Meta-Analysis and Time Series Modeling Allow a Systematic Review of Primary HIV-1 Drug-Resistant Prevalence in Latin America and Caribbean

Antonio Victor Campos Coelho*, Ronald Rodrigues De Moura, Ronaldo Celerino Da Silva, Anselmo Jiro Kamada, Rafael Lima Guimarães, Lucas André Cavalcanti Brandão, Hemílio Fernandes Campos Coelho and Sergio Crovella

Department of Genetics, Universidade Federal de Pernambuco, Avenida da Engenharia, Cidade Universitária, Recife, PE, Postal Code: 50740-600, Brazil


Antonio V. C. Coelho

Abstract: Here we review the prevalence of HIV-1 primary drug resistance in Latin America and Caribbean using meta-analysis as well as time-series modeling. We also discuss whether there could be a drawback to HIV/AIDS programs due to drug resistance in Latin America and Caribbean in the next years. We observed that, although some studies report low or moderate primary drug resistance prevalence in Caribbean countries, this evidence needs to be updated. In other countries, such as Brazil and Argentina, the prevalence of drug resistance appears to be rising. Mutations conferring resistance against reverse transcriptase inhibitors were the most frequent in the analyzed populations (70% of all mutational events). HIV-1 subtype B was the most prevalent in Latin America and the Caribbean, although subtype C and B/F recombinants have significant contributions in Argentina and Brazil. Thus, we suggest that primary drug resistance in Latin America and the Caribbean could have been underestimated. Clinical monitoring should be improved to offer better therapy, reducing the risk for HIV-1 resistance emergence and spread, principally in vulnerable populations, such as men who have sex with men transmission group, sex workers and intravenous drug users.

Keywords: Drug resistance mutation, HAART, primary drug resistance, meta-analysis, systematic review, time series.

1. INTRODUCTION

Estimations indicate that around 35 million people are living with human immunodeficiency virus type 1 (HIV-1) globally. Among these, around 1.75 million live in Latin America and Caribbean. The implementation of highly active antiretroviral therapy (HAART) in 1996 saved 6.6 million lives [1]. However, the fight against HIV-1 is far to be over. One of the most challenging aspects in the management of HIV-1 infection is the emergence of strains resistant to antiretroviral drugs.

*Address correspondence to this author at the Department of Genetics, Universidade Federal de Pernambuco, Avenida da Engenharia, Cidade Universitária, Recife, PE, Postal Code: 50740-600, Brazil;
Tel: 55 81 2126 8522;
E-mails: avccbio@gmail.com, antonio.victor@ufpe.br

HIV-1 typically produces high levels of viral particles. As its reverse transcriptase (RT) is error-prone, it consequently generates high degree of genetic diversity. Poor adherence to HAART regimens leads to suboptimal drug levels, which are insufficient to maintain persistent virus suppression. The virus then continues to replicate, albeit at lower replication rates. Thus, if a mutation conferring resistance to drugs arises, this will turn into a selective advantage for resistant *quasispecies*. In some cases, a single mutation can cause cross-resistance against all members of an antiretroviral drug class [2, 3].

A resistant HIV-1 strain may be transmitted to other persons. This is defined as primary HIV-1 drug resistance (PDR), and it complicates the choice of which regimen a patient with PDR should receive, because it increases the risk of

therapy failure (incomplete viral replication suppression), and consequently, disease progression [4].

Latin America and Caribbean countries have been improving HAART coverage in the past few years [1]. However, as more people are being treated, resistant strains could occur more frequently [4], and, since pre-treatment HIV-1 genotyping for resistance evaluation is generally not available in low/medium-income countries, PDR could turn into a significant threat to the HIV/AIDS programs from these countries.

Therefore, we decided to perform a systematic review of Latin America and Caribbean literature to overview PDR prevalence in the past years through meta-analysis and time-series modeling.

2. METHODS

2.1. Literature Search Strategy and Study Selection

We searched Latin America and Caribbean literature (from 1980 to June 2014) focusing HIV primary antiretro-viral resistance through PubMed database using MeSH (Medical Subject Headings) search terms. For example: ((“Country name” [Mesh]) AND “Antiretroviral Therapy, Highly Active” [Mesh]) AND “Drug Resistance” [Mesh]; ((“Country name” [Mesh]) AND “HIV/genetics” [Mesh]) AND “Drug Resistance” [Mesh] – in which “Country name” stands for each country from the geopolitical aspect defined above; or combinations of search terms with country names in PubMed search tool: “HIV DRM”; “HIV ARV resistance”.

After full text retrieval, the authors reviewed all potentially relevant studies. The inclusion criteria were (1) be an observational study, (2) include primary resistance prevalence estimation or available data so we could estimate it and (3) preferentially (though not mandatorily) include a list or description of the detected major mutations (mutations that relate to high resistance to antiretroviral drugs). We excluded studies that dealt with secondary resistance and pediatric or vertical transmission patients. For studies in which both types of resistance were reported, we included only the primary resistance data if they could be clearly distinguished from the secondary resistance information.

We extracted study characteristic data – HAART naïve HIV positive individuals sample number, number and percentage of females among the total sample size, age and demographics. We classified the demographics in six categories: pregnant women, men who have sex with men (MSM), intravenous drug users (IDU), sex workers, inmates and general population (defined as a cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors – such as anonymous blood bank donors).

2.2. Statistical Analysis

We performed a meta-analysis in a per country basis, but only if there were at least three studies, not performed by the same research group, for each country. All meta-analysis were performed through “meta” package [5] for R software version 3.1.1 [6].

Briefly, prevalence estimates were log-transformed for meta-analysis. Heterogeneity between studies was assessed by τ^2 statistic and I^2 measure and if they were significantly different from zero as evaluated by Cochran’s Q test with n-1 degrees of freedom (in which n is the number of studies included and with significance level α=0.10 for this test). A random effects model was assumed if heterogeneity was detected (DerSimonian and Laird method [7]). A fixed effects model was chosen if otherwise [8]. Ninety-five percent confidence intervals (95% CI) were calculated for each pooled prevalence.

Additionally, we performed a time-series analysis using Brazilian studies’ data, since more data were available for this country, allowing us to model PDR prevalence change over time, as mentioned in Results and Discussion section. Concisely, a time series is a sequence of measurements taken at ordered points in time. We performed an auto-regressive moving average (ARIMA) model, as proposed by Box and Jenkins (1970, 2013) [9]. We denote the model in the form ARIMA (p, d, q), where p, d, and q are non-negative integers numbers that represents, respectively, the autoregressive, integrated and moving average orders of the model. These orders represent the number of estimated regression parameters during model fitting.

First, we sorted studies chronologically in calendar years according to sample collection starting period. We used month ranges as stated in the studies whenever possible to order studies conducted in the same years range. Thus, we considered each study as an independent time point.

After that, we used the test proposed by Dickey and Fuller (1979) [10] to check whether the PDR prevalence series was stationary (i.e. to check if data parameters such as mean and variance did not change over time). Subsequently, we used the "forecast" package [11], also from R software, to choose the ARIMA model that best fitted our original data, using Akaike Information Criterion (AIC). After choosing the model, we performed the following diagnostics tests: standardized residuals test, autocorrelation function (ACF) of residuals test and Ljung-Box statistics [12], to check whether the assumptions of the model have been satisfied. If all the assumptions were met, the model was deemed useful for describing the PDR prevalence change over time according to Brazilian data and forecast the prevalence a few years further from the most recent collection sample date found during the literature search.

We interpreted the PDR prevalence estimates in three levels: low (prevalence lower than 5.0%), moderate (between 5.0% and 15.0%) and high (higher than 15.0%) based on the World Health Organization consensus [13].

3. RESULTS AND DISCUSSION

3.1. Studies Selection

The search produced 655 unique abstracts, from which 206 were potentially eligible for our review according to our criteria. Further 123 studies were excluded due to different outcomes being investigated (study not focused on primary resistance), inappropriate populations studied (pediatric patients, secondary resistance) or because we were not able to extract suitable data for statistical analysis due to the way they were reported by the authors. Fig. (**1**) depicts the flowchart detailing studies search, inclusions and exclusions.

Thus, 83 studies were suitable for the statistical analysis, but 14 of them could not be included since had fewer studies than the threshold defined in the Methods session (three studies).

Finally, 24 studies (nine from Argentina, three from Cuba, four from Chile, four from Mexico and four from Venezuela) were meta-analyzed. Forty-five studies from Brazil were analyzed through time-series.

The 83 studies included original articles, short communications and sequence notes, reporting epidemiological and/or phylogenetical findings. The median sample number was 76 (interquartile range, IQR=44-126.5; minimum and maximum 16 and 1655, respectively). The median age of the recruited individuals (for those studies with available information) was 34 years old (IQR=30.7-35.8). In average, 39.6% of recruited individuals by the studies were women. The majority (64 studies) dealt with HIV general population; MSM samples were recruited in seven studies; six dealt with pregnant women cohorts; sex workers were sampled in two; male inmates, IDU and persons involved with occupational exposure were sampled in one study each. A single study sampled both MSM and IDU. Table **1** details the information about each report, except for Brazilian studies, which are displayed chronologically according to the sample collection period on Table **2**.

3.2. Prevalence Summaries

Sixty-nine studies (among the total 83) reported which drug resistance interpretation algorithm was used. Since the majority of them (45 studies) used Stanford University HIVdb algorithm [14], we also used this algorithm (surveillance mutation list, June 2013 version) to improve consistency between studies. We recalculated prevalences according to Stanford algorithm major mutation list, thus disregarding minor (accessory) mutations whenever possible. Thus, please note that the reported prevalences in this review may not reflect the same published by the original authors.

