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Drug resistance in children and adolescents with HIV in Panama

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Objectives: The inadequacy of resistance monitoring in Latin America leads to circulation of HIV strains with drug resistance mutations (DRMs), compromising ART effectiveness. This study describes the DRM prevalence in HIV-infected paediatric patients in Panama.

Methods: During 2018–19, plasma was collected from 76 HIV-infected children/adolescents (5 ART-naive, 71 treated) in Panama for HIV-1 DRM *pol* analysis, predicted antiretroviral (ARV) susceptibility by Stanford, and HIV-1 variant phylogenetic characterization.

Results: HIV-1 *pol* sequences were recovered from 67 (88.2%) of 76 children/adolescents (median age 12 years), carrying 65 subtype B, 1 subtype G and 1 unique recombinant URF_A1B. Five were ART-naive and 62 ART-treated under virological failure (viraemia >50 copies/mL) with previous exposure to NRTIs, (100%), NNRTIs (45.2%), PIs (95.2%) and integrase strand transfer inhibitors (INSTIs, 17.7%). Among the treated patients, 34 (54.8%) carried resistant strains, with major DRMs to one (40.3%), two (9.7%) or three (4.8%) ARV families. Most of them harboured DRMs to NRTIs (58.5%) or NNRTIs (39%), but also major DRMs to PIs (4.9%) and INSTIs (6.5%). We also found dual-class NRTI + NNRTI (12.2%) and NNRTI + PI (2.6%) resistance. Two naive subjects carried viruses with DRMs to NRTIs and NRTI + NNRTI, respectively. Sequenced viruses presented high/intermediate resistance mainly to emtricitabine/lamivudine (48.9% each) and efavirenz/nevirapine (33.3% each). Most participants were susceptible to PIs (91.3%) and INSTIs (88.1%).

Conclusions: The high DRM prevalence to NRTIs and NNRTIs observed among treated HIV-infected children/adolescents in Panama justifies the need for routine resistance monitoring for optimal rescue therapy selection in this vulnerable population.

Introduction

HIV infection is one of the major causes of mortality and morbidity in resource-limited countries. ART in HIV-infected patients has reduced HIV transmission and AIDS-associated deaths worldwide. WHO guidelines recommending the 'treat all' approach and pre-exposure prophylaxis have succeeded in reaching more people with ART, but carry a risk of the emergence of drug-resistant viruses.[1] The use of ART in HIV-infected mothers and prophylaxis in HIV-exposed newborns to prevent mother-to-child transmission of HIV can result in the selection of viruses carrying drug resistance mutations (DRMs) to antiretrovirals (ARVs) in infected neonates.[2] Due to this and other causes, such as adherence failures, suboptimal blood drug levels or inadequate regimens, the DRM prevalence and the risk of virological failure is higher in children and adolescents than in adults.[3] The high risk of acquiring drug-resistant viruses is of particular concern in settings where ARV options are limited and correct HIV monitoring absent, as in low-middle income countries, where most HIV-infected children and adolescents live.[4] Without resistance monitoring, patients can spend months or even years on a failing ARV regimen, resulting in DRM accumulation and increased rates of morbidity and mortality.[1,5] Thus, determining the rate of resistant viruses circulating in a country is

essential to establish the most effective first-line ART in naive patients and optimize second-line ART in patients who, despite being on treatment and having good adherence, suffer virological failure due to a lack of viraemia control.

With more than 27 million people on ART around the world,[4] WHO considers that the selection of DRMs would compromise treatment efficacy of initial and rescue regimens if adherence or regimens are inappropriate.[6] Consequently, WHO recommends regular monitoring of HIV infection and routine surveillance implementation of HIV drug resistance (HIVDR) in both treated and naive patients in ART programmes in all countries to control the HIV epidemic, mainly in key populations, such as infants and adolescents.[7,8] For children, HIVDR should be monitored at the time of diagnosis and after treatment failure with good adherence,[9] since approximately one-third of the HIV-infected children in the world present virological failure within 2 years of ART.[10]

A large population of children infected with HIV perinatally over the last decade are growing into adolescence.[11] In addition, adolescents are highly vulnerable to HIV infection, mainly those living in settings with a generalized HIV epidemic.[4,11] According to the most recent UNAIDS estimations, adolescents aged between 10 and 19 years living with HIV-1 in the world accounted for over 10% of all new HIV infections globally.[12] However, suboptimal virological suppression fosters the emergence of viruses carrying DRMs, with important consequences for children and adolescents as they require ART for longer periods than adults.[13]

Nowadays, HIV infects over 38 million people worldwide, of whom 1.7 million are children (0–14 years old) and 1.75 million are adolescents (10–19 years old).[11,12] UNAIDS estimated that 31 000 people were living with HIV in Panama in 2020 (1% prevalence in adults and 0.1% in young people).[4] Among them, 59% knew their status, 51% were on ART, 53% maintained a suppressed viral load (VL) and 38% have been diagnosed with <200 T CD4-lymphocyte counts (late HIV diagnosis). In 2020, 1800 new infections and fewer than 500 AIDS-related deaths were estimated in Panama.[4] Since 2010, new HIV infections in Latin America and Panama have increased by 21% and 13%, respectively.[12] Regarding the paediatric population, the most up-to-date data from 2018 revealed that <500 children were HIV-infected in the country, of whom 81% knew their infection, 76% were on ART, and only 58% achieved infection control.[14] Moreover, an alarming 42% of the infected paediatric population in Panama appears to be under ART failure, with unsuppressed VL.[14] However, due to the lack of updated HIVDR surveillance studies in that country,[1] the proportion of therapeutic failures due to poor adherence or to the selection of ARV-resistant viruses is unknown. Knowledge of these results is important to decide the best intervention: strengthening adherence or changing ART.

Since the molecular epidemiology of HIV-1 is constantly changing worldwide,[15] mainly as a result of human movements, it remains important to monitor the arrival of new variants in countries since HIV variability is a real challenge for future vaccine development and the efficiency of ART and molecular tests for HIV diagnosis and quantification.[16–21]

Although HIV-1 subtype B is the most prevalent variant in Latin America,[15] non-B subtypes and recombinant variants have been introduced due to population movements among countries where these variants predominate, such as sub-Saharan Africa or Asia. In Panama, the predominance of subtype B and the introduction of new HIV-1 variants has also been previously described in samples from 2004 to 2013, but no data related to the past decade have been reported.[22–24]

Considering the lack of periodic resistance surveillance and the lack of updated studies on resistance surveillance and molecular epidemiology of HIV-1 in the paediatric population in Panama, the decision was taken to carry out this study. Its main objective was the evaluation of HIVDR in the HIV-infected paediatric population in Panama and the identification of HIV-1 variants that affect this group and its viral evolution.

Materials and methods

Ethics

This research was conducted in accordance with the Declaration of Helsinki and was approved by local Ethics Committees for Clinical Investigation from Hospital Universitario Ramón y Cajal (Madrid, Spain) (ID-117/19) and from Hospital del Niño Doctor José Renán Esquivel (Panamá) (ID-CBIHN-M-201909-003). Informed consent was required for all parents or legal tutors for inclusion in the cohort.

