HIV transmitted drug resistance in adult and pediatric populations in Panama

Juan Castillo,[1] *Griselda Arteaga,*[1] *Yaxelis Mendoza,*[1] *Alexander A. Martínez,*[1] *Rigoberto Samaniego,*[2] *Dora Estripeaut,*[3] *Kathleen R. Page,*[4] *Rebecca E. Smith,*[1] *Nestor Sosa,*[1] *and Juan M. Pascale*[1]

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ABSTRACT

Objective. *To investigate the prevalence of transmitted drug-resistant HIV among adults in Panama by using a modified World Health Organization Threshold Survey (WHO-TS) and to investigate rates of initial resistance among HIV-positive infants in Panama.*

Methods. *At the Gorgas Memorial Institute, 47 HIV-positive adults were genotyped for mutations associated with transmitted drug resistance (TDR) in the reverse transcriptase and protease genes of HIV-1, according to WHO-TS guidelines, modified to include patients ≤ 26 years old. Prevalence rates for drug-resistance mutations against three classes of antiretroviral drugs—nucleoside analog reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors—were calculated as low (< 5.0%), moderate (5.0%–15.0%), and high (> 15.0%). Twenty-five infant patients were also genotyped and prevalence rates for drug-resistance mutations were calculated.*

Results. *TDR among Panamanian adults was moderate: 6 of 47 HIV-positive adults showed one or more mutations associated with TDR. Horizontal TDR mutations were moderate for NRTIs and NNRTIs and low for protease inhibitors. Vertical transmission of HIV in Panama has decreased for 2002–2007, but vertical HIV TDR prevalence is moderate (12.0%) and is emerging as a problem due to incomplete antiretroviral coverage in pregnancy.*

Conclusions. *The prevalence of HIV TDR indicated by this study, combined with known rates of HIV infection in Panama, suggests more extensive surveys are needed to identify risk factors associated with transmission of HIV drug resistance. Specific WHO-TS guidelines for monitoring vertical transmission of drug-resistant HIV should be established.*

Key words HIV-1; drug resistance; infectious disease transmission, vertical; protease inhibitors; antiretroviral therapy, highly active; Panama.

[1] Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama City, Panama. Send correspondence to: Juan M. Pascale, jpascale@gorgas.gob.pa
[2] Hospital Santo Tomás, Departmento de Enfermedades Infecciosas, Panama City, Panama.
[3] Hospital del Niño, Departmento de Enfermedades Infecciosas, Panama City, Panama.
[4] Johns Hopkins University School of Medicine, Division of Infectious Diseases, Baltimore, Maryland, United States of America.

Throughout the developing world, access to antiretroviral therapy (ART) for treatment of HIV infections is increasing. As a consequence, drug-resistant HIV (HIVDR) is emerging and diminishing treatment options. In developing countries, first-line options for treatment are limited, second-line treatment regimens are much more expensive than first-line drugs, and the opportunity to perform drug-resistance genotyping is restricted (1). In Latin America, there are a number of studies of secondary drug resistance, initial drug resistance (IDR), and primary or transmitted drug resistance (TDR) (2–15), but discerning continental trends for antiretroviral (ARV) resistance in this region is complex.

Prolonging the lifetimes of ARVs is vital to the sustainability of HIV treatment programs in developing nations (1). Recommendations for limiting HIVDR in resource-limited countries are defined in the World Health Organization (WHO) global strategy for prevention and assessment of HIVDR (16). Surveillance of HIV TDR in recently infected individuals is key to the WHO strategy (16).

HIV/AIDS was first observed in Panama in 1984. There are approximately 20 000 HIV-positive persons in a concentrated epidemic (17) and there have been a total of 10 381 AIDS cases since 1984.[5] In 2006, 0.5% of pregnant women were HIV positive, and the share dropped to 0.3% for 2007–2009.[5]

Panama has provided free diagnosis, monitoring, and ART to 70.0% of all eligible patients since 1999 and to all patients since 2001 (18). Currently, 4 463 (19) to 8 700 (Panama Ministry of Health, personal communication, 30 March 2011) adult and minor patients receive ART. In the absence of a national database of patients receiving ARV, 70.0%–80.0% are estimated to receive first-line therapy, 15.0%–20.0% receive second-line therapy, and 5.0%–10.0% receive salvage therapy (Panama Ministry of Health, personal communication, 30 March 2011).

First-line ART for adults in Panama follows WHO guidelines (20): one nonnucleoside reverse transcriptase inhibitor (NNRTI), efavirenz, combined with two nucleoside analog reverse transcriptase inhibitors (NRTIs), for which recommendations have recently changed (21, 22). Since 2007, NRTIs were lamivudine (3TC) and zidovudine (AZT) (21) and, since July 2011, tenofovir with 3TC or emtricitabine. Current recommendations for second-line therapy are two NRTIs with a ritonavir-boosted protease inhibitor, usually lopinavir (22). Since 2007, Panama's ART to prevent mother-to-child-transmission has involved AZT and 3TC, with lopinavir (21, 22). From 2007 to July 2011, ART for infants born to HIV-positive mothers was AZT until 6 weeks of age (21). Current guidelines recommend this regimen only for babies born to mothers receiving ART prenatally and perinatally (22). Babies born to mothers not taking ART prenatally and perinatally should receive AZT, 3TC, and nevirapine (22).