3.2.1. Caribbean

The literature search identified nine studies conducted in Caribbean and associated countries, published between 1999 and 2013. Due to the low number of studies, formal statistical analysis could not be performed according to our methodological criteria, with exception for three Cuban studies.

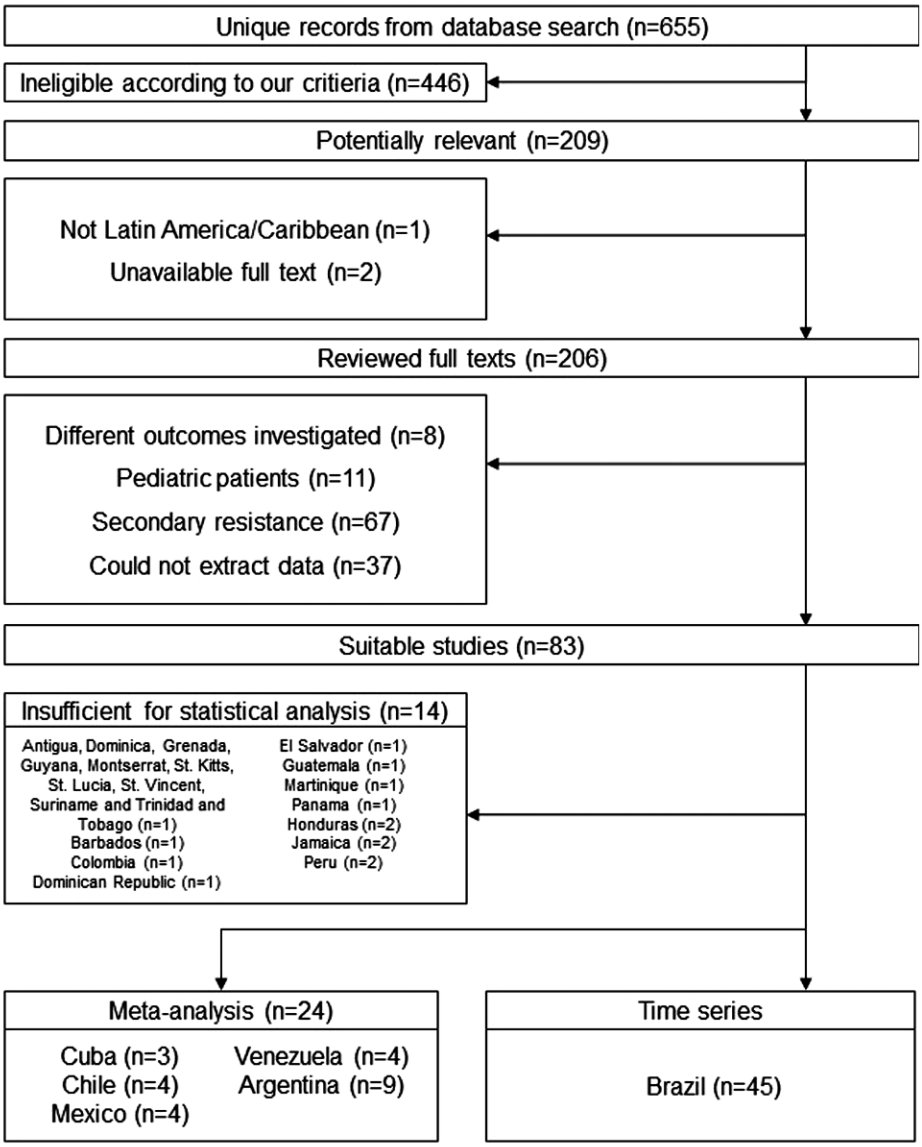

Fig. (1). Flow chart of studies selection for reviewing and inclusion on analysis.

Overall, PDR was found to be low to moderate in Caribbean countries, ranging from zero to 18.5%. A survey with samples from nine countries (Antigua and Barbuda, Dominica, Grenada, Guyana, Montserrat, St. Kitts and Nevis, St. Lucia, St. Vincent, Suriname and Trinidad and Tobago) did not report any major mutations [15].

Barbados, Dominican Republic and Martinique all had reported PDR prevalence lower than 10%: 2.8%, 7.8% and 7.2%, respectively [16-18]. Recent studies from Jamaica and Cuba reported moderate PDR prevalence. Data from Hamilton *et al.* (2012) [19] allowed us to estimate major mutations frequency of 18.5%, whereas data from Barrow *et al.* (2013) [20] yielded 12.6%, both for Jamaican populations.

Data from three Cuban studies apparently show a trend for PDR prevalence increment in the last twelve years. Ruibal-Brunet *et al.* (2001) [21] observed a prevalence of 7.4%. Later, Perez *et al.* (2007) [22] reported the close estimate of 5.2%. However, the most recent study from Machado *et al.* (2013) [23] in newly diagnosed individuals, reported a prevalence of 21.5%, the highest among Caribbean countries. According to our meta-analysis estimate, PDR pooled prevalence in Cuba

Table 1. Studies characteristics and reported primary drug resistance prevalence in Caribbean and Latin American countries.

Region, *Countries* and Studies	HIV-Infected Individuals Demographics	N Female (%)	Age (Years)	Reported Primary Drug Resistance Prevalence (%)
Caribbean				
Barbados				
[16]	General population	14/36 (38.9)	34.5 [30-41.3][a]	1/36 (2.8)
Cuba				
[21]	General population	NR	NR	2/27 (7.4)
[22]	General population	NR	NR	13/250 (5.2)
[23]	General population	30/200 (15.0)	35.3 [11.3][b]	43/200 (21.5)
Dominican Republic				
[17]	General population	56/103 (54.4)	38.6 [NR][b]	8/103 (7.8)
Jamaica				
[19]	General population	NR	NR	17/92 (18.5)
[20]	General population	64/103 (62.1)	37.3 [NR][b]	10/79 (12.6)
Martinique				
[18]	General population	23/70 (32.9)	NR	5/69 (7.2)
Nine countries*				
[15]	General population	54/94 (57.5)	22 [NR][b]	0/94 (0.0)
Continental Latin America				
Argentina				
[39]	(Transsexual) sex workers	0 (0.0)	29 [24-35][a]	12/62 (19.4)
[40]	General population	NR	NR	16/214 (7.5)
[41]	MSM, IDU	0 (0.0)	NR	2/23 (8.7)
[42]	(Female) sex workers	16/16 (100.0)	NR	3/16 (18.8)
[43]	General population	33/107 (30.8)	33 [NR][a]	4/98 (4.1)
[44]	General population	13/52 (25.0)	36 [11][b]	4/52 (7.7)
[45]	General population	33/152 (21.7)	37 [NR][b]	12/152 (7.9)
[46]	Pregnant women	78/78 (100.0)	25 [19–34][a]	7/78 (9.0)
[83]	General population	71/284 (25.0)	NR	12/284 (4.2)
Brazil				
See Table 2				
Chile				
[35]	General population	6/60 (10.0)	37.1 [23-60][a]	1/60 (1.7)
[36]	General population	NR	NR	2/79 (2.5)
[37]	MSM majority	0/25 (0.0)	35 [25-45][a]	3/25 (12.0%)
[38]	MSM majority	8/74 (10.8)	32 [18-58][a]	3/74 (4.1%)
Colombia				
[28]	General population	18/103 (17.5)	34 [18–59][a]	6/103 (5.8)
El Salvador				
[29]	General population	40/88 (45.5)	35.5 [NR][b]	5/88 (5.7)
Guatemala				
[84]	General population	64/145 (44.1)	37.3 [NR][b]	4/145 (2.8)

(Table 1) contd…..

Region, *Countries* and Studies	HIV-Infected Individuals Demographics	N Female (%)	Age (Years)	Reported Primary Drug Resistance Prevalence (%)
Honduras				
[31]	General population	NR	NR	18/239 (7.5)
[30]	General population	95/200 (47.0)	31 [15–64][a]	14/200 (7.0)
Mexico				
[25]	General population	NR	NR	41/1655 (2.5)
[51]	General population	9/96 (9.4)	NR	7/96 (7.3)
[85]	General population	4/42 (9.5)	33 [20-58][a]	1/42 (2.4)
[86]	Pregnant women majority	38/46 (82.6)	27 [6][b]	1/41 (2.4)
Panama				
[32]	General population	17/47 (36.2)	22 [20-24][a]	6/47 (12.8)
Peru				
[26]	MSM	0 (0.0)	NR	12/359 (3.3)
[27]	General population	46/112 (41.1)	NR	1/96 (1.0)
Venezuela				
[47]	General population	NR	NR	1/31 (3.2)
[48]	General population	3/20 (15.0)	NR	2/20 (10.0)
[49]	General population	14/65 (21.5)	32.6 [18-58][a]	4/62 (6.5)
[50]	General population	NR	NR	7/63 (11.1)

*Antigua and Barbuda, Dominica, Grenada, Guyana, Montserrat, St. Kitts and Nevis, St. Lucia, St. Vincent, Suriname and Trinidad and Tobago.
General population – cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors.
MSM – men who have sex with men.
NR – not reported.
[a]Median [interquartile range].
[b]Mean [standard deviation].

is around 10.0%, and can be as high as 28.0% (95% CI=3.0-28.0). These findings are summarized in Table **3**.

