Antiretroviral Drug Resistance in a Respondent-Driven Sample of HIV-Infected Men Who Have Sex With Men in Brazil

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Background: There are few studies on HIV subtypes and primary and secondary antiretroviral drug resistance (ADR) in community-recruited samples in Brazil. We analyzed HIV clade diversity and prevalence of mutations associated with ADR in men who have sex with men in all five regions of Brazil.

Methods: Using respondent-driven sampling, we recruited 3515 men who have sex with men in nine cities: 299 (9.5%) were HIV-positive; 143 subjects had adequate genotyping and epidemiologic data. Forty-four (30.8%) subjects were antiretroviral therapy-experienced (AE) and 99 (69.2%) antiretroviral therapy-naïve (AN). We sequenced the reverse transcriptase and protease regions of the virus and analyzed them for drug resistant mutations using World Health Organization guidelines.

Results: The most common subtypes were B (81.8%), C (7.7%), and recombinant forms (6.9%). The overall prevalence of primary ADR resistance was 21.4% (i.e. among the AN) and secondary ADR was 35.8% (i.e. among the AE). The prevalence of resistance to protease inhibitors was 3.9% (AN) and 4.4% (AE); to nucleoside reverse transcriptase inhibitors 15.0% (AN) and 31.0% (AE) and to nonnucleoside reverse transcriptase inhibitors 5.5% (AN) and 13.2% (AE). The most common resistance mutation for nucleoside reverse transcriptase inhibitors was 184V (17 cases) and for nonnucleoside reverse transcriptase inhibitors 103N (16 cases).

Conclusions: Our data suggest a high level of both primary and secondary ADR in men who have sex with men in Brazil. Additional studies are needed to identify the correlates and causes of antiretroviral therapy resistance to limit the development of resistance among those in care and the transmission of resistant strains in the wider epidemic.

Key Words: HIV-1, men who have sex with men, respondent-driven sampling, Brazil, antiretroviral resistance

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INTRODUCTION

The Brazilian epidemic of HIV-1 is biologically complex and includes several subtypes and recombinant genomes as well as regional differences in the distribution of these clades.[1–3] HIV-1 develops extensive genetic diversity and can acquire mutations to develop antiretroviral drug resistance (ADR). The presence of ADR is a major obstacle to successful treatment, affecting the efficacy of drug regimens and mortality rates.[4–6] Since the introduction of universal access to highly active antiretroviral therapy in Brazil in 1996, a substantial decrease in HIV-related morbidity and mortality has been observed.[7] However, there have been concerns about the emergence and transmission of ADR,[8] especially in specific subpopulations such as men who have sex with men (MSM).

Based on worldwide studies, the prevalence of transmitted drug-resistant HIV-1 variants ranges from 1.4% to 28.9%.[9,10] In some South American and Caribbean countries, a low prevalence of primary drug resistance mutation has been reported.[11–13] Recent studies from different sites in Brazil have found the prevalence of primary ADR to range from 1.4% to 12.7%.[14–16] Some studies have reported even higher prevalences[17] with the highest 25% in antiretroviral therapy (ART)-experienced patients and 37% in ART-naïve patients.[18] As a result, in the last years, the Brazilian Ministry of Health began monitoring ADR in samples in the most populated state capitals.[19]

Although the occurrence of primary ADR is well documented, less is known about mechanisms and risk factors for transmission. Possible ways that may facilitate transmission of resistant virus include reductions in immune function, repeated exposure by the same partner, specific high-risk sexual practices, or concentration of resistant HIV in certain sexual networks.[20,21]

A relatively recent systematic review of MSM in the global HIV epidemic found a ninefold higher prevalence of ADR compared with other groups in middle- to low-prevalence countries. In Brazil, MSM are 29 times more likely to be infected than the general population.[22] MSM in Latin America as a whole are the principal risk group for HIV infection and account for the greater part of incident infection. Despite the fact that MSM have accounted for most new cases for many years in Brazil and surveillance is a central part of the government's strategy, the MSM population has been relatively understudied to date. Part of the reason is the difficulty of reliably sampling this population. Recently respondent-driven sampling (RDS) has become available to access hard-to-reach populations in studies focusing on behavioral and biologic surveillance, epidemiologic studies, and evaluation of program interventions.[23] RDS generates a quasiprobability sample, and although there is controversy about its analysis and interpretation,[24] it is a significant advance over snowball sampling in limiting the selection biases inherent in relying on a few participants to recruit substantial numbers of others, in reaching diverse social networks, and in providing a basis for statistical adjustment of recruitment patterns to produce population estimates. Since RDS was introduced in South America in 2005, there have been relatively few studies conducted among MSM.[25] None of these surveys explored transmission patterns based on HIV genotype analysis nor did they determine the occurrence of ADR in participants.

