A national cross-sectional analysis of surveillance drug resistance mutations among recently diagnosed antiretroviral naïve Brazilian people with HIV

Debora Bellini Caldeira,[a] Tania Regina Constant Vergara,[a,b] Mauro Schechter,[c,l] Eliana Lima Bicudo,[d] Carmen Andréa Freitas Lopes,[e] Carlos Brites,[f,l] Eduardo Sprinz,[g] Juliana Corvo Fernandes,[h] Orival Silva Silveira,[i] Ricardo Durães-Carvalho,[a] Juliana Galinskas,[a] Mauro Sérgio Treitsman,[b] Marcos Davi Gomes de Sousa,[j] Márcio de Figueiredo Fernandes,[c] Mateus Swarovsky Helfer,[g] Ney Almeida Grilo,[i] Marcos Montani Caseiro,[k] Rodrigo Lopes Sanz Duro,[a] Alessandra Simões Bassini,[a] James R. Hunter,[a] Luiz Mário Janini,[a,m] and Ricardo Sobhie Diaz[a,*,m]

[a]Infectious Diseases Division, Federal University of São Paulo, São Paulo, SP, Brazil
[b]ONCOHIV, Rio de Janeiro, RJ, Brazil
[c]Department of Infectious Diseases, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[d]Secretaria de Saúde do Distrito Federal, Brasilia, DF, Brazil
[e]Infectious Diseases Division, Federal University of Pará, Belém, PA, Brazil
[f]Department of Medicine, Federal University of Bahia, Salvador, BA, Brazil
[g]Infectious Diseases Division, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[h]Centro de Referência em Doenças Infecciosas, Itajaí, SC, Brazil
[i]Secretaria Municipal de Saúde, Guarujá, SP, Brazil
[j]Department of Immunology, Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[k]Department of Microbiology, Lusíada University, Santos, SP, Brazil

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Summary

Background Brazil pioneered free and universal HIV antiretroviral therapy (ART) access in 1996. This study evaluated the prevalence of surveillance drug resistance mutations (SDRM) among individuals newly diagnosed with HIV and initiating ART.

Methods Using WHO HIV Threshold Survey methodology, participants were recruited from seven major cities, representing all Brazilian macro-regions. Dried blood spots collected between August 2021 and November 2023 were transported at room temperature to a central laboratory for genotyping.

Findings A total of 327 samples were analyzed, 271 (82.9%) were males. The self-reported ethnic distribution was obtained from 257 people, including 92 white (35.8%), 115 brown (44,7%), 46 black (17.9%), 2 indigenous (0.8%), and 2 Asian (0.8%) people. Mean/median CD4+ T-cell counts were 377/325 cells/μL, and mean/median viral load was 468,000/71,800 copies/mL. Overall SDRM prevalence was 11.6%, with regional values of 16.0% (Rio de Janeiro), 11.5% (Santos), 13.0% (Belém), 11.6% (Porto Alegre), 10.6% (Itajaí), 10.6% (Brasília), and 9.0% (Salvador). Resistance prevalence by class was 3.4% for nucleoside reverse transcriptase inhibitors, 5.2% for non-nucleoside reverse transcriptase inhibitors, 1.5% for protease inhibitors, and 1.8% for integrase strand transfer inhibitors (INSTI). One individual harbored the dolutegravir-associated R263K mutation. Compared to a prior survey conducted a decade earlier, subtype C prevalence increased markedly, and a probable new circulating recombinant form was detected.

Interpretation Nationwide SDRM prevalence in Brazil remains stable relative to data from 10 years ago. However, the emergence of low-level INSTI SDRM, including dolutegravir-specific resistance, is noteworthy. These results emphasize the importance of continuous SDRM and HIV genetic diversity surveillance. Sustained molecular monitoring is essential for optimizing prevention strategies and preserving long-term treatment effectiveness.

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*Corresponding author. Retrovirology Laboratory, Paulista School of Medicine, Federal University of São Paulo, Brazil, R. Pedro de Toledo, 669, 6° andar, São Paulo, SP 04039-032, Brazil.
E-mail address: rsdiaz@unifesp.br (R.S. Diaz).
[l]full professors.
[m]co-senior authors.
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Keywords: Surveillance drug resistance mutations; Dried blood spots; Brazil; Genotyping; Antiretroviral therapy; HIV-subtype; Recent HIV diagnosis

Research in context

Evidence before this study

We searched PubMed for HIV Surveillance Drug Resistance Mutation (SDRM) data in Brazil, focusing on systematic prospective studies with systematic sampling nationwide, which we found to be scarce. We identified only four. The first survey, conducted in 2001, analyzed 535 samples from voluntary counseling and testing centers across several states, including Rio Grande do Sul (138 samples), Paraná (147), São Paulo (100), Rio de Janeiro (83), Mato Grosso do Sul (7), Pará (17), Bahia (12), and Ceará (30). A 2007-8 study used World Health Organization (WHO) Threshold Survey methodology with 210 samples from individuals of the six most densely populated state capitals of Brazil: São Paulo (47 samples), Rio de Janeiro (47), Salvador (34), Porto Alegre (34), Brasília/Belém (48). In 2012, a study with a design similar to our current investigation, also using the WHO Threshold Survey methodology, evaluated samples from 329 individuals initiating antiretroviral therapy (ART) from seven densely populated cities representing all Brazilian macro-regions. A fourth study analyzed 1568 samples collected between October 2013 and January 2015 from 72 laboratories of the HIV viral load Brazilian Ministry of Health Network. All these studies, however, did not investigate resistance mutations to Integrase Inhibitors (INSTIs), a critical class of the modern HIV armamentarium. To address this gap, we reviewed a smaller survey conducted within a Brazilian city focused on INSTI resistance.

Added value of this study

This study utilized dried blood spots (DBS), enabling the collection of samples in remote areas and ensuring safe and easy transportation at room temperature. This is the first survey to report the prevalence of SDRM to INSTIs across Brazil, a level of SDRM to INSTIs previously unreported on a global level. We also report a case of SDRM associated with a second-generation INSTI. Notably, differently from resistance to the other classes of antiretrovirals, INSTI SDRMs were detected in all the cities assessed in the study. We provide unequivocal evidence of an increasing prevalence of subtype C throughout Brazil, which may be attributed to higher viral loads compared to subtype B, although other factors were not evaluated.

Implications of all the available evidence

The widespread distribution of INSTI resistance, coupled with the increasing use of dual therapy with dolutegravir and lamivudine as first-line therapy, as well as the upcoming roll-out of the cabotegravir PrEP program, underscores the importance of continuous monitoring of INSTI SDRMs in Brazil. Moreover, the growing prevalence of subtype C throughout the country and the possible emergence of recombinant strains highlight the need to continually reassess the effectiveness of diagnostic tools that rely on the diversity of HIV, including viral load, genotropism, and resistance testing.

Introduction

Brazil was the first middle-income country to provide free and universal access to antiretroviral therapy (ART) to all people with HIV (PWH) who qualified for treatment under locally developed and periodically updated guidelines. It is estimated that, at the end of 2023, there were approximately one million PWH in Brazil, of which 91% had been diagnosed, 83% had been linked to a health service, 76% were retained in care, 73% were on ART, and 65% had undetectable plasma viral loads (<50 copies/mL).[1]

According to local guidelines, genotypic resistance testing is free of cost and conducted in a central laboratory in the public health system. Pre-treatment genotyping within the Brazilian public health system is generally not allowed, with exceptions for children up to 12 years old, those with confirmed recent seroconversion, those with a sexual partner on ART, pregnant women, and patients diagnosed with tuberculosis. PWH who had at least six months of ART and confirmed virologic failure are entitled to HIV genotypic resistance testing.