The relatively low number of studies in Caribbean region is a matter of concern, because, with the exception from Dominican Republic, Cuba and Jamaica, we could not find recently published studies on PDR prevalence (five among the nine studies were published five or more years ago) in the other Caribbean countries. Thus, these PDR prevalences may be actually underestimated (maybe even unknown in other countries in the region).

In the past few years, international task forces have been created for implement and improve PDR monitoring in the Caribbean and Latin America [24]. Therefore, it is possible that in the near future this knowledge gap regarding the Caribbean HIV population will be fulfilled, and ideally stabilize potential increasing PDR rates.

3.2.2. Continental Latin America

As for Caribbean countries, PDR prevalence in continental Latin America seems to be low to moderate. Data from some countries reported prevalences under 6.0%, such as Guatemala (2.8%) [25], Peru (two estimates: 3.3% and 1.0%) [26, 27], Colombia (5.8%) [28] and El Salvador (5.7%) [29].

Honduras apparently has moderate prevalence around 7.0% and 7.5% (Murillo *et al.* (2010) [30] and Lloyd *et al.* [31], respectively). A study from Panama observed a prevalence of 12.8% [32].

Noteworthy, Lama *et al.* (2006) [26] surveyed Peruvian MSM populations, whereas Soria *et al.* (2012) [27] sampled from HIV general population also from Peru. The estimates were close, but nonetheless PDR was higher in MSM individuals. Interestingly, previous evidence in European populations showed that MSM individuals infected

Table 2. Studies' characteristics and reported primary drug resistance prevalence in Brazil. The studies are ordered chronologically according to sample collection starting period.

Study	Sample Collection Period (Calendar Years)	Study Characteristics			Reported Primary Drug Resistance Prevalence (%)
		HIV-Infected Individuals Demographics	N Women (%)	Age (Years)	
[70]	1989 to 2005	General population	124/240 (51.7)	NR	6/290 (2.1)
[87]	1996	General population	NR	NR	3/32 (9.4)
[55]	1996 to 2012	MSM	0/64 (0.0)	30.6 [19-54][a]	9/64 (14.1)
[88]	1997	General population	19/48 (40.0)	35 [NR][b]	2/48 (4.2)
[89]	1998	General population	NR	NR	0/44 (0.0)
[90]	1998 to 2002	General population	NR	NR	21/341 (6.2)
[56]	1998 to 2003	MSM	NR	NR	10/50 (20.0)
[91]	1999 to 2001	IDU	NR	NR	3/38 (7.9)
[92]	1999 to 2001	General population	24/71 (33.8)	NR	1/71 (1.4)
[93]	2000	General population	11/56 (19.6)	28.4 [NR][b]	7/50 (14)
[94]	2000 to 2001	Occupational exposure	20/44 (45.5)	35.8 [21-62][a]	2/16 (12.5)
[61]	2000 to 2001	General population	52/129 (40.3)	31 [NR][b]	3/76 (3.9)
[53]	2001	General population	155/380 (40.8)	30.7 [9.1][b]	22/409 (5.4)
[95]	2001	General population	61/112 (54.5)	31 [25-37][a]	0/112 (0.0)
[96]	2001 to 2005	General population	15/27 (55.6)	30.1 [NR]	0/27 (0.0)
[97]	2002	General population	40/85 (47.0)	35.2 [11.0][b]	2/25 (8.0)
[64]	2002 to 2003	General population	30/84 (35.7)	NR	3/84 (3.6)
[98]	2002 to 2006	General population	34/123 (27.6)	37 [NR][b]	8/123 (6.5)
[99]	2003	Pregnant women	35/35 (100.0)	24 [17–35][a]	0/35 (0.0)
[100]	2003 to 2004	General population	NR	37 [NR][b]	9/56 (16.1)
[101]	2004 to 2006	General population	81/209 (38.8)	33 [27-40][a]	18/204 (8.8)
[102]	2004 to 2006	General population	NR	NR	7/50 (14.0)
[103]	2005	General population	12/44 (27.3)	35 [30–37][a]	2/62 (3.2)
[104]	2005 to 2006	General population	NR	NR	3/32 (9.4)
[105]	2005 to 2007	General population	116/246 (47.2)	NR	39/246 (15.9)
[62]	2005 to 2008	General population	89/205 (43.4)	35.4 [11.7][b]	7/205 (3.4)
[106]	2005 to 2008	Pregnant women	197/197 (100.0)	26 [NR][b]	21/197 (10.7)
[107]	2005 to 2008	General population	25/82 (30.5)	34.1 [NR][b]	6/82 (7.3)
[108]	2006 to 2007	General population	45/99 (45.4)	35 [10.01][b]	8/99 (8.1)
[109]	2006 to 2008	General population	15/33 (45.4)	35 [NR][b]	6/33 (18.2)
[110]	2007	General population	135/400 (33.8)	36 [15–66][a]	22/387 (5.7)
[111]	2007 to 2008	General population	32/103 (31.1)	32 [15–71][a]	10/103 (9.7)
[54]	2007 to 2008	General population	122/223 (54.7)	36 [8.0][b]	17/210 (8.1)
[65]	2007 to 2009	General population	61/130 (46.9)	NR	8/130 (6.1)
[72]	2008 to 2009	General population	21/52 (40.4)	30 [14–65][a]	6/52 (11.5)
[63]	2008 to 2009	General population	42/82 (51.2)	37.8 [NR][b]	8/82 (9.8)
[112]	2008 to 2009	General population	NR	32.15 [NR][b]	17/225 (7.6)
[77]	2008 and 2010	General population	19/49 (38.8)	36 [19-64][a]	3/49 (6.1)
[57]	2008 to 2009	MSM	0/44 (0.0)	NR	10/44 (22.7)
[113]	2008 to 2009	General population	38/92 (41.3)	36 [NR][b]	5/92 (5.4)
[114]	2008 to 2010	Pregnant women	30/30 (100.0)	25 [NR][b]	4/30 (13.3)
[115]	2009	General population	28/48 (58.3)	35.1 [11.2][b]	2/48 (4.2)
[116]	2009	(Male) inmates	0/38 (0.0)	31.5 [NR][b]	4/38 (10.5)
[117]	2010 to 2011	Pregnant women	16/16 (100.0)	25 [15–38][a]	4/16 (25)
[118]	2011	MSM (majority)	11/101 (10.9)	31 [NR][b]	14/101 (13.9)

General population – cohort composed of people with different HIV risk behaviors, or sometimes unknown/unspecified by the authors.
MSM – men who have sex with men.
NR – not reported.
[a]Median [interquartile range].
[b]Mean [standard deviation].

Table 3. Meta-analysis results summary.

Country	Number of Studies (Included/ Reviewed)	Heterogeneity				Model Selection	Pooled Primary Drug Resistance Prevalence (%), [95% CI]
		Estimation (τ^2)	I^2	Q Statistic	*p-Value*		
Argentina	9/17	0.21	59.4%	19.7	0.01	random effects	8.4 [5.7-12.0]
Chile	4/5	0.01	21.8%	3.84	0.28	fixed effects	3.3 [1.1-6.2]
Cuba	3/6	1.08	91.8%	24.5	<0.001	random effects	10.0 [3.0-28.3]
Mexico	4/8	0.29	58.3%	7.20	0.066	random effects	3.5 [1.7-7.1]
Venezuela	4/7	0	0%	1.95	0.58	fixed effects	7.5 [3.8-12.2]

95% CI - 95% confidence interval.

with HIV-1 subtype B (the same population profile as in the Peruvian sample) tended to be more likely infected with a resistant HIV-1 strain than individuals reporting other types of risk behavior/transmission route [33, 34].

Studies from Chile followed a similar trend. Whereas Afani *et al*. (2005) [35] and Rios *et al*. (2007) [36] reported prevalence between 1.7% and 2.5% in the general population, Acevedo *et al*. (2007) [37] and [38] observed higher prevalence among samples with MSM majority (12.0% and 4.1%, respectively). Nonetheless, overall PDR prevalence in Chile seems to be low. Our meta-analysis estimates a prevalence of 3.3% (95% CI=1.1-6.2).

PDR prevalence also appears to be higher among Argentinian MSM. Carobene *et al.* (2014) [39] reported a prevalence of 19.4% among transsexual sex workers infected with recombinant BF and B subtypes from Buenos Aires and major Argentinian cities. Pando *et al.* (2011) [40] sampled from HIV general population infected with the same subtypes, also in Buenos Aires during approximately the same period as did Carobene *et al.* They found an overall PDR prevalence of 8.4% (7.5% if considering only major mutations as defined by Stanford University HIVdb algorithm [14]). Andreani *et al.* (2011) [41] found a similar prevalence (8.7%) in MSM and IDU men at risk to HIV-1 re-exposure. Thus, subtype B and MSM transmission route may also be risk factors for transmitted antiretroviral resistance in Latin America. Female sex workers may also be at risk, since a sample from Argentinian BF and B subtypes-infected sex workers had a relatively high PDR prevalence – 18.8% [42]. Certainly, more studies are necessary to address this issue.

Aside from these studies, other data point to a rise in PDR prevalence in Argentina in the past few years. Kijaj *et al.* (2001) [43] reported a prevalence of 4.1% between 1997 to 2000 period. Dilernia *et al.* (2007) observed a prevalence of 4.2% between 2003 and 2005, whereas Petroni *et al.* (2006) [44], Rodriguez-Rodrigues *et al.* (2013) [45] and Pando *et al.* (2011) [40] reported prevalences above 7.0% between 2003 and 2009 (7.7%, 7.9% and 7.5%, respectively). Cecchini *et al.* (2013) [46] recently reported a PDR prevalence of 9.0% in a cohort of pregnant women sampled between 2008 and 2011. Including all the Argentinian studies cited above in a meta-analysis, we estimate a pooled PDR prevalence of 8.4% (95% CI=5.7-12.0), which is considered moderate.

