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# The challenge of antiretroviral drug resistance in HIV-1-infected children

**Robert W. Shafer***

The World Health Organization (WHO) estimates that two million children younger than 15 years are infected with HIV-1.[1] Although most HIV-1-infected children are in Sub-Saharan Africa, more than 10,000 of the estimated 730,000 HIV-1-infected persons in Brazil are likely to be children.[1,2] The extent of the HIV-1 epidemic in children reflects the risk factors for HIV-1 infection in the adult population and the frequency with which maternal HIV-1 infection is not detected and mother-to-child transmission (MTCT) not prevented. The treatment of HIV-1 in children is more challenging than its treatment in adults and is associated with an increased risk of virological failure.

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In this issue of Jornal de Pediatria, Almeida et al.[3] report the prevalence of HIV-1 drug resistance in 47 children treated between 2000 and 2004 at one hospital in São Paulo, Brazil. Twenty-four of the children were newly HIV-1 diagnosed and had not received antiretroviral (ARV) therapy or been perinatally exposed to ARVs. Twenty-three of the children were receiving ARV therapy and had detectable HIV-1 levels. The number of successfully treated children (i.e., having undetectable HIV-1 levels) at the same hospital during the study period was not reported.

Among the 24 ARV-naïve children, none had genotypic evidence for significantly reduced ARV susceptibility. One child had a virus with the reverse transcriptase (RT) mutation K219N, a mutation typically selected by nucleoside analog RT inhibitor (NRTI) therapy. By itself, this mutation is unlikely to reduce ARV susceptibility or the success of ARV therapy. Whether this mutation emerged as a result of ARV selection pressure prior to being transmitted to the child or from genetic drift within the child cannot be known for certain. Not all ARV-naïve children, however, have drug susceptible viruses. Indeed transmission of drug-resistant viruses occurs frequently to infants of women receiving incompletely suppressive ARV therapy or unsuccessful MTCT prophylaxis.[4-6]

The fact that 24 ARV-naïve children were diagnosed at a median age of 21.5 months should be cause for concern. The lack of an earlier diagnosis suggests two missed opportunities. First, had the mothers of these children been diagnosed during pregnancy, it is probable that HIV-1 infection would have been prevented.[7,8] Indeed, infections resulting from MTCT in Brazil have decreased from about 2,000 per year between 1999 and 2003 to about 1,100 per year between 2004 and 2005. This success has been attributed to an increased frequency of HIV-1 testing during pregnancy in Brazil which was up to 63% by 2006 and to a modestly successful MTCT prophylaxis program which by 2004 had reduced the risk of MTCT to 7%.[2]

Second, the failure to diagnose HIV-1 during pregnancy places an HIV-1 infected newborn at high risk of morbidity and

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mortality during the first year of life – a time in which the immune system is immature and the risk of rapid HIV-1 progression is high.[9] In the recent South African CHER trial, the risk of death in HIV-1-infected infants younger than 12 months during a median follow-up of 40 weeks was four times lower (16 vs. 4%) than in infants randomized to immediate ARV therapy compared with those randomized to CD4-guided therapy.[10]

Among the 23 ARV-experienced children, most had begun therapy prior to the availability of highly active ARV treatment (HAART) regimens. Genotypic evidence of HIV-1 resistance to NRTIs, non-nucleoside RT inhibitors (NNRTIs), and protease inhibitors (PIs) was present in 96, 61 and 17%, respectively. The most common drug-resistance mutations were the NRTI resistance mutations at RT positions 41, 67, 184, 215, and 219; the NNRTI-resistance mutations at RT positions 103 and 181; and the PI-resistance mutations at protease positions 46, 54, 82, and 90.

The RT mutations at positions 41, 67, 215, and 219 are called thymidine analog mutations (TAMs) because they are selected primarily by the thymidine analogs AZT and stavudine (d4T). However, the TAMs, particularly the combination of M41L and T215Y with another TAM L210W, also confer cross-resistance to abacavir, didanosine, and tenofovir. The RT mutation M184V confers high-level phenotypic resistance to the cytidine analogs lamivudine (3TC) and emtricitabine (FTC) and low-level cross-resistance to abacavir and didanosine. Despite the high level of phenotypic 3TC and FTC resistance caused by M184V, there is often some benefit in including 3TC or FTC in a salvage therapy regimen because M184V increases susceptibility to AZT, d4T, and tenofovir and causes a modest decrease in HIV-1 replication capacity. The RT mutations K103N and Y181C are the two most commonly occurring NNRTI-resistance mutations. Protease mutations at positions 46, 54, 82, and 90 are among the most commonly occurring PI-resistance mutations. Six different PI-resistance mutations at position 54 (I54V/L/M/T/A/S) and seven different PI-resistance mutations at position 82 (V82A/T/F/S/L/M/C) have been reported. Different mutations at these two positions often have divergent effects on PI susceptibility.[11]