While this survey aims to represent nationwide surveillance of drug resistance mutations (SDRM) in Brazil, prior comprehensive studies have been infrequent. A 2001 survey (n = 535) across Ministry of Health sites offered initial insights, though its regional sampling was uneven, heavily skewed towards the South and Southeast.[2] Despite the lack of unified SDRM definitions, it reported resistance to PIs (2.2%), NRTIs (2.4%), and NNRTIs (2.1%). Notably, subtype C HIV dominated the South (nearly 45%). In contrast, most non-B subtypes in the rest of the country were subtype F.

A 2007-8 WHO Threshold Survey (n = 210) focused on six major capitals, but with unequal city representation and the combined analysis of geographically distant Belém/Brasília.[3] This study found varying

resistance rates (highest in Rio de Janeiro at 14.9%) and an overall resistance pattern of NRTI (2.4%), NNRTI (4.3%), and PI (1.9%). Subtype B was generally dominant (72%), except in Porto Alegre, where subtype C prevailed (69%).

Another study mirroring the current design used the WHO methodology on ART-initiating individuals (n = 329) from seven key cities representing Brazil's regions.[4] Regional resistance varied (Northeast highest at 17.0%), with overall SDRM at 6.9% (NRTI), 4.9% (NNRTI), and 3.9% (PI). Subtype B was most common, with higher subtype C in the South and F/BF recombinants in the North.

A larger study (2013–2015, n = 1568) across 72 labs showed a national SDRM prevalence of 10.2%, with regional variations.[5] Resistance to NRTIs was 3.6%, NNRTIs 5.8%, and PIs 1.6%. Subtype B was most frequent nationally (66.8%), followed by C (14.2%) and F (10%). However, the South showed a distinct pattern with subtype C as the most prevalent (53.8%). A key limitation of this study was the inclusion of numerous cities with limited HIV epidemic representation.

Since January 2017, dolutegravir has replaced efavirenz as the anchor drug for initial therapy. This manuscript describes changes in the prevalence of HIV-SDRM and HIV-1 subtypes among Brazilian adults with newly diagnosed HIV for whom ART was prescribed since the last nationwide survey done ten years ago.

Methods

The HIV Threshold Survey methodology (HIV-THS, World Health Organization, WHO) was used.[6] According to WHO guidelines, SDRM prevalences are divided into three categories: low (<5%), intermediate (between 5 and 15%), and high (>15%). WHO's initial treatment strategies, both at the individual and the public health level, are based on the prevalence of SDRM.[7,8] The present report complies with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (www.strobe-statement.org).

As previously done,[4] for a convenient sampling, we selected seven highly populated cities to represent the five Brazilian macro-regions. We chose two additional sentinel cities because of their different epidemiological characteristics. The selected cities were Belém (North region), Salvador (Northeast region), Brasília (Central region), Rio de Janeiro and Santos (South-east region), and Porto Alegre (South region). As sentinel cities, Itajaí (South area) was selected due to the previously reported high frequency of AIDS, while Santos (Southeast region) was chosen for its previously documented high prevalence of SDRM and HIV recombinant forms.[9] In the preceding article, we selected the city of Manaus to represent the North region.[4] We replaced Manaus with Belém due to the COVID-19 pandemic constraints. Data on gender, including transgender people, were collected. Self-reported data on ethnicity included white, black, brown, indigenous, Asian, or unavailable. Data on baseline HIV viral load and CD4+ T cell counts were obtained from the Network of Laboratories of the Brazilian Public System or tests performed in the Federal University of São Paulo Retrovirology Laboratory (São Paulo Retrolab).

Approximately 50 samples were collected from each city. Included participants were consecutive ART-naïve PWH aged 18 years or older with a recent (≤6 months) HIV diagnosis for whom ART was prescribed. Previous use of PrEP was an exclusion criterion for this study. Blood samples were collected on SS903 collection cards after a needle puncture of a finger and sent at room temperature to the São Paulo Retrolab for genotyping. After arriving in the Retrolab, volunteers were checked in the Brazilian Data Bank for Antiretroviral Dispensation (SICLON) to confirm the absence of prescription of antiretrovirals before being enrolled in the study. Samples were collected between August 2021 and November 2023. Previous studies have shown the reproducibility and accuracy of HIV genotyping using samples collected in dried blood spots (DBS) and transported at room temperature.[4]

HIV total DNA was extracted using a QIAamp Blood kit (Qiagen Inc., Chatsworth, CA, U.S.A.). Integrase, protease, and reverse transcriptase regions of the *pol* gene were amplified and sequenced as previously described.[10] Sequences were acquired for each sample following two distinct PCR amplifications and sequencing to eliminate PCR carryover. SDRM was evaluated according to the WHO Surveillance Drug Resistance Mutations List, an algorithm that excludes common polymorphisms,[11] with an update analyzing the temporal trends for SDRM.[12] We also submitted the sequence data set to the Stanford database's Calibrated Population Resistance (CPR) tool (https://hivdb.stanford.edu/). We included references' support for one unusual mutation detected (N155K) that was suggested as an SDRM. Phylogenetic analysis was performed for subtype assignment. Sequences were aligned with HIV-1 subtypes A, B, C, F, and G reference sequences retrieved from the Los Alamos database using BioEdit v.7.2.3[13] and, alternatively, the UGENE v.50.0 bioinformatics platform.[14] Phylogenetic reconstructions based on the protease, reverse transcriptase, and integrase protein-encoding genes were conducted using the maximum likelihood (ML) method implemented in FastTree v.2.1.7 software.[15] The analysis employed the standard GTR + CAT model with 20 gamma distribution parameters and a combination of nearest-neighbor interchange (NNI) and subtree-prune-regraft (SPR) approaches, using 1000 replicates of the Shimodaira-Hasegawa (SH-like) test. The Specificity and Quality of generated sequences were evaluated as

previously described.[16] Gene Bank accession numbers are PQ270759-PQ271561.

Statistical tests included the Mann–Whitney U test to determine the difference between two groups and the Chi-Squared Test to determine differences in levels of categorical variables. These tests were executed using GraphPad Prism, version 9, and the R Statistical system, version 4.2.1.

Ethical Approvals

The study was approved by the Institutional Review Board of the Federal University of São Paulo (#0457/2020). Informed consent was obtained from all participants.

Role of the funding sources

FAPESP and CNPq (the founders of this study, Brazil) had no role in the writing of the manuscript or the decision to submit it for publication. These agencies were not involved in data collection, analysis, or interpretation; study design; patient recruitment; or any aspect pertinent to the study. No agencies have paid authors to write this article. The authors were not precluded from accessing data in the study, and they accept responsibility for submitting it for publication.

Results

DBS specimens were collected from 410 patients at local sites and sent to the São Paulo Retrolab for HIV genotyping. The direct line distance between the São Paulo Retrolab and Santos in km is 85, Rio de Janeiro 352, Itajaí 425, Brasília 871, Salvador 1484, and Belém 2478. Among 410 samples, 19 were identified as originating from treatment-experienced patients following verification with the Brazilian Data Bank for Antiretroviral Dispensation, and 6 were duplicate samples from the same persons. Therefore, samples from 385 recently diagnosed treatment-naïve adult PWH were included in the study. Amplification was successful in 327 (84.9%): 272 (70.6%), 279 (72.5%), and 305 (79.2%) in the reverse transcriptase, the protease, and the integrase regions, respectively (Study flow chart, Fig. 1). The average difference between each sample's two distinct PCR amplifications was 1.43 (range 0.45–2.4%). There were no discordant SDRM mutations between sequence pairs.