Despite broad and prolonged ART coverage in Panama, few studies have examined HIVDR prevalence (2) and there are no reports of HIV TDR from horizontal or vertical transmission. HIVDR studies in this country are important, but financial, human, and laboratory capacities to manage HIVDR are limited in developing countries. In recognition of this problem and as a pillar of the WHO global strategy against HIVDR (16), the WHO Threshold Survey (WHO-TS) surveillance and classification strategy was developed (23, 24). WHO-TS allows for low-cost classification of the prevalence of HIV TDR in adults to individual drugs or drug classes as low (< 5.0%), moderate (5.0%–15.0%), or high (> 15.0%) (24).

Forty-one WHO-TS studies have been conducted in Africa, Asia, and Mexico (1, 23, 25–32).[6] An adaptation of the WHO-TS strategy was used in Brazil (33). Combined analysis of WHO-TS from 20 countries showed a low overall level of HIV TDR (3.7%), although 17.0% of surveys showed moderate levels of HIV TDR (1). In Central America, the WHO-TS has not been applied.

Our first objective is to apply WHO-TS, with modifications, to investigate HIV TDR among Panamanian adults. For the second objective, we investigate IDR in Panama, for the first time at the molecular level (34). While WHO clearly describes its goal in surveying IDR (35), it does not have a formal IDR surveillance strategy.

MATERIALS AND METHODS

WHO-TS methodology and its adaptations

The WHO-TS strategy (23, 24) focuses on regions where ART has been available to ≥ 20.0% of eligible individuals for ≥ 3 years; this study focuses on Panama City. WHO-TS methodology requires collection and analysis of 47 eligible specimens, preferably within 12 months. Samples were collected March 2008 to October 2010 at the clinic of the Gorgas Memorial Institute for Health Studies (ICGES), where all HIV patients in Panama City were referred for baseline viral load measurement after their initial HIV diagnoses elsewhere.

In this descriptive study, plasma samples for 47 HIV-positive, ART-naive adults from the general population were collected and genotyped for drug resistance at ICGES and their resistance levels were evaluated (24). A median of 3 months had elapsed between initial HIV diagnosis and genotyping. Mean $CD4^+$ count was 400 cells per µL. At ICGES, two of the WHO-TS mandatory eligibility criteria for patient inclusion were met: laboratory confirmation of HIV-positive status and, if female, no previous pregnancies (23). The third criterion, patient age < 25 years, was extended to ≤ 26 years: patients ≥ 25 years had a confirmed HIV-negative serology in the previous 3 years. The sample collection period was extended from the recommended 12 months.

Infant samples

All infant patients from the general population with an HIV-positive diagnosis in the Panama City region attend ICGES for molecular confirmation of HIV status and measurement of viral load. Twenty-five infant patients were confirmed as HIV positive at ICGES and their samples, collected February 2007 to October 2009, were genotyped. Some data were available on the ART regimens of the children and their mothers.

Genotyping methods

An in-house method was used to genotype and analyze reverse transcriptase and protease genes for specific mutations associated with drug resistance in HIV-1. HIV-1 RNA was extracted with the QIAamp viral RNA mini kit (Qiagen) and reverse-transcribed, amplified, and sequenced with the primers indicated in Table 1 (36).

Analysis

Sequences were edited and analyzed with Sequencher software, version 4.5. Consensus sequences were analyzed with the Stanford University Calibrated Population Resistance tool to identify

[5] Nuñez Maitin AE, Mastelari M, Guerrero G, Pascale JM. Panama HIV/AIDS epidemiological situation: 1984–2009 [conference presentation]. At: XVIII International AIDS Conference, Vienna, 18–23 July 2010.

[6] Bertagnolio S, Kelley K, Saadani Hassani A, Obeng-Aduasare Y, Jordan M. World Health Organization surveys of transmitted and acquired HIV drug resistance in resource limited settings [conference presentation]. At: 18th Conference on Retroviruses and Opportunistic Infections, Boston, 27 February to 2 March 2011.

TABLE 1. Primer sequences used in genotyping methods (36)

Process	Primer	Sequence
Reverse transcription	JA272	5'-GGATAAATCTGACTTGCCCART-3'
Polymerase chain reaction	JA272	5'-GGATAAATCTGACTTGCCCART-3'
	JA269	5'-AGGAAGGACACCARATGAARGA-3'
Nested polymerase chain reaction	JA270	5'-GCTTCCCTCARATCACTCTT-3'
	JA271	5'-CCACTAAYTTCTGTATRTCATTGAC-3'
Sequencing	2A	5'-GGGTCGTTGCCAAAGAGTG-3'
	JA270	5'-GCTTCCCTCARATCACTCTT-3'
	JA276	5'-TGTATATCATTGACAGTCCA-3'
	JA305	5'-ATTCCTAATTGRACYTCCCA-3'
	JA311	5'-AAAATCCATAYAAYACTCCA-3'

mutations in HIV-1 reverse transcriptase and protease genes (37–40). The 47 genotypes were classified with the WHO-TS binomial sampling and classification scheme, which classifies HIV TDR prevalence to individual drugs or drug classes but is not powered for punctual estimates of prevalence (24).

