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Publication date
2013

Link to publication

Citation for published version (APA):
Sigaloff, K. C. E. (2013). *HIV drug resistance among adults and children in sub-Saharan Africa*. [Thesis, fully internal, Universiteit van Amsterdam].

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Chapter 11

HIV-1-resistance-associated mutations after failure of first-line antiretroviral treatment among children in resource-poor regions: a systematic review

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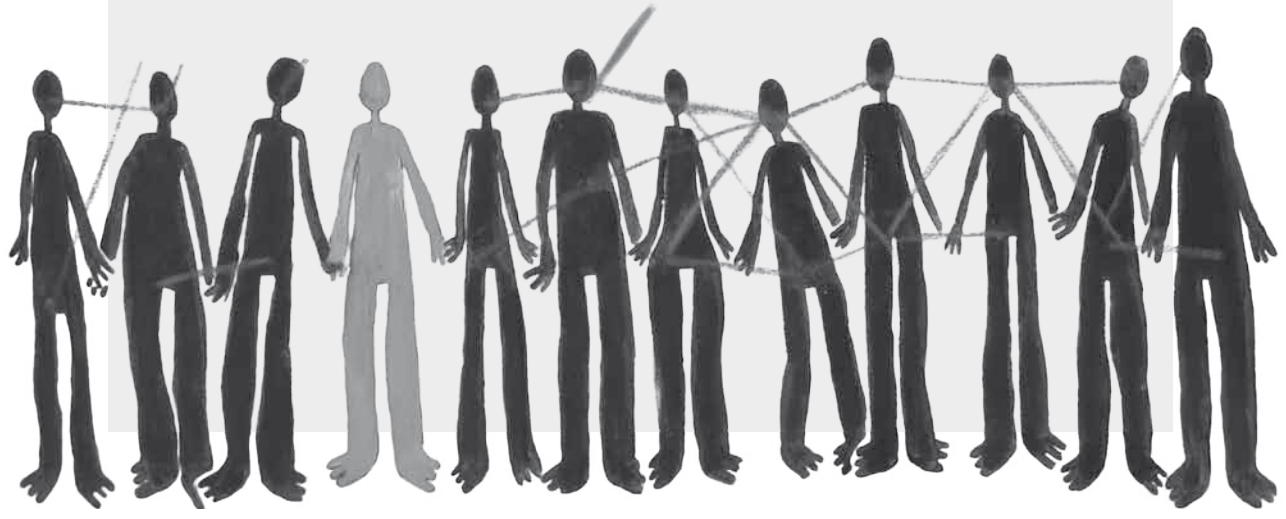
The Lancet Infectious Diseases 2011; 11(10): 769-79.



ABSTRACT

HIV-positive children are at high risk of drug resistance, which is of particular concern in settings where antiretroviral options are limited. In this Review we explore resistance rates and patterns among children in developing countries in whom antiretroviral treatment has failed. We did a systematic search of online databases and conference abstracts and included studies reporting HIV-1 drug resistance after failure of first-line pediatric regimens in children (<18 years) in resource-poor regions (Latin America, Africa, and Asia). We retrieved 1312 citations, of which 30 studies reporting outcomes in 3241 children were eligible. Viruses with resistance-associated mutations were isolated from 90% (95% CI 88–93%) of children. The prevalence of mutations associated with nucleoside reverse transcriptase inhibitors was 80%, with non-nucleoside reverse transcriptase inhibitors was 88%, and with protease inhibitors was 54%. Methods to prevent treatment failure, including adequate pediatric formulations and affordable salvage treatment options are urgently needed.

INTRODUCTION

Children are highly vulnerable to HIV infection, which can be transmitted from mother to child before, during, or after delivery.[1] Rates of disease progression and death in vertically infected infants are higher than those in older children or adults.[2,3] More than half of HIV-positive children who do not receive treatment are estimated to die before age 2 years.[4] Antiretroviral therapy (ART) has strikingly reduced morbidity and mortality in children.[5–7] In 2008, an estimated 38% of all children in need of treatment worldwide were receiving ART.[8] In view of treatment guidelines, which recommend that all HIV-positive children younger than 24 months should start treatment, irrespective of clinical or immunological status,[9] the uptake of ART is expected to increase.

The probability of virological suppression and long-term treatment success is lower in children than in adults.[10,11] Younger children typically have poorer virological responses than do older children,[12] which is associated with high viral loads before treatment[13] and the risk of sub-therapeutic drug concentrations (<90% of current recommended dose) owing to limited pediatric formulations, variable pharmacokinetics, and changes in bodyweight because of growth.[14] Furthermore, adherence is frequently hindered by multiple practical and social barriers throughout childhood and adolescence.[15] Suboptimum virological suppression might lead to the emergence of drug resistant virus, the consequences of which are particularly important for children who will need ART for longer periods of time than adults.

Most children with HIV infection live in resource-poor settings[16] and could, therefore, be most at risk of developing resistance to ART. Exposure to nevirapine for the prevention of vertical HIV transmission, especially for long periods of time, increases the risk of infection with drug resistant virus in infants and of treatment failure with standard first-line regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTIs).[17] Virological monitoring should be used to establish the optimum first-line therapy and time points for regimen switching in children. Identification of resistance mutations by genotype testing could further aid appropriate sequencing and rational use of antiretroviral medication. Financial and technical constraints, however, prevent routine use of viral load tests in developing countries, and information on resistance rates associated with pediatric antiretroviral treatment in these settings is scarce. At the same time, preservation of the first-line regimen is essential for patients living in resource-poor settings, as options are limited to two lines of ART.