This study describes HIV-1 diversity and the prevalence of antiretroviral resistance mutations based on sequence analyses of the protease (PR) and reverse transcriptase (RT) genes in HIV-1 strains isolated from MSM recruited by RDS in all five regions of Brazil.

MATERIALS AND METHODS

Study Population

We conducted a cross-sectional study using RDS in nine Brazilian cities located in all five macroregions of the country designated by the Ministry of Health for a population-based

FIGURE 1. Geographic distribution of the study population.

national evaluation: Manaus (north), Recife and Salvador (northeast), Campo Grande (central west), Belo Horizonte, Rio de Janeiro, and Santos (southeast), and Itajaí and Curitiba (south) as shown in Figure 1. In brief, the RDS methods followed standardized protocols developed by the US Centers for Disease Control and Prevention for behavioral surveillance. In each city, several study-eligible seeds are selected who recruit up to three other MSM and so on until the sample size is met and equilibrium is achieved on key variables. The recruitment began in October 2008 and ended in July 2009. The overall sample included 3515 men who reported having sex with another man in the last 12 months, were 18 years of age or older, and resided in one of the nine cities. The rapid test for HIV was offered to all participants and HIV infection was measured by finger stick rapid HIV test. Whole blood was used in all the diagnostic tests, and the study followed the national algorithm for rapid testing (Portaria no. 34 de 28/07/05 da Secretaria de Vigilância em Saúde): first the Rapid Check HIV-1 & 2 (Núcleo de Doenças Infecciosas [NDI], Vitória, Espírito Santo, Brazil) and Bio-Manguinhos HIV-1 & 2 (Instituto de Tecnologia em imunobiologicos, Bio-Manguinhos, FIOCRUZ, Rio de Janeiro, Brazil) were administered at the same time followed by a third test in case the previous two tests were discrepant. Those who tested HIV-positive were invited to give a sample to proceed with genotype testing. The HIV-1 serostatus of each of those subjects who agreed to genotype testing was further confirmed by commercial enzyme-linked immunoassay and Western blot. Of the 299 (9.5%) found to be infected with HIV, 178 (59.5%) samples were available and sufficient for genotype testing. The study was approved by the Brazilian National Ethical Committee (CONEP 14494) and written informed consent for all the procedures was obtained from each participant.

Molecular Analyses

The DNA of the 178 samples was extracted from the buffy coat using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's procedure. The HIV-1 *pol* gene was amplified using nested polymerase chain reaction (PCR) to obtain one fragment containing the entire protease (PR) gene and approximately 700 base pairs of the reverse transcriptase (RT) gene as previously described.[26] Amplified products from the second-round PCR were purified using the QIAquick PCR purification kit (Qiagen); the concentrations were quantified by ultraviolet absorbance spectrophotometry using the Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE). Purified PCR products were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems, Foster City, CA) according to the manufacturer's protocol in an Applied BioSystems ABI 3100 automatic sequencer (Applied BioSystems). All sequences obtained were assembled with Sequencher 4.7 (Gene Codes, Ann Arbor, MI) software and manually edited to identify mixtures. To define the subtype of each strain, we used interface software[27] that automatically submits the sequences to two programs: recombinant identification program and basic local alignment tool. Recombinant strains and sequences that did not yield concordant subtype results were manually reviewed using the bootscanning technique in SIMPLOT. The RT and PR regions of the virus were analyzed for drug-resistant mutations according to World Health Organization guidelines using the latest update of the Stanford Surveillance Drug Resistance Mutations Calibrated Population Resistance Tool (http://hivdb.stanford.edu/) according to the latest update.[28]

Genbank accession numbers of the sequences presented in this study are HQ127459 to HQ127620.

