Long-term effects of HIV treatment in sub-Saharan Africa: from access to quality
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High levels of HIV drug resistance in treatment-naïve children in Lagos, Nigeria: original data and a systematic review in sub-Saharan Africa.


* Both first and both senior authors contributed equally to the work.

Submitted.
ABSTRACT

Background
HIV-infected children are at risk of HIV drug resistance (HIVDR) prior to antiretroviral treatment (ART) initiation. Data on pediatric HIVDR prevalence, especially from sub-Saharan Africa, are scarce.

Methods
HIV-1 infected antiretroviral drug (ARV)-naïve children ≤12 years were enrolled at the Lagos University Teaching Hospital, Nigeria. Pre-ART viral load and population based pol genotypic testing was performed. HIVDR mutations were identified using the World Health Organization list for transmitted drug resistance. We conducted a systematic review and meta-analysis on pre-treatment HIVDR prevalence in children in sub-Saharan Africa.

Results
Thirteen of 82 (15.9%) ARV-naïve Nigerian children had pre-treatment HIVDR. All 13 harbored non-nucleoside reverse-transcriptase inhibitor (NNRTI) mutations, of whom seven also had nucleoside reverse-transcriptase inhibitor (NRTI) resistance. No protease inhibitor (PI) mutations were detected. We included 16 studies from 11 different African countries (2,057 children) in a meta-analysis. The pooled pre-treatment HIVDR-prevalence among ARV-naïve children was 10.8% (95%CI: 4.4-17.1). Meta-regression showed an increase in prevalence from 0.6% (95%CI 0.6-5.4) in 2004 to 36.2% (95%CI 25.5-46.9) in 2011 (p=0.05).

Conclusion
One in six Nigerian children had HIVDR. This high rate corroborates with other African data, which indicate a significant pre-treatment HIVDR increase over the past decade. Our findings stress the importance of PI-based regimens in all children <3 years of age. Overcoming practical barriers to implement PI-based regimens, and introduction of a population-based HIVDR surveillance system among children should receive priority to ensure optimal treatment for HIV-infected children in sub-Saharan Africa.
Introduction

Since the roll-out of antiretroviral treatment (ART) in sub-Saharan Africa in the beginning of this millennium, ART coverage has increased substantially. Nearly 12 million people in low- and middle-income countries (LMIC) were receiving ART at the end of 2013, including 740,000 children [1]. Coverage of prevention of mother-to-child transmission (PMTCT) programs has also increased, and new PMTCT strategies, including lifelong ART for pregnant women (option B+), are currently being implemented [1].

The increased usage of ART, however, is likely to come at a cost, as the levels of HIV drug resistance (HIVDR) are expected to rise [2,3]. Due to the scale-up of PMTCT, the absolute number of children born with HIV will decrease. When PMTCT is unsuccessful, however, children are prone to develop HIVDR, because of intra-uterine or perinatal exposure to antiretroviral drugs (ARV), usually containing a non-nucleoside reverse transcriptase inhibitor (NNRTI) [4,5].

The most recent World Health Organization (WHO) ART guidelines recommend all children under three years of age to start protease inhibitor (PI)-based treatment, regardless of previous PMTCT exposure [6]. In Nigeria, as in many other LMIC, national guidelines recommend PI-based treatment only for children under three years who are known to be PMTCT-exposed, because of high costs and logistic barriers of PI-treatment. All other children are recommended to start NNRTI-based treatment [7]. Determining the rate of resistance against NNRTIs will help answering the question to what extent this policy is still defensible.