Table 1 shows the SDRM prevalence and demographic, virologic, and immunologic characteristics of participants according to the geographic region of residence. The overall mean and median CD4+ T-cell counts were 377 and 325 cells/μL, ranging from 4 to 1940 cells/μL. The mean and median for females were 367.1 and 316 cell/ul, respectively, and 380.2 and 345 for males (p = 0.77 and 0.88). Also, there were no statistically significant differences between participants with or without SDRM; 107 (32.7%) had CD4+ T-cell counts <200 cells/μL. The overall mean and median viral loads were 467,577 and 71,814 copies/mL, ranging from <50 to >10 million copies/mL. The mean and median viral loads for females were 623,562.6 and 95,436, and 393,197.2 and 54,218 for males, respectively (p = 0.3 and 0.1). No statistically significant differences in HIV viral load were observed between individuals with or without SDRM. Four of 327 (1.22%) individuals (all males) presented with viral loads repeatedly <50 copies/mL, therefore being considered Elite Controllers, which are PWH who maintain undetectable viral loads without the use of antiretrovirals (Fisher's exact test not significant). The prevalence of SDRM among males and females was 11.1% (n = 30) and 15.7% (n = 8), respectively (Chi-Square, p = 0.49). Recent HIV diagnosis was predominantly in males, who accounted for 269 (82.3%) of all cases overall. However, almost half of the participants were females in Porto Alegre in the Southern region (Table 1). The mean age of the participants was 36 years. However, men were younger than women, with a mean age of 31 versus 42 years, respectively (Mann–Whitney, p < 0.0001). The self-reported ethnic distribution was obtained from 257 people, in which 92 were reported as white (35.8%), 115 brown (44.7%), 46 black (17.9%), two indigenous (0.8%), and two Asian (0.8%) people. Ethnic information was not reported for 70 people, and we recognize it as a limitation of this survey.

The overall prevalence of SDRM was 11.6% (n = 38), being 16.0% (n = 8) and 11.5% (n = 6) in Rio de Janeiro and Santos, both in the Southeast, and 13.0% (n = 7), 10.6% (n = 5), 9.4% (n = 3), 10.6% (n = 5), and 9.0% (n = 4) in Belém (North), Porto Alegre (South), Itajaí (South), Brasília (Central), Salvador (Northeast), respectively. The prevalence of nucleoside reverse transcriptase inhibitors (NRTIs) resistance-associated mutations (RAM) was 3.4% (n = 11), ranging from zero in Itajaí to 8.9% (n = 4) in Salvador; for non-nucleoside reverse transcriptase inhibitors (NNRTIs) was 5.2% (=17), ranging from zero in Salvador to 9.4% (n = 3) in Itajaí; for protease inhibitors (PIs) was 1.5% (n = 5), from zero in Salvador, Brasília, Rio de Janeiro and Itajaí to 4.3% n = 2) in Porto Alegre, whereas for integrase inhibitors (INSTIs), prevalence was 1.83% (n = 6). Two-class resistance was found only in Salvador (2.2%, n = 1, Table 1). There were no PWH with three-class resistant HIV. Fig. 2 depicts the prevalence of RAM to the distinct antiretroviral classes, showing in red the amino acid substitutions that are unequivocally considered SDRM mutations and in blue other mutations that antiretrovirals can also select.

The prevalence of HIV subtypes presenting the same clade profile in the *pol* gene's protease, reverse transcriptase, and integrase regions was 58.7% (n = 192) for B, 26.3% (n = 86) for C, and 4.3% (n = 14) for F (Table 2 and Fig. 3). The regional distribution of viral subtypes is shown in Fig. 3. Table 2 illustrates the

clade profile based on genomic regions. No association was found between resistance and the more prevalent subtypes B and C (data not shown). Viral loads were significantly higher among PWH infected with clade C viruses than with clade B viruses (Mann–Whitney p = 0.03). In addition, viral loads were also higher in comparisons that included clade C viruses plus Circulating Recombinant Form (CRF) 31 (Mann–Whitney p = 0.02) or clade C viruses and any clade C recombinant versus clade B viruses (Mann–Whitney p = 0.03). Viral loads among PWH harboring HIV of subtype F or F recombinants were similar to PWH harboring HIV from subtype B (Mann–Whitney p = 0.66). The prevalence of clade C viruses was higher in females than males (Chi-square, p = 0.0003). A recombinant with subtype F in the protease and the reverse transcriptase regions and B in the integrase region was identified in 14 samples (4,3%): 7 in Belém, 2 in Salvador, 4 in Rio de Janeiro, and one in Santos (bottom part of Fig. 3).

Table 3 shows all SDRM mutations identified by city/region and PWH. According to previous definitions,[11] SDRMs are highlighted in red, whereas other mutations, also selected by antiretrovirals, are displayed in black.

Fig. 4 illustrates the phylogenetic reconstruction of the sequenced samples based on the genomic region. Utilizing genetic distances of less than 1.5% between sequences to detect clusters, a phylogenetic analysis employing neighbor-joining reconstruction and excluding resistance-related codons indicated no clustering of samples containing SDRM sequences (data on file – refer to *Data Access statement*).

Discussion

Recent HIV diagnoses in Brazil reveal a concentrated epidemic, primarily affecting males. Interestingly, Porto Alegre shows a different trend, with nearly half of new diagnoses in women and a predominance of HIV subtype C – the most common subtype in sub-Saharan Africa, where women represent half of those living with HIV.[17] This study also highlights that most individuals were diagnosed with intermediate to high viral loads, and a median CD4+ T cell count was 325 cells/mm^2, one-third presenting <200 cells/mm^3.

Drug resistance significantly hinders effective HIV treatment, and SDRM can lead to virologic failure.[18] Interestingly, SDRM often persists,[19] owing to the frequent clonal HIV transmission.[20] While some individuals might harbor a mix of resistant and non-resistant virus strains,[21] research suggests that even minor populations of drug-resistant viruses can lead to treatment failure among people with inadequate treatment adherence.[22] It is still an open question whether next-generation sequencing or single-genome amplification assays are needed to identify these minority SDRM populations to predict if treatment will fail.[21]