Statistical Analyses

We used the chi-square test for comparing proportions and the Student *t* test for continuous variables as appropriate; $P < 0.05$ was considered significant. These analyses were performed using Stata Version 10.0 (Stata Corp, College Station, TX). For producing population prevalence estimates, adjustments were made to account for the sampling design. RDS is a chain referral sampling method that collects information on the size of each participant's network (degree) and uses this to calculate the probability of selecting for each participant. Additionally, as implemented here for a national sample, we combined individual recruitment weight (W) for each participant (i) from each city (h) as

$$W_{ih} = (1/R_{ih})/\sum(1/R_{ih}) \times n) \times M_h$$

where R_{ih} is the degree of the network, M_h is the proportion of MSM in the city related to the total sample size, and n is the total sample size. We estimated M_h as follows: 1) we used the proportion of adult men (18 years or older) who reported ever having sex with a man in the last 12 months as reported in a population-based survey conducted by the Brazilian government for each city listed in this study or region of the country where the city was located; 2) we multiplied this proportion multiplied by the adult male population 18 years old or older in that city found in the Brazilian national census to derive the estimated number of MSM for each city; and 3) we summed the numbers of MSM for all the nine cities and calculated the proportion that those MSM (M_h) in each city would represent in the total sample (n).

RESULTS

Of the 178 samples tested, we were able to sequence 162 (91.0%) successfully.

Sociodemographic Profiles of the Study Participants

Socioepidemiologic data could be linked to 143 (88.3%) of the 162 samples sequenced; they are presented in Table 1. The mean age of participants was 32.6 years, 40.2 years for the ART-experienced group and 29.1 years for the ART-naïve group. The regions with the largest number of samples were the south and southeast followed by the northeast.

Sexual activity for 10 or more years was reported by 78.8% in the ART-naïve group and 99.3% in the ART-experienced group ($P < 0.001$). Always using condoms

TABLE 1. Demographic and Behavioral Characteristics According to Antiretroviral Exposure, Men Who Have Sex With Men, Brazil, 2009

	Naïve*	Treated†	
	Percent (95% CI)	Percent (95% CI)	*P*
Age (years)			
Younger than 30	46.4 (27.0–66.9)	1.5 (0.3–7.7)	< 0.001
30 or older	53.6 (33.1–73.0)	98.5 (92.3–99.7)	
Schooling (years)			
Less than 11	52.3 (32.2–71.6)	35.4 (14.8–63.2)	0.330
11 or more	47.7 (28.4–67.8)	64.6 (36.8–85.2)	
Geographic region			
North/central west	6.6 (4.0–10.7)	0.2 (0.1–0.9)	< 0.001
Northeast	17.0 (9.3–29.1)	0.9 (0.2–3.6)	
South/south east	76.4 (63.7–85.7)	98.8 (96.4–99.6)	
Income			
Minimum wage	47.8 (29.2–67.1)	48.3 (23.6–73.8)	0.979
More than Minimum wage	52.2 (32.9–70.8)	51.7 (26.2–76.4)	
Sexual activity (years)			
Less than 10	21.2 (10.8–37.4)	0.7 (0.2–3.1)	< 0.001
10 or more	78.8 (62.6–89.2)	99.3 (96.9–99.8)	
Number of partners (last 12 months)			
Less than 10	60.9 (39.7–78.6)	59.8 (31.2–83.0)	0.951
10 or more	39.1 (21.4–60.3)	40.2 (17.0–68.8)	
Frequency of condom use (last 12 months)			
Sometimes / never	78.7 (61.4–89.5)	72.0 (49.6–87.0)	0.573
Always	21.3 (10.5–38.6)	28.0 (13.0–50.4)	
Previous STDs (last 12 months)			
No	76.1 (57.8–88.1)	58.7 (32.1–81.0)	0.248
Yes	23.9 (11.9–42.2)	41.3 (19.0–67.9)	

*n = 44.
†n = 99.
CI, confidence interval; STDs, sexually transmitted diseases.

regardless of the type of partner was reported in 21.3% of the ART-naïve group and 28.0% of the ART-experienced group. Additionally, exchange of sex for money in the last 12 months was reported by 28 (18.5%) subjects; 22 (84.6%) of them were in the ART-naïve group. Thirty (19.7%) participants reported that they had paid for sex; 58.6% of these were in the ART-naïve group (results not shown).