Monitoring pre-treatment HIVDR is especially important in children as they have fewer ART options than adults, and will require ART for more years. However, data on HIVDR in African children are scarce [8]. This study documents the prevalence of HIVDR before treatment initiation in ARV-naïve HIV-infected children in Lagos, Nigeria, the country with the second highest number of people living with HIV worldwide: 3.2 million, of whom 260,000 are children [9]. Additionally, we conducted a systematic review and meta-analysis of the literature on pre-treatment HIVDR in children in sub-Saharan Africa. The outcomes of this review put the findings of our study into perspective and estimate the extent and trends of HIVDR since large-scale implementation of ART in sub-Saharan Africa.
METHODS

Study design and population
This study forms part of the Monitoring Antiretroviral Resistance in Children (MARCH) cohort, an observational prospective cohort study conducted in Nigeria and Uganda. HIV-infected children, eligible for ART, were enrolled at the pediatric HIV clinic and through HIV screening at the emergency ward of the Lagos University Teaching Hospital, Nigeria.

Inclusion criteria were: age ≤12 years, confirmed HIV-1 test (positive HIV antibody test if age >18 months, or positive HIV nucleic acid PCR if age ≤18 months), eligibility for initiation of first-line ART according to national guidelines (all HIV-infected children <2 years of age, CD4 count <750 cells/m3 in children 2-5 years, and CD4 count <350 cells/mm3 in children >5 years) [10], and written informed consent by the parent or guardian. If the child was eight years or older and had disclosed HIV status, assent was required. Exclusion criteria were: HIV-2 co-infection, anticipated non-compliance with the protocol, and current participation in another study or clinical trial. All children received routine care according to national pediatric HIV treatment guidelines [10]. Clinical and socio-demographic data of mother and child, and laboratory (hematology, immunology, virology) data of the child were collected on standardized case report forms. Previous PMTCT exposure was documented as reported in the child’s medical files or reported by the child’s caregiver. For the current analysis of our cohort study, we included only ARV-naive children, that is, children without any prior exposure to ART or PMTCT. All data were source-data-verified by monitors and transferred to a study-specific database. Programmed queries were used to rule out common data errors and inconsistencies.

Laboratory methods
Before ART initiation, a study blood sample (6 mL EDTA tube) was collected for HIV viral load testing using the Roche Cobas AmpliPrep TaqMan® (Cobas Amplicor; Roche Diagnostics, Switzerland). If viral load was >1,000 cps/ml, population based sequencing of the HIV-1 pol gene was performed by the reference laboratory of the Institute of Human Virology in Abuja, Nigeria, using an in-house method and primers designed and optimized for subtype CFR02_AG and G. Genotypic sequence data were submitted to ViroScore® [11]. Major drug-resistance mutations were identified based on the 2009 WHO list for surveillance of transmitted resistance [12] using the Stanford Calibrated Population Resistance analysis tool [13]. Susceptibility of the prescribed ART regimen was determined through calculation of the genotypic sensitivity score (GSS) using the Stanford algorithm (Version 7.0) [14]. Reduced susceptibility to
the prescribed regimen was defined as GSS <3; i.e. <3 fully susceptible drugs. HIV-1 subtyping was performed using the REGA HIV-1 subtyping tool V3 [15]. The study has received ethical clearance from the Health Research & Ethics Committee of the Lagos University Teaching Hospital, and was conducted in compliance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All laboratory procedures were conducted according to Good Laboratory Practice guidelines.

**Systematic review**

A literature review was conducted to identify relevant studies reporting on the prevalence of pediatric pre-treatment HIVDR in sub-Saharan Africa. A search was done in Medline through PubMed using the search terms ‘transmitted’, ‘pre-treatment’, ‘naïve’, or ‘primary’ in combination with ‘drug resistance’, ‘hiv’ and ‘child’ or ‘infant’. Additionally, the conference abstracts of the last two editions of the Conference on Retroviruses and Opportunistic Infections (2014-2015), the IAS (2013-2014), and the International Workshop on HIV Pediatrics (2013-2014) were electronically searched for relevant studies using the search term ‘resistance’. The references of retrieved studies were screened for any additional relevant articles using snowballing techniques. Article selection and data extraction was performed by two individual reviewers (RB and JC), and discrepancies were resolved by discussion between both reviewers.