Venezuela also seems to have moderate PDR prevalence (pooled prevalence=7.5%; 95% CI=3.8-12.2). Some authors of the four Venezuelan studies included in our meta-analysis acknowledge that PDR prevalence has been increasing in the country. Delgado *et al.* (2001) [47] initially reported a prevalence around 3.0% (considered low). Later, other authors reported prevalences higher than 5.0%: Bouchard *et al.* (2007) [48] observed 10.0%, Castillo *et al.* (2009) [49] reported a 6.5% prevalence, and the most recent survey, by Rangel *et al.* (2009) [50], found a prevalence of 11.1%.

In contrast to Argentina and Venezuela, Mexico apparently has low PDR prevalence. Three among four studies, including a relatively recent national survey with the highest sample number among all reviewed studies [25] reported rates around 2.5%. A single study [51] reported a prevalence of 16.0%

(7.3% if considering only high-level resistance). Our meta-analysis estimates that major mutations frequency in Mexico is low (3.5%; 95% CI=1.7-7.1). This can be consequence of the delay of implementation of universal access to HAART in Mexico, which started around 2004 [25]. Thus, it is possible that effective HAART coverage was low before this period and in the few years later, resulting in low selection rates for resistant strains. All meta-analysis results are summarized in Table **3**.

3.2.3. Brazil

We included Brazilian studies in a specific session due to the extensive data published throughout the years. Among the 83 papers discussed in this review, 45 were conducted on Brazilian samples. HIV/AIDS epidemiological notification and prevention programs started already in the first decade of AIDS discovery and detection in Brazil, and in 1996 it was one of the first developing countries to provide free-of-charge HAART for all eligible patients attending the public healthcare system; this is considered as a quite successful model of program against HIV/AIDS [52].

The higher number of studies allowed us to model through time series analysis how PDR prevalence evolved during more than 20 years of research and forecast changes for the next few years.

According to Dickey-Fuller test results, our data were not stationary (p=0.13), thus requiring additional differentiation before fitting to a non-seasonal ARIMA model. The best model (AIC=284.9) was an ARIMA (3, 1, 3), and diagnostic tests results showed that the assumptions of the model were met, i.e. residual errors were randomly distributed and not auto-correlated (data not shown). Therefore, this model was suitable to describe PDR prevalence change over time in Brazil.

Thus, our model defined a rising trend in PDR prevalence in Brazil between 1989 and 2011, for which data are available. Using the model to predict three forward time points, we estimate that 2014 actual PDR prevalence is around 20.6% (95% CI=10.7-30.6). The estimates for 2012 and 2013 were 7.3% (80% CI=1.0-13.6) and 15.3% (95% CI=5.6-25.0), respectively. Note that the CI for the 2012 estimate was set at 80% because the 95% CI lower bound yielded a spurious result (a negative number; prevalence is only expressed by positive numbers); so we were less strict for the definition of CI for this estimate. We did not estimate too much forward time points to avoid excessive speculation, since it would generate more imprecision at each new forecast. Fig. (**2**) shows a graphical representation of PDR prevalence changes over time in Brazil.

Brazil is politically divided in five regions: “Central-West”, North, Northeast, South and Southeast. Two previously published national surveys sampling in cities from almost all of these regions found overall intermediate levels of PDR prevalence. The 2002 survey [53] found a prevalence of 6.6% and the 2009 survey [54] observed a prevalence of 8.1%. Nonetheless, the authors acknowledge that major cities from Southeast Brazil, such as São Paulo and Rio de Janeiro, have moderate levels of PDR. It is also important to note that Brazilian MSM populations, similarly as discussed about Argentinian and Peruvian studies, seem to be more at risk to be infected with resistant strains, with reported prevalences between 14% and 22% [55-57].

Our data came predominantly from studies that sampled cities in Southeast and South Brazil (20 studies from Southeastern cities; nine from Southern cities; one with cities from both Southeast and South; one with cities from three regions: Southeast, South and Central-West; seven in Central-Western cities, two from Northeast and a single one from North Brazil. The remaining four were global national surveys). Thus, our estimate could be biased by the observations made in these regions, yielding overestimates.

Understandably, these regional “oversampling” may reflect the fact that the majority of Brazil total HIV cases since 1980 are concentrated in Southeast and South (55.5% and 20.0% of the cases, respectively) [58]. The Southeast was the region where the first Brazilian AIDS cases emerged in the 1980’s decade. Thus, it is reasonable to suppose that the HIV therapy implementation in this region started earlier than in other regions, thus favoring the early selection of resistant strains among patients with suboptimal adherence during the monotherapy period or in the early HAART years.

The relatively low number of studies in other regions are a matter of concern, since previous

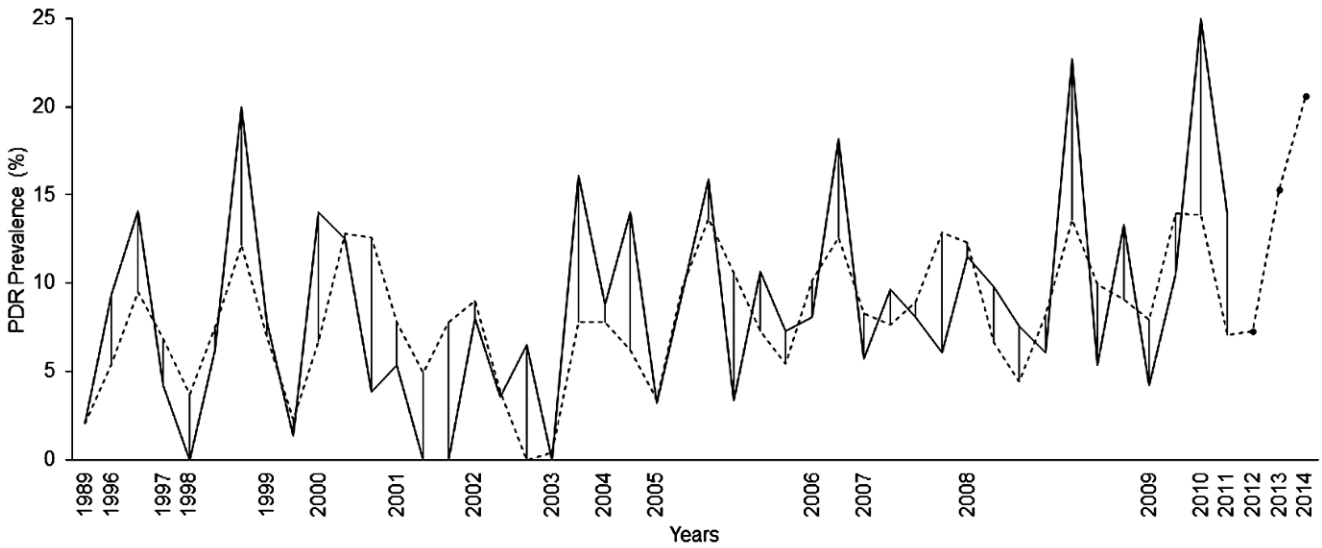

Fig. (2). Primary HIV drug resistance (PDR) prevalence time series and predicted values in Brazil. The solid line represent the prevalences at each reviewed study/time point, starting at 1989 and ending in 2011. The dashed line represents fitted predictions according to the ARIMA (3,1,3) model. The three filled circles represent three forward time points. The first circle is the 2012 estimate (7.3%, 80% CI=1.0-13.6), the second is the 2013 estimate (15.3%, 95% CI=5.6-25.0) and the third the 2014 estimate (20.6%, 95% CI=10.7-30.6).

analysis pointed out that Brazil's HIV epidemics has been in the process of "interiorization" and "pauperization" [59], meaning that HIV/AIDS cases are migrating to the countryside and affecting more people with lower socioeconomic status. Indeed, recent data show that detection rate of AIDS cases in Central-West (countryside Brazil - the only landlocked Brazilian region), Northeast and North regions (the most impoverished areas in the country) arose, whereas somewhat decreased in Southeast and South, between 2003 and 2012.

Thus, it is virtually unknown how many of these new cases in Central-West, Northeast and North regions were caused by resistant strains, which could further complicate treatment choices for populations already underserved in basic healthcare, or even favor selection of more resistant strains due to poor clinical/adherence monitoring.

To conclude, we should interpret cautiously the estimates we observed, but we may highlight that PDR prevalence is apparently rising in Brazil, and it is (at least) at moderate levels now. Moreover, the Southeast region is a possible "hotspot" of resistant strains circulation, which could influence significantly the spread of resistant strains to other regions in the future.

3.3. Subtypes and Mutations

HIV-1 B subtype was the most frequent among the individuals sampled in the 83 reviewed studies (mean percentage 69.2% of the samples), followed by subtype C (8.6%), B/F recombinants (7.5%) and subtype F (5.4%). Other subtypes and recombinant forms such as CRF or URF were 9.0% of the samples in average.

The majority of individuals from Chile, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Peru and Venezuela were infected by subtype B. Presumably, subtype distribution in the other Caribbean countries reviewed here also follows this pattern, as suggested by previous evidence [60]. Maybe Cuba is an exception, where other forms may prevail over subtype B, as reported elsewhere [22, 23].

