

ORIGINAL ARTICLE

**ACQUIRED ANTIRETROVIRAL DRUG RESISTANCE
MUTATIONS UPON TREATMENT FAILURE IN HIV-1
INFECTED PEDIATRIC PATIENTS
IN CENTRAL BRAZIL**

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ABSTRACT

Antiretroviral drug-resistance mutations compromise the successful treatment of children and adolescents infected with human immunodeficiency virus type 1 (HIV-1). We describe the clinical, virological, and immunological follow-up of a cohort of children and adolescents perinatally infected with HIV-1 treated at Hospital Estadual de Doenças Tropicais Dr. Anuar Auad – HDT, in Central Brazil, after therapeutic failure related to drug resistance mutations. We analyzed the results of the genotypic test (protease codons 1–99 and reverse transcriptase codons 1–325) performed from 2003 to 2015. The ARV susceptibility profile was analyzed according to Stanford HIV drug resistance database. A total of 65 patients (median age of 10 years; range, 18 m–18 y) with therapeutic failure (after a median of 55 months of follow up; range, 9 m–13 y) and plasma levels of HIV-1 RNA greater than 1,000 copies/mL which were included and demonstrated mutations in: nucleoside reverse transcriptase inhibitors (NRTIs), 98.5%; non nucleoside reverse transcriptase inhibitors (NNRTIs), 75.4%; and protease inhibitors (PI), 44.6%. The most frequent NRTI mutations were found in codon T215 (83.1%) with a predominance of T215Y (56.9%), followed by M184V (69.3%). In the NNRTI class, mutations K103N (36.9%) and I90A (23.1%) were predominant, and, in the protease, mutations 54VL (35.4%) and 82ASTL (32.3%) were found in approximately the same proportion, with a predominance of the M54V mutation. These results demonstrate the high levels of resistance to different classes of antiretrovirals in HIV-infected children and adolescents and the importance of genotypic resistance tests in this population.

KEY WORDS: HIV; drug resistance; genotypes; child; adolescent.

INTRODUCTION

The first fifteen years of the AIDS epidemics were marked by extreme morbidity and mortality caused by the absence of adequate antiretroviral therapy for viral suppression, as well as being associated with numerous

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antiretroviral (ARV) adverse events. Since 1996, with the introduction of a combination of antiretroviral therapy with new therapeutic options, there has been a huge improvement in survival and quality of life rates (Violari et al., 2008; Cohen et al., 2011; INSIGHT STUDY., 2015; Luzuriaga & Monfenson, 2016). By the end of 2019, a total 37.9 million people living with HIV and AIDS was estimated, of which 1.7 million were children under the age of 15 (UNAIDS, 2019). The majority of HIV-infected children acquired the virus through maternal exposure. Subsequently, prophylactic measures during pregnancy and in the neonatal period have caused the rate of maternal fetal transmission to drop significantly, reaching rates close to zero in several parts of the world (Connor et al., 1994; Nielsen-Saines et al., 2012; Mandelbrot et al., 2015; Luzuriaga & Monfenson, 2016). In Brazil, rates of vertical HIV transmission varied from 1.8% to 27.8% (Guimarães et al., 2019). However, children require long-term therapy, allowing inadequate adherence, among other factors, selecting mutation of HIV resistance to drugs due to incomplete viral suppression (Gupta et al., 2009; Tang & Shafer, 2012; Rossouw et al., 2015; Sher et al., 2020). Drug resistance mutations compromise the success of ARV treatment in children and adolescents infected with HIV-1, resulting in limited therapeutic options for these age groups (Gupta et al., 2009; Katlama et al., 2013; Wensing et al., 2017; Nuttall & Pillay, 2019). The genotypic resistance test, if performed early on after the therapeutic failure, avoids an unnecessary and erroneous exchange of ARV (Pallela et al., 2014; McCluskey et al., 2019). In Central Brazil, some authors have highlighted the molecular epidemiology, HIV-1 drug resistance and vertical transmission in adult and pregnant women which evidenced up to 16.7% of ARV drug resistance and 1.0 to 2.01% of vertical transmission (Cardoso et al., 2010; Carvalho et al., 2011; Ferreira et al. 2011; Alcântara et al., 2012; Lima et al., 2016). Previous studies reported a wide range of resistance rates (ranging from 34% to 99%) in children with first and second line ARV treatment failure, mainly consisting of NNRTI resistance and M184V NRTI mutation, leaving few treatment options available in resource-limited regions (Rogo et al., 2015; Huerta-Garcia, 2016; Collier et al., 2019).