We report a systematic review of the literature on acquired drug resistance among children in resource-poor settings after failure of ART. We explore the current knowledge of

resistance rates and patterns in relation to first-line pediatric regimens to gain insight into optimum switching strategies.

METHODS

Search strategy and selection criteria

We systematically searched PubMed, Embase, and Web of Science for articles available before March 1, 2010, with the following terms: "HIV", or "AIDS" or "human immunodeficiency virus" or "acquired immunodeficiency syndrome" AND "antiretroviral*" or "ARV" or "ART" or "HAART" AND "resistance" or "genotyp*" AND "paediatric" or "pediatric" or "infant*" or "child*" or "adolescent*". We manually searched the abstract databases of the following selected conferences held between Jan 1, 2008, and March 1, 2010: the International AIDS Society Conference (2008 and 2009); the Conference on Retroviruses and Opportunistic Infections (2008, 2009, and 2010); the International HIV Drug Resistance Workshop (2008 and 2009); and the International Workshop on HIV Pediatrics (2009). Retrieved reports were also manually screened for further potentially relevant full articles and abstracts.

We included original studies in which HIV-1-infected children were treated with first-line ART in Africa, Latin America, and Asia. Included studies had to report data on the frequency of HIV drug resistance in children who had been treated for at least 6 months. Clinical trials, observational cohort studies, and cross-sectional studies published in English were eligible.

We excluded studies in which patients were infected with HIV-2, were older than 18 years, lived in resource rich regions, were still receiving monotherapy or dual therapy at the time of study, or who had started second-line ART. Studies reporting HIV drug resistance in children before ART was started (i.e., transmitted or acquired resistance related to the use of antiretroviral drugs for the prevention of mother-to-child transmission) were not eligible. Previous use of drugs for prophylaxis of transmission or treatment was allowed.

After removal of duplicates, reports were initially assessed by title and abstract. For those not excluded, full-length versions were retrieved and assessed. If the description of the study was unclear in terms of eligibility criteria, we contacted the authors for further information. Eligible reports were independently reviewed by two authors (KCES and JCJC) and any disputes about inclusion were resolved by discussion between these authors.

Data extraction

We extracted data on locations and time frames of studies, type of study, method of analysis (intention to treat or on treatment), number of participants and age at entry, duration of follow-up, ART regimens, definitions of treatment failure, proportion of children with virological failure, virological cutoff for genotype tests, number of HIV genotype resistance tests, and proportion of children infected with HIV-1 and with at least one resistance-associated mutation.

Statistical analysis

We summarized drug resistance data. Mutations were analyzed according to the International AIDS Society 2009 update of drug resistance mutations.[18] Polymorphisms occurring in wild-type viruses and minor mutations were not included. We classified resistance mutations as being associated with nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs, or protease inhibitors (PIs). We calculated frequencies of specific mutations.

The proportions of children infected with HIV-1 that had at least one drug resistance mutation were pooled, irrespective of the type of first-line regimen. We used the Cochran Q test to assess heterogeneity between eligible studies.[19,20] In case of heterogeneity, random effects models were used to pool data. We used fixed effects models in homogeneous study sets. Virological outcomes were not pooled but were reported as the median (range) of failure rates at follow-up per study. Most studies presented on-treatment analyses and, therefore, the virological failure percentage for all studies was calculated with this approach.

RESULTS

1312 articles and abstracts were deemed potentially eligible after duplicate retrieved articles were removed. On the basis of the title and abstract, 1183 reports were excluded (figure 1) because they dealt with obviously distinct issues, such as comorbidity or adverse drug reactions, or because they did not report original data. Of the remaining 129 studies, 30 met the inclusion criteria, including 15 full-text papers and 15 conference abstracts (figure 1) and involved 3241 children. Among these, treatment was deemed to have failed in 1307 (40%).

15 studies reported virological failure and associated drug resistance levels in longitudinal cohorts of children receiving ART (n=2545). The other 15 studies described the cross-sectional analyses of HIV drug resistance rates and patterns of treatment failure

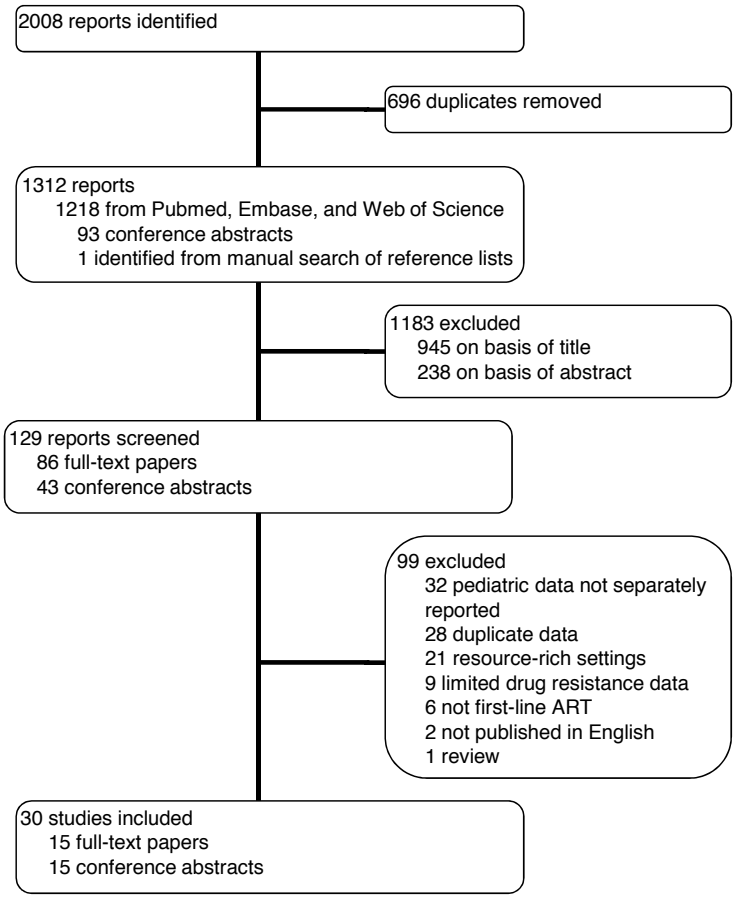


Figure 1. Search strategy and results. ART, antiretroviral therapy.

in children (n=696). None of the studies reported a clinical trial. Studies were done in 15 countries: nine in sub-Saharan Africa, three in Latin America, and three in Asia (table 1, table 2). Most of the included studies were published after 2007; six reports were published between 2003 and 2007 (table 1, table 2).

