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Resistance is common in paediatric patients failing ART in South Africa

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Background: Minimal data exist on HIV drug resistance patterns and prevalence among paediatric patients failing ART in resource-limited settings. We assessed levels of HIV drug resistance in children with virological failure.

Methods: This cross-sectional study, performed from March 2017 to March 2019 in South Africa, enrolled HIV-positive children aged ≤19 years, receiving ART through public health facilities with recent evidence suggestive of virological failure (at least one viral load ≥1000 copies/mL), across 45 randomly selected high-volume clinics from all nine provinces. Resistance genotyping was performed using next-generation sequencing technologies. Descriptive analysis taking into account survey design was used to determine outcomes.

Results: Among 899 participants enrolled, the adjusted proportion of HIV drug resistance among children with virological failure was 87.5% (95% CI 83.0%–90.9%). Resistance to NNRTIs was detected in 77.4% (95% CI 72.5%–81.7%) of participants, and resistance to NRTIs in 69.5% (95% CI 62.9%–75.4%) of participants. Overall, resistance to PIs was detected in 7.7% (95% CI 4.4%–13.0%) of children.

Conclusions: HIV drug resistance was highly prevalent in paediatric patients failing ART in South Africa, with 9 in 10 patients harbouring resistance to NNRTIs and/or NRTIs. PI-based regimens are predicted to be highly efficacious in achieving virological suppression amongst patients failing NNRTI-based regimens. Scaling up resistance testing amongst patients would facilitate access to second- and third-line regimens in South Africa.

Introduction

Children on ART in resource-limited settings are at an increased risk of developing HIV drug resistance (HIVDR) due to fewer drug formulations, frequent dosing changes following weight gain, and possible prior exposure to antiretroviral agents through prevention of mother to child transmission (PMTCT).[1–3] These complications are coupled with a developing immune system with limited innate ability to control viral replication, resulting in rapid disease progression and high viral loads.[4–6] Psychosocial issues and misconceptions about HIV infection and treatment in children, stigma and fear of disclosure to peers and sexual partners during childhood and adolescence can contribute to non-adherence and development of drug resistance.[7,8]

South African treatment guidelines for the management of HIV infection in children (2013) included abacavir and lamivudine as the NRTI backbone combined with the ritonavir-boosted PI lopinavir for children <3 years of age and efavirenz for children over 3 years of age.[9] Tenofovir was introduced for children >15 years of age. Children failing NNRTI-based regimens were switched to ritonavir-boosted lopinavir, and those failing PI-based regimens were referred to a specialist for further care. Viral load (VL) monitoring is recommended at 6 and 12 months after initiating ART and annually thereafter if VL remains <50 copies/mL. For VL between 50 and 999 copies/mL, adherence to ART was encouraged and VL repeated in 6 months. For VL ≥1000 copies/mL, the adherence package was reinforced and the VL repeated after 2 months. Virological failure (VF) is defined as two consecutive

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plasma VLs ≥1000 copies/mL within a 2 month interval after a minimum of 6 months of ART with good adherence.

Minimal data exist reporting prevalence of drug resistance in paediatric patients in South Africa. Recent studies report >60% of infants <18 months of age infected with HIV, despite PMTCT exposure, harboured resistance to NNRTIs, primarily driven by resistance to nevirapine, whilst resistance to NRTIs was relatively infrequent (<10%).[10,11] Studies from children failing ART show very high rates of NNRTI-based (65%–95%) and NRTI-based resistance (52%–93%) amongst children aged ≤15 years failing non-PI-based regimens between 2008 and 2015.[12–16] Resistance to PIs was reported in 2%–11% of children failing PI-based regimens,[13,15–17] but higher rates (36% and 49%)[12,18] have been reported in children receiving ritonavir-boosted PI regimens. These studies were conducted in small cohorts of children or restricted regions or healthcare facilities. There was a need for updated and more nationally representative surveillance of acquired drug resistance (ADR) in children failing ART in South Africa.

A cross-sectional study was performed between March 2017 and March 2019 assessing levels of HIVDR in children with VF, by age group and regimen selection. The objective of this manuscript is to describe the primary findings of this survey.

Methods

Study design and sample size criteria

A cross-sectional facility-based study with retrospective record review was conducted using a stratified cluster sampling approach. The study targeted HIV-positive children aged ≤19 years who were receiving ART at public health facilities across South Africa and had suggestive evidence of VF (defined as at least one recent VL test performed that was ≥1000 copies/mL). The sample size was determined assuming a prevalence of HIVDR of 80% among children with VL ≥1000 copies/ml, with prevalence assumed to be the same in all four age groups, 0–4, 5–9, 10–14 and 15–19 years, a 95% CI, a precision level of 5%, and a design effect of 1.5 for possible clustering of paediatric HIVDR at clinic level. The final sample size for the study was 1485 participants.

As the sample was stratified by province, a two-stage selection approach was used. Firstly, 45 participating facilities were randomly selected based on a historical record of having ≥100 specimens with VLs ≥1000 copies/mL from children or adolescents ≤19 years of age in 2014, as obtained from the National Health Laboratory Services (NHLS) Laboratory Information Systems database, and between 2 and 18 sites per province were then selected using the probability proportional to size. The number of sites that were selected from Eastern Cape (EC), Free State (FS), Gauteng (GP), Kwazulu-Natal (KZ), Limpopo (LP), Mpumalanga (MP), North West (NW), Northern Cape (NC) and Western Cape (WC) provinces was 2, 1, 10, 20, 3, 4, 2, 1 and 2 respectively. Thirty-three specimens with VLs ≥1000 copies/mL were then required from each participating facility.