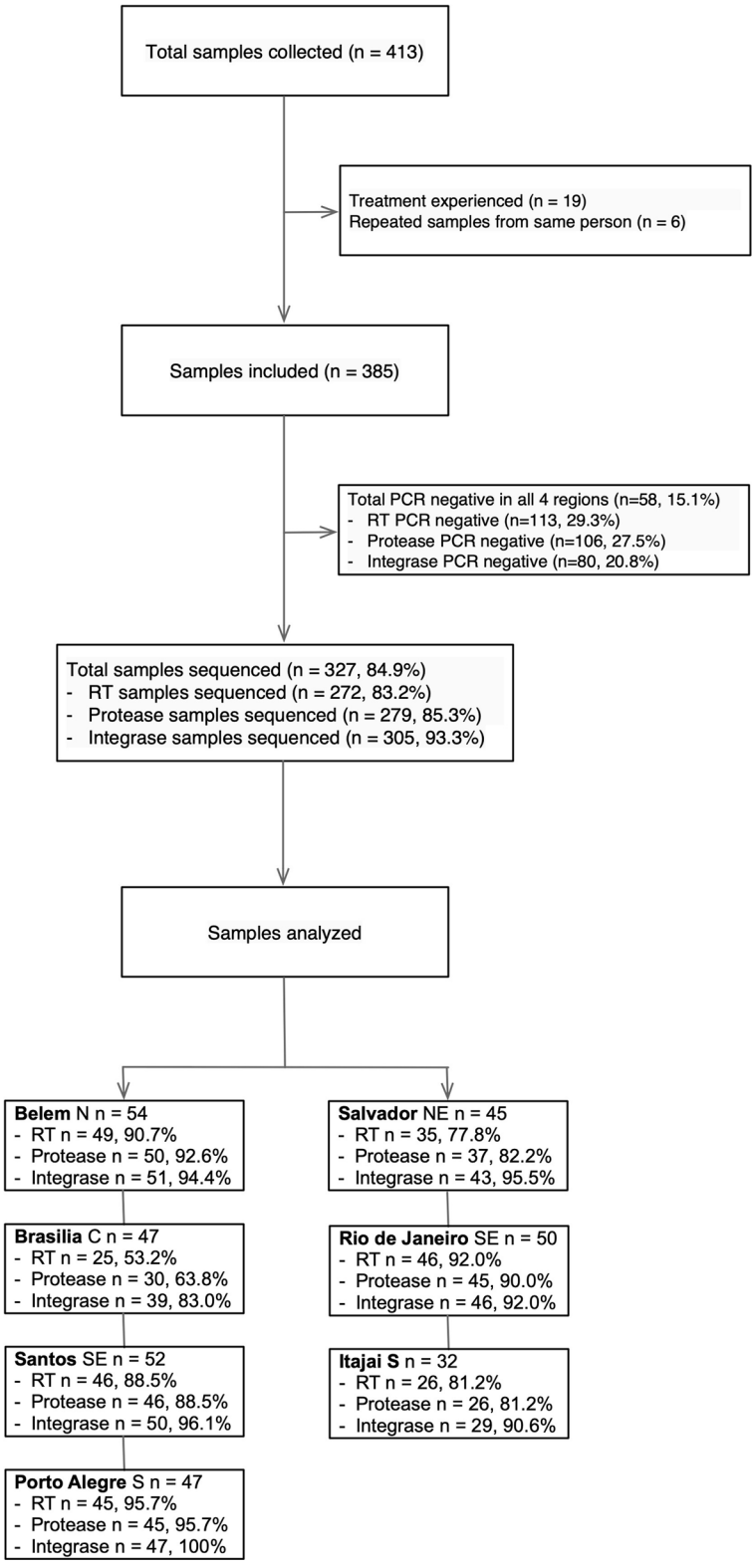


Fig. 1: Study Flow Chart. RT, reverse transcriptase.

City/Region	Mean/Median Age (Range)	Males n (%)	Mean/Median CD4 n (Range)	Mean/Median VL n (Range)	SDRM n (%)	PI n (%)	NRTI n (%)	NNRTI n (%)	INSTI n (%)	2 classes resistance n	Total n
Belem/N	30/26 (19–59)	50 (92.6)	367/323 (7–1010)	439,000/93,000 (46–5,820,000)	7 (12.96%)	2 (3.7%)	2 (3.7%)	3 (5.56%)		–	54
Salvador/NE	35/30 (21–72)	39 (86.7)	245/179 (4–1010)	305,000/92,200 (771–1,600,000)	4 (8.89%)		4 (8.89%)		1 (2.22%)	1	45
Brasília/CR	30/29 (18–57)	45 (95.8)	564/441 (36–1940)	274,000/15,600 (52–7,950,000)	5 (10.64%)		1 (2.13%)	3 (6.38%)	1 (2.13%)	–	47
Rio de Janeiro/SE	35/33 (20–68)	43 (86.0)	355/353 (21–820)	250,000/70,900 (<40–3,650,000)	8 (16%)		2 (4%)	4 (8%)	2 (4%)	–	50
Santos/SE	34/33 (18–74)	42 (80.7)	472/473 (26–1180)	203,000/27,800 (102–1,950,000)	6 (11.54%)	1 (1.92%)	1 (1.92%)	3 (5.77%)	1 (1.92%)	–	52
Itajaí/S	38/34 (19–71)	25 (78.1)	483/388 (35–1330)	638,000/88,730 (<40–5,270,000)	3 (9.38%)			3 (9.38%)		–	32
Porto Alegre/S	45/46 (19–76)	25 (53.2)	211/153 (5–1080)	1,060,000/299,000 (1974–10,000,000)	5 (10.64%)	2 (4.26%)	1 (2.13%)	1 (2.13%)	1 (2.13%)	–	47
Total	36/33 (18–76)	269 (82.3)	377/325 (4–1940)	468,000/71,800 (<40–10,000,000)	38 (11.62%)	5 (1.53%)	11 (3.36%)	17 (5.2%)	6 (1.83%)	1	327
				95% Confidence Interval–Upper Bound	14.20%	6.32%	6.57%	8.80%	3.22%		
				95% Confidence Interval–Lower Bound	9.20%	0.27%	1.02%	3.61%	1.53%		

VL, HIV RNA Viral Load; PI, Protease Inhibitor resistance-associated mutations; SDRM, Surveillance Drug Resistance Mutations; NRTI, Nucleoside Reverse Transcriptase Inhibitors resistance-associated mutations; NNRTI, Non-nucleoside Reverse Transcriptase inhibitors resistance-associated mutations; INSTI, Integrase Strand Transfer Inhibitors resistance-associated mutations.

Table 1: **Surveillance Drug Resistance Mutation (SDRM) prevalence, demographic, virologic, and immunologic characteristics of participants according to geographic region of residence.**

The WHO has set clear levels for HIV SDRM prevalence: low (under 5%), moderate (5–15%), and high (over 15%). These thresholds are crucial for guiding public health actions. The WHO recommends changing the standard initial treatment if SDRM to any drug class exceeds 10%. To stay ahead, the WHO urges countries to regularly track HIV SDRM across different populations to shape effective policies and treatment guidelines.

This study used DBS, a clever method that makes samples non-infectious and easy to transport at room temperature, opening doors to collecting samples from distant areas. Mirroring our study from a decade ago,[4] we chose five major cities representing Brazil's five regions. We also added two more cities in the South and Southeast due to their unique HIV patterns and high infection rates. The main change from our previous survey was that Belém took the place of Manaus as the representative city for the North region, purely for logistical reasons. Belém and Manaus are the capitals of the two largest states in the Amazon rainforest, with high indigenous influence, both sharing an HIV/AIDS epidemic concentrated in young male adults, predominantly through sexual transmission, with challenges in early diagnosis and healthcare access.

Although the overall proportion of SDRM remained stable and at the intermediate category level according to WHO definitions, there were significant differences. Arguably, the most important differences were the absence of PI resistance in four of the seven regions, with an overall decline from 3.6 to 1.5%, and the appearance of integrase resistance-associated mutations (INRAMs) in all cities, albeit at low levels.