In the cohort studies the median duration of treatment ranged from 6 months to 50 months (table 1). Virological failure was defined as an HIV-RNA rebound of more than 50 copies per mL (one study), 400 copies per mL (seven studies), 500 copies per mL (one study), or 1000 copies per mL (six studies). Across the studies with the cutoff of 1000 copies per mL, treatment failure was seen in a median of 26% of patients (range 14–40%). Among the studies with more stringent definitions of virological failure, the median proportion of virological failure was 36% (range 13–71%). The highest proportion of failure (71%) was seen in an Argentinean cohort with the smallest sample size

Table 1. Summary of virological outcome and HIV-1 drug resistance data in cohort studies

	Number of patients	Median age at entry (years)	Regimen		Median follow-up (months)	Previous ART	Analysis	Viral detection cut-off (cps/ml)	Proportion with virological failure (%)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI							Any	NRTI	NNRTI	PI
Argentina 2008[21]	21	1	100%		6	NA	OT	>400	71%	15	100%	NA	NA	NA
Cambodia 2003-2005[63]	212	6	100%		16.6	Unspeci-fied 3%	OT/ITT	>400	19/26%*	36	94%	86%	89%	0
Thailand, 2002-2006[64]	202	7.1†	100%		50	sdNVP 2%	OT/ITT	>1000	21/26%*	39	97%	87%	95%	0
Central African Republic 2008[39]	52	7.9	81%	19%	6	NA	OT	>500	52%	26	77%	77%	54%	12%
Côte d'Ivoire 2000-2003[65]	115	6.35	30%	70%	10.2	Dual therapy 3%	OT	>1000	34%	38	71%	66%	24%	42%
Kenya 2001-2008[23]	90	7.9†	92%	8%	24	None	OT	>400	36%	32	67%	56%	38%	9%
Mali 2006-2007[66]	97	2.5	100%		6	NA	OT/ITT	>400	44/61%*	30	73%	70%	70%	0
Mozambique 2003-2007[67]	495	4	100%		18.5	sdNVP 0.2%, ZDV 0.6%	OT	>50	27%	84	92%	88%	92%	0
South Africa 2004-2008[68]	335	8.6†‡	74%	26%	>6	NA	OT	>1000	14%	46	87%	NA	NA	NA
South Africa 2009[53]	257	all < 2		100%	12	sdNVP 100%	OT	>400	13%	34	76%	74%	6%	35%
Uganda 2007[22]	104	0.5-1.8**	100%		12	sdNVP 34%	OT	>400	42%	15	100%	100%	100%	0
Uganda 2010[69]	126	NA	100%		24.8	NA	OT	>1000	14%	14	100%	100%	86%	0

Table 1 (continued)

	Number of patients	Median age at entry (years)	Regimen		Median follow-up (months)	Previous ART	Analysis	Viral detection cut-off (cps/ml)	Proportion with virological failure (%)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI							Any	NRTI	NNRTI	PI
Uganda 2004-2005[70]	250	9.2†	100%		12	NVP or ZDV 4%	OT/ITT	>400	26/34%*	3	100%	100%	100%	0
Uganda 2004-2006[71]	86	5.4-5.5**	100%		12 (n=59) 24 (n=27)	NA	OT	>1000	40%	33	88%	88%	85%	0
Zambia 2003-2005[72]	103	8	100%		24	NA	OT (n=74)	>1000	30%	26	92%	>77%	92%	0

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NA, not available; sdNVP, single dose nevirapine; NVP, nevirapine; ZDV, zidovudine; OT, on treatment; ITT, intention to treat.* Virological failure in on-treatment or intention-to-treat analysis; ** range of medians; † mean; ‡ at time of treatment failure.

Table 2. Summary of HIV-1 drug resistance profiles in cross-sectional studies

	Number of patients	Median age (years)	Regimen			median follow-up (months)	Previous ART	Viral detection cut-off (cps/ml)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI	NNRTI + PI					Any	NRTI	NNRTI	PI
Argentina 2006-2008[73]	36	7.7	39%	61%		62.4	NA	NA	36	89%	89%	50%	53%
Brazil[74]	12	5.5	8%	75%	17%	36	NA	> 1000	10 RT, 12 PR regions	90%	80%	30%	75%
Brazil 2000-2001[30]	41	5.5	56%,	37%	7%	12	Mono/dual therapy 78%, ART 12 %	> 80	36 RT, 38 PR regions	94%	61%	56%	37%

Table 2 (continued)