We searched for original studies reporting pre-treatment HIVDR prevalence in children with a median age ≤12 years, in any country in sub-Saharan Africa [16]. We excluded articles in which only a selection of specific mutations were genotyped, articles reporting a very small sample size (<20 patients), and articles in which HIVDR was not reported separately for children and adults. The HIVDR prevalence was extracted separately for PMTCT-exposed and -unexposed children, and by drug class (NNRTI, NRTI or PI), if reported. If only the HIVDR prevalence per drug class was reported, we used the prevalence of NNRTI resistance as a conservative estimate of the total HIVDR prevalence.

**Statistical analysis**

Patient characteristics were summarized for children with and without pre-treatment HIVDR separately. Continuous variables were analyzed using a student’s t-test or Mann-Whitney U test, and categorical variables using a \( \chi^2 \) test or Fisher’s exact test; 95% confidence intervals were calculated using the exact method. Nutritional status was assessed using WHO Anthro (version 3.2.2, January 2011) for children <5 years and WHO Reference 2007 for children ≥5 years [17]. Weight-for-age z-scores and weight-for-height z-scores were only calculated for children <10 years and<5 years of age, respectively.
Univariate and multivariate logistic regression was performed to identify factors associated with pre-treatment HIVDR. Explanatory variables considered in the analysis were: age, sex, WHO clinical stage, nutritional status, hemoglobin level, immunodeficiency for age (defined as CD4 percentage<25% in children under five years of age, and CD4 count<350 cells/ml in children of five years and older), viral load, HIV-1

Table 1. Population characteristics of 90 included children

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>No HIV drug resistance</th>
<th>HIV drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years (median, IQR))</td>
<td>4.6 (1.8-8.4)</td>
<td>4.5 (1.7-8.7)</td>
<td>4.8 (2.5-6.5)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41/90 (45.6)</td>
<td>31/69 (44.9)</td>
<td>8/13 (61.5)</td>
</tr>
<tr>
<td>WHO clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III or IV</td>
<td>55/90 (61.1)</td>
<td>44/69 (63.8)</td>
<td>7/13 (53.9)</td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stunted, HAZ&lt;-2</td>
<td>21/72 (29.2)</td>
<td>15/53 (28.3)</td>
<td>3/12 (25.0)</td>
</tr>
<tr>
<td>Wasted, WHZ&lt;-2*</td>
<td>12/36 (33.3)</td>
<td>9/25 (36)</td>
<td>3/7 (42.9)</td>
</tr>
<tr>
<td>Underweight, WAZ&lt;-2**</td>
<td>23/78 (29.5)</td>
<td>19/60 (31.7)</td>
<td>3/12 (25.0)</td>
</tr>
<tr>
<td>Hemoglobin g/dL (mean, SD)</td>
<td>9.8 (1.5)</td>
<td>9.7 (1.3)</td>
<td>10.7 (2.2)</td>
</tr>
<tr>
<td>CD4+ cell percentage*</td>
<td>14.9 (8.1-26.1)</td>
<td>16.2 (8.3-26.9)</td>
<td>12.9 (7.9-25.1)</td>
</tr>
<tr>
<td>CD4+ cell count***</td>
<td>395 (137-618)</td>
<td>370 (137-662)</td>
<td>454 (289-587)</td>
</tr>
<tr>
<td>HIV RNA load log10/ml (median, IQR)</td>
<td>5.2 (4.7-5.9)</td>
<td>5.3 (4.8-5.9)</td>
<td>5.0 (4.4-5.6)</td>
</tr>
<tr>
<td>HIV-1 subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2/82 (2.4)</td>
<td>2/69 (2.9)</td>
<td>0/13 (0.0)</td>
</tr>
<tr>
<td>C</td>
<td>2/82 (2.4)</td>
<td>2/69 (2.9)</td>
<td>0/13 (0.0)</td>
</tr>
<tr>
<td>G</td>
<td>31/82 (37.8)</td>
<td>25/69 (36.2)</td>
<td>6/13 (46.2)</td>
</tr>
<tr>
<td>CRF02_AG</td>
<td>31/82 (37.8)</td>
<td>28/69 (40.6)</td>
<td>3/13 (23.1)</td>
</tr>
<tr>
<td>Other</td>
<td>16/82 (19.5)</td>
<td>12/69 (17.4)</td>
<td>4/13 (30.8)</td>
</tr>
<tr>
<td>Mother currently on ART</td>
<td>Yes</td>
<td>46/77 (59.7)</td>
<td>37/64 (57.8)</td>
</tr>
<tr>
<td>ART regimen child</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT+3TC+EFV</td>
<td>4/90 (4.4)</td>
<td>3/69 (4.4)</td>
<td>0/13 (0.0)</td>
</tr>
<tr>
<td>AZT+3TC+NVP</td>
<td>78/90 (86.7)</td>
<td>59/69 (85.5)</td>
<td>13/13 (100)</td>
</tr>
<tr>
<td>ABC+3TC+NVP</td>
<td>7/90 (7.8)</td>
<td>7/69 (10.1)</td>
<td>0/13 (0.0)</td>
</tr>
</tbody>
</table>

