International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, 2018, pp.-1406-1410.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

LOW HIV-1 SUBTYPES DIVERSITY BASED ON THE POL GENE AND TRANSMITTED DRUG RESISTANCE IN ANTIRETROVIRAL-NAIVE PATIENTS FROM BELÉM, PARÁ, AMAZON REGION OF BRAZIL

IRIAS S.D.F.[1,2], AVELINO M.E.S.[1,2], TORRES M.K.S.[1,2], SANTOS M.B. [1,2], QUEIROZ, M.A.F. 1,2, PEREIRA, D.L.A. [1,2], BURLAMAQUI T.C.T.[3], FREITAS F.B.[4], VALLINOTO A.C.R.[1,2], FEITOSA R.N.M.[1,2], AZEVEDO V.N. [1,2], MACHADO L.F.A.[*1]

[1]Programa de Pós-Graduação em Biologia de Agentes Infecciosos e Parasitários, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil
[2]Laboratório de Virologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil
[3] Museu Paraense Emilio Goeldi, Belém, Pará, Brazil
[4]Laboratório de Virologia, Serviço de Vigilância em Saúde, Ministério da Saúde, Instituto Evandro Chagas, Ananindeua, Pará, Brazil
*Corresponding Author: Email - lfam@ufpa.br

Received: October 14, 2018; Revised: November 26, 2018; Accepted: November 27, 2018; Published: November 30, 2018

Abstract- The number of people living with HIV/AIDS (PLHA) is growing in Brazil, which also has increased the prevalence of transmitted drug resistance (TDR). Besides that, there is limited information about the HIV-1 diversity in Amazon region of Brazil. The objective of this cross-sectional study was to identify HIV-1 subtypes as well as the TDR profile in antiretroviral-naive PLHA from Belém, Pará, Amazon region of Brazil. Blood samples were collected from 41 drug-naive patients to determine the virus subtypes according to the HIV-1 protease (PR) gene and part of the HIV-1 reverse transcriptase (RT) gene by sequencing the nucleotides. The HIV-1 subtypes were determined using REGA HIV-1 Subtyping tool and phylogenetic analysis. Drug resistance profile was analyzed at the Calibrated Population Resistance. The most prevalent HIV-1 clade observed in Belém was the subtype B (80.5%), followed by subtype F1 (12.2%) and BF1 (7.3%). The prevalence of transmitted drug resistance was 9.7% and 4.9% was related to nucleoside reverse transcriptase inhibitors; 2.4% was related to protease inhibitors and 2.4% to non-nucleoside reverse transcriptase inhibitors. This study has shown the low genetic diversity and the high prevalence of HIV-1 subtype B and a moderate prevalence of protease/reverse transcriptase primary antiretroviral resistance mutations in Belém, Pará. This preliminary data is important to reveal the occurrence of primary TDR, reinforces the necessity of epidemiological surveillance and monitoring of primary antiretroviral drug resistance genotypic mutations in this region.

Keywords- *HIV-1, antiretroviral therapy, molecular Epidemiology, transmitted drug resistance, Brazil*

Citation: Irias S.D.F., *et al.*, (2018) Low HIV-1 Subtypes Diversity Based on the Pol Gene and Transmitted Drug Resistance in Antiretroviral-Naive Patients from Belém, Pará, Amazon Region of Brazil. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, pp.-1406-1410.