Subtypes F and B/F recombinants had a significant frequency among subjects from Argentina and Brazil. B/F recombinants were even the majority in some Argentinian samples [39, 44].

Subtype C is frequent in the South Brazil region, being the majority among some samples [61-63]. Subtype F seems to have substantial frequencies (over 20.0%) in the Northeast region [64, 65].

Some authors evidence that different HIV-1 subtypes develop drug resistance mutations in unique ways, each favoring different patterns and frequency of mutations when challenged with antiretroviral therapy pressure [66-70].

Our study design does not allow us to elaborate further on this topic, since it is retrospective. Prospective studies with individuals matched by subtype and drug regimen would be more informative. However, we acknowledge that it can be influential in the PDR prevalence over time in countries with complex epidemics, such as Argentina and Brazil. Of note, some authors even highlight the spread of subtype C from South Brazil to major cities in Brazil and regions such as Central-West, reflecting the trend of "interiorization" mentioned earlier [59, 71, 72]. Thus, the spread of different subtypes into new areas could hypothetically change the local PDR prevalence, in a founder effect-like manner. Table **4** summarizes the subtypes frequencies for those studies in which this information was available.

In relation to the types of mutation reported by authors, we observed that among 53 studies from which we could extract mutation lists with fair accuracy, we could count 922 mutation events in 567 individuals (1.6 mutations per individual, on average) infected with HIV-1 resistant strains. Seven-hundred and twelve mutations (77.2%, 1.2 RT mutations per individual) were located at RT region, whereas 210 (22.8%) were distributed in protease (PR) codons (0.4 PR mutations per individual). Further analysis showed that among the 712 mutation events on RT region, 399 were associated to nucleoside analog RT inhibitor (NRTI) resistance and 313 were related to non-nucleoside analog RT inhibitor (NNRTI) resistance.

The NRTI resistance-associated mutations frequency distribution was T215F/Y (112 events), M184V/I (84 events), M41L (62 events), K219Q/E (47 events), D67N (41 events), K70R (19 events), T210W (22 events each) and L74V/I (12 events). NNRTI resistance-associated mutations were distributed in this manner: K103N/S (145 events), G190A/S/E (46 events), Y181C/I/V (44 events), K101E/P (23 events), V106A/M (25 events), E138A/G/K/Q (six events), Y188L/C/H (14 events), M230L (seven events) and L100I (three events). Finally, for Protease inhibitor (PI) resistance-associated mutations: M46I/L (64 events), V82A/T/F/S/L (42 events), L90M (35 events), I54V/T/A/L/M (27 events), D30N (13 events), N88S/D (10 events), I84V (six events), L76V (seven events), I47V/A (two events), G48V/M (two events), V32I and I50L/V (one event each).

Interestingly, these observations seem to confirm the concept of low "genetic barrier" for reverse transcriptase inhibitors (both NRTI and NNRTI). Just one or two mutations are sufficient to induce resistance against these drug classes, values similar to the average mutation number (1.2 RT mutations, as mentioned earlier). In contrast, more mutations are needed to induce resistance against PI (high "genetic barrier"). As expected, thymidine analog mutations were the most frequent in the samples, since low/medium-income countries (as is the case of Latin America and Caribbean) tend to use thymidine analogs, such as zidovudine, more frequently, favoring the emergence of these mutations [3].

Studies dealing with RT and PR mutations were the majority of the works surveyed before final inclusion in this review. We also found some studies in Brazil [73-78], Venezuela [79] and multinational surveys including Latin America/Caribbean countries [80, 81] that investigated mutations possibly related to resistance against integrase or fusion inhibitors. However, to our knowledge, these drug classes are not commonly used in first-line drug regimens, at least in Brazil clinical setting, being preferred as salvage regimen choices [58]. Thus, we did not include these studies in the final review because, in our opinion, these mutations, even if present, would have no clinical relevance for HAART naïve individuals.

CONCLUSION

We reviewed literature data concerning PDR prevalence in Latin American and Caribbean. We observed that (1) these regions have been reporting low to moderate levels of PDR prevalence; (2) subtype B dominates the epidemics, but Argentina and Brazil have significant contributions of B/F recombinants and subtype C and (3) NRTI and NNRTI resistance-associated mutations were more frequent, corresponding to more than 70% of mutational events observed. PI resistance-

Table 4. Summary of HIV-1 subtypes detected by each study and mean mutation number per individuals with primary HIV-1 drug resistance.

Country, Study	HIV-1 Subtypes					Mean Mutation Number Per Individual	
	B	C	F	B/F Recombinant	Other Forms	Protease Gene	Reverse Transcriptase Gene
Argentina							
[39]	38.7	4.8	0.0	54.8	1.6	1.0	2.3
[40]	57.9	2.3	0.5	39.3	0.0	0.8	2.1
[43]	NR	NR	NR	NR	NR	1.3	4.5
[44]	40.0	0.0	0.0	60.0	0.0	3.0	1.0
[45]	46.0	0.0	0.0	50.6	3.4	0.7	1.3
Brazil							
[53]	NR	NR	NR	NR	NR	ND	0.7
[54]	17.0	0.0	7.0	0.0	0.0	0.3	0.4
[55]	68.8	6.3	17.2	0.0	7.8	ND	1.6
[56]	91.4	2.5	1.2	3.7	1.2	0.5	1.1
[57]	81.8	7.7	0.0	0.0	6.9	0.3	1.2
[62]	22.0	64.4	0.0	0.0	13.7	0.7	0.7
[63]	13.4	65.9	0.0	0.0	0.0	0.3	1.3
[64]	72.6	1.2	22.6	3.6	0.0	ND	1.0
[65]	56.9	3.1	37.7	2.3	0.0	0.4	1.4
[70]	72.8	0.0	27.2	0.0	0.0	2.0	ND
[72]	78.8	5.8	1.9	0.0	13.5	0.2	1.3
[77]	65.3	10.2	8.2	8.2	8.2	ND	0.3
[88]	NR	NR	NR	NR	NR	ND	1.0
[93]	70.9	1.8	5.5	0.0	21.8	ND	0.6
[98]	82.0	5.7	6.5	0.0	5.8	0.4	0.8
[100]	78.6	21.4	0.0	0.0	0.0	ND	1.8
[102]	81.0	0.0	8.4	7.4	3.2	ND	0.3
[105]	78.0	0.0	9.8	5.7	6.5	0.3	0.5
[106]	81.0	1.0	10.0	0.0	0.0	0.2	0.4
[107]	85.3	3.7	3.7	7.3	0.0	0.2	0.7
[108]	26.2	39.4	1.1	0.0	33.3	0.3	0.9
[109]	66.7	6.1	12.1	15.2	0.0	ND	1.2
[110]	66.0	12.8	0.0	0.0	21.3	0.2	1.1
[111]	82.5	3.1	6.2	7.2	1.0	0.2	1.2
[112]	76.0	7.0	6.0	0.0	11.0	0.2	1.1
[113]	71.7	5.4	3.3	0.0	19.6	ND	0.8
[114]	61.2	12.2	4.1	20.4	0.0	0.3	0.8
[115]	39.6	25.0	8.3	12.5	14.6	1.0	0.5
[116]	13.2	34.2	0.0	0.0	0.0	0.5	0.3
[118]	77.9	2.7	1.8	10.6	0.0	0.6	0.9

(Table 4) contd…..

Country, Study	HIV-1 Subtypes					Mean Mutation Number Per Individual	
	B	C	F	B/F Recombinant	Other forms	Protease Gene	Reverse Transcriptase Gene
Chile							
[35]	NR	NR	NR	NR	NR	ND	1.0
[36]	85.0	0.0	0.0	15.0	0.0	ND	5.5
[37]	NR	NR	NR	NR	NR	ND	1.3
[38]	NR	NR	NR	NR	NR	0.3	1.3
Colombia							
[28]	NR	NR	NR	NR	NR	0.2	1.5
Cuba							
[21]	77.8	3.7	0.0	0.0	18.5	ND	0.5
[22]	43.6	4.0	0.0	0.0	52.4	0.5	0.8
[23]	36.5	0.0	0.0	0.0	63.5	0.02	1.4
El Salvador							
[29]	100.0	0.0	0.0	0.0	0.0	ND	0.8
Guatemala							
[84]	96.6	0.7	0.7	0.0	2.1	ND	0.6
Honduras							
[30]	99.0	0.0	0.0	0.0	1.0	ND	1.1
[31]	99.1	0.0	0.3	0.0	0.6	0.8	2.2
Jamaica							
[19]	100.0	0.0	0.0	0.0	0.0	ND	2.4
[20]	NR	NR	NR	NR	NR	ND	0.8
Mexico							
[25]	99.9	0.0	0.0	0.0	0.1	0.5	2.7
[51]	NR	NR	NR	NR	NR	0.9	2.9
Panama							
[32]	NR	NR	NR	NR	NR	ND	2.2
Peru							
[26]	100.0	0.0	0.0	0.0	0.0	0.8	0.9
Dominican Republic							
[17]	100.0	0.0	0.0	0.0	0.0	ND	1.0
Venezuela							
[49]	100.0	0.0	0.0	0.0	0.0	ND	1.3
[50]	100.0	0.0	0.0	0.0	0.0	0.1	1.0

NR – not reported.
ND – not detected.

associated mutations were the minority, reflecting the choice of first-line drug regimens in the area, which have thymidine analogs and NNRTI, drug classes with low "genetic barrier".

Even though PDR in Latin America and Caribbean appears to be not widespread, we still consider it a challenge for HIV clinicians due to few, relatively "outdated" studies. This, associated to the delay between sample collection dates and results publishing, lead us to hypothesize that PDR prevalence could be, in principle, underestimated.

