

Alarming increase in pretreatment HIV drug resistance in children living in sub-Saharan Africa: a systematic review and meta-analysis

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Background: Children have an augmented risk of pretreatment HIV drug resistance (PDR) due to exposure to antiretroviral drugs for the prevention of mother-to-child transmission (PMTCT). Paediatric data are essential to evaluate the effectiveness of the restricted number of paediatric regimens currently available, but these data are scarce.

Methods: We conducted a systematic review of the literature on PDR in children (median age ≤ 12 years) in sub-Saharan Africa. We separately extracted the proportion of children with PDR for children with and without prior PMTCT exposure, used random-effects meta-analysis to pool proportions and used meta-regression to assess subgroup differences.

Results: We included 19 studies representing 2617 children from 13 countries. The pooled PDR prevalence was 42.7% (95% CI 26.2%–59.1%) among PMTCT-exposed children and 12.7% (95% CI 6.7%–18.7%) among PMTCT-unexposed children ($P = 0.004$). The PDR prevalence in PMTCT-unexposed children increased from 0% in 2004 to 26.8% in 2013 ($P = 0.009$). NNRTI mutations were detected in 32.4% (95% CI 18.7%–46.1%) of PMTCT-exposed children and in 9.7% (95% CI 4.6%–14.8%) of PMTCT-unexposed children; PI mutations were uncommon ($< 2.5\%$). PDR was more common in children aged < 3 years compared with children aged ≥ 3 years [40.9% (95% CI 27.6%–54.3%) versus 17.6% (95% CI 8.9%–26.3%), respectively ($P = 0.025$)].

Conclusions: The PDR prevalence in African children is high and rapidly increasing. Even in PMTCT-unexposed children, the most recent reports indicate that PDR is present in up to a third of children starting first-line therapy. Our data underscore the importance of initiating PI-based first-line ART in young children (< 3 years of age) and suggest that older children may also benefit from this approach.

Introduction

In sub-Saharan Africa (SSA), an estimated 2.3 million children are living with HIV. Access to ART for children in SSA has increased exponentially: from 18000 in the year 2000 to > 800000 children in 2014.¹ Since the large-scale rollout of ART, concerns have been raised about increasing levels of HIV drug resistance (HIVDR).^{2,3} HIVDR can develop in individuals while on treatment, but can also already be present prior to ART initiation: pretreatment HIV drug resistance (PDR). PDR is clinically important as it is associated with a poor response to first-line therapy and further accumulation of drug resistance mutations.^{4,5} The WHO generic protocol on HIVDR

in resource-limited countries proposes to use dried blood spot specimens of children < 18 months of age to assess the paediatric PDR prevalence.⁶ In older children, drug resistance mutations might have waned to below the detection limit in the absence of selective drug pressure⁷ and the HIVDR prevalence might be underestimated. We will refer to HIVDR in children < 18 months as initial HIVDR, as opposed to PDR in children ≥ 18 months of age.

A large meta-analysis of PDR among adults showed a pooled PDR prevalence of 2.8% in SSA in samples collected between 2000 and 2013.⁸ It has been shown that this prevalence is increasing over time and a recent study in Nairobi, Kenya, reported a prevalence of 10.9% PDR in 2014.^{2,3,9} Large-scale data on PDR

prevalence in African children are currently lacking. Children are at increased risk of developing PDR due to previous exposure to anti-retroviral drugs taken by the mother or child for the prevention of mother-to-child transmission (PMTCT). Drugs for PMTCT usually contain an NNRTI to which resistance is known to develop rapidly.^{10,11} Data on the prevalence of PDR in children are essential to develop and improve guidelines for paediatric first-line ART in resource-limited settings. This study aims to assess the prevalence of PDR in PMTCT-exposed and PMTCT-unexposed children in SSA over the past decade.