Introduction

In Brazil, the number of people living with human immunodeficiency virus infection (HIV) or the acquired immune deficiency syndrome (AIDS) under combined antiretroviral therapy (ART) increased from 231,000 in 2009 to almost 498,000 by the end of 2017 [1]. The Brazlian public health system guarantees an universal access to all HIV-infected patients and enforces their treatment, measure that has been very effective in reducing morbidity and mortality in people living with HIV/AIDS (PLHA) in this country. However, the conventional treatment for newly infected/detected HIV-1 patients and ART-naive individuals may be compromised if resistant strains are responsible for the cause of HIV infection [2]. The development of primary ART resistance can lead to treatment failure and secondary resistance if conventional ART is used, which may require alternative and/or the development of new schemes of ARTs [3], with a possible social economic burden to the public health system [4]. The prevalence of transmitted drug resistance (TDR) is classified as low (< 5%), moderate (between 5% and 15%) or high (> 15%) within a specific population in a defined geographic region and using a small number of eligible individuals [5]. Low to moderate prevalence of TDR has been reported in Brazil, mainly in South and Southeast regions [6-10], although some studies have also reported a similar prevalence in the Northeast [11-13] and Central regions [14,15]. Soares *et al* (2003) [16] reported a high prevalence of TDR in the Northeast (17%), and a moderate in the Southeast (12.8%), Central (10.6%), South (8.5%) and in the North regions (Manaus city; 8.5%) of Brazil. The prevalence of resistance to nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI) were 6.9%, 4.9% and 3.9%, respectively. There are only three studies about the prevalence of TDR in the largest in area and complex in populational area in the North region of Brazil, which were performed between 2015 and 2017. The first study reported a high prevalence (16.2%) of TDR in ART-naive children newly diagnosed with HIV-1 in Manaus, Brazil (13.7% for NNRTI, 1.7% for NRTI and 3.4% for PI) [17]. The second study was carried out in Amapá in drug-naive HIV-1-infected individuals, which showed a low TDR rate (1%) to NNRTI (K103N) [18]. Finally, a third very small study investigated only 12 drug-naive HIV-1-infected individuals from Roraima, and only one (8.3%) presented a TDR to NNRTI (G190A) [19]. Belém, the capital of the state of Pará, is one of the main cities in the North region of Brazil and hosts the second largest population in this region with 1,446,042 inhabitants in 2016. Between 2007 and 2017, there were 194,217 cases of infection HIV reported in the whole country. The North Region was the fourth (out of five) geographic region in number of cases (7.4%) and the state of Pará presented the highest number of individuals (5,648 cases) in the North region, followed by the state of Amazonas (4,875 cases), and Rondônia (1006 cases) [1]. Belém is the main entrance for tourists all over the world travelling to the Amazon region, local epidemiology of HIV diversity showed a predominance of HIV-1 subtype B infection, followed by F, C, D and CRF02_AG [20]. This study aimed to perform a cross-sectional analysis of the HIV-1 subtypes and the rate of TDR among newly diagnosed and ART-naive HIV-infected patients from Belém, Pará, Amazon Region of Brazil.

Materials and Methods

Study Design and Population Examined

A cross-sectional study in HIV-1-positive newly diagnosed and treatment-naive consecutive patients (n=41) was conducted between May and September of 2016. Recruitment was performed with patients who were attending the Centro de Atendimento em Doenças Infecciosas Adquiridas (CASA-DIA), a reference center that provides treatment for HIV/AIDS-infected patients on the local public health system. All participants were invited to participate in this study and asked to sign a written informed consent form. Demographic data was collected using a semi-structured interview. The Ethics Research Committee of the Federal University of Pará approved this study (CAAE number: 55698216.9.0000.0018).

Eligible participants were adults (> 18 years old) who attended the HIV/AIDS reference center above described and were newly diagnosed with HIV, ART-naive, and living in the city of Belém, Pará, Brazil. Peripheral blood samples (10 mL) were collected and transported to the Virology Laboratory of Biological Sciences Institute of Federal University of Pará. Plasma and peripheral blood cells were stored at −80°C. The count of CD4+ and CD8+ T cells was determined by flow cytometry (BD TrucountTM Tubes) in a FACSCalibur flow cytometer (Becton-Dickinson, New Jersey, USA). Viral load was evaluated by the Abbott Real-Time PCR assay using m2000sp (Abbott Molecular Inc., Des Plaines, IL, USA) according to the manufacturer's instructions in the same Laboratory.