Participant identification and enrolment

To be eligible for inclusion in the study, the children had to be aged ≤19 years, on ART for a minimum of 1 year (±3 months), and with suggestive evidence of VF performed within the previous 6 months. Participation in the study required consent from a responsible adult for all children aged less than 18 years, and children aged ≥7 years were required to assent into the study. Participants who had a regimen change following their most recent VL test were not included.

Healthcare workers (HCWs) at participating sites identified eligible candidates through registers maintained at the participating facilities, and/or through the NHLS Weekly Result-for-Action reports, where available. Once enrolled, the HCW completed the study data collection form that abstracted minimal clinical and sociodemographic data from the patient medical file and drew a fresh blood sample. Laboratory results were returned to the participating clinic for patient management.

Blood specimen collection and HIVDR genotyping

Whole blood was collected using standard phlebotomy methods into a 6 mL EDTA tube. A minimum of 500 µL plasma was ultra-centrifuged and total nucleic acid extracted using the MagNA Pure 2.0 automated extraction system (Roche Applied Science, Penzberg, Germany). PCR amplification was performed using an in-house method resulting in a single amplicon spanning 1.5 kb of *pol* encompassing the protease gene and the first 250 amino acids of the reverse transcriptase gene (HXB2 2358–3882). Amplicon concentrations were adjusted to a final concentration of 0.5 ng/µL and libraries prepared using the 96-sample Nextera® XT DNA Library Preparation Kit (Illumina, USA). Quantified amplicons were sequenced using the MiSeq V3 sequencing Kit (Illumina, San Diego, USA). MiSeq FastQ files were analysed using DeepChek® (Advanced Biological Laboratories, Luxembourg, Luxembourg), and results were generated at a 20% mutation detection threshold. In the event of PCR non-amplification or next-generation sequencing failure, a smaller amplicon was attempted as previously described[19] and sequenced using Sanger sequencing technologies. Consensus sequences were analysed using the Stanford HIVDB Algorithm V8.8. Presence of HIVDR was defined as ≥1 drug resistance mutation associated with high-level resistance (HLR), intermediate-level resistance (IR) or low-level resistance (LLR) per genotype. Resistance to PIs, NRTIs or NNRTIs was limited to drug resistance mutations associated with resistance to members of that drug class only (PIs: ritonavir-boosted atazanavir, ritonavir-boosted darunavir, ritonavir-boosted lopinavir; NRTIs: abacavir, stavudine, didanosine, emtricitabine, lamivudine, tenofovir disoproxil fumarate; NNRTIs: doravirine, efavirenz, etravirine, nevirapine, rilpivirine.

Description of outcomes and key exposure variables

Key variables collected included age, sex, weight, relationship of caregiver, disclosure status, school grade, date of diagnosis, date of ART initiation, current regimen, previous regimen, other medicines taken, maternal and paediatric PMTCT received, current or prior TB diagnosis, recent CD4 test results and recent VL test results. Time with virological failure was determined as time between enrolment and most recent VL, or time between enrolment and first VL result ≥1000 copies/mL.

Statistical methods

The sample was selected in two stages as described above. This approach provided estimation of both the within- and between-clinic variability, which provided appropriate standard errors for 95% CI. Descriptive statistics were presented using frequencies and proportions for categorical variables and medians with corresponding IQRs for continuous variables. All prevalence analyses accounted for the sample structure by stratifying by province, after weighting for survey design and non-response at the site level (design weight/contribution weight). All analyses were performed using the survey module in STATA, using the participating facility as the primary sampling unit. Significance was set at a *P* value of less than 0.05. All analyses were conducted using STATA version 14 (STATA Corp., College Station, TX, USA).

Ethics

The protocol was approved by the Wits Human Research Ethics Committee (M151146). The protocol was also reviewed in accordance with the US CDC human research protection procedures and was determined to be research, but CDC investigators did not interact with human

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subjects or have access to identifiable data or specimens for research purposes (CGH; 2016–290).

Results

Demographic characteristics of enrolled participants

At study closure, 1000 children had participated in the study. Of these, 899 participants from 40 facilities from eight provinces (EC, FS, GP, KZ, LP, MP, NC and WC) were included in the study analysis, comprising 61% of the anticipated sample size (Figure 1). One hundred and one participants were excluded: 74 were excluded for incomplete study forms or missing data, 8 were >19 years of age, and 19 did not submit a blood specimen or the specimen was haemolysed on receipt. The final number enrolled per province were 44, 33, 135, 455, 58, 107, 37, 0 and 30 in EC, FS, GP, KZ, LP, MP, NC, NW and WC, respectively. The adjusted median age of all participants was 12.9 (IQR 8.8–15.4) years and the median most recent VL result was 24 594 (IQR 6020–99 994) copies/mL. Most participants were aged 10–14 (41%) or 15–19 years (28%); 18% were aged 5–9 years and 14% aged 4 years or less. Participants had been failing ART for a median of 1.0 (IQR 0.5–2.0) years. The majority were male (54.2%, Table 1) and attending primary school (46.8%). Two thirds of all participants were aware of their HIV status; not unexpectedly, these proportions varied across the age groups, with disclosure rates of 6.4%, 30.1%, 72.9% and 93.6% amongst children 0–4, 5–9, 10–14 and 15–19 years, respectively. Just over half of all participants were cared for by a parent, the remainder were primarily cared for by grandparents or extended family members, whilst 2% were in a foster-care environment. Documented PMTCT exposure was reported in 163/433 (37.6%) of participants, and almost two in five children reported previous or current coinfection with TB.