	Number of patients	Median age (years)	Regimen			median follow-up (months)	Previous ART	Viral detection cut-off (cps/ml)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI	NNRTI + PI					Any	NRTI	NNRTI	PI
Brazil 2000-2006[75]	103	9†	22%	14%	63%	NA	NA	>1000	92	>90%	90%	70%	55%
Mexico[76]	25	10.9†		64%	36%	NA	NA	NA	25	72%	72%	24%	60%
Cambodia 2008[77]	53	10.9	100%			26.4	Unspecified 8%	NA	38	97%	>79%	97%	0
China 2005-2007[78]	41	11	100%			12	adult formulations 51%	> 1000	34	100%	>79%	100%	0
Thailand 2000-2007[79]	21	4.1	100%			6.3	NA	> 1000	21	57%	52%	43%	0
South Africa 2007-2008[38]	30	2.3		LPV/r 73%, RTV-sPI 27%		NA	RTV-sPI 30%	> 400	30	83%	83%	0	43%
South Africa[80]	25	7.3†	92%	8%		29.6	NA	NA	25	92%	>80%	>44%	4%
South Africa 2000-2003[81]	39	6	38%	62%		NA	Unspecified 28%	NA	39	92%	>37%	>20%	>20%
South Africa 2007[82]	86	NA	71%	29%		19.2	NA	> 1000	86	>79%	>79%	64%	3%
South Africa[83]	109	7.6†*	59%	41%		NA	NA	> 5000	109	92%	>71%	47%	14%
Senegal[84]	40	NA	100%			6	NA	> 1000	40	73%	68%	78%	13%
Uganda[85]	35	NA	100%			>13	NA	NA	35	100%	100%	100%	0

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NA, not available; RT, reverse transcriptase; PR, protease; LPV/r, Lopinavir/ritonavir; RTV-sPI = ritonavir as single protease inhibitor. † mean; * n=41.

(n=21) and a short follow-up time of 6 months.[21] For children receiving regimens that included NNRTIs or PIs the median failure rates were 29% (range 14–71%) and 54% (range 13–90%), respectively.

Virological failure in the cross-sectional studies was defined as an HIV-RNA rebound of more than 80 copies per mL (one study), 400 copies per mL (one study), 1000 copies per mL (six studies), or 5000 copies per mL (one study); six studies did not define the cutoff values (table 2). The duration of ART before treatment failure was available in ten studies and ranged from 6 months to 62 months (table 2).

Across all studies, antiretroviral regimens were specified for 2952 (91%) children. Most children (n=2303, 78%) were treated with an NNRTI-based regimen that contained either nevirapine or efavirenz. PI-based regimens were reported in 569 (19%) patients. In southern Africa, ritonavir-boosted lopinavir and full-dose ritonavir were the most commonly used PI agents. Children in Latin America received nelfinavir and, occasionally, ritonavir or indinavir. In this region, regimens combining a PI and an NNRTI plus one or two NRTIs were used in 79 (3%) children. In west Africa, nelfinavir and indinavir were used. In all regions, the NRTI backbones were stavudine, zidovudine, and lamivudine, and occasionally didanosine. One child received a triple NRTI regimen. Previous use of antiretroviral drugs was reported in 12 studies and included single-dose nevirapine or long-term nevirapine or zidovudine for the prevention of perinatal transmission (seven studies) and use of these drugs in monotherapy or dual therapy regimens (two studies). Maternal exposure to antiretroviral drugs was only reported in two studies and involved single-dose nevirapine.

HIV-1 subtypes were determined for 598 children in 13 (43%) studies (table 3). The most prevalent was subtype C, which was noted in 252 (42%) patients and seen mainly in southern Africa. Subtype B was detected 108 times (18%) in Latin America and Asia. The recombinant form CRF02_AG was found in 91 patients (15%) and occurred almost uniquely in central and west Africa. Subtypes A and D were found in 23 (4%) and 14 (2%) patients, respectively, mostly in east Africa. In Latin America subtypes F and BF mosaics were found in ten (2%) and 33 (6%) patients, respectively.

The reverse transcriptase and protease regions were sequenced, respectively, in 1127 (86%) and 1131 (87%) of 1307 children. Genotype tests were done with commercial assays in 29 (97%) studies; in one study an in-house assay was used.[31] The proportion of patients in which the virus had at least one resistance-associated mutation ranged from 57% to 100%. The pooled proportion of children with virus with any resistance-associated mutation was 90% (95% CI 88–93). In a subgroup analysis done according

Table 3. Number of children receiving antiretroviral treatment, by HIV-1 subtype and region

HIV-1 subtypes	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
A	23 (4%)			5 (4%)	16 (26%)	2 (1%)
B	108 (18%)	74 (62%)	34 (100%)			
C	252 (42%)	2 (2%)			2 (3%)	248 (98%)
D	14 (2%)			3 (2%)	8 (13%)	3 (1%)
A/D/C*	35 (6%)				35 (56%)	
F	10 (2%)	10 (8%)				
BF mosaics	33 (6%)	33 (28%)				
CRF02_AG	91 (15%)			90 (70%)	1 (2%)	
CRF11_cpx	11 (2%)			11 (8%)		
CRF06_cpx	5 (<1%)			5 (4%)		
CRF01_AE	4 (<1%)			4 (3%)		
Other CRF	12 (2%)			12 (9%)		
Total	598	119	34	130	62	253

* Subtypes not specified further; CRF, circulating recombinant form.

to geographical regions, the pooled proportions of drug resistant HIV among children in whom treatment had failed varied from 75% (67–82) in central and west Africa to 96% (91–100) in Asia (figure 2). A second subgroup analysis to test the frequency of resistance-associated mutations in relation to duration of ART showed at least one mutation at the time of treatment failure in 76% (69–83) of children who had received ART for less than 1 year, and that this proportion notably increased with longer duration of treatment (97%, 94–99; figure 3)

