Drug resistance in the HIV-1-infected paediatric population worldwide: a systematic review

Patricia Rojas Sánchez and Africa Holguín*

HIV-1 Molecular Epidemiology Laboratory, Microbiology Department, Hospital Ramón y Cajal-IRYCIS and CIBERESP, Madrid, Spain

*Corresponding author. Tel/Fax: +34-91-3368153; E-mail: africa.holguin@salud.madrid.org

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Background: Drug resistance monitoring of the paediatric HIV-1-infected population is required to optimize treatment success and preserve future treatment options.

Objectives: To explore the current knowledge of HIV drug resistance (HIVDR) in naive and pretreated HIV-1-infected paediatric populations across diverse settings and sampling time periods.

Methods: PubMed database screened until May 2013. We selected publications including data on transmitted (TDR) and acquired drug resistance mutation (DRM) rates and/or pol sequences for HIVDR testing in paediatric patients. We recorded the children’s country, age, study period, number of children with pol sequences, presence or absence of antiretroviral treatment (ART) at sampling time, viral region sequenced, HIVDR rate to the three main drug classes (single, double or triple), the considered resistance mutation list and performed assay, specimen type, HIV-1 variants and subtyping methodology when available.

Results: Forty-one selected studies showed HIVDR data from 2538 paediatric HIV-1-infected patients (558 naive and 1980 pretreated) from 30 countries in Africa (11), Asia (6), America (10) and Europe (3). Both TDR and DRM prevalence were reported in 9 studies, only TDR in 6 and only DRM in 26. HIVDR prevalence varied across countries and periods. Most studies used in-house resistance assays using plasma or infected cells. HIV-1 non-B variants were prevalent in 18 paediatric cohorts of the 24 countries with reported subtypes. Only five countries (Uganda, Spain, the UK, Brazil and Thailand) presented resistance data in ≥200 patients.

Conclusions: Systematic and periodic studies among infected children are crucial to design a more suitable first- or second-line therapy.

Keywords: HIV-1, children, resistance mutations, antiretroviral therapy

Introduction

By the end of 2013, 35 million people were living with HIV infection, including 2.5 million children below the age of 15 years.1 Increasing access to services for prevention of mother-to-child transmission (PMTCT) of HIV and the higher availability of antiretroviral therapy (ART) has reduced the number of AIDS-related deaths by 20% and new HIV infections by 50% among children.1–6 According to current guidelines,6–10 the ART of choice for HIV-1-infected children and adults is a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) (backbone treatment) and a third agent from a different class, either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI). New drug families have already been used in multidrug-resistant paediatric patients,11 but their use is still limited in most countries due to the cost, the lack of appropriate formulation and pharmacokinetic data for children.11 In fact, not all antiretrovirals (ARVs) used in adults and adolescents12 are approved for use in children and not all are available in paediatric formulations.8

The use of ARVs for treatment in mothers and as prophylaxis in newborns for PMTCT can select HIV drug resistance (HIVDR) in infected infants.14 Furthermore, children require long-term therapy and can also develop HIVDR due to suboptimal plasma drug levels or regimens, non-complete compliance and inadequate dosing guidelines for specific ARVs and age groups.15,16 Children present more HIVDR than adults with heterosexually transmitted HIV12,17 and have a 2-fold higher risk of virological failure (VF) than adults after 5 years on ART.12,18,19 Thus, HIVDR genotyping has become a standard of care in HIV infection management.8

The presence of HIV-resistant strains in naive populations threatens the effectiveness of ART and transmitted HIVDR (TDR) increases with the greater coverage of ART.20,21 Previous studies have reported resistance levels (ranging from 34% to 99%) in children failing first- and second-line regimens, primarily consisting of NNRTI12 resistance and the NRTI mutation M184V, leaving scarce
treatment options available in resource-limited settings.\textsuperscript{18,22 – 24}
In developed settings with long-term access to ART, perinatally acquired HIV-1 infection has become a chronic childhood disease, although their long history of therapy with many ARV switches and the variable levels of adherence to ART increases the risk of HIVDR and VF.\textsuperscript{25 – 29}