As presented in Table 1, characteristics were generally equivalent between the two groups, except for age and the number of years of sexual activity, which were higher in the ART-experienced group. In addition, we found a higher proportion of treated patients in the south/southeast region.

HIV Subtyping

Table 2 shows overall data on subtypes and mutations. Clade B was the most common in the two groups: 66.8% (ART-naïve) and 91.1% (ART-experienced). We found recombinants in 10 (6.1%) samples, nine of which classified as BF (5.5%) and one as a BC mosaic (0.6%). The BF recombinants were all classified as F for the PR region and as B for the RT region. The BC sequence was C for the PR and B for the RT region. The BF recombinants were from seven ART-naïve and two ART-experienced subjects and from all regions except the central west. The sequence classified as BC was from an ART-naïve individual from the southeast region.

Geographically, we found that clade B predominated in all regions except for the south, where non-B samples constituted 50% (10 of 20). The prevalence of the B clade was 92.3% in the north, 90.6% in the northeast, 89.5% in the central west and 80.0% in the southeast. Recombinant samples were obtained from all the regions except the central west; the largest number was from the south region.

Circulation of Resistance Mutations

The distribution of the three most common types of mutations is shown in Figure 2. The most common mutations were 184V (17 cases) in 6.1% of ART-naïve and 25.0% of ART-experienced cases and 103N (16 cases) in 10.1% of ART-

naïve and 13.6% of ART-experienced cases. Drug resistance mutations were present in 21.4% of ART-naïve and in 35.8% of ART-experienced participants. The prevalence of protease inhibitor-resistant mutations was 3.9% in ART-naïve and 4.4% in ART-experienced, of nucleoside reverse transcriptase inhibitor-resistant mutations 15.0% in ART-naïve and 31.0% in ART-experienced and for nonnucleoside reverse transcriptase inhibitor 5.5% in ART-naïve and 13.2% in ART-experienced participants. The mutations for RT at positions 77, 100, 106, 179, and 230 were not detected in any of the samples nor were they detected for PR at positions 23, 32, 36, 47, 50, 53, 76, 83, and 85. Mutations did not cluster geographically. Table 3 summarizes the demographic characteristics of ART-naïve subjects according to their drug resistance status. Of all factors considered, greater than 11 years of schooling and clade B genotype more common among MSM with ADR mutations.

DISCUSSION

Most studies on HIV diversity and primary and secondary resistance in Brazil are based on convenience samples collected at facilities such as AIDS referral clinics, anonymous HIV testing services, or blood banks. In this study, we described the molecular characteristics of part of the HIV-1 *pol* genomic region isolated from MSM recruited from the community at large in each of the five geographic regions in Brazil using RDS sampling. Although we are not able to claim that the sample represents all Brazilian MSM, this is the first study in Brazil to focus on samples from diverse geographic areas and collected with an appropriate technique to sample a hard-to-reach population. We found high rates of both primary and secondary ADR (21.4% and 35.8%, respectively) with great potential impact on the prevention of the spread of resistance and on the health outcomes for persons under HIV care.

Our results support previous findings. All the major Brazilian circulating subtypes (B, C, and F) of group M were found with predominance of the B clade except in the south region where subtype C was found in half of the samples.[29,30] Less common HIV clades were also detected in different geographic regions such as subtype C in the north region, which argues for the need to track the simultaneous circulation of less common HIV forms as part of a more extensive characterization of the Brazilian MSM HIV epidemics. Diverse clades in circulation may denote different points of introduction, modes of transmission, and different networks requiring specifically targeted outreach programs.