Methods

We performed a systematic review of the literature, in accordance with PRISMA standards,¹² to identify studies on paediatric PDR in SSA in both PMTCT-exposed and PMTCT-unexposed children. We defined PDR as HIVDR to any drug class, detected by genotypic resistance testing, in children who have not yet initiated first-line ART, with or without previous exposure to PMTCT. We used the search terms ‘transmitted’, ‘pretreatment’, ‘naive’, ‘initial’ or ‘primary’ in combination with ‘drug resistance’, ‘hiv’ and ‘child’ or ‘infant’ in MEDLINE through PubMed, up to May 2016. Additionally, we electronically searched conference abstracts of the last three editions of the Conference on Retroviruses and Opportunistic Infections, the International AIDS Society Conference and the International Workshop on HIV Pediatrics for relevant studies using the search term ‘resistance’. The references of retrieved studies were screened for additional relevant articles using snowballing techniques.

Article selection and data extraction was performed by two individual reviewers (R. S. B. and J. C. C.) and discrepancies were resolved by discussion between both. We searched for original studies reporting the proportion of children (median age ≤ 12 years) with PDR in any country in SSA.¹³ We excluded articles about acquired, rather than pretreatment, drug resistance and articles in which PDR was not reported separately for children and adults. To reduce the risk of bias, we excluded articles in which only a selection of specific mutations was genotyped and articles reporting a very small sample size (< 20 patients). The following information was extracted for each study: country of study; median year of sample collection; median age of children included; PMTCT regimens used by mother and/or infant; number of children with genotypic resistance testing results; number of children with virus harbouring any drug resistance mutation; or to NNRTIs, NRTIs and PIs and with dual- and triple-class resistance, separately for PMTCT-exposed and PMTCT-unexposed children. We defined PMTCT as any drugs taken by the mother during pregnancy or breastfeeding and/or by the infant after birth to prevent the transmission of HIV from the mother to the child. We contacted the corresponding authors to request additional information, if needed. When we only had information on the prevalence of PDR per drug class, we used the prevalence of NNRTI resistance as a conservative estimate of the total PDR prevalence.

Meta-analysis was conducted to pool the reported PDR prevalence using a random-effects model, because of expected heterogeneity among studies. 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. Random-effects meta-regression was used to compare the PDR prevalence between groups. HIVDR prevalence was categorized as low ($< 5\%$), moderate (5%–15%) or high ($> 15\%$), according to WHO guidelines.¹⁴ Data were analysed using Stata 12® (StataCorp, TX, USA) with a two-sided P value of ≤ 0.05 considered significant.

Results

Our literature search and snowballing process retrieved 626 articles and 502 conference abstracts, of which we excluded 614

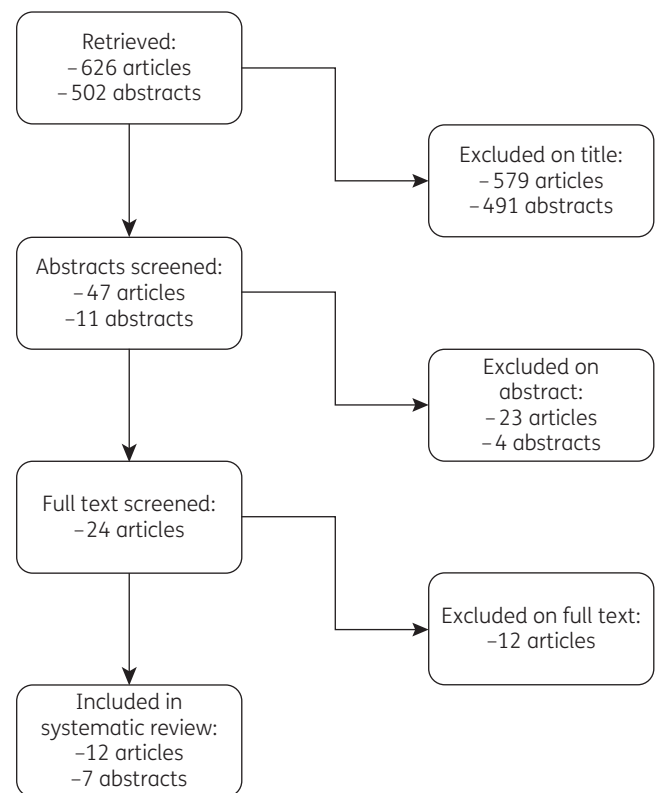


Figure 1. Flow chart of study selection.

articles and 495 conference abstracts based on title, abstract and full text (Figure 1). Main reasons for exclusion were: not conducted in SSA; adult population; or concerning acquired rather than PDR. We included 12 articles and 7 conference abstracts in a meta-analysis, representing 2617 children in 13 African countries. The median year of sample collection in each study ranged from 2003 to 2014. The median age of the children included in the studies ranged from 2.5 months to 8 years (Table S1, available as Supplementary data at JAC Online).