Ethics approval and patient consent

Panama's National Institutional Review Board approved this study as part of the ICGES HIV Epidemiology Study (RV165) and informed consent regarding sample use was obtained.

RESULTS

Adults

Forty-seven HIV-positive men (n = 30) and women (n = 17), aged 16–26 years (mean 21.6 years), all residing in Panama City, were included (Table 2). Six patients showed one or more mutations associated with TDR: one patient showed multiple mutations associated with NNRTI resistance (patient 3) and another showed multiple resistance-associated mutations against NRTIs and NNRTIs (patient 15). The prevalence of TDR mutations overall and against NRTIs and NNRTIs was moderate (5.0%–15.0%). The prevalence of TDR mutations against protease inhibitors was low (< 5.0%) (Table 2). Polymorphisms not associated with drug resistance were also observed (Table 2).

Neonates and infants

Twenty-five blood samples from male (n = 12) and female (n = 13) patients aged between 9 days and 1 year were collected (Table 3). HIV IDR was observed in 1 of 6 HIV-positive babies tested in 2007, in 0 of 11 in 2008, and in 2 of 8 in 2009. Two patients had mutations associated with NRTI resistance (patients 3 and 5) and one patient had an NNRTI-resistance mutation (patient 13). On the basis of genotyping results and information about ART given to the infant patients and their mothers, the overall prevalence of vertically transmitted HIVDR was 12.0%.

DISCUSSION

The WHO-TS guidelines were adapted to investigate HIV TDR in Panamanian adults. The overall prevalence was moderate and justifies more advanced surveillance for drug-resistant HIV among newly infected patients, especially from groups at high risk of HIV infection.

The prevalence of TDR mutations against protease inhibitors (< 5.0%) and NRTIs and NNRTIs (5.0%–15.0%) in Panama is similar to findings from a Brazilian adaptation of the WHO-TS, reporting a moderate level of HIV TDR (33), and to other regional HIV TDR studies: low to moderate HIV TDR has been identified in Argentina (2.4%–8.8%), Brazil (4.2%–11.0%), Chile (1.7%–12.0%), Colombia (5.8%), the Dominican Republic (7.8%) (7), Honduras (7.0%) (5), Peru (3.0%–3.4%), and Venezuela (3.2%–11.0%) (41). In the region, therefore, Panama's prevalence classification is not unusual, but Latin America's overall rates of HIV TDR appear to be higher than in Africa and Asia, according to the combined analysis of WHO-TS from 20 African and Asian countries (1). This general conclusion justifies closer surveillance of HIV TDR in the region and adoption of methodologic adaptations, which will increase Latin American participation in the WHO-TS.

Panama's moderate levels of HIV TDR reflect 10 years of using ART and the types of ART available. K103N, P225H, and Y181C mutations alter the effectiveness of efavirenz, which is used as a first-line drug for nonpregnant Panamanians; I85V is associated, in ART-naive patients, with resistance to atazanavir (42), a second-line ART in Panama (21); and M41L and T215F reduce the susceptibility of HIV-1 to stavudine and AZT, first-line drugs in Panama for pregnant women and newborns and formerly for adults and young people. M41L, one of the few transmitted mutations not observed to revert over time (43), was seen in four patients. Its high prevalence is particularly important in Panama: until July 2011, first-line therapy was efavirenz with AZT and 3TC, a regimen whose efficacy would have been compromised by a high prevalence of the M41L mutation, but the therapy has since changed to efavirenz, tenofovir with 3TC, or emtricitabine, a regimen compromised only by the M41L mutation combined with other thymidine analog mutations.[7] The prevalence of TDR mutations found here agrees with our work showing that mutations associated with resistance to NRTIs and NNRTIs, singularly or combined, are the most prevalent causes of HIV secondary drug resistance in Panama.[8]

The 47 samples in this study were collected from Panama City, where ART has

[7] Pingen M, Nijhuis M, Boucher C, Wensing A. The frequently transmitted M41L mutation in RT does not affect the in vitro selection of resistance pathways against TDF and FTC [conference presentation]. At: 6th International Workshop on HIV Transmission, Rome, 14–15 July 2011.

[8] Arteaga G, Castillo J, Martínez A, Mendoza Y, Meléndez J, Mojica D, et al. Prevalence of HIV drug resistance in treatment experienced, chronically HIV-infected individuals from Panama [conference presentation]. At: XVIII International AIDS Conference, Vienna, 18–23 July 2010.