A total of 418 children were receiving PI-based regimens, constituting 48.4% (95% CI 41.4%–55.3%) of all participants after adjusting for survey design; the median age of these participants was 11.7 (IQR 5.5–15.2) years, and the median most recent VL was 36 800 (7800–116 200) copies/mL. The majority were receiving ritonavir-boosted lopinavir-based regimens (97.1%), whilst 2.9% were receiving ritonavir-boosted atazanavir. The proportions of children aged 0–4 years who were receiving PI-based regimens was 83.5%, 47.1% of children aged 5–9 years, 40.4% of children aged 10–14 years, and 44.7% of children 15–19 years.

Four hundred and twenty-two participants were receiving NNRTI-based regimens [(45.0% (95% CI 38.2%–52.1%)]; the median age of these participants was 13.4 (IQR 10.5–15.6) years and the median most recent VL was 18 500 (IQR 4530–66 881) copies/mL. The majority were receiving efavirenz-based regimens (98.7%); seven participants were receiving nevirapine.

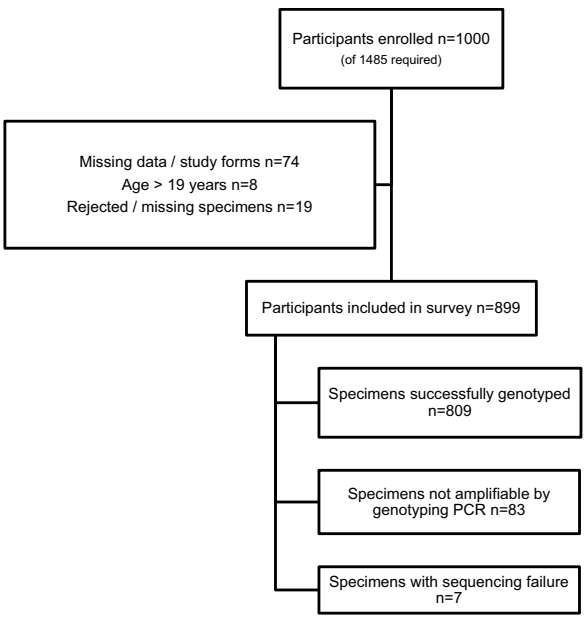


Figure 1. Final enrolment study flow chart.

Table 1. Unadjusted and adjusted demographic and clinical characteristics of enrolled participants

	n	Unadjusted %	Adjusted %	95% CI
Male sex	460	54.2	55.6	50.5–60.5
Age category (years)				
0–4	122	13.6	12.7	9.4–17.0
5–9	159	17.7	16.5	13.0–20.7
10–14	365	40.6	41.9	35.9–48.2
15–19	253	28.2	28.9	23.1–35.4
Grade at school				
Not attending school	178	19.9	19.3	14.7–24.9
Pre-school	33	3.7	2.7	1.6–4.4
Primary school	418	46.8	47.9	42.8–52.9
Secondary school/tertiary training	265	29.6	30.2	24.7–36.7
Aware of HIV status				
All children	545	62.9	66.1	58.9–72.6
Children aged 0–4 years ($n=110$)	7	6.4	16.7	4.5–45.9
Children aged 5–9 years ($n=153$)	46	30.1	39.9	26.2–55.4
Children aged 10–14 years ($n=354$)	258	72.9	70.9	59.7–79.9
Children aged 15–19 years ($n=250$)	234	93.6	94.1	89.3–96.9
Relationship of primary caregiver				
Parent	415	59.5	56.8	48.1–65.1
Grandparent or extended family	270	38.7	41.6	33.4–50.3
Foster care	13	1.9	1.6	0.7–3.5
Current regimen				
PI-based regimen	418	53.3	48.4	44.7–58.5
NNRTI-based regimen	422	47.2	45.0	47.9–61.8
NRTI-based regimen	55	6.2	6.6	3.9–11.1
Documented poor adherence	116	14.0	12.3	8.5–17.3
Previous or current coinfection with TB	318	37.7	36.9	30.5–43.8
Documented exposure to PMTCT	163	37.6	37.9	25.6–51.9

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Table 2. Adjusted proportions of participants with HIVDR by age group, $n=809$