Genotypic data were available for 82/90 children. Drug resistance mutations were identified based on the 2009 WHO list for surveillance of transmitted drug resistance [12]. HIV-1 subtyping was performed using the REGA HIV-1 subtyping tool V3 [15]. Nutritional status was assessed using WHO Anthro (version 3.2.2, January 2011) for children <5 years and WHO Reference 2007 for children ≥5 years [17]. Results for hemoglobin, CD4 count, CD4 percentage, and HIV RNA load were available for 87, 40, 48, and 82 children, respectively.

* Only for children <5 years of age.
** Only for children <10 years of age.
*** Only for children ≥5 years of age.
ART, antiretroviral therapy; HAZ, Height for age z-score; WAZ, weight for age z-score; WHZ, weight for height z-score; WHO, World Health Organization.
HIV drug resistance in children in Lagos, Nigeria

subtype, mother’s use of ART, current living situation of the child, and mother’s health status. If explanatory variables were associated with the outcome variables (p<0.10) in the univariate analysis, they were forwarded to the multivariate model using a step forward procedure.

Meta-analysis of studies of the systematic review was conducted to pool the reported HIVDR prevalence using a random-effects model, because of expected heterogeneity among studies. Random-effects meta-regression was used to assess the reported HIVDR in the included studies by year of ART initiation and for PMTCT-exposed versus -unexposed children. The variance of the raw proportions was stabilized using a Freeman-Tukey arcsine square root transformation, and was subsequently back-transformed to the original scale. A two-sided p-value of ≤0.05 was considered significant. Data were analyzed using Stata 12® (StataCorp LP, TX, USA).

RESULTS

Between March 2012 and October 2013, 100 children were enrolled in the MARCH-Nigeria cohort, of which 90 children were PMTCT-unexposed and were included in the analysis. Most (N=78; 86.7%) children started an ART regimen consisting of zidovudine/lamivudine/nevirapine (AZT+3TC+NVP) as a fixed-dose combination, and none started a PI-based regimen. The median age was 4.6 years (IQR 1.8-8.4), 46% were male, and 61% were classified as WHO clinical stage III or IV (Table 1). The child’s primary caregiver was the mother for 70 (78%) of the children. The mother was sick in six (7%) and deceased in 15 (17%) cases, including five children (6%) of whom both parents died. The median viral load before treatment initiation was 160,000 cps/ml (IQR 45,700-730,000); one child had a viral load <1,000 cps/ml. HIV-1 sequencing of the pol gene was successful in 82 of 89 children with viral load >1,000 cps/ml. The children with and without sequencing results did not differ significantly regarding sex, age, and clinical characteristics (data not shown).