The extensive drug resistance reported by Almeida et al.[3] is typical for HIV-1-infected children and adults who begin therapy with incompletely suppressive non-HAART regimens. Indeed similar results have been reported from other studies of heavily treated children from Brazil, as well as from the United States, France, and the U.K. For example, Machado et al. analyzed RT and protease sequences from 37 children at a different São Paulo hospital and reported that 84, 35, and 14% had viruses with mutations associated with resistance to at least one, two, or three drug classes, respectively.[12]

Delaugerre et al. analyzed RT and protease sequences from 119 children in Paris between 2001 and 2003 and reported that 82, 32, and 27% had viruses with genotypic evidence of resistance to at least one, two, and three drug classes, respectively, with three-class resistance significantly more common in boys compared with girls.[13]

Although the risk of virological failure and the extent of ARV resistance are lower among children receiving a first-line HAART therapy, both virological failure and resistance are also frequent problems in these children. Although the immune defense against HIV-1 improves after the first year of life,[14] ARV treatment remains challenging because of the availability of fewer drugs, less convenient formulations, unpredictable pharmacokinetics, and greater barriers to adherence. The risk of ARV treatment failure appears to be higher in children than in adults. For example, in a group of 134 children in Abidjan, Cote d'Ivoire, receiving two NRTIs + one NNRTI or nelfinavir between 1998 and 2003, 44% experienced virological failure. Similarly, from 2004 to 2005, virological failure occurred in 26% of 250 children compared with 14% of 526 adults in a university clinic in Kampala, Uganda, treated for 6 months with d4T + 3TC + nevirapine or AZT + 3TC + efavirenz. Virological failure was significantly higher in males than females, children with a CD4% < 5, and in children receiving the combination of d4T + 3TC + nevirapine. Among 212 Cambodian children receiving one of three fixed-dose combinations of two NRTIs plus one NNRTI for 12 months between 2003 and 2005, ongoing viremia was detected in 19%.[15] In these studies, virological failure was nearly always accompanied by the emergence of both NRTI and NNRTI resistance.

Subtypes B, C, and F are the most common HIV-1 subtypes in Brazil. Almeida et al. report that 18 of 23 children were infected with subtype B viruses. It is therefore not surprising that the RT and protease mutations they report were typical of those observed in other resistant subtype B viruses from Brazil.[16-19] By contrast, certain mutations such as K20T, V82L, N88S, and L89V may be more common in subtype F viruses from Brazil,[20,21] while slight variations in the pattern of PI-resistance mutations have been described in subtype C viruses in Brazil.[22,23]

Brazil's National STD/AIDS Program, which has coordinated the response to the HIV epidemic, offers generic drugs for first-line therapy and patent-protected drugs for second-line therapy.[24] In 2006, an estimated 180,000 HIV-1-infected individuals in Brazil were receiving therapy representing about 90% of the patients for whom therapy has been indicated.[2,24] Heavily-treated individuals, such as the children described in the study by Almeida et al., will stress the finances of Brazil's treatment program because it is likely that one or more of the most recently approved ARVs such as the PIs darunavir and tipranavir, the NNRTI etravirine, the integrase inhibitor raltegravir, and the cell entry inhibitors enfuvirtide and maraviroc would be needed for the patients with the greatest ARV experience and the highest levels of

drug resistance. Judicious use of such newer drugs under close supervision to ensure adherence and increased efforts to prevent MTCT and the emergence of virological failure during initial HAART are needed to maintain the health of HIV-1-infected children and to minimize new infections.