In all but one study (in which mothers were randomized to receive either PI- or NNRTI-based ART for PMTCT), NNRTI mutations were most prevalent (median 25.0%, IQR 7.5%–49.5%, reported in all 19 studies) followed by NRTI mutations (median 5.4%, IQR 2.3%–9.3%, reported in 17 studies) and PI mutations (median 1.3%, IQR 0.3%–2.2%, reported in 6 studies) (Table S1). The pooled proportion of children with NNRTI mutations was 32.4% (95% CI 18.7%–46.1%) in PMTCT-exposed children and 9.7% (95% CI 4.6%–14.8%) in PMTCT-unexposed children ($P=0.01$). Twelve studies reported children with dual resistance to both NRTIs and NNRTIs (range 0.0%–16.7%) and two studies reported children with triple-class resistance to NNRTIs, NRTIs and PIs (0.3% and 0.4%, respectively).

Figure 2 shows the overall PDR prevalence in all studies included. The PDR prevalence was almost 4-fold higher in PMTCT-exposed children compared with PMTCT-unexposed children: 42.7% (95% CI 26.2%–59.1%, $I^2=84.7\%$) compared with 12.7% (95% CI 6.7%–18.7%, $I^2=93.6\%$), respectively ($P=0.004$) (Figure 3). The pooled proportion of initial HIVDR (in children with a median age < 18 months) was higher than in children ≥ 18 months: 43.6%