Thirteen of 82 (15.9% [95%CI: 9.5-25.3]) children had HIVDR; all 13 children carried NNRTI mutations, and seven (8.5%) also had NRTI mutations. No PI mutations were identified. G190A/S (n=7) and M184V/I (n=6), were the most prevalent mutations (Figure 1). For all 13 children with HIVDR, the drug regimen prescribed was predicted to be only partially active (mean GSS=1.5, SD 0.18). All 13 had mutations associated with high NVP resistance. For all seven children with NRTI mutations, these mutations resulted in an only partially active regimen with regards to the NRTI backbone; five had mutations associated with 3TC resistance and three had thymidine analogue
mutations (TAMs). Children with HIVDR had a median of two mutations (range 1-7) per sequence. We did not find significant associations between sex, age, nutritional or immunological status, viral load, subtype, mother’s ART use, child’s living situation, or mother’s health status and the presence of HIVDR (data not shown).

**Systematic review sub-Saharan Africa**

We performed a literature search on pediatric pre-treatment HIVDR in sub-Saharan Africa. We retrieved 467 articles and 378 conference abstracts, of which we included 16 studies: 11 articles, four conference abstracts and our current study in Nigeria (Figure 2), representing 2,057 children. Studies were performed between 2003 and 2012, in 11 African countries (three in east Africa, four in southern Africa, and four in west/central Africa). The median age of the children included in the studies ranged from 3.7 months to 8 years (Table 2). All studies except one [18] reported a higher prevalence of HIVDR towards NNRTI compared to NRTI. In that study, mothers were randomized to receive either NVP- or nelfinavir (NFV)-based triple therapy for PMTCT. All HIV-infected children in the NFV-arm developed NRTI resistance, but no NNRTI or PI resistance [18]. Only four studies reported data on PI mutations which were found in less than 2% of the cases (Supplementary Table S1).

Random-effects meta-analysis yielded a pooled HIVDR prevalence of 28.1% (95%CI 18.5-37.7) among PMTCT-exposed and unexposed children, with a high level of between-study heterogeneity (I² =97.7 %). (Figure 3). The HIVDR prevalence was almost a fourfold higher in PMTCT-exposed compared to PMTCT-unexposed children, 40.0% (95%CI 24.1-56.0) versus 10.3% (95%CI 4.2-16.4), respectively (p=0.009). In PMTCT-unexposed children, we found an increase in HIVDR prevalence by year of ART initiation from 0.6% (95%CI -1.8-3.0) in 2004 to 36.2% (95%CI 25.5-46.9) in 2011, p=0.05 (Figure 4).
Figure 1. Drug resistance mutations detected in this cohort (n=82).
NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Figure 2. Flow diagram of included articles and conference abstracts.
Table 2. Studies included in systematic review of pediatric pre-treatment HIV drug resistance in sub-Sahara Africa