Sample collection

Around 275 paediatric patients are living with HIV in Panama. Paediatric patients are mainly followed up in two hospitals in the country, with a high rate (5%) of perinatal infections. The 58% HIV-infected paediatric population is followed up at the Hospital del Niño Doctor José Renán Esquivel, attending to 160 patients (2 months to 18 years old), with 50% between 15 and 18 years old. Most (95%) were infected perinatally and all are under ART, 43% of them reaching HIV viral suppression. Plasma samples from 76 HIV-positive naive or ART-treated children and adolescents with therapeutic failure under clinical follow-up at this hospital were collected during 2018–19. The samples were kept at −20°C until transported on dry ice to the HIV-1 Molecular Epidemiology Laboratory in Madrid, Spain, where they were stored at −80°C until processing.

Resistance analysis

HIV-1 RNA was extracted from plasma by automated magnetic silica extraction using the EasyMAG extractor (bioMérieux). The extracted RNA was amplified by RT–PCR and nested PCR to obtain the HIV-1 *pol* region, using primers designed by WHO for protease (PR) and reverse transcriptase (RT) amplification and ANRS primers for integrase (IN) amplification,[25,26] as previously described.[27,28] PCR amplicons were purified with illustra™ ExoProStar™ (Cytiva) and sequenced by Macrogen Inc. Lasergene software was used to assemble and manually edit the sequences. Viral sequences included the complete HIV-1 PR (codons 1–99), partial RT (1–345) and IN (48–285) for genotyping study of DRMs to PIs, NRTIs, NNRTIs and integrase strand transfer inhibitors (INSTIs). Stanford HIVdb Program v9.0 (Stanford University, Palo Alto, CA, USA) (https://hivdb.stanford.edu/hivdb/by-sequences/) was used to characterize DRMs in pre-treated children/adolescents and predict the resistance level to 25 ARVs in *pol* genotypes. Transmitted drug resistance (TDR) mutation prevalence was established among the ART-naive population by the WHO TDR list 2009 implemented in the Calibrated Population Resistance (CPR) tool v8.0 (https://hivdb.stanford.edu/cpr/) and by the Stanford algorithm v9.0, both available on the Stanford HIV website (https://hivdb.stanford.edu/).[7]

HIV-1 variant characterization

PR, RT and IN nucleotide sequences were aligned using the ClustalW algorithm implemented in MEGA6 to characterize HIV-1 variants. Phylogenetic trees were reconstructed as previously described,[29] considering a branch support of >70%. For HIV-1 variant classification,

Table 1. Epidemiological and virological features of HIV-1-infected children and adolescents from the complete study cohort at sampling (2018–19)

Epidemiological and clinical features	Naive children	Treated children	Total cohort
N (%)	5 (6.6)	71 (93.4)	76 (100)
Female, *n* (%)	3 (60)	42 (59.2)	45 (59.2)
Median age, years (range)			
At HIV diagnosis in PA	2 (0.04–13)	1 (0.08–7)	1 (0.04–13)
At first ART experience[a]	3 (0.08–13)	1 (0.08–13)	1 (0.08–13)
At sampling[a]	3 (0.58–13)	12 (1–18)	12 (0.58–18)
Route of infection, *n* (%)			
Vertical	4 (80)	67 (94.4)	71 (93.4)
Sexual	1 (20)	1 (1.4)	2 (2.6)
Unknown	0 (0)	3 (4.2)	3 (4)
Prophylaxis, *n* (%)			
Yes[b]	2 (40)	22 (31)	24 (31.6)
No	3 (60)	42 (59.1)	45 (59.2)
Unknown	0 (0)	7 (9.9)	7 (9.2)
CD4 count, cells/mm^3, *n* (%)			
<200	1 (20)	9 (12.7)	10 (13.2)
200–350	1 (20)	5 (7)	6 (7.8)
350–500	0	11 (15.5)	11 (14.5)
500–1000	1 (20)	22 (31)	23 (30.3)
>1000	1 (20)	23 (32.4)	24 (31.6)
Unknown	1 (20)	1 (1.4)	2 (2.6)
Comorbidities, *n* (%)			
0	3 (60)	37 (52.1)	40 (52.6)
1	2 (40)	13 (18.3)	15 (19.8)
2	0	2 (2.8)	2 (2.6)
Unknown	0	19 (26.8)	19 (25)
Current number of ART regimens at sampling, *n* (%)			
1	—	24 (33.8)	—
2	—	21 (29.6)	—
3	—	12 (16.9)	—
4	—	5 (7)	—
5	—	2 (2.8)	—
6	—	3 (4.3)	—
7	—	2 (2.8)	—
8	—	2 (2.8)	—
NRTI experience, *n* (%)		71 (100)	
3TC	—	70 (98.6)	—
TDF	—	12 (16.9)	—
ZDV	—	70 (98.6)	—
D4T	—	8 (11.3)	—
ABC	—	15 (21.1)	—
ddI	—	6 (8.5)	—
FTC	—	8 (11.3)	—
NNRTI experience, *n* (%)		32 (45.1)	
EFV	—	32 (45.1)	—
PI experience, *n* (%)		68 (95.8)	
LPV/r	—	68 (95.8)	—
DRV/r	—	5 (7)	—
NFV	—	4 (5.6)	—
INSTI experience, *n* (%)		11 (15.5)	
RAL	—	11 (15.5)	—
Therapeutic delay, *n* (%)			
Immediate	3 (60)	45 (63.4)	48 (63.2)
1–6 months	1 (20)	21 (29.6)	22 (29)
7–11 months	0 (0)	2 (2.8)	2 (2.6)
1–7 years	1 (20)	2 (2.8)	3 (3.9)
Unknown	0 (0)	1 (1.4)	1 (1.3)
HIV-1 viral load, copies/mL, *n* (%)			
<50	0	0	0
51–199	0	0	0
200–499	0	7 (9.9)	7 (9.2)
500–999	0	4 (5.6)	4 (5.3)
1000–4999	0	21 (29.6)	21 (27.6)
5000–9999	0	10 (14.1)	10 (13.2)
10 000–49 999	1 (20)	15 (21.1)	16 (21)
50 000–499 999	2 (40)	12 (16.9)	14 (18.4)
>500 000	2 (40)	2 (2.8)	4 (5.3)
200–1000	0	11 (15.5)	11 (14.5)
>1000	5 (100)	60 (84.5)	65 (85.5)
PCR amplification success according to VL, *n* (%)			
200–1000	0	6 (54.5)	6 (54.5)
>1000	5 (100)	56 (93.3)	61 (93.8)
Patients with available *pol* HIV-1 sequence, *n* (%)	5 (100)	62 (87.3)	67 (88.2)
PR	5 (100)	41 (66.1)	46 (68.7)
RT	4 (80)	41 (66.1)	45 (67.2)
IN	5 (100)	62 (100)	67 (100)
Only PR	0	0	0
Only RT	0	0	0
Only IN	0	19 (30.7)	19 (28.4)
PR + RT	0	0	0
PR + IN	1 (20)	2 (3.2)	3 (4.5)
RT + IN	0	2 (3.2)	2 (3)
PR + RT + IN	4 (80)	39 (62.9)	43 (64.2)
HIV-1 variant prevalence, *n* (%)			
B subtype	5 (100)	60 (96.8)	65 (97)
G subtype	0	1 (1.6)	1 (1.5)
URF_A1B	0	1 (1.6)	1 (1.5)

PA, Panama; cells/mm^3, T CD4 + lymphocyte count/mm^3 of blood; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; D4T, stavudine; ABC, abacavir; ddI, didanosine; FTC, emtricitabine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; DRV/r, darunavir/ritonavir; NFV, nelfinavir; RAL, raltegravir; copies/mL, copies of HIV-1 RNA/mL; IN, integrase.