In 27 (90%) studies the rates of resistance associated with specific drug classes were reported for 1027 children. All these children had been exposed to NRTIs and 80% (95% CI 75–86; range 52–100) had at least one related resistance mutation associated. NRTI resistance varied by geographical region and ranged from 70% (95% CI 62–78) in central and west Africa to 95% (95% CI 89–100) in east Africa (figure 2). For children exposed to NNRTIs (n=774), HIV-1 with mutations conferring resistance was seen in 88% (95% CI 84–92; range 43–100). Within the different regions the respective prevalence ranged from 79% (95% CI 71–87) in central and west Africa to 91% (95% CI 83–99) in Asia (figure 2). Among children who had used PIs (n=320) 54% (95% CI 39–69; range 12–86) were infected with HIV-1 that had at least one major protease mutation. These mutations were detected in 76% (95% CI 39–69) of children in Latin America, 51% (95% CI 35–67) in central and west Africa, and 31% (95% CI 18–43) in southern Africa. No children in Asia received PIs. In one study in east Africa,[23] three (60%) of five children exposed to PIs had major associated mutations (figure 2).

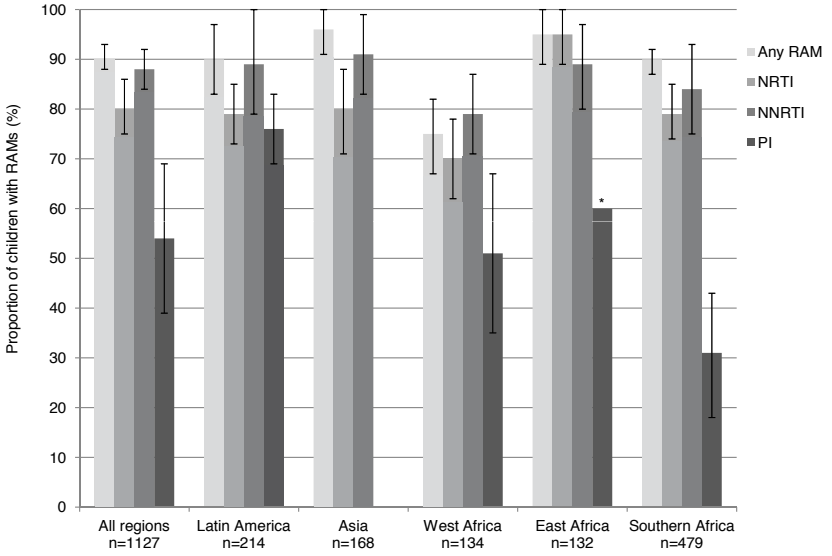


Figure 2. Frequency of at least one resistance-associated mutation, by region and drug class. Error bars indicate 95% CI. RAM, resistance-associated mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. * Proportion reported in one study.

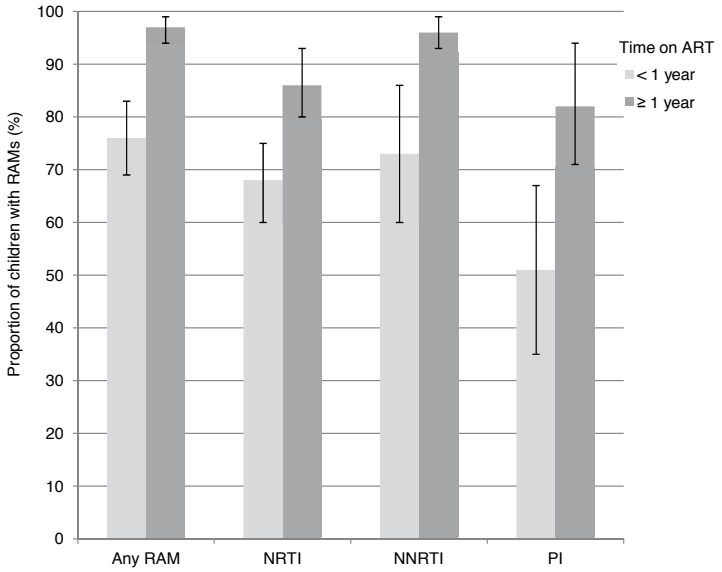


Figure 3. Frequency of resistance-associated mutations in relation to duration of ART. Error bars indicate 95% CI. ART, antiretroviral therapy; RAM, resistance-associated mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

The frequencies of specific resistance-associated mutations were provided in 24 (80%) studies, for 934 children, all of whom had been exposed to NRTIs. 695 (74%) patients had also been treated with NNRTIs and 297 (32%) had received a PI. Major mutations occurred at 36 different codons (table 4). All 16 NRTI-associated mutations listed in the 2009 update of the International AIDS Society drug resistance mutations were reported.[18] The most prevalent mutation was the M184V mutation, which was recorded in 587 (63%) patients. Thymidine analogue mutations were seen in 522 (56%) patients. The mutations K65R and Q151M were reported in 25 (3%) and 21 (2%) patients, respectively. Mutations at nine different codons associated with NNRTI resistance were reported. The K103N mutation was the most frequently detected (260 [37%] occurrences). Other frequently reported mutations were Y181C/I (112 [16%]), G190S/A and G190A (each 90 [13%]), and V106A/M (67 [10%]). 11 different major PI-associated protease mutations were noted. The most frequent were V82A (51 [17%] occurrences), L90M (44 [15%]), and I54V (36 [12%]).

By region, the most frequent NRTI-associated mutation was M184V (40% in Latin America, range 60–76% across regions), and of the thymidine analogue mutations it was T215F/Y (57% in Latin America, 16% in Asia, and 5% in Africa). In the NNRTI class, the most frequent overall was K103N (60% in Latin America, range 23–42% across regions), although V106A/M was predominant in southern Africa (21%), and Y181C/I was predominant in Asia (31%). Protease mutations were seen predominantly in Latin America and southern Africa; the D30N, M46I/L, I84V, and L90M mutations occurred most frequently in Latin America.