We are aware of the review's limitations, including the fact that it was not possible to distinguish between recently and chronically infected individuals in a suitable manner for statistical analysis. As far as we know, methodologies for infection period estimation were introduced around 2008 [82]. Since most data reviewed here were published before this date, no information regarding infection time is available. We suggest that future studies regarding PDR on Latin America and Caribbean should include infection time estimation to better assess resistance transmission in HIV-1 infected individuals.

Moreover, PDR is simply an aspect of the broader field of HIV-1 drug resistance. We decided not to include secondary resistance because we reasoned that most studies regarding this topic are retrospective, which tend to sample patients that already had therapy failure. However, to perform secondary resistance prevalence estimation, the studies needed to be prospective: including a sample of individuals starting therapy and then performing follow-up and subsequently observing how many of them presented resistance/treatment failure. This kind of study design is not generally present in primary resistance investigations. Therefore, we reasoned that reviewing secondary resistance prevalence would raise too many biases and we feared that we could obtain inaccurate results; thus, we focused our efforts only on PDR.

As pre-treatment HIV-1 genotyping is not generally available in low/medium-income countries, close monitoring of patient clinical history and treatment adherence, principally in vulnerable populations (MSM, IDU, sex workers), is still the best way to favor the therapy success, reducing the emergence and spread of HIV-1 resistant strains.

CONFLICT OF INTEREST

The authors declare that there has been no conflict of interest.

ACKNOWLEDGEMENTS

Antonio Coelho designed the study, collected, analyzed data and wrote the manuscript; Ronald Moura, Ronaldo da Silva, Anselmo Kamada, Rafael Guimarães and Lucas Brandão collected and summarized the data; Hemílio Coelho analyzed the data and Sergio Crovella critically revised the manuscript. All authors reviewed and approved the final manuscript version.

We thank Drs. Marcelo Rodrigo Portela Ferreira and Tatiene Correia de Souza, both from the Department of Statistics from Federal University of Paraíba (UFPB), Brazil, for additional support during time series analysis and Jailson Araújo Júnior for manuscript proofreading. We also would like to thank CAPES and FACEPE for financial support.

REFERENCES

[1] UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2013: UNAIDS 2013.

[2] Beerenwinkel N, Däumer M, Sing T*, et al.* Estimating HIV Evolutionary Pathways and the Genetic Barrier to Drug Resistance. Journal of Infectious Diseases 2005; 191(11): 1953-60.

[3] Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS reviews 2008; 10(2): 67.

[4] Vella S, Palmisano L. The Global Status of Resistance to Antiretroviral Drugs. Clinical Infectious Diseases 2005; 41(Supplement 4): S239-S46.

[5] Schwarzer G. meta: Meta-Analysis with R. R package version 3.7-1. 2014.

[6] R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2014; Viena, Austria. URL http: //www.R-project.org/.

[7] DerSimonian R, Kacker R. Random-effects model for meta-analysis of clinical trials: An update. Contemporary Clinical Trials 2007; 28(2): 105-14.

[8] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. 1959.

[9] Box GE, Jenkins GM, Reinsel GC. Time series analysis: forecasting and control. 4th ed. Hoboken, New Jersey: John Wiley & Sons 2013.

[10] Dickey DA, Fuller WA. Distribution of the estimators for autoregressive time series with a unit root. Journal of the American statistical association 1979; 74(366a): 427-31.

[11] Hyndman RJ, Athanasopoulos G, Razbash S*, et al.* forecast: Forecasting functions for time series and linear models. R package version 5.5. 2014.

[12] LJUNG GM, BOX GEP. On a measure of lack of fit in time series models. Biometrika 1978; 65(2): 297-303.

[13] Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antiviral therapy 2008; 13 Suppl 2: 25-36.

[14] Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006; 42(11): 1608-18.

[15] Vaughan HE, Cane P, Pillay D, Tedder RS. Characterization of HIV type 1 clades in the Caribbean using pol gene sequences. AIDS research and human retroviruses 2003; 19(10): 929-32.

[16] Gittens MV, Roth WW, Roach T, *et al.* The molecular epidemiology and drug resistance determination of HIV type 1 subtype B infection in Barbados. AIDS research and human retroviruses 2003; 19(4): 313-9.

[17] Myers JE, Taylor BS, Rojas Fermin RA, *et al.* Transmitted drug resistance among antiretroviral-naive patients with established HIV type 1 infection in Santo Domingo, Dominican Republic and review of the Latin American and Caribbean literature. AIDS research and human retroviruses 2012; 28(7): 667-74.

[18] Cesaire R, Dos Santos G, Abel S, *et al.* Drug resistance mutations among HIV-1 strains from antiretroviral-naive patients in Martinique, French West Indies. Journal of acquired immune deficiency syndromes 1999; 22(4): 401-5.

[19] Hamilton CL, Eyzaguirre LM, Amarakoon, II, *et al.* Analysis of protease and reverse transcriptase genes of HIV for antiretroviral drug resistance in Jamaican adults. AIDS research and human retroviruses 2012; 28(8): 923-7.

[20] Barrow GJ, Hylton-Kong T, Rodriguez N, Yamamura Y, Figueroa JP. HIV-1 drug resistance in treatment-naive chronically infected patients in Jamaica. Antiviral therapy 2013; 18(7): 941-4.

[21] Ruibal-Brunet IJ, Cuevas MT, Diaz-Torres H, *et al.* Genotypic resistance mutations to antiretroviral drugs in HIV-1 B and non-B subtypes from Cuba. Revista panamericana de salud publica = Pan American journal of public health 2001; 10(3): 174-80.

[22] Perez L, Alvarez LP, Carmona R, *et al.* Genotypic resistance to antiretroviral drugs in patients infected with several HIV type 1 genetic forms in Cuba. AIDS research and human retroviruses 2007; 23(3): 407-14.

[23] Machado LY, Dubed M, Diaz H, *et al.* Transmitted HIV type 1 drug resistance in newly diagnosed Cuban patients. AIDS research and human retroviruses 2013; 29(2): 411-4.

[24] Jack N, Ravasi G, Schrooten W, *et al.* Implementing Early-Warning Indicators of HIV Drug Resistance in the Caribbean. Clinical Infectious Diseases 2012; 54(suppl 4): S290-S93.

[25] Avila-Rios S, Garcia-Morales C, Garrido-Rodriguez D, *et al.* National prevalence and trends of HIV transmitted drug resistance in Mexico. PloS one 2011; 6(11): e27812.

[26] Lama JR, Sanchez J, Suarez L, *et al.* Linking HIV and antiretroviral drug resistance surveillance in Peru: a model for a third-generation HIV sentinel surveillance. Journal of acquired immune deficiency syndromes 2006; 42(4): 501-5.

[27] Soria J, Bull M, Mitchell C, *et al.* Transmitted HIV resistance to first-line antiretroviral therapy in Lima, Peru. AIDS research and human retroviruses 2012; 28(4): 333-8.

[28] DiazGranados CA, Mantilla M, Lenis W. Antiretroviral drug resistance in HIV-infected patients in Colombia. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2010; 14(4): e298-303.

[29] Holguin A, Yebra G, Martin L, *et al.* Transmitted drug-resistance in human immunodeficiency virus-infected adult population in El Salvador, Central America. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2013; 19(12): E523-32.

[30] Murillo W, Paz-Bailey G, Morales S, *et al.* Transmitted drug resistance and type of infection in newly diagnosed HIV-1 individuals in Honduras. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2010; 49(4): 239-44.

[31] Lloyd B, O'Connell RJ, Michael NL, *et al.* Prevalence of resistance mutations in HIV-1-Infected Hondurans at the beginning of the National Antiretroviral Therapy Program. AIDS research and human retroviruses 2008; 24(4): 529-35.

[32] Castillo J, Arteaga G, Mendoza Y, *et al.* HIV transmitted drug resistance in adult and pediatric populations in Panama. Revista panamericana de salud publica = Pan American journal of public health 2011; 30(6): 649-56.

[33] Vercauteren J, Wensing AMJ, van de Vijver DAMC, *et al.* Transmission of Drug-Resistant HIV-1 Is Stabilizing in Europe. Journal of Infectious Diseases 2009; 200(10): 1503-08.

[34] Booth CL, Garcia-Diaz AM, Youle MS, *et al.* Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection. Journal of Antimicrobial Chemotherapy 2007; 59(3): 517-24.

[35] Afani A, Ayala M, Meyer A, Cabrera R, Acevedo W. Primary resistance to antiretroviral therapy in patients with HIV/AIDS in Chile. Revista medica de Chile 2005; 133(3): 295-301.

[36] Rios M, Delgado E, Perez-Alvarez L, *et al.* Antiretroviral drug resistance and phylogenetic diversity of HIV-1 in Chile. Journal of medical virology 2007; 79(6): 647-56.

[37] Acevedo W, Gallardo AM, Galaz J, Afani A, Cortes E. Detection of primary antiretroviral resistance in Chilean patients recently infected with human immunodeficiency virus (HIV). Revista medica de Chile 2007; 135(11): 1406-13.

[38] Afani A, Beltran C, Maria Gallardo A, *et al.* Prevalence of primary antiretroviral resistance among HIV infected patients in Chile. Revista medica de Chile 2010; 138(6): 669-76.

[39] Carobene M, Bolcic F, Farias MS, Quarleri J, Avila MM. HIV, HBV, and HCV molecular epidemiology among trans (transvestites, transsexuals, and transgender) sex workers in Argentina. Journal of medical virology 2014; 86(1): 64-70.