Surveillance of primary resistant strains will become increasingly important for countries where ART is scaling up rapidly. The worldwide prevalence of transmitted drug-resistant HIV-1 variants fluctuates according to the characteristics of the population and the methodology of the studies.[9] Of note, Brazil was one of the first countries in the world to offer universal access to ART and achieved a high level of coverage. Previous convenience samples in Brazil demonstrate drug resistance levels range below 13%[14–16] with the exception of one study in the harbor city of Santos where ADR reached 37%.[18] Most of these studies did not assess rates of resistance

TABLE 2. Virologic Characteristics According to Antiretroviral Drug Exposure, Men Who Have Sex With Men, Brazil, 2009

	Naïve*		Treated†	
	Percent	(95% CI)	Percent	(95% CI)
Genotype				
B	66.8	(44.4–83.5)	91.1	(76.9–96.9)
C	4.8	(1.0–20.9)	5.1	(1.3–17.6)
F	16.1	(4.3–45.0)	2.8	(0.4–18.6)
Recombinants	12.3	(4.3–30.4)	1.0	(0.5–2.1)
Mutations to NRTI	15.0	(5.5–34.7)	31.0	(11.9–59.8)
Mutations to NNRTI	5.5	(1.7–16.3)	13.2	(4.4–33.7)
Mutations to PI	3.9	(1.5–9.6)	4.4	(1.1–16.0)
Any antiretroviral resistance mutation	21.4	(9.9–40.3)	35.8	(15.2 to 63.5)

*n = 44.
†n = 99.
CI, confidence interval; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
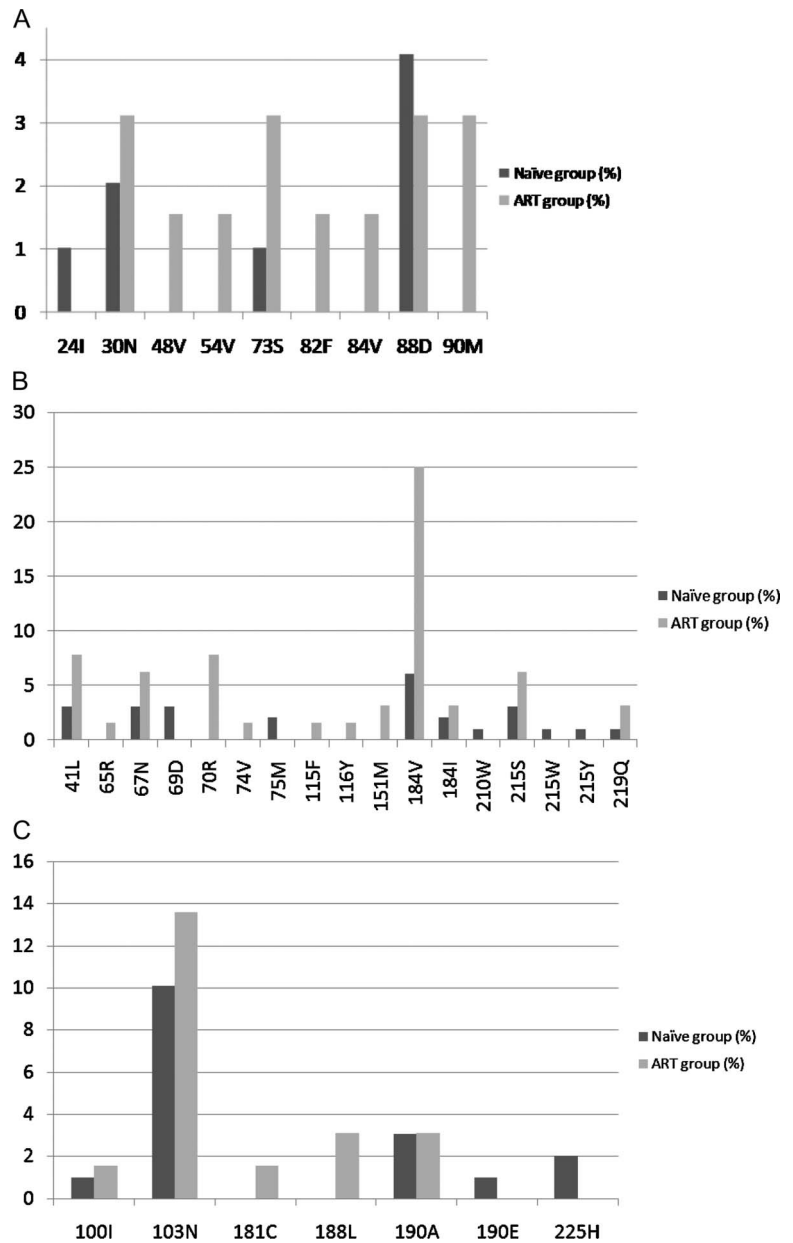
FIGURE 2. Distribution of mutations according to antiretroviral exposure.