DISCUSSION

We identified 15 peer-reviewed papers and 15 abstracts that provided information on the emergence of drug resistance to ART among children in resource-poor regions. Most studies originated from sub-Saharan Africa. At least one resistance-associated mutation was found in 90% of children in whom first-line ART had failed. The drug-class-specific resistance rates were 88% for NNRTIs, 80% for NRTIs, and 54% for PIs. Despite geographical and virological differences, the drug resistance rates are similar to those reported for children living in Europe.[51–53] The risk of drug resistance increased with the duration of ART.

When we compared the frequencies and patterns of mutations between the regions included in this systematic review we noted several important differences. The frequencies of reverse transcriptase and protease mutations in Latin America contrasted greatly with those in other regions: the M184V mutation, which is rapidly selected by lamivu-

Table 4. Number of children with reported major drug-associated mutations in children who experienced treatment failure, by drug class and region

Associated with NRTIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
M41L	92 (10%)	66 (48%)	10 (8%)	3 (2%)		13 (3%)
A62V	6 (<1%)			2 (1%)	1 (1%)	3 (<1%)
K65R	25 (3%)		8 (6%)	3 (1%)	3 (3%)	11 (3%)
D67N	113 (12%)	55 (40%)	19 (15%)	4 (3%)	4 (4%)	31 (7%)
T69ins	5 (<1%)		4 (3%)	1 (<1%)		
T69S/A/D/N	27 (3%)	17 (12%)		2 (1%)	3 (3%)	5 (1%)
K70R	79 (8%)	32 (23%)	9 (7%)	8 (6%)	5 (5%)	25 (6%)
L74V	14 (1%)	2 (1%)	2 (2%)	2 (1%)	1 (1%)	7 (2%)
V75I	12 (1%)		4 (3%)		2 (2%)	6 (1%)
V75A/L/M	3 (<1%)	3 (2%)				
F77L	3 (<1%)		3 (2%)			
Y115F	4 (<1%)		3 (2%)		1 (1%)	
F116Y	3 (<1%)	1 (<1%)				2 (<1%)
Q151M	21 (<1%)		9 (7%)			12 (3%)
M184V	587 (63%)	55 (40%)	92 (71%)	80 (60%)	75 (76%)	285 (66%)
M184I	25 (3%)					25 (6%)
L210W	61 (7%)	40 (29%)	10 (8%)	4 (3%)	1 (1%)	6 (1%)
T215F/Y	134 (14%)	79 (57%)	21 (16%)	7 (5%)	5 (5%)	22 (5%)
T215I/D/N	4 (<1%)			2 (1%)	1 (1%)	1 (<1%)
K219Q/E	43 (5%)	1 (<1%)	12 (9%)	2 (1%)	4 (4%)	24 (6%)
K219N/K	1 (<1%)			1 (<1%)		
Total*	934	138	130	134	99	433
Associated with NNRTIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
L100I	18 (3%)	15 (15%)	2 (2%)	1 (1%)		
K101E/H	47 (7%)	13 (13%)		6 (6%)	5 (5%)	23 (8%)
K103N	260 (37%)	61 (60%)	38 (29%)	22 (23%)	25 (27%)	114 (42%)
V106A/M	67 (10%)	5 (5%)		3 (3%)	1 (1%)	58 (21%)
V106V/I	2 (<1%)			2 (2%)		
V108I	20 (3%)	2 (2%)	9 (7%)	1 (1%)	4 (4%)	4 (1%)
Y181C/I	112 (16%)	14 (14%)	40 (31%)	16 (16%)	11 (12%)	31 (11%)
Y188L	22 (3%)	2 (2%)	3 (2%)	3 (3%)	2 (2%)	12 (4%)
Y188H	1 (<1%)			1 (1%)		
G190A	38 (5%)	2 (2%)	10 (8%)	9 (9%)	10 (11%)	7 (3%)
G190A/S	52 (7%)	15 (15%)	7 (5%)			30 (11%)
G190S/E	1 (<1%)			1 (1%)		
P225H	17 (2%)		2 (2%)	1 (1%)		14 (5%)
Total*	695	101	130	97	94	273

Table 4 (continued)

Associated with PIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
D30N	13 (4%)	12 (13%)				1 (<1%)
V32I	3 (1%)	2 (2%)				1 (<1%)
M46I	20 (7%)	3 (3%)		3 (8%)	2 (40%)	12 (8%)
M46I/L	27 (9%)	26 (27%)		1 (3%)		
I47V	5 (2%)	5 (5%)				
I50V	1 (<1%)					1 (<1%)
I54V	36 (12%)	11 (12%)		1 (35)		24 (15%)
I54V/L	1 (<1%)			1 (3%)		
L76V	1 (<1%)					1 (<1%)
V82A	51 (17%)	12 (13%)				39 (24%)
V82F	1 (<1%)			1 (3%)		
V82I	5 (2%)			5 (14%)		
V82A/F/T/S	31 (10%)	24 (25%)				7 (4%)
I84V	11 (4%)	11 (12%)				
N88S	11 (4%)			8 (22%)	2 (40%)	1 (<1%)
N88D	5 (2%)	4 (4%)				1 (<1%)
L90M	44 (15%)	28 (29%)		8 (22%)	1 (20%)	7 (4%)
L90A	3 (1%)					3 (2%)
Total*	297	95		37	5	160

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. Thymidine analogue mutations are indicated in **bold**; * Number of children with exposure to specific drug class.