The aim of this study was to analyze, through genotyping, the profile of antiretroviral resistance mutations in children and adolescents infected with HIV-1 with therapeutic failure, as well as to describe their clinical and laboratorial aspects.

METHODS

This was a descriptive study based on a retrospective cohort of HIV-infected children and adolescents diagnosed between 1992 and 2013. Of the 160 pediatric patients undergoing ARV treatment, 65 were eligible for the study. The following 95 patients were excluded from the analysis: a) those

who only had primary genotyping n=30; b) patients who had no viral load and/or CD4 counts after changing ARV n=2; c) those who did not undergo the genotyping test n=9; d) patients without therapeutic failure n=40; e) those over 20 years of age n=2; f) transferred patients n=7. The pattern of resistance mutations in 65 HIV-infected children and adolescents (median 10 years of age) with therapeutic failure were analyzed by polymerase sequencing using TrueGene HIV-1 Genotyping Test® (Siemens Diagnostics, USA) and ViroSeq HIV-1 Genotypic Test® (Celaera Diagnostics, USA). The patients were followed up as of their HIV diagnosis at the pediatric infectious disease outpatient clinic of the “Hospital Estadual de Doenças Tropicais Dr. Anuar Auad – HDT” (Central Brazil) reference in treatment of HIV infected children. Virological failure was defined as the presence of HIV viral load (RT-PCR Abbott RealTime HIV-1 – Abbott Molecular) > 50 copies/mL after the use of ARV for 6 months and the genotypic test was performed with viral load above 1,000 copies/mL (Brazil, 2014 and 2015). In each genotype test, partial amplification of HIV polymerase was performed (codons 1-99 of the protease and codons 1-325 of the reverse transcriptase). For the interpretation of antiretroviral resistance, the Brazilian algorithm (RENAGENO- Algorithm version 12, 2012) and the Stanford University HIV Drug Resistance Database (Stanford HIV drug resistance database, 2015; available at <http://hivdb.stanford.edu>, summarized at <http://hivdb.stanford.edu/pages/drugSummaries.html>) were used. ARVs were classified as having low-, intermediate- or high-level resistance, according to the algorithms. A total of 44 HIV-1 subtypes were identified using the REGA HIV subtyping tool. The present study was approved by the Ethics Committee (Plataforma Brasil protocol 1,210,496) and, as it was carried out as a retrospective model, did not require the Free and Informed Consent from patients and/or parents or guardians. A total of 92 genotypic resistance tests were carried out from 2003 to 2015. 45 patients had a sample genotyped once, 17 twice, 2 were genotyped three times and one four times due to treatment failure. Genotypic tests were collected at the Central Laboratory (LACEN) and performed by the RENAGENO laboratory through the National STD and AIDS Program of the Brazilian Ministry of Health. For resistance interpretation, the ARV algorithm (RENAGENO - Algorithm version 13, 2015) and the algorithm from the Stanford University HIV Drug Resistance Database (Stanford HIV drug resistance database, 2015) were used. The study was approved by the ethics committee of participating institutions (Federal University of Goiás and State of Goiás Health Secretary). The statistical analysis was performed using the Microsoft Excel version 2010 software and Statistical Package for the Social Sciences (SPSS®) 20.0 for Windows. Descriptive and bivariate analyzes were performed, considering a level of significance of 5%.