## References


1. Joint United Nations Programme on HIV/AIDS (UNAIDS). 2008 Report on the Global HIV Epidemic. http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_G . Access: 05/03/2008.
2. Brazilian Ministry of Health. Health Surveillance Secretariat. National Programme on STD and AIDS. Targets and commitments made by the member-states at the United Nations General Assembly Special Session on HIV/AIDS. UNGASS - HIV/AIDS. Brazilian Response 2005/2007 Country Progress Report. http://data.unaids.org/pub/Report/2008/brazil_2008_country_progress_report_en.pdf . Access: 05/03/2008.
3. Almeida FJ, Berezin EN, Rodrigues R, Sáfadi MA, Arnoni MV, Oliveira C, et al. Diversity and prevalence of antiretroviral genotypic resistance mutations among HIV-1-infected children. J Pediatr (Rio J). 2009;85(2):104-9.
4. Eshleman SH, Hoover DR, Chen S, Hudelson SE, Guay LA, Mwatha A, et al. Resistance after single-dose nevirapine prophylaxis emerges in a high proportion of Malawian newborns. Aids. 2005;19:2167-9.
5. Vignoles M, Barboni G, Agosti MR, Quarleri J, García MK, Giraudi V, et al. High frequency of primary mutations associated with antiretroviral drug resistance in recently diagnosed HIV-infected children. Antivir Ther. 2007;12:1133-7.
6. Persaud D, Palumbo P, Ziemniak C, Chen J, Ray SC, Hughes M, et al. Early archiving and predominance of nonnucleoside reverse transcriptase inhibitor-resistant HIV-1 among recently infected infants born in the United States. J Infect Dis. 2007;195:1402-10.
7. Succi RC, Figueiredo EN, Zanatta Lde C, Peixe MB, Rossi MB, Vianna LA. Evaluation of prenatal care at basic health units in the city of Sao Paulo. Rev Lat Am Enfermagem. 2008;16:986-92.
8. Stringer EM, Chi BH, Chintu N, Creek TL, Ekouevi DK, Coetzee D, et al. Monitoring effectiveness of programmes to prevent mother-to-child HIV transmission in lower-income countries. Bull World Health Organ. 2008;86:57-62.
9. Bolton-Moore C, Mubiana-Mbewe M, Cantrell RA, Chintu N, Stringer EM, Chi BH, et al. Clinical outcomes and CD4 cell response in children receiving antiretroviral therapy at primary health care facilities in Zambia. JAMA. 2007;298:1888-99.
10. Violari A, Cotton MF, Gibb DM, Babiker AG, Steyn J, Madhi SA, et al. Early antiretroviral therapy and mortality among HIV-infected infants. N Engl J Med. 2008;359:2233-44.
11. Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev. 2008;10:67-84.
12. Machado DM, Fernandes SC, Succi RC, Babiker AG, Steyn J, Madhi SA, et al. Analysis of HIV-type 1 protease and reverse transcriptase in Brazilian children failing highly active antiretroviral therapy (HAART). Rev Inst Med Trop Sao Paulo. 2005;47:1-5.
13. Delaugerre C, Warszawski J, Chaix ML, Veber F, Macassa E, Buseyne F, et al. Prevalence and risk factors associated with antiretroviral resistance in HIV-1-infected children. J Med Virol. 2007;79:1261-9.
14. Leal E, Janini M, Diaz RS. Selective pressures of human immunodeficiency virus type 1 (HIV-1) during pediatric infection. Infect Genet Evol. 2007;7:694-707.
15. Janssens B, Raleigh B, Soeung S, Akao K, Te V, Gupta J, et al. Effectiveness of highly active antiretroviral therapy in HIV-positive children: evaluation at 12 months in a routine program in Cambodia. Pediatrics. 2007;120:e1134-40.
16. Couto-Fernandez JC, Silva-de-Jesus C, Veloso VG, Rachid M, Gracie RS, Chequer-Fernandez SL, et al. Human immunodeficiency virus type 1 (HIV-1) genotyping in Rio de Janeiro, Brazil: assessing subtype and drug-resistance associated mutations in HIV-1 infected individuals failing highly active antiretroviral therapy. Mem Inst Oswaldo Cruz. 2005;100:73-8.
17. Cavalcanti AM, Lacerda HR, Brito AM, Pereira S, Medeiros D, Oliveira S. Antiretroviral resistance in individuals presenting therapeutic failure and subtypes of the human immunodeficiency virus type 1 in the Northeast Region of Brazil. Mem Inst Oswaldo Cruz. 2007;102:785-92.
18. de Sa-Filho DJ, Soares Mda S, Candido V, Gagliani LH, Cavaliere E, Diaz RS, et al. HIV type 1 pol gene diversity and antiretroviral drug resistance mutations in Santos, Brazil. AIDS Res Hum Retroviruses. 2008;24:347-53.
19. Eyer-Silva WA, Couto-Fernandez JC, Silva-de-Jesus C, Morgado MG. Prevalence of HIV type 1 drug resistance mutations in treatment-naive and experienced patients from resource-limited settings with universal access to antiretroviral therapy: a survey in two small Brazilian cities. Mem Inst Oswaldo Cruz. 2008;103:143-9.
20. Calazans A, Brindeiro R, Brindeiro P, Verli H, Arruda MB, Gonzalez LM, et al. Low accumulation of L90M in protease from subtype F HIV-1 with resistance to protease inhibitors is caused by the L89M polymorphism. J Infect Dis. 2005;191:1961-70.
21. Dumans AT, Barreto CC, Santos AF, Arruda M, Sousa TM, Machado ES, et al. Distinct resistance mutation and polymorphism acquisition in HIV-1 protease of subtypes B and F1 from children and adult patients under virological failure. Infect Genet Evol. 2009;9:62-70.
22. Gonzalez LM, Brindeiro RM, Aguiar RS, Pereira HS, Abreu SM, Soares MA, et al. Impact of nelfinavir resistance mutations on in vitro phenotype, fitness, and replication capacity of human immunodeficiency virus type 1 with subtype B and C proteases. Antimicrob Agents Chemother. 2004;48:3552-5.