The guidelines for the use of ARVs in paediatric HIV infection recommend drug resistance genotyping before initiation of therapy in all treatment-naive infants, children and adolescents to select the initial ARV combination and also before changing therapy among pretreated children with VF.\textsuperscript{7} Therefore, paediatric populations in high- and middle-income countries usually have frequent resistance testing. However, in resource-limited settings, although gathered by the WHO (WHO HIVResNet, the Global HIV Drug Resistance Network),\textsuperscript{30} resistance genotyping data are still limited\textsuperscript{21} and often restricted to children and adolescents failing second-line regimens, since cost and complexity limit its full implementation.\textsuperscript{31} The WHO has published a generic protocol for surveillance to assess HIVDR among children <18 months of age using dried blood spots (DBS) in resource-limited countries.\textsuperscript{32}

HIV-1 subtype-specific variability can have implications for resistance monitoring\textsuperscript{33} and for resistance development.\textsuperscript{34,35} In fact, certain HIV-1 subtypes and recombinants prevalent in most low-resource countries present specific substitutions in positions related to drug resistance, which can accelerate the emergence of drug-resistant viruses and change or induce alternative resistance pathways.\textsuperscript{34 – 37}

Although HIVDR prevalence has been well documented in naive and pretreated adult cohorts worldwide,\textsuperscript{21,38 – 41} studies of HIVDR mutations in naive and pretreated paediatric HIV-infected populations still remain limited in most countries. In this review, we explore the current knowledge of HIVDR in naive and pretreated children across diverse settings and sampling time periods, including high-, middle- and low-income countries. We also summarize available TDR and drug resistance mutation (DRM) rates of the three main ARV classes reported in each country, providing the HIV-1 variant information for each selected study when available.

**Methods**

**Data source and searches**

PubMed (www.ncbi.nlm.nih.gov/pubmed) was screened until May 2013 to identify studies showing HIVDR data from naive and pretreated HIV-1-infected paediatric populations worldwide. We searched using the following terms: ‘HIV-1 infected paediatric resistance’, ‘HIV-1 infected children resistance’, ‘HIV-1 infected newborn resistance’, with additional PubMed filter regarding age ‘Child: birth–18 years’ and ‘Infant: birth–23 months’. We then selected only those studies showing TDR and/or DRM rates and/or HIV-1 pol sequence data derived for resistance testing. From each study we recorded the children’s country, age, study period, number of children with available pol sequences, presence or absence of ART at sampling time, protease (PR) or/and reverse transcriptase (RT) viral region sequenced, TDR and/or DRM rates to NNRTIs, NRTIs and PIs and the considered resistance mutation list. We considered double resistance when resistance mutations affected two of these drug classes (NNRTI + NRTI, NRTI + PI or NNRTI + PI) and triple resistance when they affected all three. We also recorded the resistance assay performed and the specimen type used in each study as well as HIV-1 variants and subtyping methodology when available. When the sample size was <200 specimens analysed, the provided TDR/DRM rates were recorded without decimal places (except those with half decimal).

**Results and discussion**

**Features of HIV-1-infected paediatric populations with resistance data**

A total of 41 articles published during 2003–13 analysing HIV-1-infected paediatric populations with available resistance data derived from PR and/or RT sequences were selected. Most (80.5%) studies were published between 2009 and 2013 (Figure 1). The 41 selected studies showed HIVDR data for the three main ARV classes in 2538 patients (558 naive and 1980