[a]Three naive subjects started the first ART at sampling.

[b]The two naive children had received zidovudine and lamivudine as prophylaxis before sampling.

we used reference sequences from each HIV-1 group M subtype, subsubtype and circulating recombinant form (CRF).[30] Sequences not clustering with any known subtype or CRF were analysed using the Recombination Detection Program (RDP3v4.13),[31] identifying the subtypes involved in eventual recombination events and hypothetical

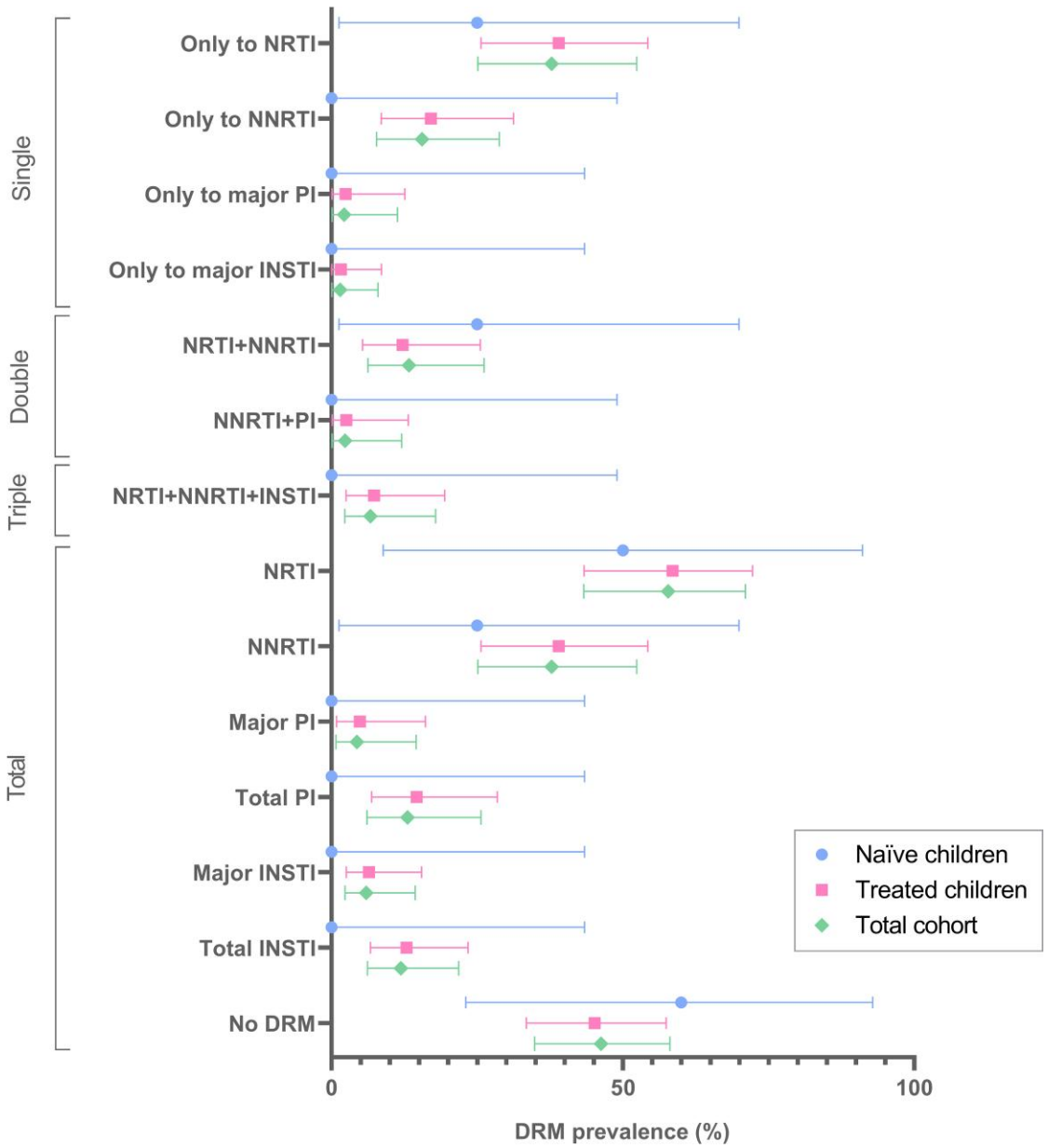

Figure 1. Percentage of HIV-infected paediatric patients carrying DRMs to the main ARV families in Panama (2018–19). Mean prevalence (coloured figures) and 95% CIs in the 67 children/adolescents under study with available *pol* sequence: 46PR, 45RT and 67IN. 'Single' is resistance to one ARV family, 'double' to two ARV families, 'triple' to three ARV families. 'Total' covers resistance by ARV family. 'No DRMs' means no major DRMs found in the available sequenced *pol* regions per patient. DRMs to PIs and INSTIs are always major unless indicated otherwise. More data are available in Table S1. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

recombination breakpoints. To further confirm the detected putative recombination events, new phylogenetic analyses were performed using the sequence fragments assigned to different subtypes according to the proposed breakpoint position(s) defined by RPD3. In the positive cases, the recombinant sequences were redefined as unique recombinant forms (URFs).

Statistical analysis

Medians were assessed for data not normally distributed. The statistical significance was calculated using Fisher's exact test or chi-squared test for categorical variables and Mann–Whitney test for continuous variables. Two-sided P values of <0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism v8.0.1. The percentage of viruses carrying DRMs was calculated with 95% CIs.

Accession numbers

HIV-1 sequences were submitted to GenBank with the following accession numbers: OM201778–OM201846.