[40] Pando MA, Gomez-Carrillo M, Vignoles 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 research and human retroviruses 2011; 27(1): 17-23.

[41] Andreani G, Espada C, Ceballos A, *et al.* Detection of HIV-1 dual infections in highly exposed treated patients. Virology journal 2011; 8: 392.

[42] Pando MA, Eyzaguirre LM, Carrion G, *et al.* High genetic variability of HIV-1 in female sex workers from Argentina. Retrovirology 2007; 4: 58.

[43] Kijak GH, Pampuro SE, Avila MM, *et al.* Resistance profiles to antiretroviral drugs in HIV-1 drug-naive patients in Argentina. Antiviral therapy 2001; 6(1): 71-7.

[44] Petroni A, Deluchi G, Pryluka D, *et al.* Update on primary HIV-1 resistance in Argentina: emergence of mutations conferring high-level resistance to nonnucleoside reverse transcriptase inhibitors in drug-naive patients. Journal of acquired immune deficiency syndromes 2006; 42(4): 506-10.

[45] Rodriguez-Rodrigues N, Duran A, Bouzas MB, *et al.* Increasing trends in primary NNRTI resistance among newly HIV-1-diagnosed individuals in Buenos Aires, Argentina. Journal of the International AIDS Society 2013; 16: 18519.

[46] Cecchini DM, Zapiola I, Fernandez Giuliano S, *et al.* Etravirine resistance mutations in HIV-infected pregnant women. HIV medicine 2013; 14(2): 125-6.
[47] Delgado E, Leon-Ponte M, Villahermosa ML, *et al.* Analysis of HIV type 1 protease and reverse transcriptase sequences from Venezuela for drug resistance-associated mutations and subtype classification: a UNAIDS study. AIDS research and human retroviruses 2001; 17(8): 753-8.
[48] Bouchard M, Masquelier B, Moreno M, *et al.* HIV type 1 drug resistance among naive patients from Venezuela. AIDS research and human retroviruses 2007; 23(3): 482-5.
[49] Castillo J, Comegna M, Quijada W, *et al.* Surveillance of HIV type 1 drug resistance among naive patients from Venezuela. AIDS research and human retroviruses 2009; 25(12): 1329-33.
[50] Rangel HR, Garzaro DJ, Torres JR, *et al.* Prevalence of antiretroviral drug resistance among treatment-naive and treated HIV-infected patients in Venezuela. Memorias do Instituto Oswaldo Cruz 2009; 104(3): 522-5.
[51] Escoto-Delgadillo M, Vazquez-Valls E, Ramirez-Rodriguez M, *et al.* Drug-resistance mutations in antiretroviral-naive patients with established HIV-1 infection in Mexico. HIV medicine 2005; 6(6): 403-9.
[52] Nunn AS, da Fonseca EM, Bastos FI, Gruskin S. AIDS Treatment In Brazil: Impacts And Challenges. Health Affairs 2009; 28(4): 1103-13.
[53] Brindeiro RM, Diaz RS, Sabino EC, *et al.* Brazilian Network for HIV Drug Resistance Surveillance (HIV-BResNet): a survey of chronically infected individuals. Aids 2003; 17(7): 1063-9.
[54] Inocencio LA, Pereira AA, Sucupira MC, *et al.* Brazilian Network for HIV Drug Resistance Surveillance: a survey of individuals recently diagnosed with HIV. Journal of the International AIDS Society 2009; 12: 20.
[55] Tupinambas U, Duani H, Martins AV, Aleixo AW, Greco DB. Transmitted human immunodeficiency virus-1 drug resistance in a cohort of men who have sex with men in Belo Horizonte, Brazil--1996-2012. Memorias do Instituto Oswaldo Cruz 2013; 108(4): 470-5.
[56] Dudley DM, Chin EN, Bimber BN, *et al.* Low-cost ultra-wide genotyping using Roche/454 pyrosequencing for surveillance of HIV drug resistance. PloS one 2012; 7(5): e36494.
[57] Bermudez-Aza EH, Kerr LR, Kendall C, *et al.* Antiretroviral drug resistance in a respondent-driven sample of HIV-infected men who have sex with men in Brazil. Journal of acquired immune deficiency syndromes 2011; 57 Suppl 3: S186-92.
[58] Brasil. Ministério da Saúde. Secretária de Vigilância em Saúde. Programa Nacional de DST e Aids. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. Brasília: Ministério da Saúde: Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST e Aids 2013.
[59] de Brito AM, de Castilho EA, Szwarcwald CL. AIDS e infecção pelo HIV no Brasil: uma epidemia multifacetada. Revista da Sociedade Brasileira de Medicina Tropical 2000; 34(2): 207-17.
[60] Nadai Y, Eyzaguirre LM, Sill A, *et al.* HIV-1 epidemic in the Caribbean is dominated by subtype B. PloS one 2009; 4(3): e4814.
[61] Rodrigues R, Scherer LC, Oliveira CM, *et al.* Low prevalence of primary antiretroviral resistance mutations and predominance of HIV-1 clade C at polymerase gene in newly diagnosed individuals from south Brazil. Virus research 2006; 116(1-2): 201-7.
[62] Santos AF, Silveira J, Muniz CP, *et al.* Primary HIV-1 drug resistance in the C-terminal domains of viral reverse transcriptase among drug-naive patients from Southern Brazil. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011; 52(4): 373-6.
[63] Graf T, Passaes CP, Ferreira LG, *et al.* HIV-1 genetic diversity and drug resistance among treatment naive patients from Southern Brazil: an association of HIV-1 subtypes with exposure categories. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011; 51(3): 186-91.
[64] de Medeiros LB, Lacerda HR, Cavalcanti AM, de Albuquerque Mde F. Primary resistance of human immunodeficiency virus type 1 in a reference center in Recife, Pernambuco, Brazil. Memorias do Instituto Oswaldo Cruz 2006; 101(8): 845-9.
[65] Cavalcanti AM, Brito AM, Salustiano DM, *et al.* Primary resistance of HIV to antiretrovirals among individuals recently diagnosed at voluntary counselling and testing centres in the metropolitan region of Recife, Pernambuco. Memorias do Instituto Oswaldo Cruz 2012; 107(4): 450-7.
[66] Carobene MG, Rubio AE, Carrillo MG, *et al.* Differences in Frequencies of Drug Resistance–Associated Mutations in the HIV-1 pol Gene of B Subtype and BF Intersubtype Recombinant Samples. JAIDS Journal of Acquired Immune Deficiency Syndromes 2004; 35(2): 207-09.
[67] Grossman Z, Istomin V, Averbuch D, *et al.* Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C. Aids 2004; 18(6): 909-15.
[68] Soares EA, Santos AF, Sousa TM, *et al.* Differential drug resistance acquisition in HIV-1 of subtypes B and C. PloS one 2007; 2(8): e730.
[69] Cunha RD, Abreu CM, Gonzalez LMF, *et al.* Differential *In Vitro* Kinetics of Drug Resistance Mutation Acquisition in HIV-1 RT of Subtypes B and C. PloS one 2012; 7(10): e46622.
[70] Dumans AT, Barreto CC, Santos AF, *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. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 2009; 9(1): 62-70.
[71] Brigido LF, Ferreira JL, Almeida VC, *et al.* Southern Brazil HIV type 1 C expansion into the state of Sao Paulo, Brazil. AIDS research and human retroviruses 2011; 27(3): 339-44.
[72] Carvalho BC, Cardoso LP, Damasceno S, Stefani MM. Moderate prevalence of transmitted drug resistance and interiorization of HIV type 1 subtype C in the inland North State of Tocantins, Brazil. AIDS research and human retroviruses 2011; 27(10): 1081-7.
[73] Passaes CB, Guimaraes ML, Fernandez SL, *et al.* Lack of primary mutations associated with integrase inhibitors among HIV-1 subtypes B, C, and F circulating in Brazil. Journal of acquired immune deficiency syndromes 2009; 51(1): 7-12.
[74] Arruda LB, Fonseca LA, Duarte AJ, Casseb J. Genetic diversity on the integrase region of the pol gene among HIV type 1-infected patients naive for integrase inhibitors in Sao Paulo City, Brazil. AIDS research and human retroviruses 2010; 26(1): 105-7.
[75] Teixeira C, de Sa-Filho D, Alkmim W, *et al.* Short communication: high polymorphism rates in the HR1 and HR2 gp41 and presence of primary resistance-related mutations in HIV type 1 circulating in Brazil: possible impact on enfuvirtide efficacy. AIDS research and human retroviruses 2010; 26(3): 307-11.
[76] Araujo LA, Junqueira DM, de Medeiros RM, Matte MC, Almeida SE. Naturally occurring resistance mutations to HIV-1 entry inhibitors in subtypes B, C, and CRF31_BC.

Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2012; 54(1): 6-10.

[77] da Silveira AA, Cardoso LP, Francisco RB, de Araujo Stefani MM. HIV type 1 molecular epidemiology in pol and gp41 genes among naive patients from Mato Grosso do Sul State, central western Brazil. AIDS research and human retroviruses 2012; 28(3): 304-7.

[78] Iamarino A, de Melo FL, Braconi CT, Zanotto PM. BF integrase genes of HIV-1 circulating in Sao Paulo, Brazil, with a recurrent recombination region. PloS one 2012; 7(4): e34324.