**Figure 1.** Selected resistance studies in HIV-1-infected paediatric populations over time including naive and pretreated patients. The number of HIV-1 naive children and under ART with available HIVDR data included in Tables 1 and 2 are indicated according to the year of publication. The number of studies published per year is indicated above each bar.
pretreated) from 30 countries in Africa (11), Asia (6), America (10) and Europe (3). The number of naive and pretreated children with resistance testing published per year is shown in Figure 1. Among the 41 selected studies, 6 included only naive infants and children, 26 only pretreated and 9 both. Patients with ART or prophylaxis were considered as treated. Thus, patients with prior ART exposure via PMTCT were included among the pretreated group. Fourteen countries reported TDR rates\textsuperscript{14,42–54} in samples from 558 naive patients collected between 1993 and 2011 (Table 1) and DRM rates in specimens from 1980 pretreated paediatric patients from 26 countries taken during 1990–2011\textsuperscript{6,16,18,19,31,43–46,48,50,52,54–77} (Table 2). Nearly a quarter of the pretreated patients had received prophylaxis close to the time of birth (Table 2). In 16 studies, we inferred the DRM rate from the available sequences carrying resistance mutations as only sequences and no resistance rates were provided. The patients’ ages ranged from newborn to 19 years, although most studies among both naive (78.6%) and pretreated (58.3%) patients included infants <2 years old (Tables 1 and 2). PR sequences were not provided in 10 (24.4%) of the 41 studies.

**HIV-1 infecting variants in paediatric populations**

In 28 (68.3%) of the 41 selected studies, the presence of HIV-1 subtypes and recombinants in infants and children was analysed using *pol* sequences derived from resistance testing. Eighteen of the 28 studies reporting HIV-1 variants used phylogenetic analysis, the gold standard method for HIV-1 subtyping. Rapid subtyping tools were used in two studies, despite their reported limitations for the correct identification of certain HIV-1 subtypes and complex recombinants.\textsuperscript{78} Both methods were used in four studies and in the remaining four, HIV-1 subtypes were provided but the subtyping methodology was not specified.

Subtype B infections were prevalent in some studies including paediatric populations from Spain, the USA, Central American

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<th>Table 1. Summary of TDR rates in drug-naive HIV-1-infected paediatric populations</th>
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TDR, transmitted drug resistance rate; PR, protease; RT, reverse transcriptase; —, data not analysed or not provided. TDR rates were recorded without decimal places.

\textsuperscript{a}Naive children with available PR and/or RT sequence.

\textsuperscript{b}Studies including data related to ARV experience in mothers.

\textsuperscript{c}Two subjects previously considered infected with viruses harbouring TDR to NRTI received zidovudine after birth and are considered as pretreated for the current analysis.

\textsuperscript{d}The provided TDR in the paper were recalculated following the WHO mutation list.\textsuperscript{115}
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<td></td>
</tr>
</tbody>
</table>

*NB: DRM = drug-resistant mutations; NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors; PI = protease inhibitors.*
### Table 2. Continued

<table>
<thead>
<tr>
<th>Country</th>
<th>Sampling period</th>
<th>Population size/years</th>
<th>TDR data</th>
<th>Age range</th>
<th>Pretreated patients with DRM (%)</th>
<th>NRTI + PI</th>
<th>NRTI + PT</th>
<th>PI PT</th>
<th>HIV-1 variant list</th>
<th>ANRS</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>2000-09</td>
<td>147,000</td>
<td>no</td>
<td>1-8</td>
<td>yes</td>
<td>0</td>
<td>6%</td>
<td>14%</td>
<td>6%</td>
<td>yes</td>
<td>88.5</td>
</tr>
</tbody>
</table>

Only naive or ARV-experienced (T) paediatric patients with available PR and/or RT sequence are included. When sample size was <200, analyses were performed on all patients. —, data not available or provided. When sample size was <200, analyses were performed on all patients. —, data not available or provided. PR, protease; RT, reverse transcriptase; TDR, transmitted drug resistance rate in drug-naive infected children; DRM, drug resistance mutations in pretreated children; seq, number of sequences; IAS, International AIDS Society; ANRS, Agence Nationale de Recherche sur le Sida (http://www.hivfrenchresistance.org); Renageno, algorithm for HIVDR interpretation provided by the Brazilian network for HIV-1 genotyping (RENAGENO) available at http://forrest/ime.usp.br:3001/resistencia, Stanford University, USA. (http://cpr.stanford.edu/cpr.cgi); for PI resistance, only major mutations were considered. Non-B variants include HIV-1 non-B subtypes and recombinants. Only indicates the rate of patients carrying M184V (70%) and K103N (13.6%) in RT and L90M (42%) in PR.