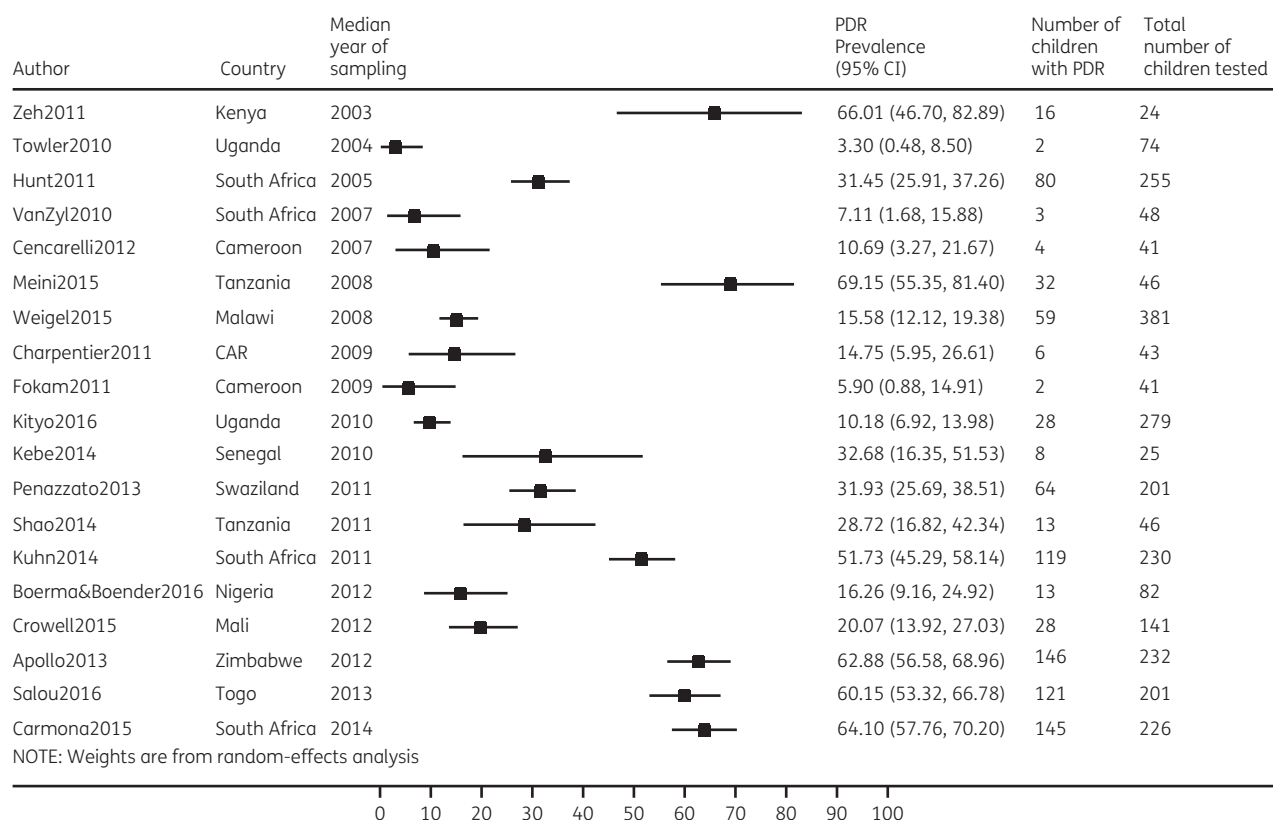


Figure 2. Proportion of children with PDR, overall. I^2 statistic: 97.1%. CAR, Central African Republic.

(95% 31.0%–56.3%, $I^2=96.4%$) versus 17.1% (95% CI 10.0%–24.2%, $I^2=92.5%$) ($P=0.009$). In addition, studies including children with a median age <3 years reported significantly higher rates of PDR compared with studies including children aged ≥ 3 years [40.9% (95% CI 27.6%–54.3%, $I^2=96.9%$) versus 17.6% (95% CI 8.9%–26.3%, $I^2=92.3%$), respectively ($P=0.025$)]. In children <3 years, 46.1% (95% CI 27.0%–65.1%, $I^2=96.4%$) of PMTCT-exposed children and 19.2% (95% CI 3.1%–35.3%, $I^2=85.5%$) of PMTCT-unexposed children had PDR ($P=0.096$); in children ≥ 3 years, 36.2% (95% CI 6.2%–66.1%, $I^2=87.3%$) of PMTCT-exposed children and 9.3% (95% CI 3.4%–15.2%, $I^2=88.5%$) of PMTCT-unexposed children had PDR ($P=0.153$).

Among PMTCT-unexposed children, there was a significant increase in PDR prevalence over time, from 0% in 2004 to 26.8% in 2013 ($P=0.009$) (Figure 4). The four most recently conducted studies all reported a high PDR prevalence of >15%, of which two were conducted in children with a median age <18 months and two in children aged ≥ 18 months.

Discussion

In this systematic review and meta-analysis, we show that PDR levels among HIV-infected children in SSA are very high. Of PMTCT-exposed children, >40% are estimated to harbour drug resistance mutations before ART initiation. In PMTCT-unexposed children, this is the first report showing an alarming increase in PDR prevalence over the past 10 years; the most recent studies report PDR levels

up to 35%. The pooled prevalence of initial HIVDR (in children <18 months of age) was >40%. According to WHO recommendations, PDR levels >15% should lead to a full-scale national survey to estimate the PDR prevalence on a national level and, if needed, to change treatment guidelines accordingly.¹⁴

To our knowledge, this is the first meta-analysis of PDR prevalence among children in SSA. It is the second review on paediatric PDR in Africa; a previous review¹⁵ included just two African studies, reporting a prevalence of 0% in Uganda¹⁶ and 5% in Cameroon.¹⁷ Our analysis, including more recent studies, shows an alarmingly high rate of 42.7% in PMTCT-exposed children and a significant increase in PDR prevalence in PMTCT-unexposed children.

The paediatric data are in contrast with data on PDR in adults in SSA. A large adult study conducted in six African countries between 2007 and 2009 found a pooled PDR prevalence of 5.6%.³ A recent meta-analysis including >11000 adults in SSA reported an overall PDR prevalence of 2.8% (median year of sampling 2007), which has been increasing since ART scale-up.⁸ Our results confirm the increasing prevalence over time in children, but show a much higher overall PDR prevalence, also in PMTCT-unexposed children.