	%	95% CI	*P* value
Total resistance			
All children	87.6	83.2–91.0	
Ages 0–4 years	89.5	76.6–95.7	0.9019
Ages 5–9 years	87.8	80.4–92.6	
Ages 10–14 years	86.5	78.0–92.1	
Ages 15–19 years	88.2	82.4–92.4	
Resistance to PIs			
All children	7.9	4.5–13.3	
Ages 0–4 years	5.8	2.0–15.6	0.5157
Ages 5–9 years	9.8	3.4–25.1	
Ages 10–14 years	10.1	3.9–23.9	
Ages 15–19 years	4.5	1.9–10.2	
Resistance to NNRTIs			
All children	77.3	72.4–81.6	
Ages 0–4 years	76.3	64.1–85.3	0.1719
Ages 5–9 years	70.0	59.4–77.2	
Ages 10–14 years	77.2	67.8–84.5	
Ages 15–19 years	82.4	75.6–87.5	
Resistance to NRTIs			
All children	69.6	62.7–75.8	
Ages 0–4 years	78.6	66.6–87.0	0.0093
Ages 5–9 years	81.9	72.3–88.8	
Ages 10–14 years	68.2	58.3–76.7	
Ages 15–19 years	61.1	50.8–70.5	
Resistance to RTIs			
All children	59.4	52.5–65.9	
Ages 0–4 years	65.4	51.3–77.2	0.5496
Ages 5–9 years	63.2	54.1–71.4	
Ages 10–14 years	58.9	48.2–68.7	
Ages 15–19 years	55.5	45.2–65.4	

Amongst children aged 0–4 years, 8.7% were receiving NNRTI-based regimens, and 47.0% of children aged 5–9 years, 52.4% of children aged 10–14 years, and 48.9% amongst children 15–19 years.

A total of 55 (6.5%, 95% CI 3.8%–11.1%) participants were receiving NRTI-only-based regimens. These included lamivudine only ($n=21$), abacavir+lamivudine ($n=18$) and abacavir only ($n=2$), whilst the remainder were combinations of zidovudine, lamivudine, emtricitabine, stavudine and/or tenofovir disoproxil fumarate. The median age of these participants was 14.6 (IQR 9.1–15.4) years and the median VL was 27 670 (IQR 11 982–263 000) copies/mL.

Prevalence of HIVDR in children on ART with VF

Of 899 specimens included in the analysis, genotyping PCR and sequencing was successful in 809 (809/899, 90.0%), whereas 7 could not be sequenced and 83 specimens were not amplifiable by genotyping PCR.

The adjusted proportion of HIVDR among children on ART with VF was determined to be 87.6% (95% CI 83.2%–91.0%, Table 2). Resistance to NNRTIs was detected in 77.3% (95% CI 72.4%–81.6%) of participants, and resistance to NRTIs in 69.6% (95% CI 62.7%–75.8%). Dual-class NRTI+NNRTI resistance was detected in 59.4% (95% CI 52.5%–65.9%), and PI resistance was present in 7.9% (95% CI 4.5%–13.3%) of enrolled participants.

Table 3. Adjusted proportions of participants with HIVDR by regimen, $n=809$

	%	95% CI
PI-based regimens		
Resistance—any	80.5	73.1–86.4
Resistance to PIs	11.5	6.4–19.8
Resistance to NNRTIs	60.9	54.4–67.1
Resistance to NRTIs	65.1	56.3–73.0
Resistance—dual RTI	45.7	39.1–53.6
NNRTI-based regimens		
Resistance—any	94.9	90.0–97.4
Resistance to PIs	0.7	0.2–2.1
Resistance to NNRTIs	94.1	89.1–96.9
Resistance to NRTIs	74.8	67.1–81.2
Resistance—dual RTI	74.1	66.5–80.5
NRTI regimens		
Resistance—any	91.2	78.4–96.7
Resistance to PIs	29.2	8.1–65.9
Resistance to NNRTIs	84.4	68.7–93.1
Resistance to NRTIs	69.6	43.5–87.2
Resistance—dual RTI	62.9	38.0–82.4

Prevalence of HIVDR in children on ART with VF by age group

Adjusted prevalence of HIVDR did not vary across the four different age groups [0–4 years: 89.5% (95% CI 76.6%–95.7%); 5–9 years: 87.8% (95% CI 80.4%–92.6%), 10–14 years: 86.5% (95% CI 78.0%–92.1%), 15–19 years: 88.3% (95% CI 82.4%–92.4%); Table 2]. Resistance to PIs was most prevalent in the 10–14 years age group [10.1% (95% CI 3.9%–23.9%)], whereas resistance to NNRTIs was most prevalent in adolescents aged 15–19 years [82.4% (95% CI 75.6%–87.5%)]. Levels of NRTI resistance were highest in the younger age groups, notably amongst children aged 5–9 years [82.0% (95% CI 72.3%–88.8%, $P=0.0093$)].

There was slightly higher levels of resistance amongst male participants; overall 55.2% (95% CI 49.4%–60.9%) of children with HIVDR were male ($P=0.8256$). The proportion of children with HIVDR and aged 0–4 years who were male was 49.9% (95% CI 31.4%–68.5%, $P=0.5819$); among children aged 5–9 years was 59.8% (95% CI 48.3%–70.4%); among children aged 10–14 years was 57.6% (47.8%–66.9%, $P=0.5800$) and among children aged 15–19 years was 51.6% (95% CI 41.0%–62.1%, $P=0.2190$).