RESULTS

The sample consisted of 65 children and adolescents, the majority female (36/65) and median age of 10 years at genotyping test (18 months to 18 years old) with a median of 4 years and 7 months of follow-up (9 to 164 months). Most of the patients acquired the infection by vertical transmission and only one by blood transfusion (Table 1). Of the analyzed samples, only 44/65 had the HIV-1 subtype identified. Subtype B was more prevalent (37/44), representing 84.1%. F1 (4/44, 9.1%), BF1 (2/44, 4.6%) and A1 (1/44, 2.7%) subtypes were also found. Approximately half of the patients presented severe immunosuppression on diagnosis of HIV, as the median age of diagnosis was around two and a half years; also 33% belonged to classes B or C, according to the CDC-1994 clinical and immunological classification. The median CD4 lymphocyte count and HIV viral load at diagnosis were, respectively, 921 cells/mm³ and 678,998 copies (Table 1). At the time of genotyping, CD4 ranged from 1 to 2,940 cells/mm³, with a median of 608 cells/mm³, and a median of 40,548 copies/mL (log 4.60) (Table 2). The most frequent ARV regimens at the time of genotyping were 2 NRTIs combined with NNRTIs (NVP or EFV) or associated with IP/rat. It is important to note that some patients had received prior AZT monotherapy (9.5%) or dual therapy (47.6%) in initial ARV therapy. Several subsequent regimens including 3rd line ARVs were used to rescue therapeutic failures, targeted or not by prior genotypic testing. Only 6 patients (9.2%) had undergone primary genotyping tests and these did not show resistance mutations.

Most mutations were found in the NRTIs class, followed by NNRTIs and PI (Table 3). The most frequent mutations in the NRTIs class were T215 codons, whereas the mutations most commonly observed in the NNRTI class were K103N/S, 190A / S, and 101E/P/Q. Mutations associated with resistance in protease occurred mainly in codons 54, 82 and 46 (Figure 1).

Dual class resistance occurred with the combination of NRTIs plus NNRTIs or NRTI plus PI/r; and triple class with NRTIs plus NNRTIs plus PIs (table 3). After rescue therapy guided by genotyping, approximately 90% of the patients analyzed had viral suppression, with HIV viral RNA levels below the detection limits (<40 or 50 copies) along with improved CD4+ cell counts, after 24 weeks of change in the combined antiretroviral regimen, compared with pre-genotyping data ($p < 0.001$) (Figure 2).

For the analysis of susceptibility to antiretrovirals, the Stanford version 2015 algorithm was used. The analysis of the entire profile of susceptibility to antiretrovirals in each patient is available in supplementary figure (<https://revistas.ufg.br/iptsp/article/view/62639/35036>).

Table 1. Clinical and laboratory characteristics of HIV-infected children and adolescents at diagnosis.

	valid n	median / min-max or %	SD
Age (months) at diagnosis	65	29.2 (2 – 120)	29.86
sex male	29/65	44.6%	-
Acquisition mode	65	-	-
Vertical transmission	64/65	98.5%	-
Blood transfusion	1/65	1.5%	-
Clinical Classification (CDC-1994)	65	100%	
Category N	7/65	10.8%	-
Category A	14/65	21.5%	-
Category B	22/65	33.8%	-
Category C	22/65	33.8%	-
Absolute CD4 count (cells/mm ³)	65	921 (34 - 4,372)	
HIV-1 RNA (copies/mL)	58	678,891.84 (400 - 10,000,000)	
HIV-1 RNA (log ₁₀ copies/mL)	58	5.83	
Initial ARV therapy	65		
AZT	4/65	6.1%	
AZT + ddI or AZT + 3TC	23/65	35.4%	
AZT + ddI (or AZT + 3TC) + RTV	7/65	10.8%	
AZT + ddI (or AZT + 3TC) + NFV	7/65	10.8%	
AZT + 3TC + (NVP or EFV)	16/65	24.6%	
AZT + 3TC + LPV / r	5/65	7.7%	