23. Soares EA, Santos AF, Sousa TM, Sprinz E, Martinez AM, Silveira J, et al. Differential drug resistance acquisition in HIV-1 of subtypes B and C. PLoS ONE. 2007;2:e730.

24. Greco DB, Simao M. Brazilian policy of universal access to AIDS treatment: sustainability challenges and perspectives. Aids. 2007;21 Suppl 4:S37-45.

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# HIV drug resistance in HIV-infected children

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The global roll-out of antiretroviral therapy (ART) in children still lags significantly behind that of adults, and data on treatment outcomes from resource-limited settings have only recently begun to emerge. As a result, while there is a wealth of literature on patterns of drug resistance in adults on ART, there is still limited data on children.

Almeida et al. report, in this issue of the journal, a retrospective study on the prevalence and patterns of antiretroviral drug resistance in 24 treatment-naïve and 23 treatment-experienced children failing therapy between 2000 and 2004 attending a clinic in São Paulo.[1] All children were vertically infected, and the median age of the naïve- and treatment-experienced children was 22 months and 102 months, respectively. In the children failing ART, the median duration of ART exposure was 60 months (range 3-120 months), and the log viral load at treatment failure was 5.04 log copies/mL. The overall distribution of subtypes among both groups of children was 78.3% subtype B, 13% subtype F, 4.4% BF mosaics and 4.3% subtype C, which reflects the distinctive molecular epidemiology of HIV-1 in Brazil, with the low-level but rising prevalence of subtype C infection in the south of the country.[2]

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There are no surprises in their findings. There was an absence of resistance mutations among vertically infected ART-naïve children, but ART-experienced children had extensive drug resistance. The absence of primary resistance largely reflects the fact that none of the mothers had received ART prior to delivery, and that only two children received zidovudine in the first 6 weeks of life. These findings also highlight the ongoing problem of suboptimal uptake of HIV testing among pregnant women that would enable access to effective prevention of mother-to-child transmission (PMTCT).

Among treated children failing therapy, there was an almost universal presence of nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations, particularly T215Y/F, M184V/I and D67N, M41L and K219Q/E, reflecting the widespread exposure to zidovudine and lamivudine. Overall, 60.8% also had resistance mutations to non-nucleoside reverse transcriptase inhibitors (NNRTIs) (K103N 39.1%) and to Y181C (17.4%), but these were present in all 12 children that were failing on NNRTIs. The most commonly used protease inhibitors (PIs) were unboosted – ritonavir and nelfinavir –, and primary PI resistance mutations were observed in 47.8%, particularly V82A, M46I, and L90M. These findings concur with previous reports from other pediatric cohorts in Brazil.[3-5]

One of the difficulties in the interpretation and generalizability of these findings is the limited information on duration of treatment failure. Although the authors state that the median duration of ART in those failing therapy was 60 months, where failure was defined either as a decrease in viral

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