dine or emtricitabine, was seen less frequently in Latin American studies, whereas more thymidine analogue mutations were reported. These differences might be explained by the use of regimens in Latin America that combine NNRTIs, PIs, and one or more NRTIs but do not always contain lamivudine or emtricitabine.[54] In other regions, where lamivudine or emtricitabine are generally included in triple ART regimens, the M184V mutation was more likely to be the first mutation to appear and the emergence of thymidine analogue mutations was delayed.[55] Furthermore, previous exposure to ART drugs in monotherapy or dual therapy regimens or for the prevention of vertical transmission, might have occurred more often in Latin America. In Brazil, guidelines formerly recommended that children with non-advanced HIV disease receive two NRTIs,[56] and this practice was reported in a Brazilian study.[38] Long-term administration of zidovudine to infants is recommended in Brazil[57] and Argentina,[58] and in Mexico zidovudine and lamivudine are recommended[59] to prevent vertical transmission of HIV. Previous research

suggests, however, that the development of resistance after zidovudine prophylaxis during pregnancy is limited.[60,61]

The overall rate of protease mutations was significantly higher in Latin America than in other regions, which is likely to be related to children in this region having high exposure to this drug class. PIs have been used for longer in Latin America than in other regions and, therefore, the degree of transmitted PI resistance might be higher. For instance, PIs became available in Brazil in 1996.[62] The type of PI used probably also affects the development of resistance. Ritonavir-boosted lopinavir only became available in around 2003, and, therefore, nelfinavir (mostly without boosting) is the most widely studied PI in Latin American studies. Adequate plasma concentrations of this drug are seldom reached in children,[63] which leads to acquired resistance. In a small study from Argentina sub-therapeutic plasma concentrations of nelfinavir were measured in most children.[21] Studies from Latin America frequently reported the D30N and N88D mutations, which are specifically selected by nelfinavir.[18]

In southern Africa, ritonavir-boosted lopinavir and full-dose ritonavir are widely used in pediatric regimens. The most frequently reported mutations from this region were V82A, I54V, and M46I, which are selected by lopinavir and ritonavir.[18] One study compared rates of major PI mutations between children exposed to ritonavir-boosted lopinavir and ritonavir alone, and found that the latter is significantly associated with an increased risk of resistance.[44] In west Africa, a small number of children received indinavir, among a high proportion of whom treatment failure and drug resistance were reported.[39] The bioavailability of indinavir is variable in children, which contributes to sub-therapeutic plasma concentrations.[64]

In Asia, where NRTI resistance after treatment failure is high among children, frequencies of the T69ins and Q151M mutations were raised. These mutations, which confer resistance to multiple NRTIs, might indicate accumulated resistance if use of a failing treatment regimen is continued. Although virological criteria were used to define treatment failure in most studies we identified from Asia, monitoring of viral load is unlikely to have been used routinely to detect early treatment failure. Another contributing factor to the high rate of resistance could be the undisclosed use of previous ART exposure through monotherapy, dual therapy, or both, which could in turn be related to the early introduction of antiretroviral treatment in the region. In Thailand, for instance, zidovudine monotherapy started being used in 1992, dual therapy of zidovudine plus didanosine or zalcitabine in 1995, and ART was introduced in 2001.[65] Lastly, the Y181C/I mutation in the NNRTI drug class also occurred more frequently in Asia than in other regions.

This difference could be due to unreported use of nevirapine to prevent mother-to-child transmission of HIV.

Some of the differences in findings between regions might be attributable to diversity in HIV-1 subtype. The V106A/M mutation was found in 21% of cases in southern Africa, compared with less than 5% in other regions. The NNRTI mutation V106M seems to be found most frequently in subtype C HIV-1, whereas V106A is more frequently seen in subtype B.[66] V106A in subtype B is mainly associated with resistance to nevirapine but not efavirenz. By contrast, V106M confers broad cross-resistance. We noted no differences in the prevalence of the K65R mutation between HIV subtypes, although this mutation develops more rapidly in populations infected with subtype C.[67,68]

Rates of drug resistance at the time of virological failure seem to be similar for children and adults in resource-poor regions.[66,69,70] A previous systematic review that compared outcomes in frequently and infrequently monitored adult patients found a prevalence of 88% resistance at time of virological failure in the latter group.[71] As the rates of virological failure are, however, higher in children,[10,11] having a similar resistance rate to that in adults translates into an overall higher rate of increased emergence of drug resistance among children.

The pattern of mutations in the reverse transcriptase region of children was largely similar to that seen in adult patients in sub-Saharan Africa.[72] The frequency of the K103N mutation in HIV-1, however, was much lower among children with NNRTI exposure than in adults (37% vs. 52%). This finding corroborates that of the HIVNET 012 study,[73] which identified that the K103N mutation was selected more frequently in women than in their infants after single-dose nevirapine.[73] Mutations in the protease region also differ between adults and children. The M46I variant of the M46I/L mutation was detected in 16% of all children with PI exposure, compared with in 8% of adults. The various codon substitutions at the V82 location were more common in children than in adults (30% vs. 16%). One possible explanation for the occurrence of mutations at these two locations in children might be the use of full-strength ritonavir as the sole PI, which is associated with the emergence of major protease mutations.[44]

We could not assess whether prophylactic antiretroviral medication during the perinatal period contributed to the frequencies of resistance mutations at the time of treatment failure. Most studies did not report perinatal prophylaxis or did not separately report results for exposed children. Only one study[31] compared outcomes in children who were exposed to perinatal single-dose nevirapine with those in children who were not. The rate of virological failure and subsequent resistance development were increased in the

exposed children. The diversity in NNRTI mutations in the exposed children was greater than that in unexposed children, and included the V106A, V108C/V, and Y181C mutations. These findings are consistent with those from other investigations, which have shown that exposure to single-dose nevirapine affects treatment outcomes.[74] Various studies are in progress to assess this effect further.[75,76]

A limitation of our study is that the reported rates of drug resistance associated with particular drug classes might not be due only to the ART regimen. Transmitted resistance, which is of particular concern in children who have become infected despite maternal antiretroviral medication or acquired resistance because of the previous prophylactic or therapeutic use, might have increased drug resistance. We decided to include studies in which children had previously received antiretroviral drugs for two reasons. First, we could not confirm that studies with no information on previous antiretroviral medication had excluded children with any previous exposure. Second, exposure before ART reflects the reality of pediatric treatment programmes.