Identifying an overall prevalence of 1.83% (95% CI 1.53–3.27) of INRAMs is likely the most critical finding of our study. A recent report of the Mediterranean consortium, evaluating 2705 samples from Portugal, Spain, Italy, France, and Greece, reported a prevalence of 0.3% of INRAMs.[23] Another survey conducted in Shenzhen, China, successfully PCR amplified 1071 sequences from 1682 people and described five SDRM to INSTIs, R263K (n = 4), E138K (n = 4), T66A/I (n = 3), N155H (n = 1) and S147G (n = 1), in 13 samples with an alleged prevalence of 1.21%.[24] Also, one survey including 192 newly diagnosed individuals from São Paulo, Brazil (2020 to March 2023), reported that SDRM to INSTI was detected in three cases (1.6%; 95% CI: 0.5%–5%), two people harboring the E138K and one harboring R263K.[25] Two much smaller studies, one from Italy and another from South Korea, also provided low levels of INSTI resistance. The SDRM survey conducted in Italy between 2009 and 2018 identified 3 SDRMs in 52 ART-naive persons (G140S + Q148H in 2 persons and E138K in 1 person).[26] The other survey conducted in Korea between 2014 and 2017 identified 3 SDRMs in 58 INSTI-naive ART-experienced persons (E92Q in 2 persons and S230R in 1 person).[27]

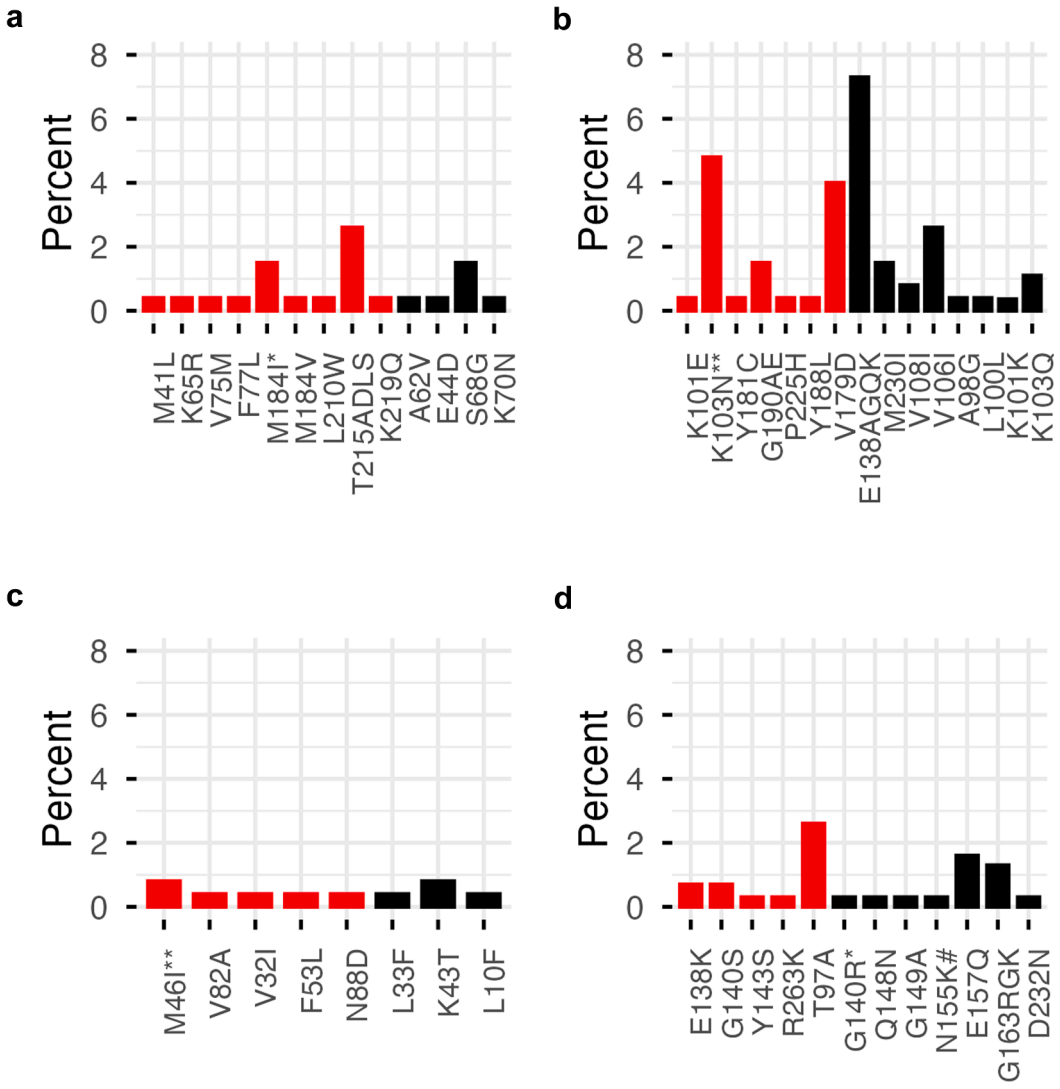


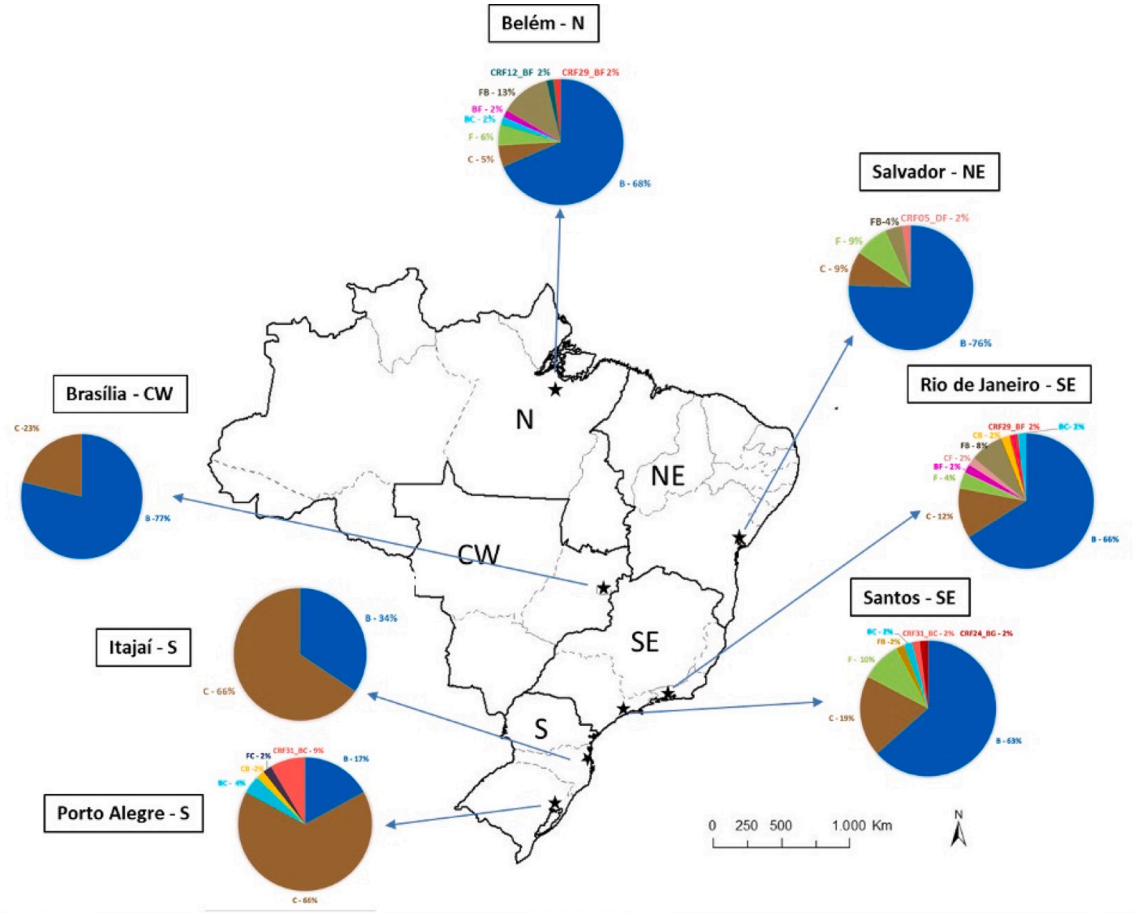
Fig. 2: Prevalence of Resistance-Associated Mutations to Nucleoside Reverse Transcriptase Inhibitors (NRTI, panel a), Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI, panel b), Protease Inhibitors (PI, panel c), and Integrase Strand Transfer Inhibitors (INSTI, panel d). In red are SDRM mutations, and in blue are the other mutations that can be either selected by antiretrovirals or HIV clade-related polymorphisms. *Mutations not counted in the prevalence of SDRM due to hypermutation. ** Only one of the sequences harboring this mutation was hypermutated and not counted in the prevalence of SDRM. #Mutation not counted in the prevalence of SDRM due to lack of confirmation of its impact on resistance.