Prevalence of HIVDR in children with VF by regimen

Amongst participants receiving PI-based regimens, adjusted total HIVDR was detected in 80.6% (95% CI 73.1%–86.4%, Table 3); resistance to PIs was present in 11.5% (95% CI 6.4%–19.8%) of participants, resistance to NNRTIs was present in 60.9% (95% CI 54.4%–67.1%) and resistance to NRTIs in 65.1%

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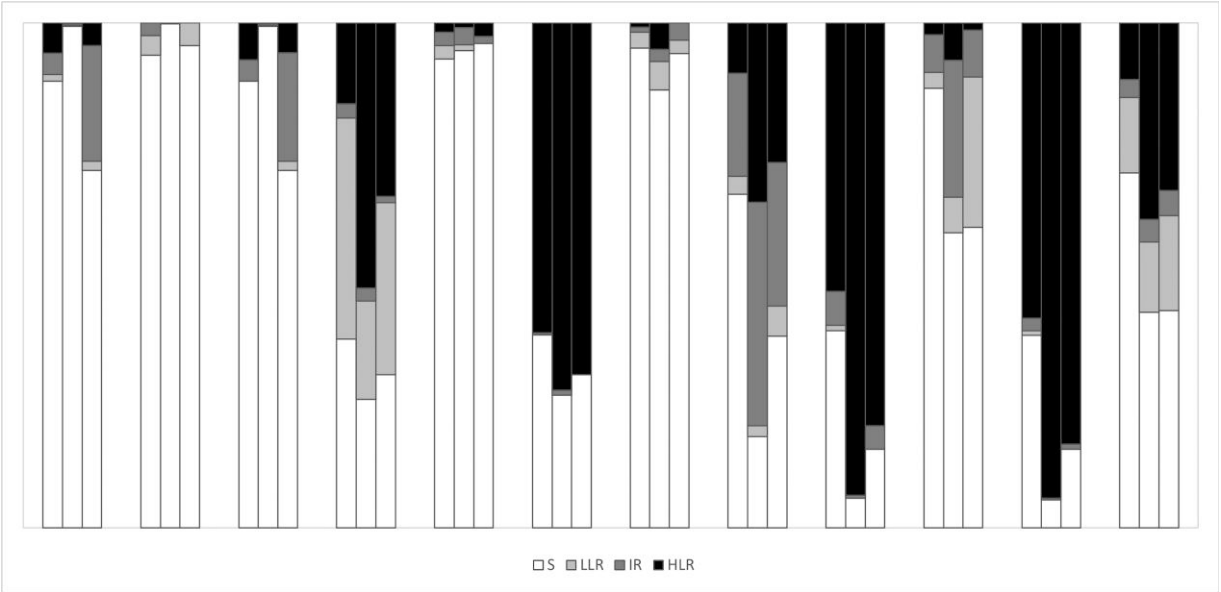


Figure 2. Unadjusted predicted resistance profiles amongst paediatric participants failing ART, categorized according to current regimen in use ($n = 805$). PI, PI-based regimen, $n = 369$ participants; NNRTI, NNRTI-based regimen, $n = 384$ participants; NRTI, NRTI-based regimen, $n = 52$ participants.

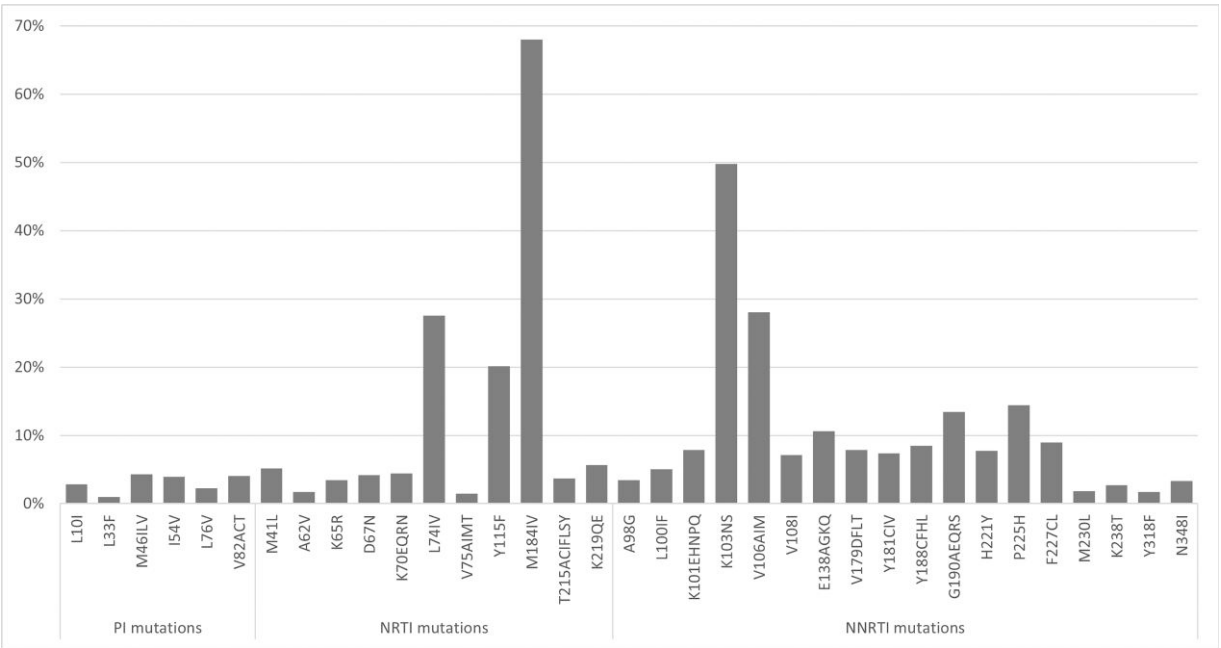


Figure 3. Unadjusted patterns of HIV drug resistance mutations in paediatric participants with virological failure, $n = 809$. Note: only mutations present at ≥1.0% are depicted in this figure.