Another shortcoming of this systematic review is that we could not analyze patient adherence as a predictor of HIV drug resistance. Four included studies assessed adherence in relation to virological outcomes and one study assessed the association with resistance. Heterogeneous adherence measures were used, however, and direct comparison was not possible. Good adherence seemed generally to be related to virological suppression. In one study drug resistance was reported to be less likely in children in whom treatment had failed than in those with good adherence,[30] which is similar to previous findings in adults.[77]

Lastly, we could not relate the selection of specific resistance mutations to the antiretroviral regimens used. The reviewed studies provided only summary data on resistance-associated mutations rather than individual patients' data. Therefore, the frequencies of mutations associated with resistance to single or multiple drugs could not be assessed.

The highest rates of resistance were identified for nevirapine and efavirenz and reflect the low genetic barriers of these drugs. The reported mutations preclude further use of first-generation and possibly even second-generation NNRTIs. Etravirine has shown promising results in children in developed countries,[78] but is not yet licensed for pediatric use. It might, however, be less effective in resource-poor settings, where widespread use of nevirapine is associated with increased risk of acquiring the Y181C and G190S/A mutations and, therefore, possibly reduced susceptibility to etravirine. In the studies we assessed these mutations were detected, respectively, in 16% and 13% of children exposed to NNRTIs.

The reported NRTI mutations selected for stavudine, zidovudine, lamivudine, and, infrequently, didanosine. Extensive NRTI resistance could jeopardize the recycling of NRTIs in second-line ART. This issue is of particular concern in young children, for whom the number of licensed NRTIs is limited. The frequencies of K65R and Q151M mutations, which confer broad NRTI cross-resistance, were low, but the various thymidine analogue mutations were reported in 56% of patients. The number of patients with multiple thymidine analogue mutations, which would be indicative of accumulated resistance, was not, however, possible to calculate. The NRTI tenofovir would be suitable to include in the NRTI backbone of second-line ART, as it retains potency despite multiple thymidine analogue mutations. This drug has been associated with promising clinical outcomes in studies,[79,80] but is not yet formulated or licensed for young children. Furthermore, the risk of toxic effects to the kidneys and bone will restrict its use in resource-poor settings, where monitoring is limited.

For children with resistance to NRTIs and NNRTIs, salvage therapy with a dual-boosted PI regimen that uses low-dose ritonavir in combination with lopinavir and saquinavir has been investigated.[81] A mean viral load lower than 400 copies per mL was seen in 39 (78%) of 50 children in Thailand after 12 weeks of therapy, but significant increases in lipid concentrations in serum were observed. Further safety and efficacy data are needed to establish the usefulness of double-boosted PI regimens for children in whom the NRTI backbone is no longer expected to be active.

The number of children with PI exposure and subsequent resistance was limited in the reports we assessed. Our findings suggest, however, that nelfinavir and single-dose ritonavir are associated with a raised risk of drug resistance, which highlights the need for effective PIs in formulations suitable for children. The mutations selected for by ritonavir and lopinavir confer cross-resistance, but HIV that is resistant to older PIs may still be susceptible to newer PIs, such as darunavir and tipranavir. The lopinavir-associated V82A mutation even has a positive impact on virological response to darunavir.[82] Darunavir boosted with ritonavir and tipranavir are among the new regimens licensed for use in children and pediatric formulations are available.[83,84] Darunavir has a low pill burden and is not associated with serious adverse events. Furthermore, the first studies of the integrase inhibitor raltegravir in children show promising results, although pediatric formulations are required.[85]

This Review reveals a high proportion of children with drug resistant HIV-1 infections after first-line ART failure. One of the underlying causes could be the shortage of appropriate formulations for children and difficulties in calculating correct doses. The occurrence of triple-class resistance is rare in resource-poor regions, especially when

WHO treatment guidelines are followed. Our subgroup analysis points to high resistance rates in regions with extensive antiretroviral exposure (i.e., Asia and Latin America). In the context of increased access to medication in all resource-poor settings, commonly without testing of viral load to detect early failures, the rate of resistance to at least two drug classes is likely to increase. The policy to start treatment immediately after diagnosis in infants will lead to greater demand for feasible sequencing of regimens early in childhood. Ideally, active second-line agents should be selected on the basis of resistance testing, but this option is seldom available outside research settings because of the high cost and complex logistics. Access to second-line options remains limited in developing countries and, therefore, children are likely to continue taking failing regimens for longer periods of time, which promotes mutation accumulation and hinders the chance of virological control with new drugs. The future of an increasing number of children will depend on the availability of new-generation antiretroviral medications, and on pharmaceutical companies, donor agencies, and policy makers prioritizing the development of second-line and salvage pediatric formulations. Moreover, to make these regimens accessible on a global scale, low-cost generic drugs or major price reductions of patented versions are necessary.

ACKNOWLEDGEMENTS

We thank the authors of the original research papers in this Review who shared additional data with us upon request. We also thank Rob de Haan for help on statistical analyses and Annette Sohn for critical review of the manuscript.

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