We submitted the HIV sequences to the CPR, which is available in the Stanford database. The sequences with APOBEC-driven hypermutations were excluded (marked * in Table 3 and Fig. 2). These included all four samples with M184I in the reverse transcriptase (one with K103N), one M46I in the protease, and one G140R in the integrase. Although hypermutations may represent artefacts, the arbitrary exclusion of these sequences may be debatable. Hypermutation is a process of genetic diversity generation in HIV. Although these proviruses may contain stop codons, when the majority of sequences in the HIV quasispecies present with them (as detected by Sanger sequencing), it may represent an opportunity for the selection of antiretroviral-resistant strains. Furthermore, recombination processes may help to expand and fix these resistance-associated mutations into viable viruses. Considering the unusual N155K mutation and the hypermutated G140R sequence, INSTI RAMs were the only class where mutations were detected in all sites. Equally important was the dolutegravir-specific resistance mutation R263K, found in a woman in South

	HIV-1 SUBTYPES n (%)										
	B	C	F	G	BC	CRF05_DF	CRF12_BF	CRF24_BG	CRF29_BF	CRF31_BC	Total
PR	167 (59.8)	73 (26.2)	30 (10.7)	–	2 (0.7)	1 (0.3)	–	1 (0.3)	–	4 (1.4)	279
RT	165 (60.7)	71 (26.1)	28 (10.3)	–	2 (0.7)	–	–	1 (0.3)	–	4 (1.4)	272
IN	189 (61.9)	94 (30.8)	17 (5.6)	1 (0.3)		–	1 (0.3)	–	2 (0.6)	–	305

PR, Protease; RT, Reverse Transcriptase; IN, Integrase.

Table 2: **HIV subtype profile according to the HIV genomic region.**



City/Region	HIV-1 SUBTYPES														*n*
	B	C	F	FB	BC	BF	CB	CF	FC	CRF31_BC	CRF29_BF	CRF05_DF	CRF12_BF	CRF24_BG	
Belem/N	68.5	5.5	5.5	13	1.8	1.8	-	-	-	-	1.8	-	1.8	-	54
Salvador/NE	75.5	8.9	8.9	4.4	-	-	-	-	-	-	-	2.2	-	-	45
Brasilia/CW	76.6	23.4	-	-	-	-	-	-	-	-	-	-	-	-	47
Rio de Janeiro/SE	66.0	12.0	4.0	8.0	2.0	2.0	2.0	2.0	-	-	2.0		-	-	50
Santos/SE	63.5	19.2	9.6	1.9	1.9	-	-	-	-	1.9	-	-	-	1.9	52
Itajai/S	34.4	65.6	-	-	-	-	-	-	-	-	-	-	-	-	32
Porto Alegre/S	17.0	65.9	-	-	4.2	-	2.1	-	2.1	8.5	-	-	-	-	47
Total	58.7	26.3	4.3	4.3	1.5	0.6	0.6	0.3	0.3	1.5	0.6	0.3	0.3	0.3	327

Fig. 3: Brazilian map showing the location of the cities and the Brazilian macro-regions where samples were collected. The prevalence of the subtype profile is indicated for each city. Regions are N, North; NE, Northeast; CW, Centralwest; SE, South-east; and S, South. At the bottom, subtype profiles are presented in percentages, where the first letter describing the recombinant subtype refers to the 5′ region of the *pol* gene, whereas the second letter refers to the 3′ region in a concatemer combining the protease + RT (first letter) and integrase regions (second letter: BC, BF, CB, etc.).

City	Sample ID	Subtype	NRTI	NNRTI	PI	INSTI
Belem	PA224	B			K43T	
Belem	PA227	B				D232N
Belem	PA230	C		V179D		
Belem	PA231	B	**M184I**[a]	M230I	**M46I**[a]	T97A
Belem	PA236	FB		**K103N**		
Belem	PA241	FB		V106I		
Belem	PA244	B		E138A		
Belem	PA248	B			**N88D**	
Belem	PA249	B		E138A		
Belem	PA251	B		E138A		
Belem	PA252	B		E138A		
Belem	PA255	B		**K103N**; V106I		
Belem	PA257	FB		**K103N**		
Belem	PA259	B			L10F	
Belem	PA263	B	**K65R**			N155K[a]
Belem	PA265	C		V108I		
Belem	PA266	B				E157Q
Belem	PA271	F	**V75M; F77L; T215S**	A98G		
Belem	PA272	B		E138A		
Belem	PA273	BF			**V82A**	
Belem	PA274	B		V106I; M230M/I		
Belem	PA280	B	E44D			
Belem	PA281	B				G149A
Belem	PA285	B		K103Q; V106I		E157Q
	PA287	F/CRF29_BF				G163K
Salvador	BA56	B		V179D		
Salvador	BA87	B				E157Q
Salvador	BA104	B	**M184V**	V179D; M230MI		**E138K**
Salvador	BA105	F		V106I		
Salvador	BA106	F		V106I		
Salvador	BA173	B	**M41L; T215D**			
Salvador	BA174	C		K103Q		
Salvador	BA261	F		E138A; V179D		
Salvador	BA262	B	**T215L**			
Salvador	BA279	B				E157Q
Salvador	BA289	B	**L210W**	V106I		
Salvador	BA291	B		V179D		
Brasilia	DF132	B	**T215A**			
Brasilia	DF133	B		V179D		
Brasilia	DF134	B		**K103N**		
Brasilia	DF146	B				T97A
Brasilia	DF149	B		E138A		
Brasilia	DF151	B				**E138K**
Brasilia	DF165	B		**K103N**		
Brasilia	DF189	B		**G190A**		
	DF206	B				G163R
Santos	SA11	F		E138Q		
Santos	SA13	B			**F53L**	
Santos	SA18	C	**M184I**[a]	**K103N**[a]		T97A
Santos	SA19	B		**K103N**		
Santos	SA45	B				G163R
Santos	SA93	B		V108I		
	SA362	B	**T215S**			
	SA380	B		**K103N**		
	SA382	B		**G190E**		
	SA386	B	S68G			

(Table 3 continues on next page)