(56.3%–73.0%). Amongst patients with PI resistance, 29.3% (95% CI 12.8%–53.9%) exhibited LLR and 15.2% (95% CI 3.0%–50.6%) exhibited IR to darunavir.

HIVDR was detected in 94.9% (95% CI 90.0%–97.4%) of participants receiving NNRTI-based regimens (Table 3), and resistance to NNRTIs was detected in 94.1% (89.1%–96.9%). Resistance to PIs was present in 0.7% (95% CI 0.2%–2.1%) of participants and resistance to NRTIs in 74.8% (67.1%–81.2%). Notably, 74.1% (95% CI 66.5%–80.5%) of children receiving NNRTI-based regimens harboured dual NNRTI + NRTI resistance; of these patients with dual NNRTI + NRTI resistance, 70.7% (95% CI 62.0%–78.2%) exhibited HLR to both abacavir and

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efavirenz, whilst 25.4% (95% CI 19.1%–32.9%) exhibited HLR to efavirenz and LLR to abacavir.

Among the 55 participants receiving NRTI-based regimens, resistance to PIs was present in 29.2% (95% CI 8.1%–65.9%), resistance to NNRTIs was present in 84.4% (95% CI 68.7%–93.1%) and resistance to NRTIs in 69.6% (95% CI 43.5%–87.2%). Overall, resistance was detected in 91.2% (95% CI 78.4%–96.7%) of participants receiving NRTI-based regimens.

Adjusted predicted resistance profiles [susceptible (S), LLR, IR or HLR] of the 809 genotypes obtained are shown in Figure 2. More than half of children with VF harboured HLR to lamivudine + emtricitabine, irrespective of current regimen, whilst resistance to zidovudine and tenofovir disoproxil fumarate was infrequent. Resistance to abacavir differed by regimen; half of children failing an NNRTI-based regimen exhibited HLR to abacavir, whilst one in six children failing PI-based regimens harboured HLR to abacavir. Resistance to efavirenz and nevirapine was prevalent in most children, with cross-resistance to rilpivirine and doravirine in >50% of children failing NNRTI-based regimens. Etravirine showed the highest susceptibility profile, with 60%–80% of all patients exhibiting susceptibility to etravirine, irrespective of current regimen. Patients with resistance to PIs showed high levels of susceptibility to darunavir.

Patterns of resistance

The unadjusted prevalence and patterns of HIVDR mutations detected are shown in Figure 3. The most prevalent NNRTI mutations detected were K103N/S (49.8%), V106A/I/M (28.1%) and P225H (14.5%). The most prevalent NRTI mutations detected were M184I/V (68.0%), L74I/V (27.6%) and Y115F (20.1%). The most prevalent PI mutations detected were M46I (4.3%), I54V (4.0%) and V82A/C/T (4.1%).

Discussion

This national cross-sectional study showed that 9 in 10 children with VF in South Africa were failing with HIVDR and have been failing with HIVDR for a substantial time. Resistance to the NNRTI drug class was most prevalent, but half of children failing ART were failing with resistance to NNRTIs and NRTIs. Resistance to efavirenz, nevirapine, lamivudine and abacavir was most prevalent, including amongst children receiving NNRTI-based and NRTI-based regimens. As NRTI and second-generation NNRTI regimen alternatives, tenofovir disoproxil fumarate, zidovudine and etravirine were predicted to be most efficacious for subsequent management of children with HIVDR. Our study showed that PI resistance remains comparatively low, and PI regimens remain likely to be highly effective in achieving virological suppression amongst patients failing NNRTI-based regimens. However, amongst children that were failing PI-based regimens with resistance to ritonavir-boosted lopinavir and ritonavir-boosted atazanavir, half exhibited susceptibility profiles to darunavir, which is typically used as part of third-line regimens in South Africa.

The prevalence and patterns reported herein were similar to those in recent reports. A recent study reported NNRTI resistance in 65.3%, NRTI resistance in 51.4% and PI resistance in 2.8% of 72 children living with HIV from five public health facilities in the EC province.[16] In this study, children aged between 0 and 12 years were monitored for 24 months between 2012 and 2014, and genotypes performed on children with VF. Data from KwaZulu-Natal collected during 2011–12 showed 82.2% and 86.3% of children aged ≤15 years failing NNRTI-based regimens, and 25.0% and 62.5% of children failing PI-based regimens harboured resistance to NNRTIs and NRTIs, respectively.[13] A retrospective analysis of resistance patterns amongst 370 genotypes collected from children aged 3–15 years and submitted for clinical management testing between 2009 and 2012, showed that up to 33% of children failing PI-based regimens harboured PI resistance.[12] More recently, resistance to NNRTIs, NRTIs and PIs was reported in 65.2%, 60.8% and 5.8%, respectively, of 69 specimens collected from paediatric patients aged 2–10 years from two sites in LP province, between 2013 and 2015.[15] Our study reports resistance prevalence findings in the largest cohort of paediatric patients, with 809 specimens successfully sequenced, and collected from eight of nine South African provinces, and shows that children failing ART are highly likely to be failing with HIVDR, and that resistance to abacavir, lamivudine + emtricitabine, efavirenz and nevirapine is frequently detected. As alternative regimens from this drug class, resistance to zidovudine and tenofovir disoproxil fumarate was less frequent, and that etravirine could be considered as an alternative second-generation NNRTI.