TABLE 2. Epidemiological and genotyping data for 47 eligible adult HIV-infected patients selected consecutively from all HIV-positive patients attending the Gorgas Memorial Institute for Health Studies, Panama City, Panama, March 2008 to October 2010

No.	Sex	Age (years)	Diagnosis date			Mutations			
			Day	Month	Year	NRTI	NNRTI	Minor protease	Major protease
1	M	25	4	3	2008	None	None	None	None
2	M	22	4	8	2008	**M41L**	None	None	None
3	M	23	1	10	2008	**A62V, T215L**	**K103N, Y181C, P225H**	None	None
4	F	20	16	10	2008	None	None	**A71T**	None
5	M	18	25	11	2008	None	None	**A71T**	None
6	M	23	1	12	2008	None	None	None	None
7	F	24	1	1	2009	None	None	**A71V**	None
8	F	18	1	1	2009	None	None	None	None
9	F	25	1	1	2009	None	None	None	None
10	F	20	26	1	2009	None	**V90I/V**	**L10V, A71V**	None
11	F	18	1	2	2009	None	None	None	None
12	M	24	5	2	2009	None	None	**L10I**	None
13	M	22	28	3	2009	None	None	None	None
14	F	21	1	4	2009	None	**E138A**	None	None
15	M	19	1	5	2009	**M41L, A62V, T215F/L**	**K103N, P225H**	None	None
16	M	20	1	6	2009	None	None	None	None
17	M	24	1	6	2009	None	None	None	None
18	M	23	1	7	2009	None	None	None	None
19	F	24	8	7	2009	None	None	None	None
20	F	23	24	7	2009	None	None	None	None
21	F	21	1	9	2009	None	**K101Q**	**V11I, A71V**	None
22	M	24	1	10	2009	None	None	None	None
23	M	23	1	10	2009	None	None	**A71V**	None
24	F	16	20	10	2009	None	None	**A71T**	None
25	M	20	1	12	2009	None	None	**A71V**	None
26	M	21	1	12	2009	None	None	**A71T**	None
27	M	24	1	1	2010	None	None	None	None
28	M	19	25	1	2010	**L210F/L**	None	None	None
29	M	26	30	1	2010	None	None	None	None
30	M	24	1	2	2010	None	**V90I/V**	None	None
31	M	19	3	2	2010	**M41L**	None	None	None
32	M	23	1	3	2010	**M41L**	None	None	None
33	M	20	1	3	2010	None	None	None	None
34	M	24	5	3	2010	None	None	None	None
35	M	20	16	3	2010	None	None	**L10I**	None
36	F	20	17	3	2010	None	None	**I85V**	None
37	M	23	25	3	2010	None	None	None	None
38	M	24	1	4	2010	None	None	None	None
39	M	26	10	4	2010	None	None	**A71V**	None
40	F	19	6	5	2010	None	None	**L10V**	None
41	F	18	6	6	2010	None	None	None	None
42	F	19	1	7	2010	None	None	None	None
43	M	24	13	7	2010	None	None	None	None
44	F	20	1	8	2010	None	**V106I**	**A71V**	None
45	F	23	1	8	2010	None	None	**L10I, A71T**	None
46	M	19	1	9	2010	None	**K238T**	None	None
47	M	21	1	9	2010	None	None	None	None
Total patients with TDR-associated mutations						4	2	1	0

Note: NRTI: nucleoside analog reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, M: male, F: female, TDR: transmitted drug resistance, bold lettering: polymorphisms not associated with transmitted drug resistance, underlined bold lettering: mutations associated with transmitted drug resistance.

TABLE 3. Epidemiological and genotyping data for 25 HIV-infected infant patients who attended the Gorgas Memorial Institute for Health Studies, Panama City, Panama, February 2007 to October 2009

			Medication applied[a]		Sampling date		Mutations			
No.	Sex	Age	Infant (AZT)	Mother	Month	Year	NRTI	NNRTI	Minor protease	Major protease
1	M	1 month	...	...	12	2007	None	None	**L33I**	None
2	M	2 months	...	...	3	2008	**V118I**	None	**A71V**	None
3	F	2 months	...	...	7	2009	**T215Y**	None	**A71V**	None
4	M	2 months	...	...	10	2007	None	None	**L10I**	None
5	M	7 months	...	...	4	2007	**K219Q**	None	None	None
6	M	9 months	...	...	8	2009	None	None	None	None
7	M	3 months	...	...	10	2008	None	None	**A71T**	None
8	F	3 months	...	No	11	2008	None	None	**L10I**	None
9	F	4 months	...	No	5	2009	None	None	None	None
10	F	1 year	...	No	10	2009	None	None	None	None
11	F	9 days	...	CK6	10	2008	None	None	None	None
12	M	6 weeks	Yes	No	3	2008	None	None	None	None
13[b]	F	6 weeks	Yes	No	10	2009	None	**K103N**	**A71V**	None
14[c]	F	1 months	Yes	No	9	2007	None	None	None	None
15	F	4 months	Yes	No	2	2007	None	None	None	None
16	F	5 months	Yes	No	10	2008	None	None	**A71T**	None
17	F	6 months	Yes	No	5	2009	None	None	**A71T**	None
18	F	7 months	Yes	No	6	2008	None	None	None	None
19	F	1 months	Yes	AZT	10	2009	**L210F**	**K101Q**	None	None
20[c]	M	3 months	Yes	CK8	7	2008	None	None	None	None
21	M	2 months	No	No	6	2008	None	None	**L10I, A71V**	None
22	M	2 months	No	No	1	2008	None	None	**L33I**	None
23	F	2 months	No	No	9	2008	None	None	**L10I**	None
24	M	6 months	No	No	4	2009	None	None	**L10I, A71T**	None
25	M	1 year	No	No	3	2007	None	None	**L33I**	None
Total patients with TDR-associated mutations							2	1	**0**	0

