SUPPLEMENT ARTICLE

Surveillance of HIV Drug Resistance in Children Receiving Antiretroviral Therapy: A Pilot Study of the World Health Organization's Generic Protocol in Maputo, Mozambique

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Between 2007 and 2008, the Mozambique Ministry of Health conducted an assessment of human immunodeficiency virus drug resistance (HIVDR) using World Health Organization (WHO) methods in a cohort of children initiating antiretroviral therapy (ART) at the main pediatric ART referral center in Mozambique. It was shown that prior to ART initiation 5.4% of children had HIVDR that was associated with nevirapine perinatal exposure ($P < .001$). Twelve months after ART initiation, 77% had viral load suppression (<1000 copies/mL), exceeding the WHO target of ≥70%; 10.3% had HIVDR at 12 months. Baseline HIVDR ($P = .04$), maternal prevention of mother-to-child transmission ($P = .02$), and estimated days of missed medication ($P = .03$) predicted HIVDR at 12 months. As efforts to eliminate pediatric AIDS are intensified, implementation of ritonavir-boosted protease inhibitor regimens in children with prevention of mother-to-child transmission exposure may reduce risk of virological failure in our setting.

The rapid scale-up of antiretroviral therapy (ART) has dramatically reduced human immunodeficiency virus (HIV)–related morbidity and mortality [1–7]. In large part, successful ART scale-up in resource-limited settings can be attributed to the use of a public health approach where standardized treatment guidelines are adapted to local circumstances and implemented on a large scale. Despite impressive gains in ART coverage in recent years, as of December 2010 only 23% of HIV-infected children aged <15 years in need of therapy were receiving it [8].

Scale-up of ART will inevitably lead to the emergence of some HIV drug resistance (HIVDR) [9, 10], which is of particular concern in the pediatric population. Although increased prevention of mother-to-child transmission (PMTCT) coverage will reduce incident infection, it is likely that children who are infected with HIV despite PMTCT will have some degree of HIVDR. Additionally, limited access to routine viral load (VL) monitoring, limited availability of pediatric drugs for second-line therapy, and unique challenges related to pediatric ART adherence raise concerns about HIVDR in children receiving ART [11–13].

Mozambique has an estimated HIV prevalence of 11.5% [14], representing approximately 1.4 million adults and 100 000 children. ART scale-up began in 2003, and as of October 2010, 211 000 adults and 16 800 children were receiving ART. ART coverage of adults and children in need of therapy is estimated to be 40% and 27%, respectively [15]. In Mozambique, ART is

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Clinical Infectious Diseases 2012;54(S4):S369–74
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DOI: 10.1093/cid/cis006

provided following a population-based model of care. Prior to December 2008, HIV-infected children with World Health Organization (WHO) clinical stage III or IV disease (regardless of CD4 cell count) or children with CD4 percentage <20% (for children aged <18 months) or <15% (for children aged >18 months), regardless of clinical stage, were eligible to initiate ART [16]. After December 2008, all infants aged<12 months with WHO clinical stage III or IV disease (regardless of CD4 cell count) or children with CD4 percentage <20% (for children aged <36 months) or <15% (for children aged >36 months), regardless of clinical stage, were eligible to initiate ART [17]. First-line pediatric ART consists of zidovudine (ZDV) or stavudine (d4T) in combination with lamivudine (3TC) and either nevirapine (NVP) or efavirenz (EFV). The vast majority of children receive d4T and NVP–containing regimens in the form of pediatric fixed-dose combinations (from December 2007 onward), with ZDV and EFV reserved for cases of toxicity. Children aged <3 years receiving concomitant tuberculosis treatment are prescribed a triple nucleoside analogue combination: ZDV, 3TC, and abacavir. Infants infected with HIV despite mother-to-child prophylaxis receive a ritonavir-boosted protease inhibitor (PI)–based regimen, lopinavir/ritonavir (LPV/r) in combination with ZDV and 3TC.