HIV-1 DNA amplification and sequencing

Genomic DNA was extracted from 200 µl of total blood using the BIOPURTM Mini Spin Plus (Biometrix Diagnóstica, Brazil), following the manufacturer's instructions. The DNA was quantified using Qubit® 2.0 fluorometer (Invitrogen, Delaware-USA). The proviral DNA was used as the target for a nested polymerase chain reaction (nested PCR) for amplification of partial HIV-1 polymerase gene, 297 bp of protease (codons 1–99) and 663 bp of partial reverse transcriptase (codons 11–231) was performed as previously described [21,22]. Both strands of the HIV RT and HIV PR gene were sequenced for three times in both directions using ABI Prism BigDyeTM Terminator Ready Reaction Cycle Sequencing kit, version 3.1 (Applied Biosystems, Foster City, CA, USA). After precipitation of the reaction product, the samples were denatured and sequenced in an automated ABI 3130 sequencer (Applied Biosystems) following the manufacturer's protocol.

Sequence analysis

For assessment of data quality and construction of the consensus sequences, the sequences obtained were analyzed on the Phred-Phrap software (http://asparagin.cenargen.embrapa.br/phph/). The sequences were aligned and edited using the Bioedit Sequence Alignment Editor, version 7.0.9.0 [23]. The HIV-1 subtypes were identified using REGA HIV-1 Subtyping tool version 3.0 [24,25] and by phylogenetic inference using reference sequences obtained from Los Alamos HIV (https://www.hiv.lanl.gov/content/index). Phylogenetic trees analysis was generated by Maximum-likelihood (ML) and Bayesian Inference (BI) with RaxML 7.2.8 [26] and MrBayes 3.2 programs [27], respectively. The best model of DNA evolution accord to the data was obtained with jModeltest 2.0 [28, 29] previously to both analyses. Branch confiability was estimated through approximate likelihood ratio test (aLRT), while the support was generated by 1,000 pseudoreplicas of bootstrap for the ML. For BI, three independent runs, each with four chains, were computed for 1,000,000 generations with one sample every 100 generations, with the first 10% of samples discarded as burn-in after checking the run in Tracer 1.5 [30]. The identification of possible recombinants was evaluated by SimPlot 3.5.1 software [31] and RIP Recombinant Identification Program 3.0 [32] when subtypes in the PR and RT fragments were discordant. TDMs were analyzed according to the Calibrated Population Resistance version 6.0 (CPR tool, http://cpr.stanford.edu/cpr.cgi).

Results

The median age of participants was 36.4 years (range: 19–66 years), with 29 males (70.7%) and 12 females (23,3%). Nineteen individuals (46.3%) reported heterosexual unprotected sex as a possible contamination, 12 (29.3%) were men who have sex with men (MSM) and one participant (2.4%) believed that got HIV infection through intravenous drug use. The first positive HIV serology to the data collection was an average of 63 days (ranging from 19–127 days). Partner HIV-infected was related by 10 participants (24.4%) and other sexually transmitted infections (syphilis, gonorrhea and herpes) were reported by 16 (30.0%) participants. Regarding the sexual partners, 58.5% (24/41) reported having just 1 partner, while 34.1% (14/41) reported having multiple partners. Ten subjects (24.4%) reported partners from other states of Brazil (Amapá, Maranhão, Tocantins, Bahia, Pernambuco, Rio de Janeiro, São Paulo and Paraná) and 12.2% (5/41) reported partners from other counties (Venezuela, Suriname, Bolivia, Mexico, Canada, France and Germany). The average CD4+ T cells count was 367 cells/µl (range: 13–1,096 cells/µl) and 29 (70.7%) participants had T cell count below 500 cells/µl. The average plasma viral load was 159,481 copies/ml (range: 5,187–744,942 copies/ml). The complete information about demographic data is available in Table 1.

Table-1 Socio-demographic and laboratory tests characteristics of ART-naive people living with HIV/AIDS from Belém, Pará, Northern Brazil, between May and September 2016.

Characteristics	n (%)
Sex	
Male	29 (70.7)
Female	12 (29.3)
Age (years)	
18 - 20	4 (9.8)
21 - 30	12 (29.3)
31 - 40	13 (31.7)
> 40	12 (29.2)
Exposure category	
HTS	19 (46.3)
MSM	12 (29.3)
Bisexual	9 (00.0)
Occupational accident	1 (2.4)
$CD4^+$ T cells	
< 200 cells/µL	10 (24.4)
201-500 cells/µL	19 (46.3)
> 500 cells/µL	12 (29.3)
HIV-1 RNA viral load	
1,000-10,000 copies/mL	3 (7.3)
10,001-100,000 copies/mL	22 (53.7)
> 100,000 copies/mL	16 (39.0)

HTS: heterosexual; MSM: man who have sex with man.