Note: AZT: zidovudine, NRTI: nucleoside analog reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, M: male, F: female, . . . : no data, CK6: Combivir (lamivudine with zidovudine) begun 6 weeks before labor, CK8: Kaletra (lopinavir/ritonavir) begun 8 weeks before labor, none: no mutations observed, TDR: transmitted drug resistance, bold lettering: polymorphisms not associated with transmitted drug resistance, underlined bold lettering: mutations associated with transmitted drug resistance.

[a] Medication applied refers to antiretroviral therapy provided to the infant from birth and to the mother during pregnancy.

[b] The mother of this patient was a known HIV-positive patient who did not take antiretroviral therapy during her pregnancy.

[c] These patients have died.

been available since 2001 (Panama Ministry of Health, personal communication, 30 March 2011). Samples were collected at ICGES, which at the time of this study received 100.0% of newly diagnosed HIV-positive patients from Panama City's wider population. Reference laboratories having the opportunity to consecutively enroll eligible HIV-positive patients from the general population may be considered for WHO-TS studies.

Ages within this study's patient population ranged from 16 to 26 years: 96.0% of the cohort was ≤ 25 years old and all patients ≥ 25 years old (25, n = 2; 26, n = 2) had an HIV-negative test in the 3 years before diagnosis. However, the age range of patients was at the limit of WHO-TS feasibility: the mandatory age-eligibility criterion stipulates < 25 years. The period of sample collection was 2.5 years: WHO recommends only 12 months.

While ICGES received 100.0% of newly diagnosed HIV-positive patients from Panama City, identifying 47 age-eligible individuals in 12 months was difficult and was the reason for adapting the WHO-TS methodology to older patients and longer sampling. Panama has the second highest HIV prevalence rate in Central America (17), but HIV infections are concentrated in specific groups (44) and absolute numbers of new HIV-positive patients from the general population attending ICGES were low.

Identifying sufficient numbers of eligible HIV-positive patients will be a problem faced by similar countries in Latin America, with small populations, low numbers of HIV infections, low prevalence, and concentrated epidemics. The WHO publication on WHO-TS (23) states: "generally no more than 50 individuals < 25 years of age, without previous pregnancies and ineligible for ART, were likely to be diagnosed with HIV . . . within 3–6 months, even in areas of high HIV prevalence." For these reasons, either WHO-TS HIV TDR studies are best applied only in populations or subpopulations with a high prevalence of HIV, or WHO could consider adapting survey guidelines to increase participation by small populations with a low prevalence of HIV and generalized epidemics.

These alterations may involve relaxing the < 25-year age criterion. In WHO-TS, patient age is an indicator of recent infection, and if some other marker of re-

cent infection can be consistently used, it should be satisfactory. The BED test has been rejected for this function (30). Alternative criteria, such as a negative HIV test or a well-defined risk event in the previous 12 months may be considered.

A Tanzanian study on ART-naive patients failing WHO-TS eligibility criteria due to age (45) reported a statistically significant difference in the prevalence of HIVDR between patients < 25 years old (0.0%) and those aged 25–63 years (19.1%). Failing to involve patients > 25 years old in threshold studies may exclude some recently infected HIV-positive patients who are at increased risk of acquiring drug-resistant HIV strains and disregards underlying social factors leading to increased risk of infection, such as older-age repartnering.

WHO-TS methodology was designed to help developing countries assess HIV TDR economically. We suggest it is important that WHO consider alternative criteria for site selection and patient eligibility to maximize WHO-TS participation, especially in Latin American countries underrepresented in this program. There is some urgency in increasing participation, as future opportunities for WHO-TS may be limited: ART for preventing transmission is increasingly discussed (46) and, if adopted, wider uptake throughout the developing world will make it more difficult to identify ARV-naive patients.

This study also represents Panama's first molecular examination of IDR in 25 HIV-positive infants. Between 2007 and 2009, the overall prevalence of IDR was 12.0% (n = 3), the prevalence of IDR mutations against NRTIs was moderate (8.0%), and the prevalence of IDR was < 5.0% for NNRTIs and protease inhibitors.