Acknowledging the importance of HIVDR surveillance and the need to optimize pediatric ART delivery, Mozambique's national HIVDR working group piloted the WHO's generic protocol to estimate acquired drug resistance in children initiating first-line ART and to assess associated factors at sentinel ART clinics. The methods described in the pediatric generic protocol are consistent with the WHO generic protocol for adult populations [18]. This standardized, minimum-resource method evaluates prevalence of HIVDR prevention (defined as VL suppression with HIV RNA <1000 copies/mL 12 months after ART initiation) during the first year of treatment in cohorts of children starting first-line ART at sentinel clinics.

Specifically, the goals of this survey were to (1) estimate the proportion of the pediatric population achieving HIVDR prevention 12 months after starting first-line ART; (2) describe specific HIVDR mutations and mutation patterns among pediatric patients not achieving VL suppression; and (3) identify patient and program factors associated with HIVDR emergence. Results from this pilot and from future surveys at clinics chosen to be representative of different models of pediatric ART treatment will support optimization of pediatric ART delivery and care in Mozambique.

METHODS

Survey Setting and Population

This survey was conducted between October 2007 and June 2008 at the Pediatric Day Hospital (HDP), Maputo Central Hospital, Maputo, Mozambique. HDP is the main pediatric ART referral center in Mozambique and has been operational since 1994. At the time of the survey, there were >4000 HIV-infected children in care and >1000 receiving ART.

Survey Design and Procedures

The survey protocol was based on the WHO generic protocol for assessment of acquired HIVDR in the pediatric population. This protocol is similar to the generic adult protocol [18] with appropriate pediatric adaptations including evaluation of previous PMTCT exposures of mother and child as well as appropriate weight-based dosing and availability of pediatric ART formulations. Per WHO guidance, an effective sample size of 96 permits estimation of clinic-level HIVDR prevention 12 months after initiation of ART [18]. The survey protocol received approval from Mozambique's National Health Bioethics Committee and the Ministry of Health.

Children aged ≤15 years initiating first-line ART and those whose legal guardians consented to participation were included, regardless of previous PMTCT exposure. Exclusion criteria included children who had previously initiated first-line ART at HDP and stopped; patients transferring from another ART clinic on a standard first-line ART regimen; and those children whose guardians declined informed consent. At initiation of ART (baseline), minimal demographic data including previous antiretroviral (ARV) exposure and specimens for HIVDR genotyping were obtained. At 12 months after ART initiation, blood for VL testing was collected from all children alive and still on ART. Specimens with VL >1000 copies were then genotyped.

Twelve months after ART initiation, the following survey endpoints were assigned: still on first-line ART, lost to follow-up (LTFU), died, transferred to another ART clinic, or stopped ART; transfers out and deaths were censored from analyses. Survey outcomes included the following:

1. HIVDR prevention: alive and on first-line ART at 12 months with a VL <1000 copies/mL. The WHO target for HIVDR prevention is ≥70% VL suppression at each clinic.
2. Possible HIVDR: alive and on first-line ART at 12 months but with VL ≥1000 copies/mL and no detected HIVDR; and children LTFU or who stopped ART.
3. Detected HIVDR*:* alive and on first-line ART at 12 months, with VL ≥1000 copies/mL and HIVDR mutations generating a high-, intermediate-, or low-level resistance classification to ARVs per the Stanford HIVDR algorithm (HIVdb program version 6.0.11) [19].

Adherence to ART

Two population-level surrogate measures of adherence were used. The estimated number of days over the entire 12-month period where each child was without ART, if the regimen had been taken according to prescription, was calculated and

expressed as a percentage. Additionally, on-time appointment keeping was used as a surrogate for adherence to ART. On-time appointment keeping was defined as attending appointments within 7 days of the scheduled appointment and was expressed as a percentage of total scheduled appointments during the first year of ART.

Clinical and Routine Laboratory Assessments

All clinical and demographic data were abstracted from existing medical records. CD4 cell counts were performed at baseline and at 6-month intervals per clinic routine. CD4 T-lymphocyte counts were performed using the FACSCalibur flow cytometer (Becton Dickinson, San Jose, California). At endpoint, plasma VL was quantified using Versan HIV RNA Assay 3.0 (Siemens Medical Solutions, Tarrytown, New York). HIVDR testing was performed at baseline and after 12 months of ART in patients with VL >1000 copies/mL. Dried blood spots and plasma were used for HIVDR genotyping at baseline and at endpoints, respectively.