[79] Rangel HR, Garzaro D, Fabbro R, *et al.* Absence of primary integrase resistance mutations in HIV type 1-infected patients in Venezuela. AIDS research and human retroviruses 2010; 26(8): 923-6.

[80] Eshleman SH, Hudelson SE, Bruce R, *et al.* Analysis of HIV type 1 gp41 sequences in diverse HIV type 1 strains. AIDS research and human retroviruses 2007; 23(12): 1593-8.

[81] Eshleman SH, Hudelson SE, Smith P, *et al.* Analysis of pol integrase sequences in diverse HIV type 1 strains using a prototype genotyping assay. AIDS research and human retroviruses 2009; 25(3): 343-5.

[82] Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2008; 13(36).

[83] Dilernia DA, Gomez AM, Lourtau L, *et al.* HIV type 1 genetic diversity surveillance among newly diagnosed individuals from 2003 to 2005 in Buenos Aires, Argentina. AIDS research and human retroviruses 2007; 23(10): 1201-7.

[84] Avila-Rios S, Mejia-Villatoro CR, Garcia-Morales C, *et al.* Prevalence and patterns of HIV transmitted drug resistance in Guatemala. Revista panamericana de salud publica = Pan American journal of public health 2011; 30(6): 641-8.

[85] Valle-Bahena OM, Ramos-Jimenez J, Ortiz-Lopez R, *et al.* Frequency of protease and reverse transcriptase drug resistance mutations in naive HIV-infected patients. Archives of medical research 2006; 37(8): 1022-7.

[86] Viani RM, Hsia K, Hubbard P, *et al.* Prevalence of primary HIV-1 drug resistance in pregnant women and in newly diagnosed adults at Tijuana General Hospital, Baja California, Mexico. International journal of STD & AIDS 2007; 18(4): 235-8.

[87] Brindeiro R, Vanderborght B, Caride E, *et al.* Sequence diversity of the reverse transcriptase of human immunodeficiency virus type 1 from untreated Brazilian individuals. Antimicrobial agents and chemotherapy 1999; 43(7): 1674-80.

[88] Pilcher CD, Perkins MD, Fiscus SA, *et al.* Genotypic resistance and the treatment of HIV-1 infection in Espirito Santo, Brazil. The Journal of infectious diseases 1999; 179(5): 1259-63.

[89] Dumans AT, Soares MA, Pieniazek D, *et al.* Prevalence of protease and reverse transcriptase drug resistance mutations over time in drug-naive human immunodeficiency virus type 1-positive individuals in Rio de Janeiro, Brazil. Antimicrobial agents and chemotherapy 2002; 46(9): 3075-9.

[90] Barreto CC, Nishyia A, Araujo LV, *et al.* Trends in antiretroviral drug resistance and clade distributions among HIV-1--infected blood donors in Sao Paulo, Brazil. Journal of acquired immune deficiency syndromes 2006; 41(3): 338-41.

[91] Maia Teixeira SL, Bastos FI, Hacker MA, Guimaraes ML, Morgado MG. Trends in drug resistance mutations in antiretroviral-naive intravenous drug users of Rio de Janeiro. Journal of medical virology 2006; 78(6): 764-9.

[92] Varella RB, Ferreira SB, de Castro MB, Zalis MG, Tavares MD. Human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns among treatment-naive patients in different stages of infection in Rio de Janeiro, Brazil. Journal of medical virology 2007; 79(8): 1033-9.

[93] Pires IL, Soares MA, Speranza FA, *et al.* Prevalence of human immunodeficiency virus drug resistance mutations and subtypes in drug-naive, infected individuals in the army health service of Rio de Janeiro, Brazil. Journal of clinical microbiology 2004; 42(1): 426-30.

[94] El-Far F, Medeiros EA, Gasparoto CT, Diaz RS. Antiretroviral drug resistance among patients with human immunodeficiency virus who act as sources or potential sources in occupational accidents involving healthcare workers. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America 2005; 26(9): 782-8.

[95] Soares MA, De Oliveira T, Brindeiro RM, *et al.* A specific subtype C of human immunodeficiency virus type 1 circulates in Brazil. Aids 2003; 17(1): 11-21.

[96] Eyer-Silva WA, Morgado MG. A genotyping study of human immunodeficiency virus type-1 drug resistance in a small Brazilian municipality. Memorias do Instituto Oswaldo Cruz 2005; 100(8): 869-73.

[97] Soares EA, Martinez AM, Souza TM, *et al.* HIV-1 subtype C dissemination in southern Brazil. Aids 2005; 19 Suppl 4: S81-6.

[98] Gonsalez CR, Alcalde R, Nishiya A, *et al.* Drug resistance among chronic HIV-1-infected patients naive for use of anti-retroviral therapy in Sao Paulo city. Virus research 2007; 129(2): 87-90.

[99] Cardoso LP, Pereira GA, Viegas AA, Schmaltz LE, Stefani MM. HIV-1 primary and secondary antiretroviral drug resistance and genetic diversity among pregnant women from central Brazil. Journal of medical virology 2010; 82(3): 351-7.

[100] Diaz RS, Vasconcelos L, Hayden RL, *et al.* Similar efficacy of lopinavir/ritonavir-containing regimens among clades B and F HIV-1-Infected individuals in Brazil. Journal of acquired immune deficiency syndromes 2008; 47(3): 399-401.

[101] Brigido LF, Nunes CC, Oliveira CM, *et al.* HIV type 1 subtype C and CB Pol recombinants prevail at the cities with the highest AIDS prevalence rate in Brazil. AIDS research and human retroviruses 2007; 23(12): 1579-86.

[102] 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. Memorias do Instituto Oswaldo Cruz 2008; 103(2): 143-9.

[103] Brigido LF, Franco HM, Custodio RM, *et al.* Molecular characteristics of HIV type 1 circulating in Sao Paulo, Brazil. AIDS research and human retroviruses 2005; 21(7): 673-82.

[104] Ferreira JL, Thomaz M, Rodrigues R, *et al.* Molecular characterisation of newly identified HIV-1 infections in Curitiba, Brazil: preponderance of clade C among males with recent infections. Memorias do Instituto Oswaldo Cruz 2008; 103(8): 800-8.

[105] Velasco-de-Castro CA, Grinsztejn B, Veloso VG, *et al.* HIV-1 diversity and drug resistance mutations among people seeking HIV diagnosis in voluntary counseling and testing sites in Rio de Janeiro, Brazil. PloS one 2014; 9(1): e87622.

[106] Pilotto JH, Grinsztejn B, Veloso VG, *et al.* Moderate prevalence of transmitted drug resistance mutations among antiretroviral-naive HIV-infected pregnant women in Rio de Janeiro, Brazil. AIDS research and human retroviruses 2013; 29(4): 681-6.

[107] Pfrimer IA, Bizinoto MC, Brandao NA, *et al.* Intermediate levels of transmitted antiretroviral drug resistance in Midwestern Brazil. AIDS research and human retroviruses 2013; 29(2): 205-6.

[108] de Medeiros RM, Junqueira DM, Matte MC, *et al.* Co-circulation HIV-1 subtypes B, C, and CRF31_BC in a drug-naive population from Southernmost Brazil: analysis of primary resistance mutations. Journal of medical virology 2011; 83(10): 1682-8.

[109] de Sa-Filho DJ, Ambar RF, Duarte NB, *et al.* HIV type 1 diversity from newly diagnosed patients in Santos metropolitan area/Brazil. AIDS research and human retroviruses 2009; 25(9): 925-9.

[110] Sprinz E, Netto EM, Patelli M, *et al.* Primary antiretroviral drug resistance among HIV type 1-infected individuals in Brazil. AIDS research and human retroviruses 2009; 25(9): 861-7.

[111] Cardoso LP, Queiroz BB, Stefani MM. HIV-1 pol phylogenetic diversity and antiretroviral resistance mutations in treatment naive patients from Central West Brazil. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2009; 46(2): 134-9.

[112] Ferreira JL, Rodrigues R, Lanca AM, *et al.* Transmitted Drug Resistance among People Living with HIV/Aids at Major Cities of Sao Paulo State, Brazil. Advances in virology 2013; 2013: 878237.

[113] Ferreira AS, Cardoso LP, Stefani MM. Moderate prevalence of transmitted drug resistance and high HIV-1 genetic diversity in patients from Mato Grosso State, Central Western Brazil. Journal of medical virology 2011; 83(8): 1301-7.

[114] Reis MN, de Alcantara KC, Cardoso LP, Stefani MM. Polymorphisms in the HIV-1 gp41 env gene, natural resistance to enfuvirtide (T-20) and pol resistance among pregnant Brazilian women. Journal of medical virology 2014; 86(1): 8-17.

[115] Gaspareto KV, Mello FM, Dias JR, *et al.* Genetic diversity and primary resistance among HIV-1-positive patients from Maringa, Parana, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 2012; 54(4): 207-13.

[116] Prellwitz IM, Alves BM, Ikeda ML, *et al.* HIV behind bars: human immunodeficiency virus cluster analysis and drug resistance in a reference correctional unit from southern Brazil. PloS one 2013; 8(7): e69033.

[117] da Costa ZB, de Lima YA, Martelli CM, Stefani MM. Transmitted HIV resistance among pregnant young women infected with HIV-1 in Brazil. AIDS patient care and STDs 2013; 27(8): 439-41.

[118] Sanabani SS, Pastena ER, da Costa AC, *et al.* Characterization of partial and near full-length genomes of HIV-1 strains sampled from recently infected individuals in Sao Paulo, Brazil. PloS one 2011; 6(10): e25869.

Accepted: February 12, 2015