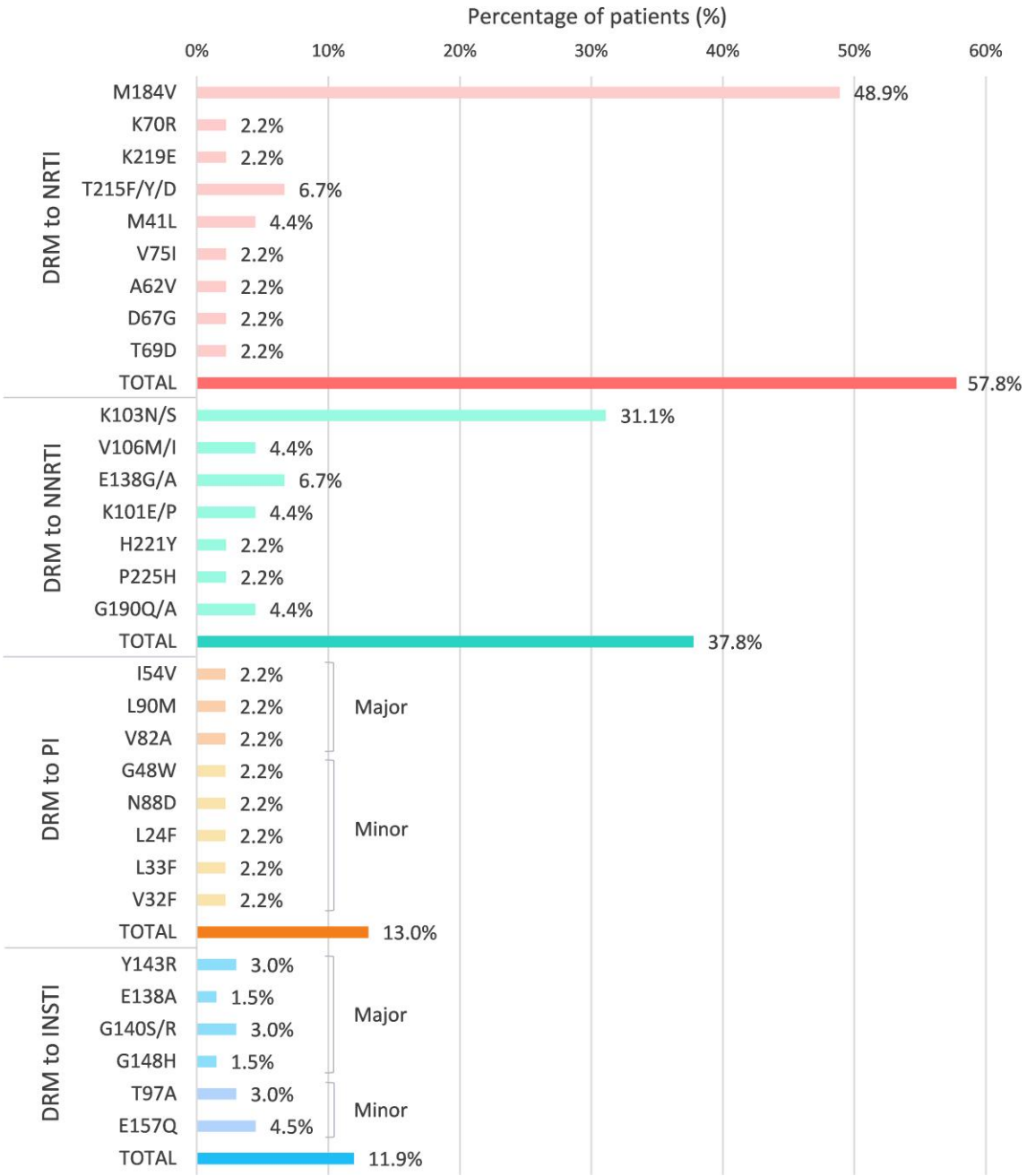

Figure 2. DRMs to the main ARV families in the study population. Available sequences in 67 children/adolescents under study: 46PR, 45RT and 67IN. We considered major and minor DRMs to PIs, INSTIs, NRTIs and NNRTIs, according to Stanford v9.0. More data are available in Table S2. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Results

Study cohort

Plasma samples were collected from 76 HIV-infected children/adolescents under clinical monitoring at the Hospital del Niño Doctor José Renán Esquivel in Panama, five of them drug-naive and 71 ART-experienced at sampling, exhibiting >50 HIV-1 RNA copies/mL of plasma. Table 1 summarizes the epidemiological and virological features of the 76 HIV-1-infected children/adolescents that made up the complete study cohort at sampling (2018–19). The median age at diagnosis and first ART was 1 year (14 days to 13 years), while it was 12 years (7 months to 18 years) at sampling. Nearly all (97.3%) the children with available information acquired HIV infection by mother-to-child transmission; only two subjects were infected by sexual transmission. Around half (65.2%) of the 69 with available data did not receive prophylaxis. Out of the 24 (34.8%) subjects with previous prophylaxis, 75% had received zidovudine, 20.8%

Table 2. DRMs to the main ARV families found in the 11 HIV-1-infected treated children/adolescents with experience of INSTIs

ID	Age at sampling (years)	Number of different ART regimens at sampling	INSTI	DRMs to NRTIs	DRMs to NNRTIs	DRMs to PIs		DRMs to INSTIs	
						Major	Minor	Major	Minor
HDN-001	18	8	RAL	—	—	—	—	Y143R	T97A
HDN-019	18	7	RAL	None	None	None	None	None	None
HDN-025	17	7	RAL	—	—	—	—	None	None
HDN-026	16	6	RAL	M184V	None	None	None	None	None
HDN-046	7	2	RAL	—	—	None	None	None	None
HDN-055	4	2	RAL	M184V	None	None	L24F	None	None
HDN-067	17	6	RAL	None	K103N	I54V, V82A	L33F	None	None
HDN-068	16	4	RAL	M184V	None	None	None	None	None
HDN-069	18	2	RAL	K70R	None	None	None	None	None
HDN-073	15	6	RAL	M184V	K103N, P225H	—	—	Y143R	None
HDN-076	16	3	RAL	None	None	None	N88D	None	None

Available sequences in 11 treated children/adolescents from Panama under study with experience to INSTIs: 8PR, 8RT, 11IN. Patients' ID codes were provided in the laboratory after sample reception to maintain their anonymity. RAL, raltegravir; —, HIV-1 *pol* region not available. ARV susceptibility by Stanford of these children can be found in Figure S1.

zidovudine + lamivudine and one patient efavirenz + lamivudine. Among the 74 (97.4%) children/adolescents with known CD4 data, 10 (13.5%) presented <200 cells/mm^3 at sampling, revealing a delay in HIV diagnosis. Most (70.2%) of the 57 subjects with documented data lacked comorbidities.

At sampling, all 71 treated children/adolescents had received NRTIs, 45.1% NNRTIs, 95.8% PIs and just 11 (15.5%) patients were INSTI-experienced (Table 1). The 71 treated subjects were under their first (33.8%), second (29.6%) or third (16.9%) ART regimen, and 14 (19.7%) had received four to eight different ART regimens at sampling, having failed at least three previous ART regimens. Most treated children (98.6%) had received lamivudine + zidovudine, followed by lopinavir/ritonavir (95.8%) and efavirenz (45.1%). About half (64.3%) of the 70 treated children with available data had received ART immediately after HIV diagnosis. However, four (5.7%) suffered a long delay in treatment, ranging from 7 months to 7 years from the first diagnosis and the start of ART. Three of the five naive children started ART immediately upon HIV diagnosis, right when the analysed sample was collected. However, the remaining two started ART 5 months and 3 years, respectively, after HIV diagnosis.

HIV-1 DRM analysis

HIV-1 *pol* sequences (46PR/45RT/67IN) were recovered from 67 (88.2%) of 76 children/adolescents (median age 12 years), five of them ART-naive and 62 treated at sampling (Table 1). Two (40%) of the five naive children had received prophylaxis with zidovudine + lamivudine, one for 27 days and the other for 1 month. Figure 1 and Table S1 (available as Supplementary data at *JAC* Online) indicate the percentage of patients carrying DRMs to the main ARV families in ART-naive, treated and the total paediatric population studied with available *pol* sequence. Table 1 shows the PR, RT and IN sequences recovered in naive (5PR/4RT/5IN) and treated (41PR/41RT/62IN) subjects. We were able to amplify 6 (54.5%) of the 11 samples with VL ≤ 1000 copies/mL and 61 (93.8%) of the 65 samples with >1000 copies/mL (Table 1).