by risk exposure or other epidemiologic data. A recent study by Sprinz and colleagues[31] evaluated 387 ART-naïve patients from 20 outpatient clinics from 13 cities in Brazil. Primary resistance was detected in only nine (5.2%) of 173 MSM, and no difference was found with regard to any risk factors. The higher prevalence of resistance among ART-naïve MSM (19.3%) in our study could be the result of differences in the recruitment methods and type of population studied. In the Sprinz study, the samples were collected from outpatients not receiving treatment, and it is likely that the time between initial infection and genotype testing was longer than in our study. Resistant strains are less fit and when there is no selection by antiretroviral drugs, ADR tends to disappear or at least diversity narrows.[32,33] Through its modified chain-referral

methods, RDS permits us to identify links between ART-naïve and ART-experienced individuals and, although these links could be friendship or acquaintance links, we can reasonably assume that some are sexual links. The proportion of sexual links between ART-experienced and ART-naïve individuals in our study may be higher than in Sprinz, accounting for our results. Alternatively, there could be some other form of selection bias in study convenience samples that resulted in underestimation of primary ADR.

Our data indicate a statistical association of ADR with higher education level and genotype B, probably reflecting an association with the network of the ART-naïve subjects of this particular sample. Drug resistance studies in Brazil need to incorporate epidemiologic and behavioral data and

TABLE 3. Sociodemographic Data in the Antiretroviral Treatment-Naïve Group, Men Who Have Sex With Men, Brazil, 2009

H	No Resistance* Percent (95% CI)	Resistance† Percent (95% CI)	P
Age (years)			
Younger than 30	47.5 (24.8–71.2)	42.3 (14.6–75.8)	0.808
30 or older	52.5 (28.8–75.2)	57.7 (24.2–85.4)	
Schooling (years)			
Less than 11	63.0 (41.1–80.6)	12.9 (4.3–32.8)	< 0.001
11 or more	37.0 (19.4–58.9)	87.1 (67.2–95.7)	
Income			
Minimum wage	44.6 (24.0–67.2)	59.6 (24.3–87.1)	0.511
More than Minimum wage	55.4 (32.8–76.0)	40.4 (12.9–75.7)	
Sexual activity (years)			
Less than 10	21.9 (10.0–41.4)	18.7 (5.0–50.1)	0.817
10 or more	78.1 (58.6–90.0)	81.3 (49.9–95.0)	
Number of partners (last 12 months)			
Less than 10	61.4 (36.5–81.5)	59.2 (23.9–87.0)	0.922
10 or more	38.6 (18.5–63.5)	40.8 (13.0–76.1)	
Frequency of condom use (last 12 months)			
Sometimes/ never	76.1 (54.3–89.5)	88.0 (63.3–96.9)	0.317
Always	23.9 (10.5–45.7)	12.0 (3.1–36.7)	
Geographic region			
North/central west	5.9 (3.1–10.8)	9.2 (3.4–22.7)	
Northeast	18.5 (9.2–33.7)	11.5 (2.9–36.0)	0.661
South/southeast	75.6 (60.2–86.4)	79.2 (54.7–92.3)	
Genotype			
B	58.0 (33.5–79.1)	98.8 (93.3–99.8)	< 0.001
Non-B	42.0 (20.9–66.5)	1.2 (0.2–6.7)	

*n = 73.
†n = 26.
CI, confidence interval.

to use these findings to support enhanced secondary prevention methods. Additionally, although numbers are small, there is a suggestion that the mutation variants are different in the ART-experienced and ART-naïve groups. This may be the result of the fact that some mutations are less fit and will disappear soon without drug selection or it may suggest that primary resistance is being transmitted by a particular subgroup of individuals under treatment. Phylogenetic analysis of these samples may give us clues on how this transmission occurs.

In conclusion, our data suggest a high level of development and transmission of HIV-resistant strains among MSM in Brazil. Additional information involving the exploration of new hypotheses that investigate the factors associated with transmission of HIV-resistant strains in MSM, including "upstream" causal factors related to ART supply, adherence, and prevention, are urgently needed. A national ADR surveillance system should be a public health priority for monitoring resistance and to guide interventions to reduce the development and transmission of resistant strains of HIV.

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