HIV Genotypic Resistance Analysis

HIVDR testing was performed at the National HIV and Retrovirology Laboratories, Public Health Agency, Ottawa, Canada. Complete protease and part of reverse transcriptase regions were sequenced using previously described procedures [20]. Sequencing was performed using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California). The neighbor-joining distance matrix method was used to assign HIV subtype based on the *pol* gene [21]; all subtypes were confirmed using the REGA HIV subtyping tool (http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html).

Statistical Analysis

Data were analyzed using STATA 11 software (StataCorp, College Station, Texas). Associations between categorical variables were determined using Pearson's χ^2 test and Fisher's exact test. Numeric variables were compared by medians using the Mann–Whitney test. To control for different risk factors associated with outcomes, including baseline HIVDR, logistic regression was performed. Only factors with significance <20% on univariate analysis were included in the multivariate model; all factors found not to be statistically significant were excluded. Age and sex were included as control variables.

RESULTS

Between October 2007 and June 2008, 119 eligible children between 0 months and 13 years of age consecutively initiating first-line ART at HDP were enrolled in the survey. Fifty percent of children were <18 months of age, 90% had advanced disease (WHO clinical stages III and IV), and 48% were severely immunocompromised.

HIVDR at Baseline

Baseline demographic data are presented in Table 1. All children were initiated on a standard first-line ART regimen as defined by national ART guidelines: ZDV, 3TC, and NVP for 62 of 119 (52.1%) children; d4T, 3TC, and NVP for 55 of 119 (46.2%) children; and d4T, 3TC, and LPV/r for 2 of 119 (1.7%) children. Thirteen children reported having received PMTCT prophylaxis.

Baseline genotypes were available for 112 of 119 (94.1%) children, with HIVDR-associated mutations observed in 6 of 112 (5.4%): 5 (4.5%) had nonnucleoside reverse transcriptase inhibitor (NNRTI)–related mutations, of whom 1 (0.9%) had both NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) mutations and 1 (0.9%) had NRTI mutations only. Four of the 13 (30.7%) children with maternal or child PMTCT exposure had baseline HIVDR (Supplementary Table 1). Perinatal exposure to NVP predicted baseline resistance (odds ratio [OR], 35.7 [95% confidence interval {CI}, 4.1–310.6]; $P < .001$). HIV subtype distribution was as follows: 98% subtype C, 1% subtype D, and 1% C/D recombinant.

HIVDR Outcomes at 12 Months

Twelve months after ART initiation, 101 (84.9%) children were alive and on ART, 6 (5.0%) died, 12 (10.1%) were LTFU, none

Table 1. Baseline Characteristics of a Cohort of 119 Children Initiating Antiretroviral Therapy at Pediatric Day Hospital, Maputo Central Hospital, Mozambique (2007–2008)

Characteristic	No. (%)
Age (months)	
Minimum–maximum	3.7–167.6
Median (IQR)	25.2 (15.2–70.8)
0–18	41 (34.5)
19–59	45 (37.8)
≥60	33 (27.7)
Area of residence	
Rural	15 (12.6)
Suburban	79 (66.4)
Urban	25 (21.0)
WHO clinical staging	
I	2 (1.7)
II	6 (5.0)
III	54 (45.4)
IV	56 (47.1)
Unknown	1 (0.8%)
CD4 percentage	
<5	10 (8.4)
5–15	56 (47.1)
≥15	45 (37.8)
Unknown	8 (6.7)

Abbreviations: IQR, interquartile range; WHO, World Health Organization.