Among the 67 subjects with sequence, 36 (53.7%) were infected with viruses carrying major DRMs to one (38.8%), two (10.4%) or three (4.5%) ARV families, while the remaining 46.3% did not show any major DRMs in the sequenced regions. When minor DRMs to INSTIs and PIs were also considered, 37 (55.2%) patients carried viruses with DRMs to one (29.9%), two (20.9%) or three (4.5%) ARV families. None of the patients with the three regions available presented DRMs to the four drug families. DRMs to NRTIs were identified in 57.8% (95% CI, 43.3–71) of the available *pol* sequences, DRMs to NNRTIs in 37.8% (95% CI, 25.1–52.4), major DRMs to PIs in 4.3% (95% CI, 0.8–14.5), minor DRMs to PIs in 10.9% (95% CI, 4.7–23), major DRMs to INSTIs in 6% (95% CI, 2.3–14.4) and minor DRMs to INSTIs in 7.5% (95% CI, 3.2–16.3). Dual-class NRTI + NNRTI resistance was present in 13.3% (95% CI, 6.3–26.2) of the available sequences (Figure 1).

All 62 children/adolescents treated with *pol* sequence had received NRTIs, 45.2% non-NRTIs, 95.2% PIs and only 11 (17.7%) INSTIs. Despite the use of ART, all treated children/adolescents with *pol* sequence showed virological failure (VL > 50 copies/mL). Among them, 34 (54.8%) carried resistant strains, with major DRMs to one (40.3%), two (9.7%) or three (4.8%) ARV families (Table S1). Most of them harboured DRMs to NRTIs [58.5% (95% CI, 43.4–72.2)] or NNRTIs [39% (95% CI, 25.7–54.3)], but also major DRMs to PIs [4.9% (95% CI, 0.9–16.1)] and to INSTIs [6.5% (95% CI, 2.5–15.4)] (Figure 1). Dual-class NRTI + NNRTI and NNRTI + PI resistance appeared in 12.2% (95% CI, 5.3–25.5) and 2.6% (95% CI, 0.1–13.2) of patients, respectively. Two naive subjects carried viruses resistant to NRTIs and NRTI + NNRTI, respectively. None of the six ART-treated subjects with VL ≤ 1000 copies/mL carried DRMs in available *pol* sequence.

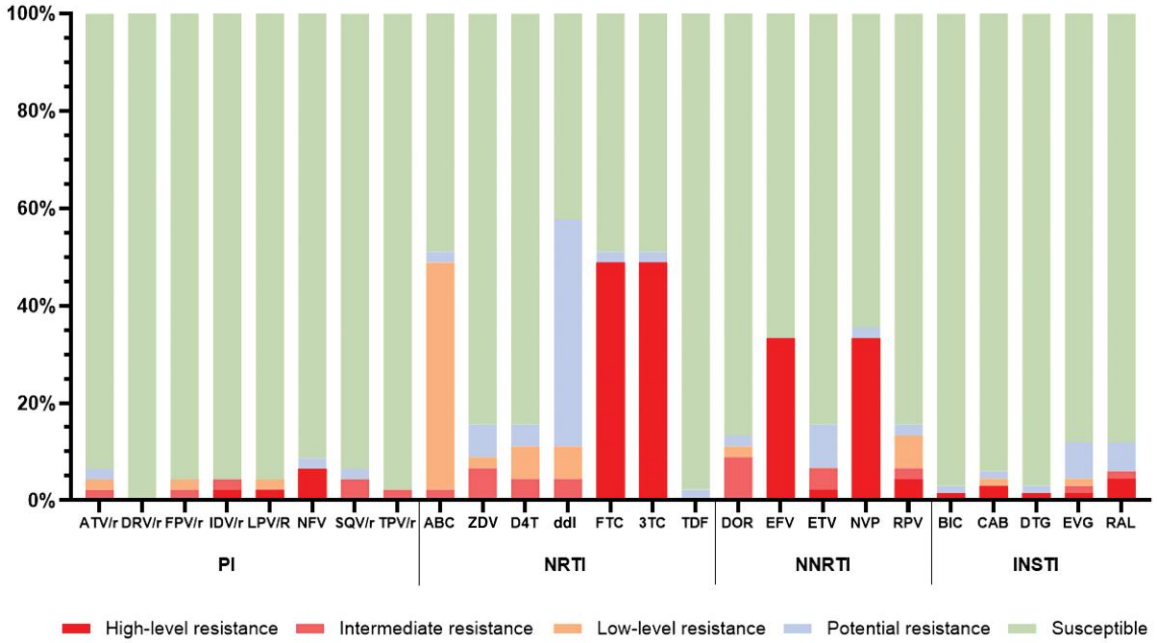

Figure 3. Predicted ARV susceptibility by Stanford in HIV-1-infected children and adolescents in Panama with available *pol* sequence from samples collected during 2018–19. Predicted ARV susceptibility in 67 available sequences (46PR/45RT/67IN) according to Stanford. ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; FPV/r, fosamprenavir/ritonavir; IDV/r, indinavir/ritonavir; LPV/r, lopinavir/ritonavir; NFV, nelfinavir; SQV/r, saquinavir/ritonavir; TPV/r, tipranavir/ritonavir; ABC, abacavir; ZDV, zidovudine; D4T, stavudine; ddI, didanosine; FTC, emtricitabine; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; DOR, doravirine; EFV, efavirenz; ETV, etravirine; NVP, nevirapine; RPV, rilpivirine; BIC, bictegravir; CAB, cabotegravir; DTG, dolutegravir; EVG, elvitegravir; RAL, raltegravir. Information per patient is shown in Figure S1. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