The subtype B (B^{PR}/B^{RT}) was the most prevalent (33/41; 80.5%), followed by sub-subtype F1 ($F1^{PR}/F1^{RT}$) in 12.2% (5/41) and recombinant forms BF1 ($B^{PR}/F1^{RT}$ or $F1^{PR}/B^{RT}$) in 7.3% (3/41). The phylogenetic analysis is showed in Figures 1A and 1B. The prevalence of TDR was 9.7% (4/41) and mutations associated with resistance to NRTI were the most frequent (4.9%; 2/41; including mutations M184I, M41L and T215E). The prevalence of NNRTI was 2.4% (1/41; mutation K103N) and to PI was 2.4% (1/41; mutations M46I and I85V). Three isolates were subtype B (B^{PR}/B^{RT}) and one was subtype F1 ($F1^{PR}/F1^{RT}$). The mutations found in the study are detailed in Table 2, which also presents the epidemiologic and laboratory data of these patients. Two or three-class resistance was no found.

Discussion

Primary ART resistance can become a problem for newly-infected HIV patients and it can result from infections with viruses caring drug resistance. The HIV TDR may compromise patients treatment and the establishment of its prevalence is fundamental for understanding the response of treatments. In this study, we identified a 9.7% TDR prevalence in newly-infected ART-naïve HIV infected adults from Belém, Pará, North region of Brazil. According to the WHO [4], this is considered a moderate prevalence, which was similar to most studies carried out in other regions in Brazil [33-40]. Simultaneous multiple resistance mutations to more than one pharmacological drug class was not found. The presence of specific mutations is known to confer resistance to certain drugs used in the ART for HIV/AIDS treatment.