Table 2. Risk Factors for Development of HIV Drug Resistance 12 Months After Initiation of Antiretroviral Therapy

Risk Factor	Patients With VL >1000 Copies/mL and Detected HIVDR Mutations at 12 Months (%)	Patients With VL <1000 Copies/mL at 12 Months (%)	Univariate Analysis			Multivariate Analysis	
			OR (95% CI)	*P* Value[a]	*P* Value[b]	OR (95% CI)	*P* Value[c]
No.	10	87					
Males	6 (60.0%)	37 (42.5%)	2.03 (.53–7.81)	.292	.236	2.00 (.41–9.73)	.391
Age at ART initiation (months)						1.0 (.98–1.02)	.997
Median (IQR)	19.1 (6.2–30.0)	33.6 (16.7–80.9)		.145			
Mother exposed to ARV for PMTCT	4 (40.0%)	10 (11.5%)	5.13 (1.17–22.54)	.016	.035	6.41 (1.21–33.88)	.029
Newborn exposed to ARV for PMTCT	3 (30.0%)	7 (8.1%)	4.90 (.98–24.38)	.031	.065	…	…
Resistance at ART initiation	2 (20.0%)	2 (2.3%)	10.65 (1.20–94.18)	.008	.052	47.0 (1.05–2111.78)	.047
CD4 (%) at ART initiation[d]	10	81					
Median (IQR)	9.1 (6.0–13.5)	14.5 (12.0–18.0)		.022		…	…
WHO stage at ART initiation				.317	1.000	…	…
I, II	0 (0.0%)	8 (9.2%)	…				
III, IV	10 (100.0%)	79 (90.8%)	…				
Estimated days of missed medication, %							
Median (IQR)	6.8 (0.0–12.7)	0.0 (0.0–4.3)		.041		1.07 (1.01–1.14)	.033
Missing clinical consultations, %						…	…
Median (IQR)	7.4 (0.0–15.4)	20.0 (7.1–23.1)		.076			

Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral; CI, confidence interval; HIV, human immunodeficiency virus; HIVDR, HIV drug resistance; IQR, interquartile range; OR, odds ratio; PMTCT, prevention of mother-to-child transmission; VL, viral load; WHO, World Health Organization.

[a] *P* value for Pearson's χ^2 categorical variables/Kruskal–Wallis median.

[b] *P* value for Fisher's exact χ^2.

[c] *P* value for adjusted odds ratio.

[d] Only 81 of 87 of patients without resistant mutations at 12 months had CD4 percentage measured.

stopped ART, no child transferred care to a different site, and none switched from an NNRTI- to a PI-based regimen. The few children who initiated boosted PI-based ART remained on the same regimen. Intraclass substitutions for toxicity or side effects were not assessed in this survey. At 12 months, VL data were available for 96% (97 of 101) of the children.

HIVDR Prevention

By excluding death and transfer out from the analysis and by treating LTFU as having virological failure, 87 of 113 (77.0%) children initiating first-line ART achieved HIVDR prevention at 12 months. In an on-treatment analysis of children alive and on ART at 12 months, 89.7% (87 of 97) of children with available VL test results at 12 months had VL <1000 copies/mL.

Possible HIVDR

By excluding death and transfer out from the analysis and by treating LTFU as having virological failure, 10.6% (12 of 113) children had possible HIVDR. In an on-treatment analysis, possible HIVDR was 0% because none of the children had VL >1000 copies/mL and no detected HIVDR.

Detected HIVDR

By excluding death and transfer out from the analysis and by treating LTFU as having virological failure, 8.8% (10 of 113) had detected HIVDR-associated mutations. In an on-treatment analysis, 10.3% (10 of 97) of the children with VL testing available at 12 months had detected HIVDR.

At 12 months, overall 8.8% (10 of 113) and 7.9% (9 of 113) of specimens had resistance mutations associated with NRTI and NNRTI, respectively. The most frequently observed mutations at endpoint were Y181C (6.1%) and M184V (7.9%). The K65R mutation was observed in 2 children (1.8%). Dual NRTI and NNRTI class resistance was present in 8.0% (9 of 113) of the specimens (Supplementary Table 1).

Age at ART initiation, mother and newborn ARV exposure for PMTCT, adherence (as determined by missing days of medication), and CD4 percentage were associated with HIVDR at 12 months of ART in univariate analyses. In the multivariate analysis, factors that remained significant included baseline HIVDR (OR, 47.0 [95% CI, 1.05–2111.78]; $P = .04$), maternal exposure to ARVs for PMTCT (OR, 6.4 [95% CI, 1.21–33.8]; $P = .02$), and estimated days of missed medication (OR, 1.07 [95% CI, 1.01–1.14]; $P = .03$ (Table 2). During the survey no ART stockouts were documented, and all children were prescribed and maintained on standard, appropriately weight-based first-line regimens (Table 2).