This study did not assess levels of resistance to the integrase strand transfer inhibitors (INSTIs), as these regimens were not recommended for management of paediatric HIV infection unless recommended for third-line ART. Whilst INSTI-based regimens are predicted to be efficacious in children,[20–22] limited evidence of dolutegravir efficacy in infants and children with NRTI resistance exists.[23]

Of concern, the highest levels of resistance to all three drug classes were reported amongst the 55 participants who were receiving NRTI-only based regimens, also referred to as 'holding regimens'. These regimens are intended to provide partial clinical and immunological benefit without accumulations of additional resistance mutations, until full suppression using alternative regimens can be obtained.[24] Most were receiving lamivudine only, or lamivudine + abacavir. Twenty-one percent of these participants harboured resistance to PIs, and 63% harboured resistance to NRTI + NNRTI regimens. Extensive resistance has accumulated in these children by the time they are switched to holding regimens, and the predicted efficacy of alternative PI regimens is lower. However, these children may benefit from INSTI-based therapy.

Study limitations

High-volume paediatric sites were included in the study. This approach possibly introduced bias to the study results, should there be significant differences in characteristics between high- and low-volume sites. In addition, participants were selected at the discretion of the site HCW, with the intention to offer a resistance test when clinically warranted. This survey did not necessarily recruit participants who were known to be poorly adherent and who were possibly less likely to harbour resistance mutations. The study also used facility HIV care providers/clinic staff to collect this data on the laboratory request form, in facilities where staff shortages are well documented. In many sites, recruitment was incomplete and subject to HCW availability to participate.

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The survey was not successful in achieving the anticipated sample size, particularly in certain provinces, primarily due to administrative delays in obtaining permission to conduct the research at the respective facilities, and reluctance of HCWs at sites to take on the additional study requirements. However, enrolment of clinics and participants was more successful in EC, FS, KZ, MP and NC provinces, four of which have a very high HIV burden. Therefore, these results are possibly not nationally representative. Complications in implementing nationally representative HIVDR surveys have previously been described, leading to considerations for alternative laboratory-based surveillance strategies or targeted cohort studies.[25] To our knowledge, this study reports on HIVDR findings in the largest number of children to date.

Conclusions

HIVDR is highly prevalent amongst children on ART and with viraemia in South Africa. Rapid change of regimen is critical after a second elevated viral load, given the predicted high efficacy of second-line and/or dolutegravir-based regimens. In addition, the implementation of routine HIVDR testing for all paediatric patients with VF irrespective of regimen should be prioritized in national treatment programmes to assist in selecting optimal treatment regimens for these patients.

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Transparency declarations

All authors have no interests to declare. Representatives of the study sponsor did not participate in the collection of data but did participate in the study design, analysis and interpretation of data, the writing of this report and the decision to submit this paper for publication.

Author contributions

Study design and protocol development: G.M.H., L.L., T.K., G.A., K.A., K.D., G.S., E.R. Study implementation, management and acquisition of data: G.M.H., M.Y., L.L., J.L., K.S., G.K., K.D., S.C., G.S. Interpretation of reported results and data analysis: G.H., M.Y., L.L., J.L., T.K., K.A., G.K., K.D., G.S., E.R. Administrative, technical and supervisory support: G.A., J.P., K.A., G.K., K.D., E.R. Drafting and revising on manuscript: G.M.H., L.L., J.L., K.S., T.K., K.A., G.K., K.D., G.S., E.R.

References

1 Adetokunboh O, Atibioke O, Balogun T *et al*. Antiretroviral treatment and resistance patterns in HIV-infected children. *Curr Infect Dis Rep* 2015; **17**: 502. https://doi.org/10.1007/s11908-015-0502-9

2 Penazzato M, Prendergast A, Tierney J *et al*. Effectiveness of antiretroviral therapy in HIV-infected children under 2 years of age. *Cochrane Database Syst Rev* 2012; CD004772. https://doi.org/10.1002/14651858.CD004772.pub3

3 Rojas Sánchez P, Holguín A. Drug resistance in the HIV-1-infected paediatric population worldwide: a systematic review. *J Antimicrob Chemother* 2014; **69**: 2032–42. https://doi.org/10.1093/jac/dku104

4 Hoernle EH, Reid TE. Human immunodeficiency virus infection in children. *Am J Heal Pharm* 1995; **52**: 961–79. https://doi.org/10.1093/ajhp/52.9.961

5 Scarlatti G. Paediatric HIV infection. *Lancet* 1996; **348**: 863–8. https://doi.org/10.1016/S0140-6736(95)11030-5

6 Tovo PA, Gabiano C, Palomba E *et al*. Prognostic factors and survival in children with perinatal HIV-1 infection. *Lancet* 1992; **339**: 1249–53. https://doi.org/10.1016/0140-6736(92)91592-V

7 Kimani-Murage EW, Manderson L, Norris SA *et al*. It's my secret: barriers to paediatric HIV treatment in a poor rural South African setting. *AIDS Care* 2013; **25**: 744–7. https://doi.org/10.1080/09540121.2012.748865

8 Coetzee B, Kagee A, Bland R. Barriers and facilitators to paediatric adherence to antiretroviral therapy in rural South Africa: a multi-stakeholder perspective. *AIDS Care* 2015; **27**: 315–21. https://doi.org/10.1080/09540121.2014.967658

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9 National Department of Health. The South African Antiretroviral Treatment Guidelines 2013. 2013. https://sahivsoc.org/Files/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf.