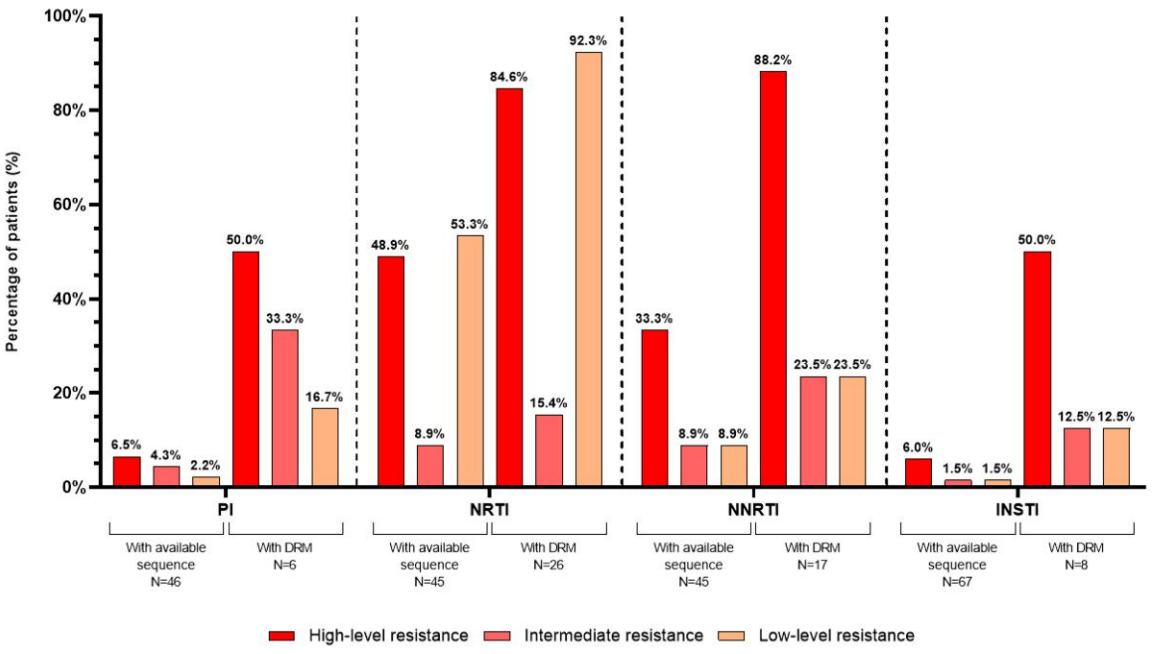

Figure 4. DRM prevalence based on predicted ARV susceptibility by Stanford in HIV-1-infected children and adolescents in Panama with available *pol* sequence or major and minor DRMs. Resistance prevalence in 67 children/adolescents under study with available sequences (46PR/45RT/67IN) and with DRMs to the main ARV families (6 PI/26 NRTI/17 NNRTI/8 INSTI). Information per patient is shown in Figure S1. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 3. Historic ARV regimens in eight HIV-1-infected treated children/adolescents carrying major and minor DRMs to INSTIs

ID	Age at sampling (years)	Route of infection	First-line ART	Second-line ART	Third-line ART	Fourth-line ART	Fifth-line ART	Sixth-line ART	Seventh-line ART	Eighth-line ART
HDN-001[a]	18	Vertical	ZDV + 3TC + LPV/r	ddI + 3TC + LPV/r	D4T + 3TC + LPV/r	TDF + 3TC + LPV/r	ZDV + 3TC + LPV/r	ZDV + 3TC + EFV	ZDV + 3TC + ABC	ZDV + 3TC + **RAL**
HDN-009	14	Unknown	TDF + FTC + EFV							
HDN-011	0.6	Vertical	ZDV + 3TC + LPV/r							
HDN-036	9	Vertical	ZDV + 3TC + LPV/r							
HDN-040	9	Vertical	ZDV + 3TC + LPV/r							
HDN-041[a]	12	Vertical	ZDV + 3TC + LPV/r	LPV/r + EFV						
HDN-044	8	Vertical	ZDV + 3TC + LPV/r							
HDN-073[a]	15	Vertical	ZDV + 3TC + LPV/r	ddI + 3TC + LPV/r	D4T + 3TC + LPV/r	ZDV + 3TC + LPV/r	ZDV + 3TC + EFV	ZDV + 3TC + LPV/r + **RAL**		

Patients' ID codes were provided in the laboratory after sample reception to maintain their anonymity. 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; D4T, stavudine; ABC, abacavir; ddI, didanosine; FTC, emtricitabine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; RAL, raltegravir (in bold).

[a]These adolescents were on a rescue regimen; the other children/adolescents were on first-line ART regimen at sampling.

Table 4. DRMs to the main ARV families found in the eight HIV-1-infected treated children/adolescents carrying viruses with major and minor DRMs to INSTIs

			DRMs to PIs		DRMs to INSTIs	
ID	DRMs to NRTIs	DRMs to NNRTIs	Major	Minor	Major	Minor
HDN-001	—	—	—	—	Y143R	T97A
HDN-009	M184V, T215F, K219E	G190Q	None	None	E138A, G140S, Q148H	None
HDN-011	M184V	None	None	None	None	E157Q
HDN-036	M184V	None	None	None	None	E157Q
HDN-040	M184V, T215D	V106I	None	None	G140R	None
HDN-041	None	K101P, K103N, H221Y	None	None	None	E157Q
HDN-044	M184V	None	None	None	None	T97A
HDN-073	M184V	K103N, P225H	—	—	Y143R	None

Available sequences in eight treated children/adolescents from Panama under study carrying DRMs to INSTIs: 6PR, 7RT, 8IN. Patients' ID codes were provided in the laboratory after sample reception to maintain their anonymity. —, HIV-1 *pol* region not available. ARV susceptibility by Stanford of these children can be found in Figure S1.

In the five ART-naive children/adolescents at sampling, no major or minor DRMs to PIs or INSTIs was found. However, two (50%) of the four naive subjects with RT sequence presented TDR to RT inhibitors, one child to NRTI + NNRTI and another to NRTIs (Figure 1). Global major TDR prevalence for both NRTIs and NNRTIs was 25% according to the WHO TDR list 2009. When analysed by Stanford v9.0, TDR prevalence to NNRTIs was maintained (25%), increasing to 50% to NRTIs. One child carried A62V, V75I (NRTIs) and K103N (NNRTIs), while the other child presented M41L (NRTIs). None had received prior prophylaxis.

Figure 2 shows DRMs to the four main ARV families found in the 67 HIV-infected children/adolescents with available *pol* sequence, and Table S2 provides data comparing naive versus treated paediatric patients. Among the 57.8% subjects carrying DRMs to NRTIs, the most frequent DRM was M184V (48.9%), followed by T215F/Y/D (6.7%) and M41L (4.4%). Among the 37.8% of those carrying DRMs to NNRTIs, the most prevalent changes were K103N/S (31.1%), E138G/A (6.7%) and V106M/I/K101E/P/G190Q/A (4.4% each). Major and minor DRMs to PIs were identified in 6 (13%) of 46 subjects with PR sequence, 2 of them presenting major DRMs (I54V, L90M and V82A) and 5 with minor DRMs. Among the 8 (11.9%) subjects carrying resistant viruses with DRMs to INSTIs of the 67 with IN sequence, 4 were infected with viruses harbouring major DRMs (Y143R, G140S/R, E138A and G148H) and 5 with minor DRMs.