DISCUSSION

Rapid ART scale-up in Mozambique began in 2003. As of 2011, >16 000 children were receiving ART in the national program following a standard public health approach. Individual HIVDR genotyping is not routinely available nor recommended; thus, understanding factors associated with successful VL suppression and the prevention of HIVDR in children receiving ART is essential for good program management.

Overall, low levels of HIVDR were observed among children about to initiate ART; however, 4.5% of children did have NNRTI mutations that predicted virologic failure at 12 months. Not unexpectedly, 5 of 6 children with baseline NNRTI resistance had perinatal exposure to NVP ($P = .04$). Perinatal exposure to NVP was the only factor significantly associated with baseline HIVDR (OR, 35.7 [95% CI, 4.10–310.61]; $P < .001$). This observation has been reported in other studies [22–24], in particular, when NVP is administered as a single-dose regimen, which was the case for one-third of the PMTCT-exposed children in our survey.

Findings from this pilot survey show that 12 months after ART initiation, prevention of HIVDR was observed in 77% of children initiating first-line ART during the survey period. Results exceeded the WHO-suggested target of ≥70% [19]. This rate of virological suppression during the first year of ART is similar to reports from other settings [25–29].

Overall, 8.8% (10 of 113) of the children initiating ART had detected HIVDR-associated mutations at 12 months. Dual class resistance, including combined NRTI and NNRTI, was present in 9 of 113 (7.9%) children. No PI mutations were detected, which reflects the very low use of PIs in this population. The most frequently detected mutations were M184V and Y181C. The mutation M184V selected by the use of 3TC has been found in similar settings in both adults and children in which 3TC is included in the first-line ART. The NNRTI mutation Y181C is frequently found in patients on NVP-containing ART regimens and has been found in infants exposed to NVP single-dose PMTCT for HIV subtypes B and C. The K65R mutation has been found in patients taking d4T and was present in 2 of 96 (1.8%) children. Similar resistance patterns have been reported among patients failing the same first-line regimens used in this cohort [11, 12, 29–33]; resistance rates reported were higher than in our cohort, but comparisons are unreliable due to significantly different methods.

Age at ART initiation, perinatal ARV exposure of the mother and newborn, adherence (determined by missing days of medication), HIVDR at ART initiation, and CD4 percentage were associated with HIVDR at 12 months of ART in univariate analyses. However, in the multivariate analysis, only maternal PMTCT exposure, adherence, and baseline HIVDR were associated with HIVDR at 12 months of ART. In this survey, all children failing ART had detected HIVDR-associated mutations, suggesting that in this cohort adherence support and counseling were probably effective.

Recent results of the P1060 study [34] showed that NVP for PMTCT and treatment with a first-line ART regimen, including a ritonavir-boosted PI, was significantly more effective in treating infants than NNRTI-containing regimens. This has led to a modification of pediatric ART initiation guidelines, which now recommend the use of a PI as first-line therapy. As efforts to eliminate pediatric AIDS are intensified, implementation of ritonavir-boosted PI regimens in children with PMTCT exposure may reduce the risk of virological failure in our setting.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank The Stephen Lewis Foundation, Fundação Ariel Glaser de Luta Contra o SIDA Pediátrico, and the Pediatric Day Hospital staff.

Disclaimer. The conclusions and opinions expressed in this article are those of the authors and do not reflect those of the World Health Organization.

Financial support. This work was supported by The Bill & Melinda Gates Foundation (National Institute of Allergy and Infectious Diseases K23 AI074423-05 to M. R. J.) and the European Community's Seventh Framework Programme (FP7/2007–2013) under the project "Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)" (grant agreement 223131 to S. B.). The Stephen Lewis Foundation has supported the work of the Hospital Dia Pediátrico staff.

Supplement sponsorship. This article was published as part of a supplement entitled "The World Health Organization HIV Drug Resistance Prevention and Assessment Strategy: Global, Regional, and Country Progress," sponsored by The Bill & Melinda Gates Foundation (38180).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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