**Paediatric HIV-1 resistance testing by region**

Figure 2 shows the 30 countries reporting HIVDR data from HIV-1-infected paediatric cohorts included in the present review and the number of included patients with available pol sequences, which varied across studies.

Since most infected children live in low- and middle-resource countries, most studies were performed in Africa, Asia and Latin America and the Caribbean, where global access to ART is increasing and routine resistance testing is being implemented. However, despite being the most affected continents, HIVDR data in children were only available in 11 (20.75%) of the 53 African countries, in 6 (13.6%) of the 44 Asian countries, in 1 (4.8%) of the 21 Caribbean islands, in 3 (23.1%) of the 13 South American countries and in 4 (57.1%) of the 7 Central American countries (Tables 1 and 2). The lack of resistance data in other resource-limited settings is probably due to very limited viral monitoring and resistance testing because of the high cost and lack of appropriate infrastructure. Moreover, in most of those settings, genotyping is not routinely provided or is often restricted for use in children and adolescents failing second-line regimens. Only three Western European countries presented HIVDR data in children (Tables 1 and 2).
Samples used and resistance assays

In our review we found insufficient sample size for providing resistance data in the paediatric population per country as no large HIV-1-infected paediatric cohorts have been analysed.

In fact, among the 30 countries with available HIVDR data in infants and children across studies, 15 analysed HIV-1 pol sequences in <50 patients (Figure 2) and 5 (16.7%) analysed <20 (Tables 1 and 2). Only 5 countries presented resistance data from ≥200 infants or children (Uganda, Spain, Brazil, Thailand and the UK). This is probably explained by several different reasons: (i) the lack of well-established paediatric cohorts including a large number of paediatric patients in high-income countries; (ii) samples are not always available from HIV-1-infected children; and (iii) sequencing of a large number of specimens, required for most drug surveys, is too expensive for low- and middle-income countries. In fact, 28 (68.3%) studies used non-commercial and the cheaper in-house PCR to amplify the pol coding region (Table S2, available as Supplementary data at JAC Online). Only 11 studies performed resistance tests using the more expensive commercial assays: ViroSeq™ HIV-1 Genotypic System (Abbott Molecular) in 7 studies18,44,49,62,63,65,70 and Trugene™ HIV-1 Genotyping Assay (Siemens HealthCare Diagnostics) in 4 studies.46,48,67,71 There was one study that did not specify the method54 and another used an oligonucleotide ligation assay.68

Most studies used plasma for HIVDR testing. In more detail, HIV-1 pol sequences were determined using viral RNA from plasma samples in 31 studies, proviral DNA from peripheral blood mononuclear cells in 246,56 or both viral RNA/proviral DNA in 35,45,47 (Table S2). Although plasma is considered the optimal specimen type for genotyping, DBS can also be used for drug resistance testing in infants, children and adults.97 In fact, DBS have been implemented in a large number of resource-limited countries for paediatric resistance studies due to their simple collection, transport, storage and the lower blood volume required.97,98 In our review, even though 32 of the 41 studies included infants <2 years of age (Tables 1 and 2), DBS specimens were only used in 4 (9.8%) of them, in patients from Peru, El Salvador, Belize and Honduras and the USA (Table 2), including data from 11 to 55 patients in each country.50,51,68,76 According to WHO guidelines, in countries where only one laboratory participates using DBS, the recommended sample size should be 245; however, the survey should still be conducted if the number of patients is smaller despite the loss in precision.32 Furthermore, genotyping ideally should be performed in a WHO HIVResNet-accredited laboratory using approved methods for DBS genotyping.