Data from Panama's Ministry of Health for the number of HIV-positive pregnancies (Panama Ministry of Health, personal communication, 30 March 2011) can be matched to reports from the Joint United Nations Programme on HIV/AIDS on ART coverage among pregnant Panamanians: in 2006, 100.0% of 153 pregnant women received Panama's recommended ART (AZT, 3TC, lopinavir) (47), resulting in no cases of vertical transmission (21); in 2007, 71.0% of 100 mothers received ART (47), resulting in one infant with NRTI resistance (K219Q); for 2008, no data on ART coverage were available and no cases of IDR were detected; and in 2009, 73.0% of 162 mothers received ART (17) and our data show two cases of HIV IDR, with one case of NRTI resistance (T215Y) and one case of NNRTI resistance (K103N). The mother of the K103N infant was a known HIV-positive patient of poor compliance who did not take ART prenatally.

Conclusion

The overall prevalence of HIV TDR was moderate. This fact, combined with known rates of HIV infection among adult Panamanians, suggests that it is important that more extensive surveys better identify factors associated with horizontal HIV TDR and assist Panama's Ministry of Health in choosing first- and second-line regimens. Applying WHO-TS to high-risk subpopulations in Panama may reveal a broader range of resistance mutations, while larger surveillance studies may more accurately indicate the prevalence of HIV TDR in the general population.

Some HIV-positive mothers in Panama transmit HIV and HIVDR to their infants. To better understand vertical infection and IDR, we also need new surveys among HIV-positive pregnant women and their newborns. This will require collection of sociodemographic data providing early warnings about mothers at increased risk of transmitting HIV, information about ART use by infants and their mothers before and during pregnancy, and introduction of genotyping tests at defined times during pregnancy and neonatal life. Establishing public health protocols in Panama whereby HIV-positive mothers and their newborns would be automatically subject to HIVDR analysis would be a useful collaboration between Panama's Ministry of Health, ICGES, and antenatal clinics. Finally, we recommend establishing WHO-TS guidelines specifically for assessing IDR.

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REFERENCES

1. World Health Organization. HIV drug resistance fact sheet. Geneva: WHO; 2011. Available from: http://www.who.int/hiv/facts/drug_resistance/en/index.html Accessed 7 September 2011.
2. Ahumada-Ruiz S, Flores-Figueroa D, Toala-Gonzalez I, Thomson MM. Analysis of HIV-1 pol sequences from Panama: identification of phylogenetic clusters within subtype B and detection of antiretroviral drug resistance mutations. Infect Genet Evol. 2009;9: 933–40.
3. Soria J, Bull M, Mitchell C, Rosa AL, Dross S, Kraft K, et al. Transmitted HIV resistance to first-line antiretroviral therapy in Lima, Peru. AIDS Res Hum Retroviruses. 2011.
4. DiazGranados CA, Mantilla M, Lenis W. Antiretroviral drug resistance in HIV-infected patients in Colombia. Int J Infect Dis. 2010;14: e298–303.
5. Murillo W, Paz-Bailey G, Morales S, Monterroso E, Paredes M, Dobbs T, et al. Transmitted drug resistance and type of infection in newly diagnosed HIV-1 individuals in Honduras. J Clin Virol. 2010;49:239–44.
6. Parham L, de Rivera IL, Murillo W, Naver L, Largaespada N, Albert J, et al. High prevalence of drug resistance in HIV type 1-infected children born in Honduras and Belize 2001 to 2004. AIDS Res Hum Retroviruses. 2011.
7. Myers JE, Taylor BS, Rojas Fermin RA, Reyes EV, Vaughan C, Jose L, et al. Transmitted drug-resistance among antiretroviral naive patients with established HIV-1 infection in Santo Domingo, Dominican Republic and review of the Latin American and Caribbean literature. AIDS Res Hum Retroviruses. 2011.
8. Pando MA, Gomez-Carrillo M, Vignoles M, Rubio AE, dos Ramos Farias MS, Vila M, et al. Incidence of HIV type 1 infection, antiretroviral drug resistance, and molecular characterization in newly diagnosed individuals in

Argentina: a Global Fund project. AIDS Res Hum Retroviruses. 2011;27:17–23.
9. Castillo J, Comegna M, Quijada W, Jauvin V, Pinson P, Masquelier B, et al. Surveillance of HIV type 1 drug resistance among naive patients from Venezuela. AIDS Res Hum Retroviruses. 2009;25:1329–33.
10. Rangel HR, Garzaro D, Fabbro R, Martinez N, Ossenkop J, Torres JR, et al. Absence of primary integrase resistance mutations in HIV type 1-infected patients in Venezuela. AIDS Res Hum Retroviruses. 2010;26:923–6.
11. Rangel HR, Garzaro DJ, Torres JR, Castro J, Suarez JA, Naranjo L, et al. Prevalence of antiretroviral drug resistance among treatment-naive and treated HIV-infected patients in Venezuela. Mem Inst Oswaldo Cruz. 2009; 104:522–5.
12. Charles M, Noel F, Leger P, Severe P, Riviere C, Beauharnais CA, et al. Survival, plasma HIV-1 RNA concentrations and drug resistance in HIV-1-infected Haitian adolescents and young adults on antiretrovirals. Bull World Health Organ. 2008;86:970–7.
13. Perez L, Correa C, Aleman J, Gonzalez I, Perez J, Martinez PA, et al. Drug-resistant HIV-1 in Cuban children and their seropositive mothers. MEDICC Rev. 2011;13:24–31.
14. Afani A, Beltran C, Maria Gallardo A, Roessler P, Acevedo W, Vasquez P. Prevalencia de resistencia primaria en pacientes con infección reciente por VIH-1 en Chile. Rev Med Chil. 2010;138:669–76.
15. Soto-Ramirez LE, Rodriguez-Diaz R, Duran AS, Losso MH, Salomon H, Gomez-Carrillo M, et al. Antiretroviral resistance among HIV type 1-infected women first exposed to antiretrovirals during pregnancy: plasma versus PBMCs. AIDS Res Hum Retroviruses. 2008; 24:797–804.
16. Bennett DE, Bertagnolio S, Sutherland D, Gilks CF. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. Antivir Ther. 2008; 13(Suppl 2):1–13.
17. Joint United Nations Programme on HIV/AIDS. Report on the global AIDS epidemic. Geneva: UNAIDS; 2010.
18. Ministerio de Salud Panamá. Informe nacional sobre los progresos realizados en la applicación del UNGASS—Panama: Enero 2006–Diciembre 2007. Panama City: Ministry of Health, National Program on HIV and AIDS, Panama, and UNAIDS; 2008. Available from: http://data.unaids.org/pub/Report/2008/panama_2008_country_progress_report_sp_es.pdf Accessed 24 March 2011.
19. Joint United Nations Programme on HIV/AIDS. Epidemiological factsheet. Geneva: UNAIDS; 2009. Available from: http://www.unaids.org/en/regionscountries/countries/panama/ Accessed 20 February 2011.
20. World Health Organization HIV/AIDS Programme. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. Geneva: WHO; 2010.
21. Ministerio de Salud Panamá y Organización Panamericana de la Salud. Normas para el manejo terapéutico de las personas con VIH en la República de Panamá. Panama City: Ministry of Health; 2007.
22. Ministerio de Salud Panamá y Organización Panamericana de la Salud. Normas para el manejo terapéutico de las personas con VIH en la República de Panamá. Panama City: Ministry of Health; 2011.
23. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antivir Ther. 2008;13(Suppl 2):25–36.
24. Myatt M, Bennett DE. A novel sequential sampling technique for the surveillance of transmitted HIV drug resistance by cross-sectional survey for use in low resource settings. Antivir Ther. 2008;13(Suppl 2):37–48.
25. Maphalala G, Okello V, Mndzebele S, Gwebu P, Mulima N, Dlamini S, et al. Surveillance of transmitted HIV drug resistance in the Manzini-babane corridor, Swaziland, in 2006. Antivir Ther. 2008;13(Suppl 2):95–100.
26. Abegaz WE, Grossman Z, Wolday D, Ram D, Kaplan J, Sibide K, et al. Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. Antivir Ther. 2008;13(Suppl 2):89–94.
27. Kamoto K, Aberle-Grasse J. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. Antivir Ther. 2008;13(Suppl 2):83–7.
28. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antivir Ther. 2008;13(Suppl 2):101–7.
29. Somi GR, Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, et al. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. Antivir Ther. 2008;13(Suppl 2):77–82.
30. Sirivichayakul S, Phanuphak P, Pankam T, O-Charoen R, Sutherland D, Ruxrungtham K. HIV drug resistance transmission threshold survey in Bangkok, Thailand. Antivir Ther. 2008;13(Suppl 2):109–13.
31. Nguyen HT, Duc NB, Shrivastava R, Tran TH, Nguyen TA, Thang PH, et al. HIV drug resistance threshold survey using specimens from voluntary counselling and testing sites in Hanoi, Vietnam. Antivir Ther. 2008;13(Suppl 2):115–21.
32. Chaturbhuj D, Hingankar N, Srikantiah P, Garg R, Kabra S, Deshmukh P, et al. Transmitted HIV drug resistance among HIV-infected voluntary counseling and testing centers (VCTC) clients in Mumbai, India. AIDS Res Human Retroviruses. 2010;26:927–32.
33. Inocencio LA, Pereira AA, Sucupira MCA, Fernandez JCC, Jorge CP, Souza DF, et al. Brazilian network for HIV drug resistance surveillance: a survey of individuals recently diagnosed with HIV. J Int AIDS Soc. 2009; 12:20–5.
34. Estripeaut D, Nieto Guevara J, De Suman O, Rodríguez-Vigil C, Mojica E, Navas CA. Efectividad de las medidas de prevención relacionadas a la transmisión vertical de VIH: cuánto hemos avanzado? Rev Pediatr Panamá. 2009; 38:20–4.
35. World Health Organization. Surveillance of initial drug resistance. Geneva: WHO; 2011. Available from: http://www.who.int/hiv/topics/drugresistance/initial_dr/en/index.html Accessed 12 September 2011.
36. Murillo W, de Rivera IL, Parham L, Jovel E, Palou E, Karlsson AC, et al. Prevalence of drug resistance and importance of viral load measurements in Honduran HIV-infected patients failing antiretroviral treatment. HIV Med. 2010;11:95–103.
37. Gifford RJ, Liu TF, Rhee SY, Kiuchi M, Hue S, Pillay D, et al. The calibrated population resistance tool: standardized genotypic estimation of transmitted HIV-1 drug resistance. Bioinformatics. 2009;25:1197–8.
38. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One. 2009;4:e4724.
39. Shafer RW, Rhee SY, Pillay D, Miller V, Sandstrom P, Schapiro JM, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS. 2007;21:215–23.
40. Shafer RW, Rhee SY, Bennett DE. Consensus drug resistance mutations for epidemiological surveillance: basic principles and potential controversies. Antivir Ther. 2008;13(Suppl 2): 59–68.
41. Petroni A. Resistencia primaria de HIV-1: estado de situación en Argentina. Actual SIDA. 2010;18(70).
42. Vora S, Marcelin AG, Gunthard HF, Flandre P, Hirsch HH, Masquelier B, et al. Clinical validation of atazanavir/ritonavir genotypic resistance score in protease inhibitor-experienced patients. AIDS. 2006;20:35–40.
43. Pingen M, Nijhuis M, de Bruijn JA, Boucher CA, Wensing AM. Evolutionary pathways of transmitted drug-resistant HIV-1. J Antimicrob Chemother. 2011;66:1467–80.
44. World Health Organization, Joint United Nations Programme on HIV/AIDS, and United Nations Children's Fund. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Geneva: WHO, UNAIDS, UNICEF; 2010.
45. Kasang C, Kalluvya S, Majinge C, Stich A, Bodem J, Kongola G, et al. HIV drug resistance (HIVDR) in antiretroviral therapy-naive patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high. PLoS One. 2011;6:e23091.
46. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365: 493–505.
47. Joint United Nations Programme on HIV/AIDS. Report on the global AIDS epidemic. Geneva: UNAIDS; 2008.

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Farmacorresistencia transmitida del VIH en poblaciones adultas y pediátricas en Panamá

RESUMEN

Objetivo. Investigar la prevalencia de farmacorresistencia transmitida del VIH en adultos en Panamá mediante un estudio del umbral modificado de la Organización Mundial de la Salud (OMS) e investigar las tasas de resistencia inicial en lactantes seropositivos para el VIH en Panamá.

Métodos. En el Instituto Conmemorativo Gorgas, en 47 adultos seropositivos al VIH se efectuó la genotipificación de las mutaciones asociadas con la farmacorresistencia transmitida en los genes de la transcriptasa inversa y la proteasa del VIH-1, según las directrices del estudio umbral de la OMS, modificadas para incluir a las personas ≤ 26 años de edad. Las tasas de prevalencia de las mutaciones farmacorresistentes contra tres clases de fármacos antirretroviral —inhibidores de la transcriptasa inversa análogos de nucleósidos, inhibidores de la transcriptasa inversa no análogos de nucleósidos e inhibidores de la proteasa— se clasificaron en bajas (< 5,0%), moderadas (5,0%–15,0%) o altas (> 15,0%). También se llevó a cabo genotipificación y se calcularon las tasas de prevalencia de las mutaciones causantes de farmacorresistencia en 25 lactantes.

Resultados. En los adultos de Panamá la farmacorresistencia transmitida fue moderada: 6 de 47 adultos seropositivos para el VIH presentaron una o más mutaciones asociadas con farmacorresistencia transmitida. Las mutaciones farmacorresitentes de transmisión horizontal fueron moderadas para los inhibidores de la transcriptasa inversa análogos de nucleósidos y los inhibidores de la transcriptasa inversa no análogos de nucleósidos, y bajas para los inhibidores de la proteasa. En Panamá la transmisión vertical del VIH ha disminuido en el período 2002–2007, pero la prevalencia de la farmacorresistencia del VIH transmitida por vía vertical es moderada (12,0%) y está surgiendo como un problema debido a la cobertura antirretroviral incompleta durante el embarazo.

Conclusiones. La prevalencia de farmacorresistencia transmitida del VIH observada en este estudio, junto con las tasas de infección por el VIH registradas en Panamá, indican que se necesitan estudios más amplios para determinar los factores de riesgo asociados con la transmisión de la farmacorresistencia del VIH. Deben establecerse directrices específicas del estudio del umbral de la OMS a fin de vigilar la transmisión vertical del VIH farmacorresistente.

Palabras clave VIH-1; resistencia a medicamentos; transmisión vertical de enfermedad infecciosa; inhibidores de proteasas; terapia antirretroviral altamente activa; Panamá.