<table>
<thead>
<tr>
<th>Year of study</th>
<th>Author, year of publication</th>
<th>Country</th>
<th>N</th>
<th>Age in months (median, IQR)</th>
<th>HIV drug resistance prevalence, overall</th>
<th>HIV drug resistance prevalence, in PMTCT unexposed children</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-2006</td>
<td>Towler, 2010</td>
<td>Uganda [2]</td>
<td>74</td>
<td>51.5 (7-148)</td>
<td>2/74 (2.7%)</td>
<td>0/39 (0%)</td>
</tr>
<tr>
<td>2007-2009</td>
<td>Van Zyl, 2010</td>
<td>South-Africa [4]</td>
<td>49</td>
<td>3.7 (range 1-17)</td>
<td>3/49 (6.1%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>2008</td>
<td>Meini, 2015</td>
<td>Tanzania [6]</td>
<td>46</td>
<td>96 (84-132)</td>
<td>32/46 (70.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>2008-2010</td>
<td>Weigel, 2014</td>
<td>Malawi [7]</td>
<td>381</td>
<td>30 (14.8-73.4)</td>
<td>59/381 (15%)</td>
<td>NR</td>
</tr>
<tr>
<td>2009-2011</td>
<td>Fokam, 2011</td>
<td>Cameroon [9]</td>
<td>41</td>
<td>72 (3-144)</td>
<td>2/41 (4.9%)</td>
<td>1/40 (2.5%)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>Kebe, 2014</td>
<td>Senegal [10]</td>
<td>25</td>
<td>5.5 (range 1.5-17)</td>
<td>8/25 (32%)</td>
<td>1/12 (8.3%)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>Sigaloff, 2012</td>
<td>Uganda [11]</td>
<td>279</td>
<td>58.8 (27.6-108)</td>
<td>28/279 (10%)</td>
<td>18/233 (7.7%)</td>
</tr>
<tr>
<td>2011</td>
<td>Kuhn, 2014</td>
<td>South-Africa [12]</td>
<td>230</td>
<td>&lt;24</td>
<td>122/230 (53.0%)</td>
<td>27/75 (36.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRTI: 7/201 (3.5%)</td>
<td></td>
</tr>
<tr>
<td>2012-2013</td>
<td>Boender &amp; Boerma (Current study)</td>
<td>Nigeria</td>
<td>90</td>
<td>51.6 (1893.6)</td>
<td>13/82 (15.9%)</td>
<td>13/82 (15.9%)</td>
</tr>
</tbody>
</table>

PMTCT, prevention of mother-to-child transmission; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; IQR, interquartile range; NR, not reported; NA, not applicable.

* Absolute numbers not reported.
In this study we found a high rate of 15.9% HIVDR among ARV-naïve children in Lagos, Nigeria. This implies that, even in children that have never been exposed to any antiretroviral drug, almost one in six children carries HIVDR mutations. All 13 children with HIVDR consecutively received suboptimal treatment. Seven (8.5%) children had mutations associated with one of the NRTIs they were prescribed.

The high [19] pre-treatment HIVDR prevalence in this study could be due to direct transmission of drug resistant HIV from the mother to her child. The prevalence of pre-treatment HIVDR among women of child-bearing age at the same hospital in Lagos was 3.8% (95%CI 1.5-9.4%) in 2008-2009 in one of our previous cohorts [20],...
and 2.9% in 2010-2012 in a study conducted in north central Nigeria [21]. We did not have data on HIVDR in the mothers of the children in our cohort, but it is possible that the rate of pre-treatment HIVDR among women of child-bearing age has risen over the past years [2,3].

Some of the children in our cohort who were reported to be ARV-naïve might actually have received PMTCT, as some mothers or caregivers might not recall perinatal PMTCT. This has been suggested before and confirms that caregiver’s report of PMTCT exposure might not be a reliable criterion to base treatment decisions on [6, 22]. Furthermore, in this cohort of relatively old children (range 0.2-12.7 years), it is possible that children have been exposed to other ARVs than those for PMTCT and, as such, are not entirely ARV-naïve. In a study among adults in seven African countries, ARVs were found in the samples of 22% of the participants who reported to be ARV-naïve [23].
Our systematic review confirms the high pre-treatment HIVDR prevalence among ARV-naïve children, and identifies a significant increase in prevalence in ARV-naïve children in sub-Saharan Africa over the past decade. Our findings are in contrast with a recent systematic review from 2014 on pediatric HIVDR worldwide, which identified only two studies conducted on the African continent, and reported a low prevalence of 0 to 5% pre-treatment HIVDR [8]. In adults, pre-treatment HIVDR has been shown to be increasing as well. Among almost 2,500 adults in six countries in sub-Saharan Africa, we previously reported that the risk of HIVDR rose by 38% each year after ART roll-out with the highest prevalence in Uganda, the country with the longest history of large-scale ART provision [2]. A meta-analysis of pre-treatment HIVDR on a global level (26,102 patients) confirmed these results, and modelled an increase in HIVDR prevalence of almost 30% per year in east Africa, 14% in southern Africa, and 3% in west and central Africa [3].