10 Jordan MR, Penazzato M, Cournil A *et al*. Human immunodeficiency virus (HIV) drug resistance in African infants and young children newly diagnosed with HIV: a multicountry analysis. *Clin Infect Dis* 2017; **65**: 2018–25. https://doi.org/10.1093/cid/cix698

11 Hunt GM, Ledwaba J, Salimo A *et al*. Prevalence of HIV-1 drug resistance amongst newly diagnosed HIV-infected infants age 4–8 weeks, enrolled in three nationally representative PMTCT effectiveness surveys, South Africa: 2010, 2011–12 and 2012–13. *BMC Infect Dis* 2019; **19**: 787. https://doi.org/10.1186/s12879-019-4339-y

12 Steegen K, Levin L, Ketseoglou I *et al*. High-level cross-resistance to didanosine observed in South African children failing an abacavir- or stavudine-based 1st-line regimen. *PLoS One* 2014; **9**: e97067. https://doi.org/10.1371/journal.pone.0097067

13 Pillay S, Bland RM, Lessells RJ *et al*. Drug resistance in children at virological failure in a rural KwaZulu-Natal, South Africa, cohort. *AIDS Res Ther* 2014; **11**: 3. https://doi.org/10.1186/1742-6405-11-3

14 Kiepiela P, Manasa J, Moosa M-Y *et al*. HIV drug resistance patterns at the epicentre of the HIV-1 epidemic in Kwazulu-Natal, South Africa 2003–2013. *J AIDS Clin Res* 2014; **5**: 299. https://doi.org/10.4172/2155-6113.1000299

15 Etta EM, Mavhandu L, Manhaeve C *et al*. High level of HIV-1 drug resistance mutations in patients with unsuppressed viral loads in rural northern South Africa. *AIDS Res Ther* 2017; **14**: 36. https://doi.org/10.1186/s12981-017-0161-z

16 Hackett S, Teasdale CA, Pals S *et al*. Drug resistance mutations among South African children living with HIV on WHO-recommended ART regimens. *Clin Infect Dis* 2020; **7**: e2217–25.

17 Meyers T, Sawry S, Wong JY *et al*. Virologic failure among children taking lopinavir/ritonavir-containing first-line antiretroviral therapy in South Africa. *J Pediatr Infect Dis* 2015; **34**: 175–9. https://doi.org/10.1097/INF.0000000000000544

18 Rossouw TM, Feucht UD, Melikian G *et al*. Factors associated with the development of drug resistance mutations in HIV-1 infected children failing protease inhibitor-based antiretroviral therapy in South Africa. *PLoS One* 2015; **10**: e0133452. https://doi.org/10.1371/journal.pone.0133452

19 Zhou Z, Wagar N, DeVos JR *et al*. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PLoS One* 2011; **6**: e28184. https://doi.org/10.1371/journal.pone.0028184

20 Winzia A, Alvero C, Fenton T *et al*. IMPAACT 1093: dolutegravir in 6- to 12-year old HIV-infected children: 48-week results. *Conference on Retroviruses and Opportunistic Infections, Boston, 2016*: Abstract 816.

21 Bruzzese E, Lo Vecchio A, Smarrazzo A *et al*. Dolutegravir-based anti-retroviral therapy is effective and safe in HIV-infected paediatric patients. *Ital J Pediatr* 2018; **44**: 37. https://doi.org/10.1186/s13052-018-0469-x

22 Semengue ENJ, Santoro MM, Ndze VN *et al*. HIV-1 integrase resistance associated mutations and the use of dolutegravir in sub-Saharan Africa: a systematic review and meta-analysis protocol. *Syst Rev* 2020; **9**: 93. https://doi.org/10.1186/s13643-020-01356-z

23 Inzaule SC, Jordan MR, Bello G *et al*. High levels of resistance to nucleoside/nucleotide reverse transcriptase inhibitors in newly diagnosed antiretroviral treatment-naive children in sub-Saharan Africa. *AIDS* 2020; **34**: 1567–70. https://doi.org/10.1097/QAD.0000000000002580

24 Fairlie L, Bernheimer J, Sipambo N *et al*. Lamivudine monotherapy in children and adolescents: the devil is in the detail. *South African Med J* 2017; **107**: 1055–7. https://doi.org/10.7196/SAMJ.2017.v107i12.12776

25 Raizes E, Hader S, Birx D. The US president's emergency plan for AIDS relief (PEPFAR) and HIV drug resistance: mitigating risk, monitoring impact. *J Infect Dis* 2017; **216**: S805–7. https://doi.org/10.1093/infdis/jix432

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