The high PDR prevalence in PMTCT-unexposed children might be explained by the following mechanisms. First, it is possible that some children who were reported to be PMTCT-unexposed actually have received antiretroviral drugs before. Caregivers might not properly recall previous PMTCT, especially in older children or if the mother passed away. Moreover, some studies might have considered children with unknown PMTCT exposure as being

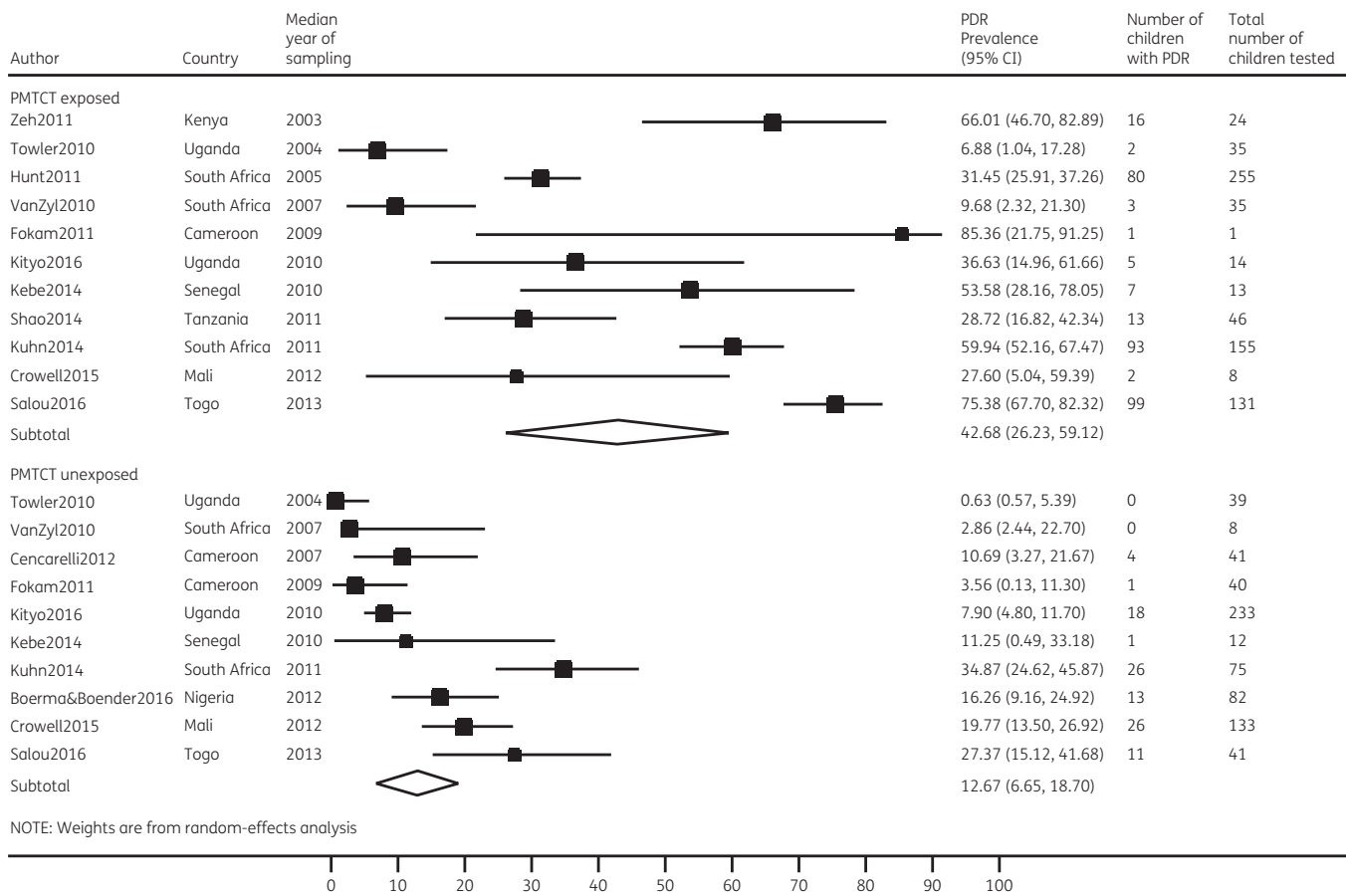


Figure 3. Proportion of children with PDR with and without prior PMTCT exposure, $P=0.004$. I^2 statistic PMTCT exposed: 84.7%; I^2 statistic PMTCT unexposed: 93.6%.