Among the 71 treated children/adolescents, 14 (19.7%) had failed more than three different ART regimens at sampling. Eleven out of 14 had available *pol* sequence and two-thirds of them (63.6%) presented DRMs to NRTIs (57.1%) and NNRTIs

Table 5. Studies reporting HIVDR data and HIV-1 variants in Panama

	Study				
	Ahumada-Ruiz *et al.* (2009)[24]	Castillo *et al.* (2011)[42]	Mendoza *et al.* (2014)[22]	Mendoza *et al.* (2016)[23]	Present study
Patients (naive/treated)	135 (53/82 with AIDS)	72 (72/0)	655	717 (250/467)	76 (5/71)
Children/adolescents	0	25	53	0	76
Adults	135	47	602	717	0
Sampling years	2004–05	2007–09 (children) 2008–10 (adults)	2007–13	2007–13	2018–19
Patients with sequences (PR/RT/IN)	135 (135/135/0)	72 (72/72/0)	655 (655/655/0)		67 (46/45/67)
TDR prevalence in naive, *n* (%)	0	3/25 (12) 6/47 (12.8)	nd	23/250 (9.2)	2/5 (40)
To NRTIs	0	2/25 (8) 4/47 (9)	nd	16/250 (6.4)	1/4 (25)
To NNRTIs	0	1/25 (4) 2/47 (4)	nd	19/250 (7.6)	1/4 (25)
To PIs	0	0/25 (0) 1/47 (2)	nd	3/250 (1.2)	0
To INSTIs	nd	nd	nd	nd	0
DRMs in treated, *n* (%)	8/82 (9.7)	nd	nd	409/467 (87.6)	34/62 (54.8)
To NRTIs	4/82 (4.9)	nd	nd	ns	24/41 (58.5)
To NNRTIs	5/82 (6.1)	nd	nd	ns	16/41 (39)
To PIs	4/82 (4.9)	nd	nd	ns	6/41 (14.6)
To INSTIs	nd	nd	nd	nd	8/62 (12.9)
HIV variants					
Subtype B, *n* (%)	133 (98)	nd	648 (98.9)	717 (100)	65 (97)
Non-B variants, *n* (%)	2 (1.5)	nd	7 (1.1)	0	2 (3)
Specific non-B variants	1 CRF02_AG/A3 1 CRF12_BF/B	nd	1 subtype F1, 1 subtype C, 3 URFs_BC, 1 CRF20_BG, 1 CRF28/29		1 subtype G 1 URF_A1B

nd, not done; ns, not specified in the paper. The percentages of resistant viruses were calculated taking into account major and minor DRMs. HIV-1 variants by phylogeny.

(28.6%) and major DRMs to PIs (33.3%) and INSTIs (18.2%) (Table S3). The rate of subjects carrying DRMs to the main ARV families was higher (63.6%) in those under four or more ART regimens at sampling (Table S3).

The DRM prevalence to the main ARV families found in the 11 HIV-1-infected treated children/adolescents with INSTI experience (all with raltegravir) is shown in Table 2. Among the sequenced viruses, 5 carried DRMs to NRTIs (M184V or K70R), 2 to NNRTIs (P225H and/or K103N), 1 to PIs (I54V and V82A, major DRMs) and 2 to INSTIs (Y143R, major DRM).

We also described the historical ART regimens until sampling of the eight HIV-1-infected treated children/adolescents harbouring major and minor DRMs to INSTIs (Table 3). The specific DRMs found to the four main ARV families are summarized in Table 4, the most prevalent changes being M184V (six patients) and K103N (two patients) in RT and Y143R (two subjects) in IN.

Predicted ARV susceptibility

The predicted ARV susceptibility to 25 ARVs by Stanford in the 67 HIV-1-infected children/adolescents from Panama with *pol* sequence is shown in Figure 3 and in each subject, including the ARV experience, in Figure S1. When considering intermediate and high resistance levels, half of the population (53.3%) were infected with viruses resistant to NRTIs, 33.3% to NNRTIs, 6.5% to PIs and 6% to INSTIs. In more detail, viruses presented high/intermediate resistance to: emtricitabine and lamivudine (48.9% each); efavirenz and nevirapine (33.3% each); doravirine (8.9%); nelfinavir (6.5%); raltegravir (6%); and zidovudine, rilpivirine and etravirine (6.7% each), among others. Most children/adolescents with available *pol* sequences in Panama were infected with viruses susceptible to PIs (91.3%) and INSTIs (88.1%), representing interesting alternatives for rescue ART regimens if required.

Figure 4 shows the DRM prevalence based on predicted ARV susceptibility by Stanford in HIV-1-infected children and adolescents in Panama with available *pol* sequence or major and minor DRMs, revealing that most subjects carrying resistant viruses presented high-resistance level to NRTIs (84.6%) and NNRTIs (88.2%), as well as 3 of 6 and 4 of 8 children/adolescents with DRMs to PIs and INSTIs, respectively. Considering all subjects with available sequence, 48.9% presented high-level resistance to NRTIs, 33.3% to NNRTIs and only 6.5% to PIs and 6% to INSTIs.

HIV-1 viral variants in the study cohort

Most (97%) paediatric subjects from Panama with *pol* sequence under study were infected with HIV-1 subtype B, except two treated subjects, one carrying subtype G and the other carrying URF_A1B. Among them, no transmission clusters were found.

Discussion

This study provides the most recent data on resistance to ARVs among HIV-infected children and adolescents in Panama and it is the first to report resistance data for INSTIs in Panama, which is appropriate after the broad implementation of dolutegravir.

Treatment options for children and adolescents lag behind those for adults, making outcomes consistently worse.[32] Regarding specific drugs, HIV treatment guidelines recommend an oral INSTI as the preferred ARV for individuals initiating therapy because of their efficacy in controlling VL, safety and ease of use.[33] Dolutegravir is a second-generation HIV INSTI that has proven effective in trials involving adults,[34,35] being rapidly rolled out in national treatment programmes. INSTI-based regimens are nowadays considered the first choice in paediatric clinical guidelines.[36,37] Recently, a clinical trial showed that dolutegravir-based therapy was superior to the standard of care in children and adolescents as first- and second-line therapy.[38] Dolutegravir-based ART also presented an excellent profile leading to a high rate of virological suppression in a Spanish cohort of HIV-infected children and adolescents.[39]

At the time of sampling, the basis of paediatric ART in Panama was triple therapy with zidovudine + lamivudine + lopinavir/ritonavir.[40] In children over 3 years old, the use of efavirenz was considered, although the majority continued to be treated with lopinavir/ritonavir. Since 2013, the combination of tenofovir + emtricitabine + efavirenz was used for older patients. They also employed darunavir and raltegravir as rescue regimens. In our study paediatric cohort, none of the 11 INSTI-exposed children and adolescents under virological failure had received dolutegravir, since it was not included in clinical guidelines in Panama at the time of the study.[40] It was not until 2020 that lopinavir/ritonavir was replaced by dolutegravir for children and tenofovir + lamivudine + dolutegravir was switched for adolescents and adults.

There is a lack of updated data regarding the circulation of resistant strains in the country. Only four studies reported data on HIVDR in Panama with samples collected during 2004–13, summarized in Table 5.[22–24,41] Only one of them analysed TDR in 25 HIV-infected naive children sampled during 2007–09, reporting TDR to RT inhibitors in 12% of them, with two children presenting TDR to NRTIs and one DRM to NNRTIs.[41] Our study found high DRM prevalence among treated subjects, showing that one in two ART-experienced patients under therapeutic failure with available *pol* sequence carried resistant strains. Moreover, one out of three subjects presented intermediate- or high-level resistance to efavirenz and nevirapine, and one in two to emtricitabine and lamivudine. None of the naive children showed high- or intermediate-level resistance to any ARV, except for one child who harboured viruses resistant to efavirenz and nevirapine. Interestingly, most patients with sequenced viruses were susceptible to INSTIs (88.1%) and PIs (91.3%). These findings support alternative ART regimens based on PIs and INSTIs instead of RT inhibitors in HIV-infected children and adolescents in this country, mainly in those under therapeutic failure. Our findings also manifest the need to detect HIVDR in both the naive and treated paediatric populations of Panama to optimize first and rescue ART for those subjects carrying resistant viruses.