One of the limitations of our study is the fact that data on PMTCT exposure of the included children were collected retrospectively. However, we attempted to collect all available data on prior PMTCT, by interviewing the mother or caregiver and by searching the medical files of all mothers and children. Children were recruited at a single
site, and the children’s age ranged from 0 to 12 years which makes comparison of HIVDR prevalence with other studies harder, as mutations might be archived in older children [22, 24]. However, our cohort directly reflects the day-to-day practice of a clinician working in a pediatric HIV clinic, and the practical challenges of HIVDR in a resource-constraint setting. One of the limitations of our meta-analysis is the relatively low number of included studies with limited sample size and the large heterogeneity, which may have affected our pooled results and meta-regression model.

**Implications**

The high rate of NNRTI resistance we found in PMTCT-unexposed children implies that one in six HIV-infected children is receiving suboptimal treatment, as current ART Nigerian guidelines still recommend NNRTI-based first-line treatment for PMTCT-unexposed children [7]. Larger studies in Nigeria are urgently needed to draw more robust conclusions on a national level. In a country with ~260,000 HIV-infected children, a pretreatment HIVDR prevalence of ~16% would have catastrophic implications for pediatric HIV treatment in Nigeria. Children with pre-treatment HIVDR are at risk of early virological failure and subsequent treatment switch. More than half of the children with NNRTI mutations in our cohort also had resistance against NRTIs, which could limit the effectiveness of a second-line PI-based regimen with an NRTI backbone. However, various studies have reported that the presence of NRTI mutations was not a risk factor for virological failure in children and adults on second-line PI-based treatment [25–27].

The alarming increase in HIVDR we report in sub Saharan Africa, including the figures in Nigeria, stress the importance of implementing the WHO recommendations of PI-based regimens for children under three years and of considering extending this policy for children up to at least 12 years. Pediatric PIs are more costly than NNRTIs and used to be only available as a liquid that requires refrigeration. Recently, however, the United States Food and Drug Administration (FDA) has approved LPV/r in pellet form for pediatric usage [28]. This is an important step towards overcoming the barriers of implementing PI-based first-line treatment in resource-constraint countries. Solving these issues should be given priority in order to control pediatric HIVDR.

Furthermore, population-based HIVDR surveillance programs on a national level could provide valuable information on pre-treatment HIVDR prevalence. This is especially important in children as we identified a high prevalence of pediatric pretreatment HIVDR, and second-line drugs options are limited in this population. Early detection of increasing levels of HIVDR in the pediatric population of a country can help policy makers to take informed decisions on national ART guidelines and on
the selection of first-line regimens. Individual genotypic resistance testing before treatment initiation as in high-income countries [29], is currently not recommended in resource constraint settings, mainly due to the high costs and the complexity of testing assays [6]. The current efforts to develop simplified tests against lower costs are a step towards population-based surveillance [30], and possibly even individualized testing in the future.

In summary, in this cohort of ARV-naïve HIV-infected children in Nigeria we found a high rate of 16% pre-treatment HIVDR. Our systematic review and meta-analysis confirmed and expanded these findings, showing a significant increase in HIVDR prevalence in ARV-naïve children in sub-Saharan Africa over the past decade. Implementation of PI-based regimens for children under three years of age in countries in sub-Saharan Africa should receive priority. Overcoming the barriers of PI-based treatment in LMIC and close monitoring of HIVDR through regular surveillance programs are essential in order to ensure optimal treatment for HIV-infected children in LMIC.

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**DECLARATION OF INTEREST**

We declare no competing interests.
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