City	Sample ID	Subtype	NRTI	NNRTI	PI	INSTI
(Continued from previous page)						
	SA389	F				T97A; G163K
	SA390	B			K43T	
	SA391	F				T97A
	SA392	F		E138A		
	SA409	B	S68G			
	SA410	B				**G140S**/Q148N
Rio de Janeiro	RJ02	B		V179D		
Rio de Janeiro	RJ05	B	**T215L**			
Rio de Janeiro	RJ07	C		E138G		
Rio de Janeiro	RJ38	B		V179D		
Rio de Janeiro	RJ42	B	**M184I**[a]	M230I		
Rio de Janeiro	RJ43	B				**Y143S**
Rio de Janeiro	RJ50	B	**K219Q**			
Rio de Janeiro	RJ51	B				**G140S**
Rio de Janeiro	RJ65	B	A62V			
Rio de Janeiro	RJ67	B				E157Q
Rio de Janeiro	RJ77	FB	**M184I**[a]	E138K[a]		
Rio de Janeiro	RJ112	B		**K103N; P225H**		
Rio de Janeiro	RJ117	FB		**K103N**		T97A
Rio de Janeiro	RJ128	CB		K103Q		
Rio de Janeiro	RJ381	BC		**K103N**		
Rio de Janeiro	RJ396	B		E138A		
Rio de Janeiro	RJ399	B	K70N			
Rio de Janeiro	RJ408	B		**G190A**		
Rio de Janeiro						
Rio de Janeiro	ITA29	C		L100L		
Itajai	ITA295	C		**Y181C**		
Itajai	ITA296	C		E138K		G140R[a]
Itajai	ITA303	C		**K103N**		
Itajai	ITA306	B				T97A
Itajai	ITA311	C		E138A		
Itajai	ITA316	C		E138A		
	ITA340	B	S68G	**K101E; K103N**; E138A		
Porto Alegre	POA297	C		E138A		
Porto Alegre	POA298	C		E138G		
Porto Alegre	POA299	C			**V32I;** L33F	
Porto Alegre	POA319	C		E138G		T97A
Porto Alegre	POA324	C				**R263K**
Porto Alegre	POA327	C		V179D		
Porto Alegre	POA331	C		E138A		
Porto Alegre	POA334	BC		K101K; **Y188L; G190A**		
Porto Alegre	POA341	B		V179D		
Porto Alegre	POA347	C			**M46I**	
Porto Alegre	POA352	B		V179D		
Porto Alegre	POA361	C	**T215S**			
Porto Alegre	POA366	B	S68G			

NRTI, mutations related to nucleoside reverse transcriptase inhibitors; NNRTI. mutations related to non-nucleoside reverse transcriptase inhibitors; PI, mutations related to protease inhibitors. In bold are the mutations unequivocally considered SDRM, and other are mutations that can be selected by antiretrovirals or can be related to clade-specific polymorphisms. [a]Mutation was not counted in the prevalence of SDRM due to hypermutation or to lack of confirmation of its impact on resistance (N155K).

Table 3: **SDRMs of specific PWH by city/region and HIV subtype.**

Brazil (Porto Alegre), which confers resistance to second-generation integrase inhibitors. Of note, the R263K mutation has also been found in one sample in the Mediterranean consortium,[23] as well as in another sample in the Metropolitan area of São Paulo, Brazil,[25] and China.[24]

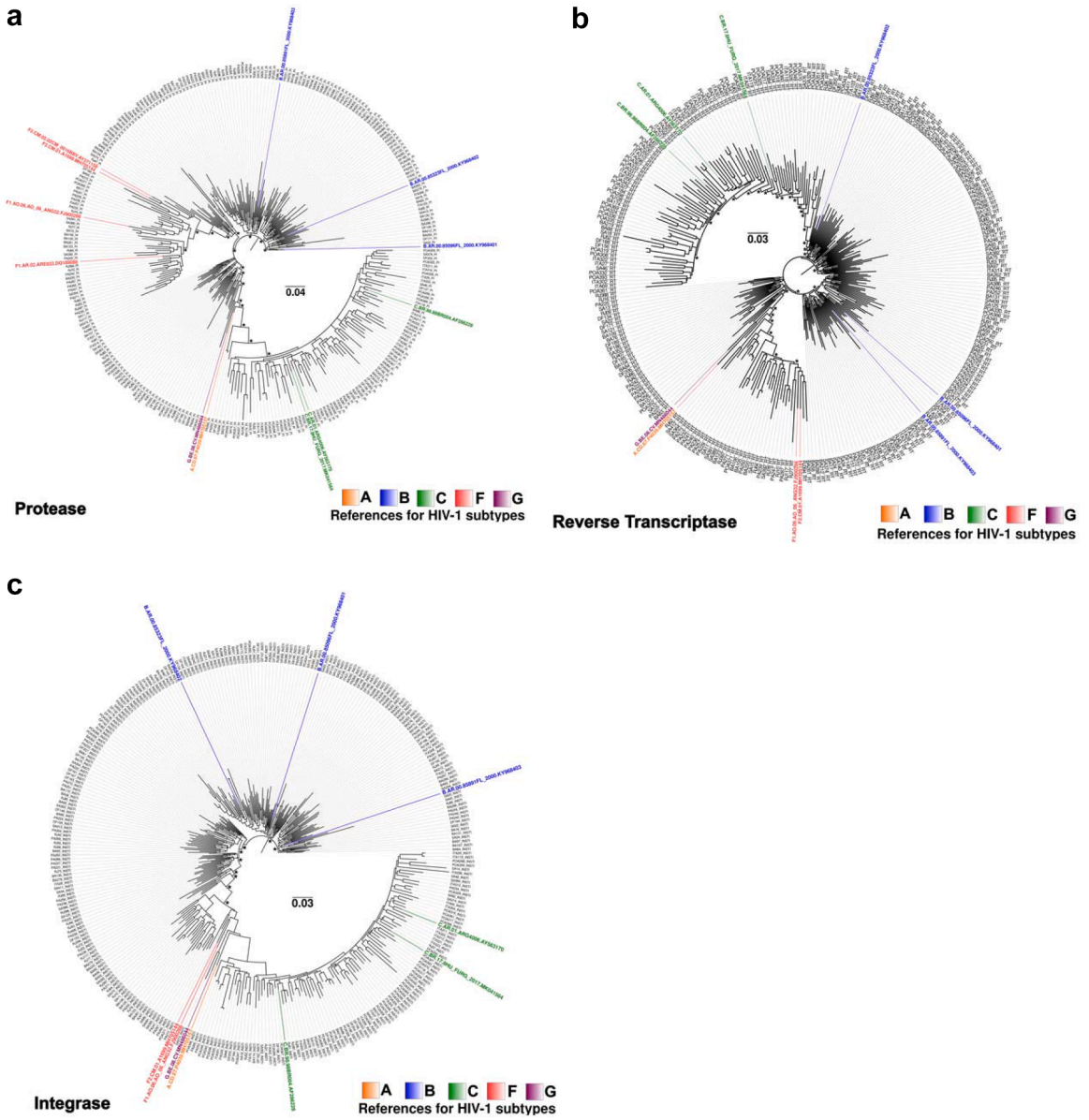


Fig. 4: Maximum-likelihood phylogenetic trees based on HIV-1 protease (294 bp) (a), reverse transcriptase (603 bp) (b), and integrase (594 bp) (c) protein-encoding genetic regions of the pol gene. Colored tips correspond to distinct HIV-1 reference subtypes. Blacktip codes represent sequences from this study. Asterisks on the main branches assume SH-like support values, with only values equal to or greater than 70% shown. Branch lengths are drawn to scale and measured in nucleotide substitutions per site, as indicated by the scale bar in the figure.