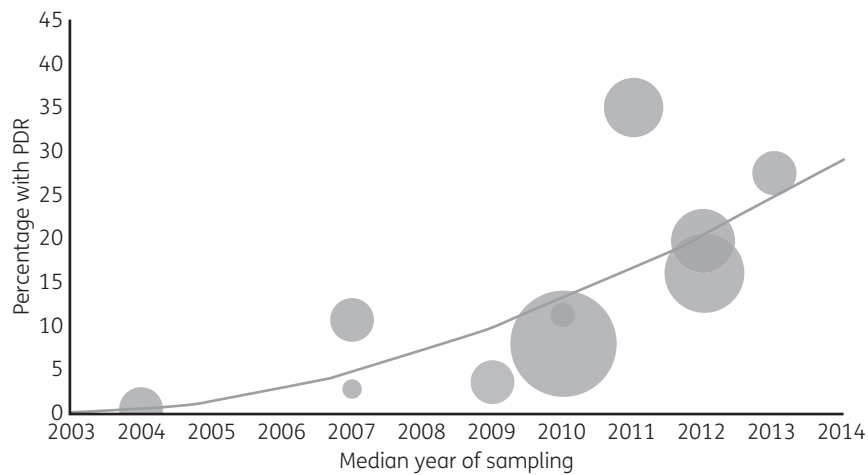


Figure 4. Prevalence of PDR in PMTCT-unexposed children by median year of sampling per study, $P=0.009$. Each circle represents a study and the size of the circle is proportional to the sample size of each study. The fitted line is plotted using meta-regression.

unexposed. Both could have led to an overestimation of the PDR rate in unexposed children. As not all studies specified how a child's PMTCT status was ascertained, we could not correct for the possible bias this might have created. Second, the paediatric PDR prevalence may simply reflect the likewise increased HIVDR prevalence in mothers due to the increased ART coverage. This resistance, either pretreatment or acquired drug resistance, is then subsequently transmitted to the child. A large study in Kenya recently showed that the adult PDR prevalence in Nairobi had increased from 3.9% in 2006 to 10.9% in 2014,⁹ which is, however, still lower than the prevalence we found in the most recent paediatric studies. Third, breastfed HIV-infected children might have been repeatedly exposed to breastmilk containing suboptimal doses of antiretroviral drugs if mothers start taking ART for their own health after delivery. This might lead to the development of PDR in the child.^{18,19}

Compared with adults, the pooled prevalence of PDR is 4 to 15 times higher in PMTCT-unexposed and PMTCT-exposed children, respectively. The PDR prevalence in African children is increasing rapidly and studies have found a prevalence of up to 35% in PMTCT-unexposed children. Urgent actions are needed to be able to optimally treat children living with HIV in SSA and are described below.

First, our results stress the importance of rapid implementation of the WHO guidelines recommending PI-based first-line ART for all young children. Although WHO has recommended that children <3 years of age should start on a PI- instead of an NNRTI-based first-line regimen since 2013,^{20,21} many resource-limited countries have not yet implemented these recommendations. The limited PI formulations are reserved for children with known prior PMTCT exposure and, in many settings, unexposed children still start on NNRTI-based first-line ART. Especially in the absence of widely available PI-based first-line ART, implementing regular viral load monitoring is very important to detect virological failure in time. In our study, the prevalence of PDR in PMTCT-unexposed children was, as expected, lower than in PMTCT-exposed children, but rates were still >15% in the most recent studies. This implies that one in six PMTCT-unexposed children and more than one in three PMTCT-exposed children who are currently born with HIV are started on a regimen to which the virus is only partially susceptible, carrying a high risk of early first-line failure and further accumulation of drug resistance mutations.^{4,22} To ensure WHO guidelines are followed and PI-based first-line ART is available for all children under 3 years of age, financial and logistic barriers must be overcome. Although drug prices have decreased over the past years, ritonavir-boosted lopinavir is still about five times more expensive than nevirapine.^{23,24} Until recently, ritonavir-boosted lopinavir for young children was only available as a syrup, which requires refrigeration and has an unpleasant taste. However, the US FDA has recently approved the use of paediatric ritonavir-boosted lopinavir oral pellets, which can be mixed with food or milk and are heat stable.^{25,26} This is an important step towards increased access to PI-based first-line treatment.