AZT: zidovudine; ddI: didanosine; 3TC: lamivudine; RTV: ritonavir; NFV: nelfinavir; EFV: efavirenz; LPV: lopinavir

Table 2. Laboratory characteristics and ARV schemes related to genotyping test (GT).

	n	median / variation or %
Age (years) when performing GT	65	10 years
<1 year	2/65	3.1%
1- 5 years	17/65	26.1%
6- 12 years	23/65	35.4%
>13 years	23/65	35.4%
Date of execution of current GT (year)	65	-
2003- 2005	21/65	32.3%
2006- 2008	18/65	27.7%
2009- 2015	26/65	40.0%
CD4 absolute count (cels / mm ³) prior to GT	65	(1 to 2.940)
HIV VL (copies/mL) prior to GT	65	40,548.52 (1,009 to 543,897)
ARV regimens prior to GT collection	65	100.0%
2RTI + 1NRTI	28/65	43.1%
2RTI + 1PI	22/65	33.8%
2RTI + 1PI + 1NRTI	4/65	6.2%
2RTI	5/65	7.7%
2PI	3/65	4.6%
Third-line schemes (RAL or T-20)	2/65	3.1%

ARV: antiretroviral; GT: genotypic test; VL: viral load; NRTIs: nucleoside analogue reverse transcriptase inhibitors; NNRTIs: non-nucleoside analogue reverse transcriptase inhibitors; PI: protease inhibitor; RAL: raltegravir; T-20: enfuvirtide.

Table 3. Resistance mutation by ARV classes.

	n	%	
Presence of any mutation of resistance to antiretroviral classes	65	100.0	
NRTIs	64/65	98.46	
NNRTIs	49/65	75.38	
PIs	29/65	44.61	
dual class mutations	NRTIs + NNRTIs	27/65	41.63
	NRTIs + PIs	8/65	12.30
triple class mutations	NRTIs + NNRTIs + PIs	23/65	35.38
Complete NRTIs resistance	36/65	55.38	
Complete NNRTIs resistance	6/65	9.23	
Complete PIs resistance	0/65	0.0	

NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: nonnucleoside reverse transcriptase inhibitors; PIs: protease inhibitors.