TDR studies are scarce among children

Although viruses carrying TDR mutations can compromise first-line ART effectiveness, increasing the rate of VF,50 we only found 14 countries reporting TDR rates among 558 naive children (Table 1). The number of analysed patients per country ranged
from 4 to 105 and the TDR rates from 0% to a maximum rate of 33.3% for NNRTIs, 16.6% for NRTIs and 18.2% for PIs (Table 1). In Uganda, Brazil, Mexico and Argentina, TDR mutations were not found. However, the number of analysed subjects was low. Different results were obtained across studies in Brazil, probably due to the different sample size and study period. Only four countries (India, Spain, Brazil and Cuba) reported naive paediatric subjects harbouring viruses with resistance to two drug families. Spain was the only country reporting paediatric infections with viruses carrying TDR mutations to three drug families, observed in one naive patient infected in 2004 with triple-resistant viruses carrying changes D30N and N88D in PR and K70R and Y181C in RT. Most selected TDR studies in paediatric populations were performed after 2008, reporting data of 495 drug-naive infants (Figure 1). The TDR mutations found in each study are included in Table S3 (available as Supplementary data at JAC Online).

Current guidelines for the use of ARV agents in paediatric HIV infection recommend that resistance testing should be considered in newly diagnosed infants <12 months of age before initiation of therapy and they also emphasize the importance of monitoring the frequency of HIVDR in newly infected infants. In regions of sub-Saharan Africa, where most infected children live, a significant increase in the prevalence of TDR over time has been reported in adults since ARV rollout, mainly driven by NNRTI resistance. TDR trends could be intimately related to the ARV programmes implemented in each region and vary according to the origin of the patients. In high-income countries, TDR rates have been estimated ~10%–17% and mainly among immigrants from regions without well-implemented ARV strategies. However, in these settings and after several years with a continuous increase in the TDR rate, the efficacy of highly active antiretroviral treatment (HAART) and the development of both new ARV drugs and classes have led to a decrease of TDR trends. There are fewer TDR studies in children from settings with viral load (VL) monitoring and resistance testing.

The transmission of a drug-resistant virus generally appears to be less efficient than that of a wild-type virus may be due to its diminished fitness in the absence of the ARV drugs. In the absence of drug pressure as happens in naive subjects, the stability of TDR varies markedly with the mutation. Some mutations leading to NNRTI resistance and VF can persist in certain subtypes for more time than in others. This can be of interest in paediatric infected populations, since the widespread use of an NNRTI for infant or maternal PMTCT prophylaxis or HAART regimens during pregnancy and breastfeeding will result in an increase in the proportion of children infected with NNRTI-resistant HIV despite prophylaxis or maternal HAART. Furthermore, although combination regimens for use in PMTCT prophylaxis have been recommended for resource-limited countries since 2006 many countries continue to offer single-dose nevirapine, which is associated with the development of high rates of NNRTI resistance among both HIV-1-infected mothers and infants who become infected, mainly due to prolonged subtherapeutic plasma nevirapine levels. In some cases, we observed pretreated children carrying viruses with resistance mutations to drug families different from those included in the perinatal prophylaxis or ART and these were considered as TDR mutations.

### Acquired resistance mutations in pretreated paediatric populations

The selected 36 studies included samples collected from 1990 to 2011 from 26 countries. The DRM rates ranged from 0% to 100% for NRTIs, from 3% to 100% for NNRTIs and from 0% to 66.7% for PIs (Table 2). Double resistance to NNRTI+RTI ranged from 0% to 100%, to NNRTI+PI from 0% to 41.7% and to RTI+PI from 0% to 58.3%. The rate of infections in children with triple-resistant viruses varied across studies (Table 2), reaching 33.3% in one study performed in Brazil with 12 paediatric patients with VF. Previous studies reported higher triple resistance rates in children than in adults. The most common associated DRMs in RT were K103N, Y181C and K101E/P for NNRTIs and K70R/E, M184V and thymidine analogue mutations for NRTIs. For PIs, the most common mutations in PR were M46I/L, D30N, V82A and L90M.

The emergence of HIVDR following access to ART in resource-limited settings resembles the pattern seen previously in high-income countries. It also reinforces the need for periodic drug resistance testing and alternative drug regimens that have proven beneficial to control the appearance of triple-class resistant viruses in children from high-income settings, which has been reported to be higher in children than in adults.

The WHO recommends virological monitoring to be performed biannually. In the absence of virological monitoring and due to the low positive predictive value of CD4 monitoring in both adults and children for diagnosing treatment failure, the VL remains the gold standard for an early virological diagnosis of VF. Without virological testing, HIV-infected African children starting ART are at risk of undetected VF and the development of resistance. Most of the selected resistance studies in our review were performed in paediatric patients under VF, although definition of VF varied across studies mainly regarding the VL values considered (HIV-1 RNA from 50 to >5000 copies/mL in plasma), despite the WHO definition of VF as two consecutive VL >1000 copies/mL after 2 months with adherence support.

The WHO recommends that countries adopt the HIVDR prevention and assessment strategy for children receiving ART to evaluate the effect of maternal and paediatric ARV history, baseline drug resistance and other factors on ART outcomes in children. Unfortunately, maternal ARV history before pregnancy was not always available, mainly in low-resource settings. In our review, we observed that only 24 of the 41 studies included data related to ART experience in mothers (Tables 1 and 2). The presence of HIVDR mutations transmitted from the mother or generated during prophylaxis in infants should be considered in paediatric HIV-1-infected populations before initiating their ART regimen, since these could affect future therapeutic options in infants and children.

### Limitations of drug resistance studies in paediatric cohorts

There are several important limitations when comparing TDR and DRM rates in HIV-1-infected paediatric populations from different countries. Due to the diversity of patient cohorts, extrapolation of the found TDR and DRM rates to broader populations cannot be made, since HIVDR transmission can vary geographically and with time in the same country and can be different among...
differently risk groups. Surprisingly, most of the studies testing HIVDR in paediatric and adult cohorts in resource-limited countries do not comply with the WHO criteria for performing HIVDR testing. In fact, neither data nor specimens routinely collected in most HIV diagnostic sites in resource-limited countries are ideal for HIVDR surveillance. Most limitations are related to the specific population subgroups and sample size tested, to the selection of survey areas and to the limited information available for included patients. Additionally, although many of the studies of treatment-naive children included infants, the average age of children in a high number of the reported studies is >2 years. These populations should have followed the participant eligibility criteria for HIVDR surveys provided by the WHO in adults from countries with scaling up ART, recommending selection of individuals for whom the duration of HIV infection is more likely to be <3 years. Moreover, some TDR mutations could revert in the oldest perinatally infected children.

Another constraint for regular surveys is the limited number of laboratories within the WHO HIV ResNet Laboratory Network (mainly those accredited for HIVDR testing using DBS) and the necessity to minimize financial and human resources. In fact, most HIVDR published studies are performed by laboratories not belonging to the WHO network and their reported HIVDR rates are not included in the official WHO HIVDR surveillance reports.

Another limitation is that ARV prophylaxis data in infants and in women during pregnancy were not always available. Furthermore, different specimens, resistance genotyping assays and mutation lists were used across studies. This could provide different results due to varied assay sensitivity, leading to a different interpretation of the resistance mutation frequency for each drug family depending on the DRM list used. Lastly, reports studying only one or two specific resistance mutations in infants and children were not included. Surveillance to assess HIVDR prevalence among naive and pretreated paediatric patients is essential for improving health outcomes and minimizing the subsequent accumulation of DRMs among HIV-infected infants, children and adolescents. It can help clinicians to select a more rational design for first- or second-line ART in this population.

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Transparency declarations
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Author contributions
P. R. S. carried out the initial analyses, drafted the initial manuscript, prepared the figures and approved the final manuscript. A. H. conceived and designed the study and reviewed, completed and revised the manuscript. A. H. wrote the final manuscript and designed the final figures and tables as submitted.

Supplementary data
Tables S1 to S3 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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