Second, the recommendation of PI-based first-line treatment might need to be extended beyond the age of 3 years. Currently, NNRTI-based regimens are recommended for children aged ≥ 3 years. In our meta-analysis, we found an 18% prevalence of PDR in children ≥ 3 years, which suggests that even in this population nearly a fifth of children will receive a suboptimal regimen if

started on NNRTI-based ART. The 18% we found may still be an underestimation of the actual PDR prevalence, because drug resistance mutations may be archived if children do not take ART and may therefore be missed by population-based sequencing methods.²⁷ The recommendation to treat all young children, regardless of PMTCT exposure, with PI-based ART is based on results of a clinical trial showing superior outcomes of children aged <3 years treated with a PI compared with those treated with an NNRTI.²⁸ In older children, two previous trials did not find a difference in treatment outcomes between NNRTI- or PI-treated children.^{29,30} However, the first trial, conducted in Europe and North and South America (2002–05) reported a low prevalence of 4% PDR in their study participants and the PDR prevalence in the second trial, conducted in Uganda (2009–11), was not reported. Given the high PDR prevalence in children ≥ 3 years in recent surveys and the increasing trend we detected in this meta-analysis, new randomized trials might need to be conducted to decide on the best first-line regimen for this age group.

Alternatively, the implementation of point-of-care resistance tests, most likely using allele-specific point mutation assays to detect a selection of (N)NRTI mutations, could be used to decide which children are likely to respond well to NNRTI-based first-line ART and which children need to start a PI-based regimen. Development of such a test, which should be reliable, inexpensive and easy to use, is currently on the way.³¹

Third, the limited number of studies included in this review indicates that paediatric PDR is a profoundly understudied topic. Given the rising prevalence we identified, we expect that PDR will be an increasingly important aspect of HIV treatment in resource-limited countries. In settings where individual resistance testing is not currently feasible, surveillance data at the population level are crucial. More studies are needed in SSA to estimate the extent of the problem of paediatric PDR. The regional prevalence and trends of PDR over time should be evaluated, both by conducting surveys in previously unstudied regions and by repeating previously conducted surveys in other regions. This information is needed to evaluate current treatment guidelines and adapt these, if necessary.

Our study has some potential limitations. First, our meta-analysis included a relatively small number of studies, combining data from 19 studies on 2617 children. In addition, the studies were heterogeneous in terms of age, geographical location and PMTCT exposure. Data were too limited to conduct separate analyses by maternal and/or infant PMTCT exposure or by PMTCT regimen. In 7 out of 12 articles, it was reported that at least some of the mothers in this study had used option B (combination ART) for PMTCT (Table S1). We could not conduct a separate analysis for infants born to these mothers, as studies did not report the prevalence of PDR separately for different PMTCT regimens. However, given that more than half of the studies had combination ART as one of its PMTCT options, it seems that the current rollout of option B (plus) might not result in lower PDR rates, which was previously also concluded by Kuhn *et al.*⁷

The limited number of studies included in our study reflects the scarcity of data on this topic and stresses the importance of additional PDR surveys to be conducted in other regions in SSA. Like all systematic reviews, our analysis is subject to publication bias. By including conference abstracts in addition to peer-reviewed articles, we attempted to limit the effect of this bias. Finally, some

studies only reported the number of children with resistance to a specific drug class and not a total PDR prevalence.

In conclusion, in this meta-analysis, we show that the PDR prevalence in children in SSA is very high, both in PMTCT-exposed and PMTCT-unexposed children. In the PMTCT-unexposed group, we found an alarming increase in prevalence over time. The pooled PDR prevalence in African children is 4–15 times higher than that reported in a recent meta-analysis among adults.⁸ Resistance to NNRTIs is most common, while PI resistance remains rare. It is critical that PI-based regimens be made available for first-line treatment of all African children aged <3 years. For children aged ≥3 years, new randomized studies evaluating the benefits of PI-based ART as first-line treatment should be considered, given our findings of high PDR prevalence that continues to increase over time. Our results identify paediatric PDR as an increasingly important issue in HIV treatment in Africa and once more stress the importance of PI-based first-line ART.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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