We also found the N155K mutation in a 23-year-old male from Belém, who also harbored a K65R mutation in the reverse transcriptase gene of a clade B virus. The N155K mutation was previously described in Cameroon in a CRF36_cpx sample, and the authors considered it a Major INSTI RAM.[28] Although this substitution was detected in an important codon for INSTI resistance, its impact on INSTI resistance remains to be determined using phenotypic assays, and this mutation is not even part of the current RAM lists.

Notably, resistance to INSTIs was not investigated in the survey we conducted in Brazil a decade ago. A notable difference was the substantial two-class resistance observed in approximately one-third of participants in the prior study, compared to 0.3% in the current one. These modifications likely reflect the substitution of efavirenz with dolutegravir as the anchor drug in first-line therapy and the extensive use of the first-generation INSTI raltegravir in salvage therapy.

Another important finding of the present study was the presence of numerous SDRM mutations associated

with antiretrovirals that have not been used in clinical practice for many years, including mutations typically selected by stavudine (V75M). Protease resistance-associated mutations associated with indinavir (M46L or V82A) and nelfinavir (N88D) were also detected. These findings suggest that the SDRM transmission chain is obscure and challenging to interrupt. Of note, all mutations detected in the reverse transcriptase codon 215 were revertants, considered a hallmark of transmitted SDRM.[29]

It is essential to distinguish the two concepts described here: RAM, and SDRM. The first relates to mutations that may contribute to drug resistance, leading to virologic failure, which are usually selected by antiretrovirals. The second refers to a subgroup of mutations analyzed with strict rules, performed by CPR, with evidence of being transmitted from person to person. In this context, the relatively high prevalence of mutations at codon 138 of the reverse transcriptase region is also significant. Although substitutions at codon 138 may not constitute definitive proof of SDRM, these mutations are a known RAM that confer resistance to rilpivirine, and their presence should be considered of clinical relevance. While uncommon and not classified as SDRM mutations, the substitutions A98G (0.4%), V106I (2.6%), and V108I (0.7%) that confer low-level resistance to doravirine were identified, none of them in combination with the M184V mutation, which would potentially impair the activity of the fixed drug combination of islatravir/doravirine, presently under development.

Although the small sample size in the studies should be taken as a limitation, significant changes occurred in the distribution of HIV subtypes, particularly a pronounced rise in the prevalence of subtype C. The prevalence of subtype C isolates increased from 34.7% to 66% in Porto Alegre (in the South), from no prevalence to 12% in Rio de Janeiro and from 7.4% to 19% in Santos (both in the Southeast), from 4.3% to 23% in Brasília (Central), and from 4.3% to 9% in Salvador (Northeast).[4] This previous survey did not evaluate the city of Belém (North), which presented 5% of subtype C. Another recent study in Brazil also reported a 16% prevalence of subtype C in the integrase region of the pol gene in the metropolitan area of São Paulo, Brazil,[25] a higher prevalence than previously reported in similar surveys.[2,5] This increase in the prevalence of subtype C infection in Brazil resembles the increase that occurred in sub-Saharan Africa, where subtype C became more prevalent than subtypes A and D that predominated before,[17] and is also in keeping with the expansion of subtype C compared to other subtypes in South America.[30] A coalescent analysis utilizing Bayesian Markov chain Monte Carlo indicated that the introduction of subtypes B, F, C, and CRF 31 in Brazil occurred in 1971, 1981, 1987, and 1992, respectively. The projected basic reproductive number of secondary infections (R0) over a decade was 3.59 for subtype B in Brazil, in contrast to 8.2 for subtype C.[30] All these observations align with our finding of higher viral loads in PWH infected with clade C viruses or C recombinants, in contrast to those infected with clade B viruses.

Limitations of the study

The current study has limitations. The WHO Threshold for survey methodology assumes a sample size of 50 for each chosen site, which was not obtained for 4 of 7 sites. Also, it was assumed that the selected cities represent entire regions of a vast and diverse country, which may or may not be accurate. For instance, the city of Santos was chosen due to a previously reported high prevalence of SDRM, which could bias our estimates. Furthermore, selection bias cannot be excluded since signing a consent form was a prerequisite for enrolment in the study, and data on individuals who met the inclusion criteria but refused to sign the consent form were not collected. Although a reported recent diagnosis of HIV infection was an inclusion criterion, no attempts were made to verify or confirm the information provided by the participant. Lastly, we failed to PCR amplify about 15% of the samples. Although a lower PCR amplification performance is expected when DBS is used, suboptimal PCR primer annealing may have missed viruses with distinct genetic profiles. Due to the lack of information on ethnicity for 70 individuals (21.4%), stratified analyses by ethnic group could not be performed. Consequently, it was not possible to explore associations between recent HIV diagnosis and SDRM prevalence across different ethnicities, which may limit the formulation of targeted prevention strategies for minority populations in Brazil.

A very significant finding of this study is the marked expansion of subtype C, which has become more widespread throughout all Brazilian regions. The dynamics of the prevalence of HIV subtypes and the emergence of CRFs should be carefully monitored through full-length genome characterization in areas with co-circulating HIV clades due to the potential emergence of new CRFs, as indicated by our study findings. Interestingly, 7 of the 14 possible CRF samples originated in a single city, Belém. The prevalence of SDRM has remained at an intermediate level. A low-level INSTI RAM was documented in all cities from all regions. A patient harboring a dolutegravir-specific transmitted resistance mutation was identified. Although at present, WHO does not suggest thresholds of INSTI SDRM that would indicate specific public health interventions, the national distribution of INSTI resistance, the increasing adoption of dual therapy with dolutegravir and lamivudine as first-line therapy, and the roll-out of the cabotegravir PrEP program indicate that the prevalence of INSTI SDRM warrants close monitoring in Brazil.

Contributors

RSD designed the study and led the project; RTCV trained the clinical sites and coordinated samples and data logistics; RSD and LMJ designed the laboratory study strategies; DBC, JG planned and performed laboratory experiments; JG, JRH, and AB supervised samples and data storage and preservation; JRH performed statistical analyses; RDC, RLSD conducted phylogenetic analysis; MS contributed to the analysis and interpretation of the data; ELB, CAFL, CRBA, ES, JCGF, OSS, MST, MDGS, MFF, ES, MSH, NAG, MMC, attended to the study participants and supervised the collection of samples; MS and RSD wrote the manuscript. DBC, JRH, and RSD have accessed and verified all the data. All authors had access to all the data reported in the study and approved the final version of the manuscript.

Data sharing statement

GeneBank accession numbers for HIV DNA sequences are provided in the manuscript. All deidentified participant data, including data dictionary, demographics, HIV RNA viral loads, CD4 and CD8+ T cell counts, statistical analysis, and the informed consent form, will be made available to researchers upon request to the corresponding author after publication of the manuscript. Publication of the shared data will be contingent on submitting a protocol approved by the researcher's IRB.

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While preparing this work, the authors used "Gemini AI" to make the text more concise. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Declaration of interests

RSD declares research Grants from Jansen, MSD, and Gilead; paid lectures from GSK, Jansen, and Pfizer; participation on Advisory Boards of GSK, Jansen, and Gilead. CB declares research grants from GSK and Gilead, and honoraria for lectures and advisory board participation from GSK. All other authors declare no competing interest in this study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2025.101283.

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