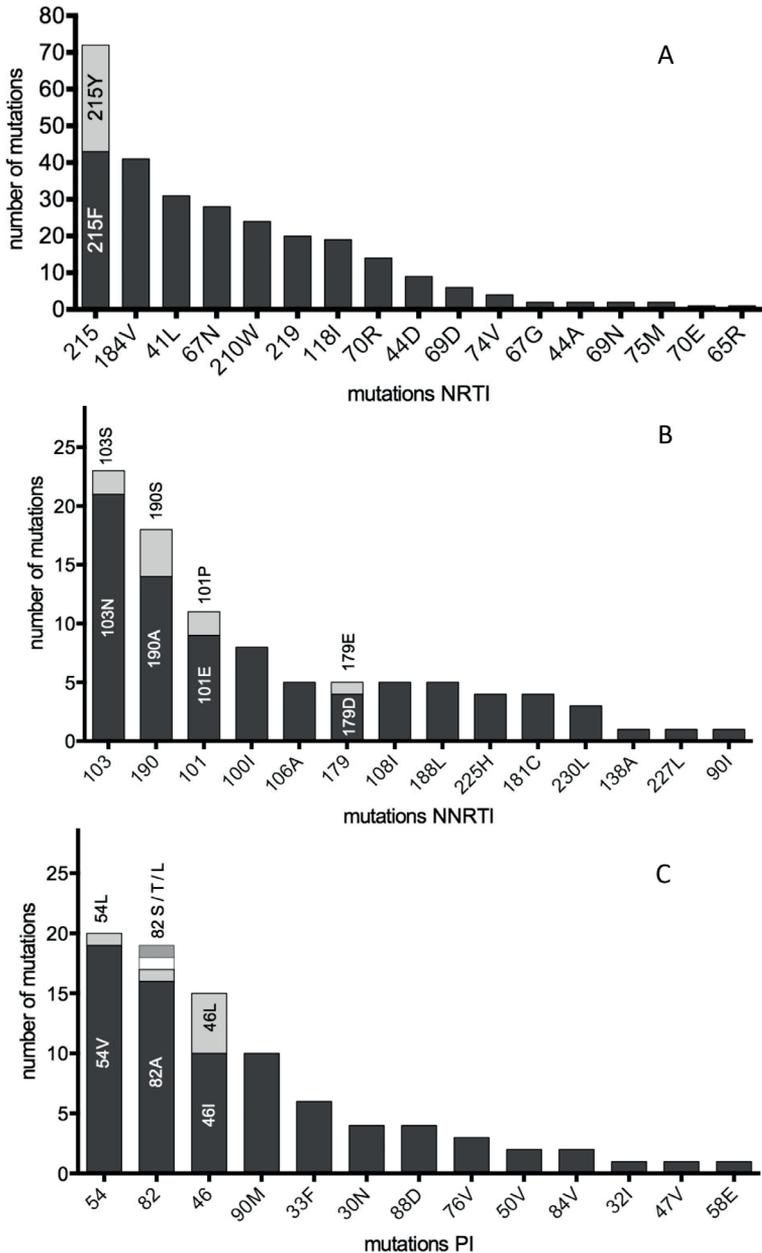


Figure 1. Pattern of resistance mutations: A) Nucleoside reverse transcriptase inhibitors (NRTIs); B) Non-nucleoside reverse transcriptase inhibitors (NNRTIs); C) and protease inhibitors (PIs).

