
grandmother	6	8.3	3	6.1	3	13.0	
Other	6	8.3	1	2.0	5	21.7	
Mother >25 years at enrollment (among those alive at enrollment)	48	71.6	31	63.3	17	94.4	.0138
Inside tap in home	50	69.4	33	67.4	17	73.9	.7844
Enrollment WAZ, median (IQR)	−1.8 (−3.1 to −0.4)		−1.8 (−3.2 to 0.0)		−1.8 (−2.7 to −1.3)		.5989
<−2	28	43.1	20	45.5	8	38.1	.0308
−2 to −1	15	23.1	6	13.6	9	42.9	
>−1	22	33.9	18	40.9	4	19.1	
Missing	7	9.7	5	10.2	2	8.7	1.00
Enrollment viral load (log), median (IQR)	5.9 (5.3–6.4)		6.2 (5.5–6.7)		5.4 (5.1–5.8)		.0037
>6.0	29	49.2	26	63.4	3	16.7	.0019
5.0–6.0	21	35.6	10	24.4	11	61.1	
4.0–4.9	8	13.6	5	12.2	3	16.7	
<4.0	1	1.7	0	0.0	1	5.6	
missing	13	18.1	8	16.3	5	21.7	.7434
Enrollment CD4 count, median (IQR)	656 (250–1216)		1078 (535–1631)		239 (106–492)		<.0001
>1000	23	37.7	21	53.9	2	9.1	<.0001
500–1000	14	23.0	11	28.2	3	13.6	
350–499	3	4.9	1	2.6	2	9.1	
200–349	9	14.8	3	7.7	6	273	
<200	12	19.7	3	7.7	9	40.9	
Missing	11	15.3	10	20.4	1	4.4	.0879

	All children		ART regimen				
			ABC+3TC+LPV/r		ABC+3TC+EFV		
	n	%	n	%	n	%	*P* value
Enrollment CD4%, median (IQR)	14.6 (9.6–24.7)		15.8 (11.2–28.5)		11.4 (6.6–18.0)		.0153
>40%	3	5.0	3	7.7	0	0.0	.2335
25–40%	12	20.0	10	25.6	2	9.5	
15–24%	13	21.7	8	20.5	5	23.8	
<15%	32	53.3	18	46.2	14	66.7	
missing	12	16.7	10	20.4	2	8.7	.20197
Time on study, median in months (IQR)	24 (17–24)		24 (14–24)		24 (23–24)		
Time on ART, median in months (IQR)	22 (14.5–24)		20 (14–23)		24 (20–24)		

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT zidovudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; HIV, human immunodeficiency virus; PMTCT, prevention of mother-to-child transmission; sdNVP single dose nevirapine; TB, tuberculosis.

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Table 2.

Drug Resistance Mutations Among South African Children 0–12 Years Living With HIV With Detectable Viremia (Viral Load >832 Copies/mL) and Successful Amplification of Dried Blood Spot Samples, 2012–2015 (n = 72)

	All children		ART regimen			
			ABC+3TC+LPV/r		ABC+3TC+EFV	
	n	%	n	%	n	%
	72	100.0	49	68.1	23	31.9
Age at ART initiation						
<3 y	47	65.3	47	95.9	0	0.0
3–12 y	25	34.7	2	1.1	23	100.0
Any DRMs among child with successful amplification	58	80.6	41	83.7	17	73.9
Any mutations by class						
NRTI	37	51.4	25	51.0	12	52.2
NNRTI	47	65.3	32	65.3	15	65.2
PI	2	2.8	2	4.1	0	0.0
NRTI + NNRTI	26	36.1	16	32.6	10	43.5
NRTI + PI	1	1.4	1	2.0	0	0.0
NNRTI + PI	2	2.8	2	4.1	0	0.0
NRTI + NNRTI +PI	1	2.1	1	2.0	0	0.0
NRTI mutations						
M184V (includes M184MV, M184MI, M184V, M184I)	33	45.8	22	44.9	11	47.8
L74I or L74V or L74LV*	4	5.6	0	0.0	4	17.4
K70E or K70KN or K70Q or K70R	4	5.6	3	6.1	1	4.4
Y115F	2	2.8	0	0.0	2	8.7
D67N	2	2.8	2	4.1	0	0.0
K219KE	3	4.2	2	4.1	1	4.4
K65R	1	1.4	0	0.0	1	4.4
T69Ins	1	1.4	1	2.0	0	0.0
T69 Deletion	1	1.4	0	0.0	1	4.4
V75M	1	1.4	0	0.0	1	4.4
M41L	0	0.0	0	0.0	0	0.0

	All children		ART regimen			
			ABC+3TC+LPV/r		ABC+3TC+EFV	
	n	%	n	%	n	%
L210	0	0.0	0	0.0	0	0.0
T215/F	0	0.0	0	0.0	0	0.0
1 TAMS	6	8.3	4	8.2	2	8.7
NNRTI mutations						
K103N, K103KN	24	33.3	19	38.8	5	21.7
Y181C, Y181YC	13	18.1	11	22.5	2	8.7
E138A	10	13.9	6	12.2	4	17.4
V106M	8	11.1	2	4.1	6	26.1
H221Y	5	6.9	3	6.1	2	8.7
G190A, G190GA	4	5.6	3	6.1	1	4.4
V179D	4	5.6	1	2.0	3	13.0
L100I, L100LI	2	2.8	1	2.0	1	4.4
F227L	3	4.2	1	2.0	2	8.7
Y188L, Y188FL	2	2.8	0	0.0	2	8.7
P225PH	1	1.4	0	0.0	1	4.4
Major PI mutations						
154V, V82C, L10F, K20T	1	1.4	1	2.0	0	0.0
Q58E	1	1.4	1	2.0	0	0.0

Abbreviations: ART, antiretroviral therapy; DRMs, drug resistance mutations; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors.