

Transmitted human immunodeficiency virus-1 drug resistance in a cohort of men who have sex with men in Belo Horizonte, Brazil - 1996-2012

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The presence of transmitted human immunodeficiency virus (HIV)-1 drug-resistance (TDR) at the time of anti-retroviral therapy initiation is associated with failure to achieve viral load (VL) suppression. Here, we report TDR surveillance in a specific population of men who have sex with men (MSM) in Belo Horizonte, Brazil. In this study, the rate of TDR was evaluated in 64 HIV-infected individuals from a cohort of MSM between 1996-June 2012. Fifty-four percent had a documented recent HIV infection, with a seroconversion time of less than 12 months. The median CD4⁺ T lymphocyte count and VL were 531 cells/mm³ and 17,746 copies/mL, respectively. Considering the surveillance drug resistance mutation criteria, nine (14.1%) patients presented TDR, of which three (4.7%), five (7.8%) and four (6.2%) had protease inhibitors, resistant against nucleos(t)ide transcriptase inhibitors and against non-nucleoside reverse-transcriptase inhibitors mutations, respectively. Two of the patients had multi-drug-resistant HIV-1. The most prevalent viral subtype was B (44, 68.8%), followed by subtype F (11, 17.2%). This study shows that TDR may vary according to the population studied and it may be higher in clusters of MSM.

Key words: HIV-1 - transmitted drug resistance - genotyping - recent HIV infection - men who have sex with men

Human immunodeficiency virus (HIV)-1 suppression through combined antiretroviral therapy (ART) has resulted in the reduction of acquired immune deficiency syndrome (AIDS)-related morbidity and mortality in countries where these medications are widely available (Hammer et al. 1997, Deeks 2008). However, those on non-suppressive therapy present an increased risk of developing resistance mutations due to unsuitable adherence, medication interactions and suboptimal treatment. This may limit future therapies and potentially increase the risk of transmission of resistant viral strains, with a possible impact on public health (Napravnik et al. 2005). Thus, HIV-1 resistance to ART may be a consequence of selective medication pressure and can be transmitted when a patient is either first infected or super-infected by a resistant strain. These individuals begin ART with a smaller genetic barrier and, consequently, a greater risk of virological failure and a higher potential for the development of new viral resistance mutations (Shafer et al. 2007, Gagliani et al. 2011).

The incidence and prevalence of transmitted drug-resistance (TDR) are difficult to estimate and data vary widely by region, population, methods and lists of mutations used to calculate these rates. Shafer et al. (2007)

outlined criteria for developing a list of surveillance drug resistance mutations (SDRM). These criteria were updated in 2009 (Bennett et al. 2009), making it possible to accurately compare TDR rates across geographic regions and times. In Europe and North America, TDR prevalence varies from 5-15% in chronically infected patients and from 10-25% in acute infections (Shafer et al. 2007). The prevalence in Asia is 13.8% and it ranges from 2.2-24% in Africa (Ceccherini-Silberstein et al. 2010). Thus, TDR is a concern for the initiation of ART, mainly in developed countries, where there is wide exposure to ART (Borrito-Esoda et al. 2004, Fox et al. 2006, Turner & Wainberg 2006). An initial study carried out by the Brazilian Ministry of Health in testing centres throughout the country found TDR in 7% of chronically infected patients, with 2.2% resistant against protease inhibitors (PIs), 2.4% resistant against nucleos(t)ide transcriptase inhibitors (NRTIs) and 2.1% resistant against non-nucleoside reverse-transcriptase inhibitors (NNRTIs) (Brindeiro et al. 2003). Two other important studies carried out in several Brazilian cities in 2009 found a TDR rate of 5.7% using the International AIDS Society (IAS) criteria in naïve HIV patients (Sprinz et al. 2009) and 8.1% (Inocência et al. 2009) in newly infected individuals using the SDRM criteria. Due to the moderate prevalence of TDR, the Brazilian guidelines recommend genotyping prior to beginning ART only for pregnant persons and those infected by patients on treatment (MS 2008, 2013). Currently, there are no robust data justifying increasing its use (Hawkins & Murphy 2009).

In addition to regional variation, other risk factors have been reported, such as intravenous drug use and homosexuality, and there is lower risk among hetero-

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sexuals (Little et al. 2002). Notably, the level of previous ART exposure and sample size may have an influence on TDR prevalence (Hirsch et al. 2008). A Swedish study performed between 2003-2010 showed that TDR prevalence was significantly higher among males having sex with males (MSM) (9.5%) and clustering of TDR strains was observed in this group (Karlsson et al. 2012). A Brazilian study found a TDR prevalence of 21.4% among MSM in community-recruited samples of nine cities (Bermúdez-Aza et al. 2011).

The objective of this study was to evaluate the TDR rate among HIV+ ART-naïve patients from a cohort of MSM in Belo Horizonte, Southeast Brazil.

SUBJECTS, MATERIALS AND METHODS

Blood samples were obtained from volunteers of Horizonte Project (PBH) in Belo Horizonte, between 1996-June 2012. This project was an open cohort of HIV-negative homosexual and bisexual men supported by the Brazilian Ministry of Health and it is one of the components of the Minas Gerais HIV-AIDS Vaccine Centre of the Federal University of Minas Gerais (UFMG) in Brazil (Carneiro et al. 2000). The project objectives included the evaluation of the role of counselling and condom distribution in the incidence of HIV infection in this population. Every six months, the volunteers were examined, received counselling and preservatives and underwent serologic tests for HIV (ELISA) and other sexually transmitted diseases, such as syphilis and viral hepatitis. Five hundred and thirteen subjects are currently being followed. The eligibility criteria for enrolment included being homosexual or bisexual, 18 years or older, consenting to HIV testing and testing negative for HIV-1. All the available HIV+ samples collected after enrolment in PBH were genotyped. From the beginning of the project in 1994 until June 2012, 1,328 subjects were followed and 119 were infected with HIV-1 during this period. Genotyping was carried out on 64 samples of seroconverters between 1996-June 2012. In the remaining 55 individuals, genotyping was not possible either because the samples were not available or the *pol* gene was not amplified with the available kits. The elapsed time was the time between the last negative HIV-1 test and the first positive HIV-1 test. We considered an infection to be a recent HIV-1 infection when the elapsed time between the last negative HIV test and the first positive test was less than 12 months.

The ViroSeq™ Genotyping System version 2.0 (Cephera Diagnostics, Alameda, California, USA) was used for genotypic analysis until 2007 and the TRUGENE® HIV-1 Genotyping Assay (Siemens Diagnostics, USA) was subsequently used.

All sequences obtained were subjected to quality control assessments using the Blast-RENAGENO Resistance Analysis Program (aids.gov.br/renageno) to exclude sample mix-up or contamination. Drug resistance was analysed using 297 nucleotides of protease (PR) (positions 1-99) and the first 1,003 nucleotides of reverse-transcriptase (RT) (positions 1-335). The amino acid sequences of the RT and PR genes were deduced from the nucleic acid sequences and compared to a subtype B consensus

sequence from the Stanford HIVdb Program Genotypic Resistance Interpretation Algorithm version 6.2.0 (hivdb.stanford.edu/) RT and the PR sequence database.

In this study, the World Health Organization SDRM criteria (Bennett et al. 2009) were used to determine the significant mutations for TDR calculation. Tests for mutations associated with at least two classes of drugs were considered in multi-drug-resistant (MDR) samples.

For all the individuals included, the initial viral load (VL) and CD4+ T lymphocyte (CD4) counts were obtained from Control System Laboratory Tests of the National Lymphocyte Count CD4+/CD8+ and Viral Load (SISCEL), a Brazilian AIDS Program Database, or from the patient files. All the seroconverters are being followed at the UFMG HIV/AIDS outpatient clinic Training and Reference Center in Infectious and Parasitic Diseases.

The data were analysed using STATA for Windows® (version 11.2, StataCorp, College Station, TX, USA). Descriptive data were presented as the mean, median and percentage. The chi-square and Mann-Whitney *U* tests were used as appropriate. Statistical significance was set at the 5% level.

This study was conducted in accordance with the Brazilian Research Ethics Guidelines and approved by the Ethical Review Committee (UFMG).

RESULTS

Demographic and laboratory data from the 64 patients are shown in Table I. All the patients lived in Belo Horizonte or in the metropolitan region. Thirty-five patients (54.7%) had documented recent infections (< 12 months). The overall TDR prevalence was 14.1% (9 patients) according to the SDRM criteria (Bennett et al. 2009). Three (4.7%), five (7.8%) and four (6.2%) patients presented PI, NRTI and NNRTI resistance mutations, respectively, as shown in Table II. Among the patients with TDR, four (44.4%) had single resistance mutations and two (22.2%) had MDR HIV-1. Resistance to three drug classes was found in one sample. Patients with and without TDR had similar CD4 cell counts (median, 531 and 476 cell/mm³, respectively, *p* = 0.355, Mann-Whitney *U* test) and VL levels (17,746 and 14,250 copies/mL, respectively, *p* = 0.952, Mann-Whitney *U* test).

Subtype B was the most prevalent (44, 68.8%), followed by F (11, 17.2%) and C (4, 6.3%). TDR was only found in subtype B, with statistically significant differences between the B and non-B subtypes and the presence of SDRM (*p* = 0.029, chi-square test).

DISCUSSION

This study presents data collected from MSM in Belo Horizonte, over a broad timespan (1996-2012) that encompasses different eras of HIV treatment. The TDR rate of 14.1% was moderate, but higher than in other studies in the general global population and in Brazil. Two large Brazilian TDR prevalence studies with random and representative samples were published. Brindeiro et al. (2003) found TDR in 7% of chronically infected patients (2.2%, 2.4% and 2.1% prevalence of resistance against PIs, NRTIs and NNRTIs, respectively), while Inocêncio

et al. (2009) reported TDR in 8.1% of recently diagnosed individuals from six Brazilian capital cities among 210 samples. There were intermediate levels of TDR (5-15%) in the cities of Belém, Brasília, São Paulo and Rio de Janeiro and the last two cities are in the Southeast Region of Brazil, which is relatively close to Belo Horizonte (Inocêncio et al. 2009). In southeastern Brazil, where the epidemic began, patients have been more exposed to ART, which is a plausible explanation for the higher rates of TDR. Our results do not differ from the overall national data (Inocêncio et al. 2009, Sprinz et al. 2009).

Studies with MSM have shown moderate rates of TDR with an apparently stable prevalence (Truong et al. 2011, Castor et al. 2012). Castor et al. (2012) showed a TDR prevalence of 14.3% in a predominantly MSM cohort with recent/acute HIV infection from 1995-2010 in New York City. A Swedish study performed in nine cities between 2003-2010 showed a 9.5% TDR prevalence (Karls-son et al. 2012). A recent study revealed a high TDR level (21.4%) in the city of São Paulo in community-recruited samples from MSM (Bermúdez-Aza et al. 2011).

TABLE I
Baseline characteristics of the subjects^a

Characteristics	Patients	Transmitted drug resistance SDRM 2009
Total [n (%)]	64 (100)	9 (14.1)
Male [n (%)]	64 (100)	9 (14.1)
Age (years)		
Mean	30.6	30.1
Range	19-54	22-42
HIV subtype [n (%)]		
B	44 (68.8)	9
F	11 (17.2)	-
C	4 (6.3)	-
BC, BF, FB	5 (7.8)	-
Elapsed time (months) ^b		
Mean	14.5	29.4
Range	3-74	6-36
Laboratory variables-		
VL (copies/mL) ^c		
Median	17.746	14.250
Range	590-610.000	700-610.000
CD4 count (cells/mL) ^c		
Median	531	476
Range	110-1.440	394-1.364

a: protease and reverse transcriptase subtypes/number of samples (BC/1, BF/3 and FB/1); b: from last negative human immunodeficiency virus (HIV) Elisa test and the first positive test. First positive HIV Elisa test sample was used for drug-resistance testing; c: for six patients there was no information on viral load (VL) and CD4⁺ T lymphocytes count; SDRM: surveillance drug resistance mutations.

According to the Brazilian genotyping interpretation system RENAGENO version 05-2012 (algoritmo.aids.gov.br) and the Stanford HIVdb Program, seven of the patients in our study would be predicted to have some degree of resistance to the initial ART regimen commonly used in Brazil (Table II), which consists of two NRTIs (AZT + 3TC or TDF + 3TC) and one NNRTI (EFV or NVP).

All three patients with single PI-related mutations were predicted to be fully susceptible to all the PIs. Interestingly, there were no PI-related mutations after 2005, most likely because ritonavir boosted the potency of this class. This is in agreement with TDR incidence studies that show a tendency of stabilisation or even reduction of resistance in this class (Vercauteren et al. 2008, 2009).

Our sample has limitations that may have affected the rate of TDR prevalence in this population. The major problem was the loss of 55 samples during the study; in some cases, the *pol* gene was not amplified with the available kits and in other cases, samples were not available after seroconversion. Another problem was that some of the patients had up to 74 months between the last HIV-negative ELISA and the first positive test and we could not determine how long they had been infected. Subsequently, more than half of the patients were found to be recently infected, that is, less than 12 months). Studies have shown that resistance mutations persist in the absence of ART, which may have been the case for those with a long elapsed time (Brenner et al. 2002, Pao et al. 2004). Other authors hypothesise that resistance may be underestimated among chronically infected individuals.

Another limitation is the relatively small sample size, which may not accurately reflect the newly HIV-infected patients in the region. However, in Belo Horizonte, the HIV epidemic is still more prevalent among MSM. We chose the SDRMs as our standard, which may have limited direct comparisons to other studies that utilised the IAS list of drug resistance mutations (IAS-DRMs). Some potentially relevant mutations are absent in the SDRM, but Green et al. (2008) showed that compared to SDRMs as the reference standard, the overall sensitivity of the IAS-DRMs was inferior for detecting TDR. In fact, when we applied the main IAS-DRMs in our study, there was no significant difference in the TDR rate (data not shown).

Viral subtype B was the most prevalent in this study, as has been observed in most regions of Brazil except the southern region, where subtype C is more common (Soares et al. 2003, Brígido et al. 2011). TDR was present only among subtype B patients, with a statistically significant difference between B and non-B subtypes and the presence of SDRM ($p = 0.029$, chi-square test). It is worth noting that subtype C was present in 6.3% (4/64) of patients, which indicated that this subtype is spreading throughout the southeast (Soares et al. 2003, Brígido et al. 2011). There were 11 patients with subtype F and an increase in its prevalence has already been reported in the northeast region of Brazil; Cavalcanti et al. (2012) showed that 37.7% of patients had subtype F in metropolitan city of Recife.

Furthermore, a study by Sax et al. (2005) showed that genotypic testing is cost-effective for ART-naïve patients when the TDR prevalence is $\geq 1\%$.

TABLE II
Horizonte Project, open cohort of human immunodeficiency virus negative males having sex with males in Belo Horizonte, Brazil between 1996-June 2012

Patient	Elapsed time (months)	Age (years)	CD4/VL	Seroconversion date	Reverse transcriptase			Subtype	Stanford ARV susceptibility ^a	RENAGENO ARV susceptibility ^b
					NRTI	NNRTI	Protease PI			
1	6	32	665/610,000	November 96	70R, 77L	-	-	B	AZT, d4T, TDF = I	AZT = I
2	22	23	688/700	August 98	-	-	90M	B	S to all drugs	S to all drugs
3	7	34	1364/-	September 2002	41L, 184V, 210W, 215Y	188L	-	B	R to all NRTI EFV, NVP = R ETV = S	R to all NRTI EFV = R ETV = S
4	6	28	-/-	August 2002	41L, 184V, 210W, 215Y	188L	90M	B	R to all NRTI EFV, NVP = R ETV = R	R to all NRTI EFV = R ETV = S
5	8	22	462/201,309	October 2003	70E	-	-	B	ABC, ddI = I, TDF = I	ddI = I, TDF = R
6	6	23	458/78,460	October 2005	-	-	73A	B	S to all drugs	S to all drugs
7	6	28	476/4,744	September 2010	41L, 67N, 69D, 210W, 215D	-	-	B	3TC = S ABC, TDF = I AZT, d4T, ddI = R	3TC = I AZT, d4T, ddI, TDF = R
8	36	33	394/14,250	September 2010	-	103N	-	B	ETV = S EFV, NVP = R	EFV, NVP = R
9	6	42	732/8,238	August 2011	-	103N	-	B	ETV = S EFV, NVP = R	EFV, NVP = R

a: susceptibility was predicted using the Stanford HIVdb Program Genotypic Resistance Interpretation Algorithm version 6.2.0 (last updated 29 Sep 2012) (hivdb.stanford.edu/); *b*: susceptibility was predicted using the RENAGENO resistance interpretation algorithm version 12 (05-2012) (algoritmo.aids.gov.br/); ARV: antiretroviral therapy; AZT: zidovudine; d4T: stavudine; ddI: didanosine; EFV: efavirenz; ETV: etravirine; I: intermediate; NNRTI: resistant against non-nucleoside reverse-transcriptase inhibitors; NRTI: resistant against nucleos(t)ide transcriptase inhibitors; NVP: nevirapine; PI: protease inhibitor; R: resistant; S: susceptible; TDF: tenofovir; VL: viral load; 3TC: lamivudine.

In our study, there were no statistically significant differences related to the CD4 count and VL with or without resistance mutations at the time of infection. However, we did not evaluate the clinical data from patients with SDRMs to verify how TDR may have affected the clinical outcome. The impact of TDR on surrogate markers of clinical progression (CD4 count and VL) remains uncertain. The EuroCoord-CHAIN project evaluated the effect of TDR on first-line ART. They estimated a virological failure of 4.7% at 12 months for those with TDR and fully active ART and 15.1% for those with TDR and resistance to at least one prescribed drug (Wittkop et al. 2011).

A slower rate of CD4 decline has been reported in patients who present TDR mutations (Bhaskaran et al. 2004), while others have not found a favourable effect on VL and CD4 counts in the presence of TDR mutations (Chan et al. 2003).

In conclusion, this study shows that the TDR prevalence among recently infected MSM in Belo Horizonte from 1996-2012 remained moderate and comparable with other similar studies. While the prevalence remained moderate in Belo Horizonte in this specific population, the profile of the transmitted mutations in the overall population may be different, underscoring the need for continued and improved measures for targeted interventions. Thus, TDR surveillance studies must be maintained over time, as recommended by WHO (WHO 2012), with additional national surveillance studies to identify the true rate of TDR and evaluate if genotyping tests should be performed prior to the beginning of ART in this specific population.

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