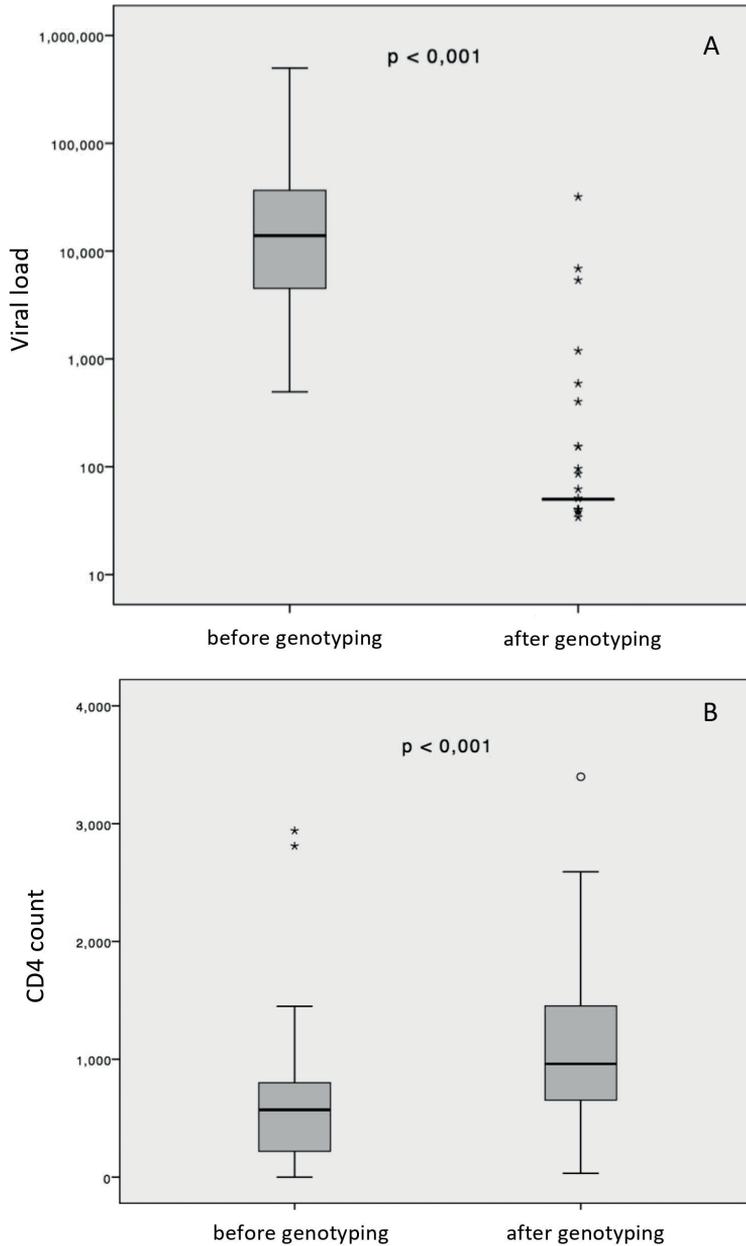


Figure 2. A) Box-plots of viral load before and after antiretroviral treatment directed by the genotyping test. B) CD4 cell count before and after antiretroviral treatment directed by the genotyping test.

DISCUSSION

This study involves genotypic resistance testing in children and adolescents perinatally infected with HIV presenting a high rate of drug resistance mutations in the 3 main classes of ARVs (NRTIs, NNRTIs, PIs). Other authors identified similar results in children and adolescents with antiretroviral therapy failure (De Mulder et al., 2012; Mutwa et al., 2014; Prasitsuebai et al., 2016). A recent study evidenced 36% of patients having at least one resistance mutation related to decreased susceptibility to ART, compared with 100% in our results. Albeit, according to the drug classes used, PIs related mutations were the least frequent leading to drug resistance (Brice et al., 2020).

Our results demonstrated that approximately 90% of patients achieved virologic suppression, i.e., HIV-1 viral load below the detection limits (40 or 50 copies) after 24 weeks of genotypic test-guided changes in combination with an antiretroviral regimen. In a review evaluating 20 studies from Africa (7), North America and Europe (8), Asia (3), and Latin America (2), the proportion of adolescents who became HIV suppressed in a specific period of ARV use ranged from 27% to 89% (Ferrand et al., 2016).

The genotypic resistance test was performed in 68 children and adolescents in a Brazilian study, showing that 58% had one or more NRTI mutations; 51% had NNRTI mutations and 35% had one or more major mutations of IP/r. Resistance was documented in 17% of cases in one class, 21% in two classes and 24% of resistance in the three ARV classes (Almeida et al., 2014; Hunt et al., 2019).

All but one of the patients in our sample had at least one major resistance mutation for the NRTI class. The high resistance rate found in this cohort probably was related to other risk factors (Prasitsuebsai et al., 2016; Salou et al., 2016; Van Dyke et al., 2016) in which patients were exposed to the backbone of the NRTIs association (AZT + ddI; d4T + 3TC or AZT + 3TC) – schemes that were recommended by the Brazilian guidelines from 1992 (MS, www.aids.gov.br/publicações). This finding may be corroborated by previous use of AZT monotherapy or dual therapy prior to the recommendation of combined ARV (Sigaloff et al., 2013).

The accumulation of 3 or more TAM mutations to thymidine analogs occurred in more than half the patients (36/65) and this accumulation was selected by the TAM1 mutational pathway in approximately 1/3 of the patients (19/65). The intensity of cross-resistance depends on specific mutations and number of mutations involved (Kuritzkes et al., 2004; Cunha et al., 2012). The mutations M41L, L210W and T215Y/F characterize the mutational pathway TAM1, which confers higher levels of cross-resistance, mainly in relation to TDF. The presence of at least 3 TAMs, including M41L and/or L210W, compromises NRTI susceptibility (Tang & Shafer, 2012; Wensing et al., 2017).