Among the 54.8% of treated children with sequence carrying DRMs in the study population during 2018–19, we found major DRMs to NRTIs (58.5%), NNRTIs (39%), INSTIs (6.5%) and PIs (4.9%). Although there are no records of DRMs in the treated HIV-infected paediatric population of Panama, the observed DRM prevalence in the treated study cohort sampled during 2018–19 (54.8%) was lower than published data in the general population during 2007–13 (87.6%),[23] which was higher than the 9.7% reported in adults between 2004 and 2005.[24] The use of NNRTI- and NRTI-based regimens as first-line ART in children

leads to an increase in the selection of resistant viruses. These two ARV families are reaching higher resistant levels in people on ART, which may jeopardize the recycling of NRTIs as second-line ART.[1] Thus, new ART guidelines promote the use of an INSTI in the first-line therapy, with less toxicity and a higher genetic barrier.

In the study cohort, two children and nine adolescents were INSTI-experienced with raltegravir on their rescue ART regimen, with only two adolescents carrying Y143R (IN), a major DRM providing high resistance to raltegravir (Table 2); all of them were susceptible to bictegravir and dolutegravir (Figure S1). However, we still found that eight treated children/adolescents carried major and minor DRMs to INSTIs (Table 4), probably transmitted by the HIV-infected mother, as most acquired HIV infection by the vertical route, compromising alternative conventional treatment options. Unfortunately, the ART data and resistance profiles of the mothers were not available to confirm INSTI exposure and the presence of DRMs. One 14-year-old female adolescent was under her first ART regimen with emtricitabine (NRTI) and efavirenz (NNRTI) but presented high resistance to the five INSTIs under study and to some NRTI and NNRTI drugs (Figure S1). This adolescent died before the results were delivered and the mother's resistance profile was unknown.

As expected, adolescents had experienced more treatment failures than children at sampling (Table S3), with a higher percentage of them on their third or more different ART regimens (62.2%). Meanwhile, all 29 treated children under study were under their first or second line. According to Table S3, one-third of adolescents under four or more ART regimens at sampling did not harbour resistance viruses, suggesting a lack of adherence to treatment.

Regarding adherence, we observed that half (45.2%) of treated subjects with *pol* sequence under study (10/25 children, 18/37 adolescents) showed adherence failure to ART, since they were under virological failure (>50 copies/mL) without resistant viruses (Table S1), suggesting the need to reinforce the adherence in these groups to prevent treatment dropouts. A previous study conducted in Panama analysed factors associated with adherence to ART in HIV-1-infected adolescents, highlighting psychological status, behaviour, adult supervision and social context, among others.[42] It also emphasizes the need to identify these factors that influence the lack of adherence in adolescents to control the HIV infection in this risk population.

NRTI and NNRTI resistance levels in people starting ART are a health concern, as their prevalence among naive infants is over 10% in some countries with NNRTI-based first-line ART.[1] Latin American countries have reported moderate levels of TDR (<10%) in adults: 5.7% in El Salvador,[27] 5.8% in Colombia,[43] 6.8% in Mexico (NRTIs: 4.2%, NNRTIs: 2.5%, PIs: 1.7%),[44] 7.0% in Honduras (NRTIs: 3%, NNRTIs: 5%, PIs: 0.5%),[45] 7.3% in Guatemala (NRTIs: 1.8%, NNRTIs: 4.9%, PIs: 1%),[46] and 9.5% in Brazil (NRTIs: 3.6%, NNRTIs: 5.8%),[47] with an overall TDR prevalence of 7.7% in Latin America.[48] Compared with previous reports, TDR prevalence in Panama has been changing from 0% in 2005,[24] 12% in 2007–09[41] and 9.2% in 2007–13[23] to 40% in 2018–19 (depending on the TDR list) in the present study. However, due to the low number of sequences for TDR analysis, new studies with larger study cohorts of ART-naive subjects should be performed in future for a better estimation of TDR prevalence in the HIV-infected paediatric population in Panama. Consequently, continuous molecular epidemiological surveillance of HIV-1 Latin American epidemics is crucial to determine whether TDR prevalence in this region and country will remain stable or not in the following years.

Our results showed that one out of four naive children/adolescents at sampling presented TDR to NRTIs according to both the WHO and Stanford v9.0 lists. However, TDR prevalence to NRTIs was twice as high by Stanford versus WHO (50% versus 25%), reinforcing the need for a WHO TDR list 2009 update. In fact, the Stanford v9.0 algorithm includes some polymorphic DRMs absent in the WHO TDR list, such as V75I and A62V at RT (present in our study cohort), both affecting NRTI, as well as others providing resistance to one or more NNRTI drugs, such as V106I, E138A, V179D/E/T and K238N, among others.

DRMs to NRTIs and NNRTIs are approaching alarming resistance levels. The increase in drug-resistant viruses leads to a lack of viraemia control, with a consequent rise in virological failure cases. The delay in ART failure identification due to resistant viruses can lead to DRM accumulation, affecting the rescue treatment efficacy, and decreasing CD4 counts in patients. Considering these facts, the WHO recommends that countries where the national prevalence of resistance to the NNRTIs efavirenz or nevirapine in populations initiating first-line ART exceeds 10% should consider transitioning to an alternative ARV drug, such as dolutegravir, as opposed to NNRTI-based first-line therapy.[49]

Regarding HIV-1 variants circulating in Panama, subtype B was the predominant variant in our study, as previously reported,[22–24] although non-B variants were also identified in samples collected before 2013: CRF02_AG/A3 and CRF12_BF/B,[24] URFs_BC, CRF20_BG and CRF28/29_BF.[22] We identified subtype G and URF_A1B strains in two children. HIV-1 non-B variants increased from 1.1% and 1.5% to 3% (present study),[22,24] suggesting that the prevalence of non-B variants in Panama may increase in the following years. Since HIV-1 genetic variability can affect ART monitoring, leading to VL underestimation or RNA detection failure in some cases,[19,50] periodic HIV-1 molecular epidemiology studies in the country could be useful.

The high (>10%) DRM prevalence to NRTIs and NNRTIs observed among treated HIV-infected children/adolescents in Panama justifies the need for routine resistance monitoring for optimal rescue therapy selection in this vulnerable population. These results also reinforce the need to fast-track the transition to dolutegravir-based first-line regimens and to use PI-based ART when dolutegravir cannot be administered, following WHO recommendations.[1] These measures would help to control the spread of resistant HIV and to reach the 95:95:95 UNAIDS targets in Panama. Furthermore, HIVDR monitoring will help to identify which treatment failure cases are due to resistant viruses or adherence failure. It will allow for a switch to an appropriate salvage therapy in patients infected by resistant strains or to strengthen adherence if ART failure is caused by poor adherence to treatment. This will also help us to optimize second-line ART in treated patients harbouring DRMs and to establish the most appropriate first-line ART in naive subjects.

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Transparency declarations

None to declare and none conflict of interest.

Supplementary data

Figure S1 and Tables S1 to S3 are available as Supplementary data at *JAC* Online.

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