Among 65 patients included in our analysis, more than 3/4 had at least one NNRTI resistance mutation. The most frequently observed in this class were K103N/S (40.0%), 190A/S (30.8%) and 101E/P/Q (23.1%). These findings are consistent with the prior use of nevirapine (NVP) or efavirenz (EFV) in our patients. EFV and NVP are low-barrier drugs, and the presence of a single mutation confers a high level of resistance (Wensing et al., 2017; Yang et al., 2019). Mutations K103N, 190A and 181C are most commonly associated with previous use of NVP and cause a high level of cross-resistance to other NRTIs (Madruga et al., 2007a e b). The Y181C mutation, in addition to causing resistance to NVP, reduces susceptibility to etravirine (ETR), rilpivirine (RPV) and EFV, whereas K103N and 190A causes a high level of EFV resistance (Wensing et al., 2017). However, K101P, the third most frequent mutation observed here, confers a high level of resistance to NVP, EFV and RPV, and intermediate resistance to ETR. On the other hand, interestingly, in the same codon 101, the 101E mutation confers a lower impact on susceptibility to non-nucleoside analogues (intermediate: NVP and RPV, low resistance level: EFV and ETR.) (Vingerhoets et al., 2010; Tudor-Williams et al., 2014; Stanford, 2015). The prevalence of described NNRTI resistance mutations in pediatric patients with therapeutic failure shows a variation from 12.5% to 95.0% (Angelis et al., 2011; Mutwa et al., 2014; Salou et al., 2016).

Resistance mutations in the protease gene (PR) were also identified and were less frequent than in reverse transcriptase. The presence of any major mutation to protease inhibitors (PI) occurred in 44.6% (29/65). Mutations associated with resistance to protease inhibitors occurred mainly at codons 54, 82 and 46, with rates of 35.4%, 32.3% and 27.7% respectively. The findings observed in the protease resistance profile were consistent with reports from other studies in children and adolescents in therapeutic failure, with prevalence ranging from 10% (Ramkinson et al., 2015) to 88.3% (Dehority et al., 2013). The susceptibility to darunavir/ritonavir (DRV/r) (96.9%) demonstrated in this study, favored therapeutic rescue in children and adolescents treated with several different ARV, presenting resistance to more than one class (Blanche et al., 2009; Salazar et al., 2014; Violari et al., 2015; Huibers et al., 2019), and was similar to that demonstrated in other studies (Rojas Sánchez et al., 2015).

In Central Brazil, several investigations described HIV-1 drug resistance and molecular epidemiology in adults, specifically in pregnant women/newborns, which evidenced up to 16.7% ARV drug resistance (Cardoso et al., 2010; Carvalho et al., 2011; Ferreira et al. 2011; Alcântara et al., 2012; Lima et al., 2016). The HIV-1 subtype B was identified in 65.3% of 49 adults (Silveira et al., 2012) and 62.5% of 142 mothers (Alcântara et al., 2012), quite similar to our results (subtype B found in 84.1% in our pediatric population).

Despite the high ARV resistance observed in our study, susceptibility to some drugs, such as non-peptidic PIs (TPV/re DRV/r) and the new generation of NNRTIs combined with enfuvirtide (T-20) or raltegravir (RAL),

enabled suppression in approximately 90% of children and adolescents. Our data are similar to those found in a Spanish cohort, where 48 adolescents with a genotype resistance test and with extensive experience of ARV therapy presented multidrug resistance in the TR and PR genes. However, in the Spanish cohort, viral suppression (<50 copies/mL) was achieved in 58.3% of regimens containing DRV/r + RAL + etravirine (ETR) (Sanchez et al., 2015).

An extensive resistance profile was noted in children and adolescents with therapeutic failure, predominantly in reverse transcriptase inhibitors. The susceptibility of protease inhibitors allowed the composition of schemes associated with the new class of antiretrovirals (integrase inhibitors and inhibitors of entry).

The increasing prevalence of resistance among children and adolescents perinatally infected by HIV-1 highlights the importance of a specific surveillance of this population regarding long-term drug susceptibility to optimize treatment regimens.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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