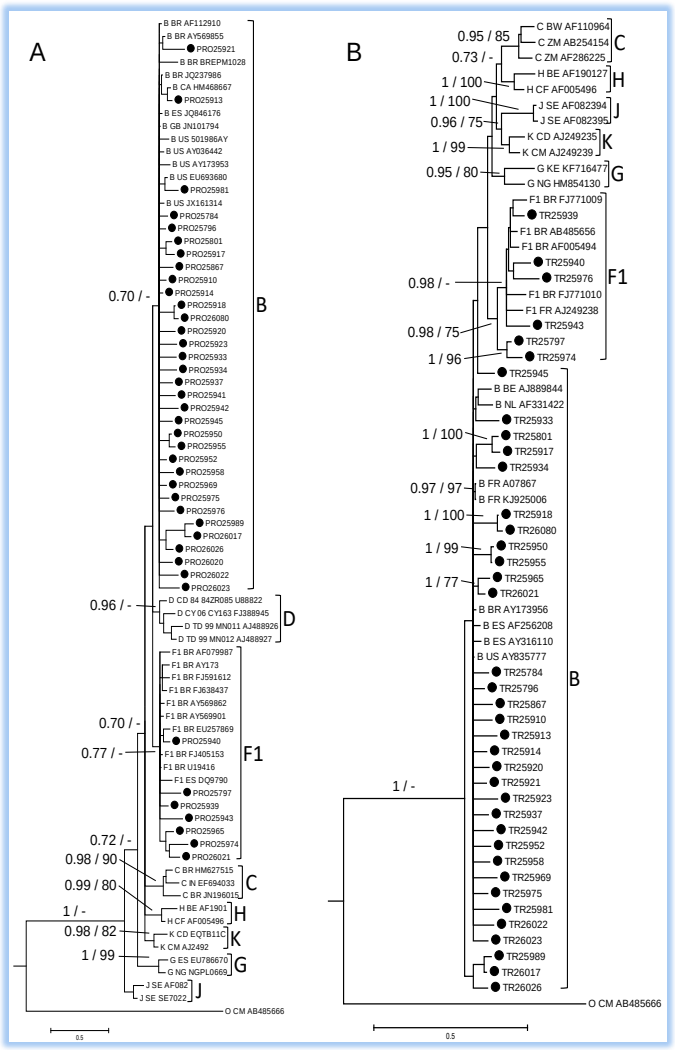

Fig-1 Phylogenetic analysis of the protease (A) and reverse transcriptase (B) fragments of HIV-1 isolates obtained from newly-diagnosed and ART-naive patients from Belém, Para, Northern Brazil. Isolates characterized in this study (black circle) were compared to HIV-1 reference sequences of group M subtypes (B, C, D, F1, G, H, J and K) available in the Los Alamos database. Support values for BI and ML, both higher than 0.7 and 70, are presented before and after the slash, respectively. The subtype O sequence (O CM AB485666) was used as an outgroup.

Table-2 Individuals with drug resistance mutations of antiretroviral-naive people living with HIV/AIDS from Belém, Pará, Northern Brazil, between May and September, 2016.

				Mutations			
Patient ID	Sex	Age	Exposure category	NRTI	NNRTI	PI	Subtype (PR/RT)
BRPA-25913	F	28	Hetero	M184I			B/B
BRPA-25920	M	38	MSM			M46I, I85V	B/B
BRPA-25940	F	44	Hetero		K103N		F1/F1
BRPA-25969	M	25	MSM	M41L, T215E			B/B

M: male; F: female; MSM: men who have sex with men; Hetero: heterosexual; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: nonnucleoside reverse transcriptase inhibitors; PI: protease inhibitor; PR: protease region; RT: reverse transcriptase region.

For instance, the mutation M184V/I causes high drug resistance to lamivudine (3TC) and emtricitabine (FTC) and a level low of drug resistance to didanosine (ddI) and abacavir (ABC) [41-43]. We found mutations associated to drug resistance to NRTI which were identified as the M184I, M41L and T21SE. M41L and T21SE were from thymidine-associated mutations (TAM) I and found in two subtype B (B^{PR}/B^{RT}) isolates. The M41L substitution generally takes place in combination with T215Y, which together provide a high resistance level to ddI and stavudine (d4T), and low/intermediate to ABC, ddI and tenofovir (TDF). Identification of the T215E variant was an important finding because of individuals infected with resistance strains containing the 215Y/F mutation develop viruses with revertant mutations at the position 215 (T215S/C/D/E/I/V/N/A/L) [44-46]. Most T215 revertant do not reduce directly the susceptibility to drugs, but when associated to a higher chance of viral failure in patients whose regimes contain AZT and d4T [47]. Other TDR studies in Brazil have reported this mutation type in antiretroviral therapy-naive patients [8,11,14,34]. There was only a single mutation resistance to NNRTI found in this study, the K103N, which provides high resistance to nevirapine (NVP) and efavirenz (EFZ). K103N mutation was found in an isolated from sub-subtype F1 ($F1^{PR}/F1^{RT}$). Its tracking in antiretroviral therapy-naive patients is highly important, taking into consideration that EFV is currently the NNRTI primarily chosen in ART top-notch in Brazil, and NVP is the alternative to EFV use [48]. The mutations to PI (M46I and I85V) were found in an isolated subtype B (B^{PR}/B^{RT}), where M46I is a primary mutation that can result in decreased susceptibility to each of the PI, except darunavir (DRV), whereas the I85V mutation produces minimal effects in PI resistance [45]. This isolate also presented the polymorphism A71V, possibly playing a compensatory role by increasing the viral replication capacity [45,49,50]. The two previous studies that analyzed virologic failure undertaken in Belém with PLHA also demonstrated mutations in ITRN (M184V, M41L, T215Y), ITRNN (K103N and P225H) and PI (M46I, Y82A and 154V) [51,52]. Majority of TDR studies in Brazil came from the Southeast region of Brazil [6,8,37,39,53,54], and just a few papers were originated in the North region [18,34,55], which demonstrates the lack of information about TDR in this region. In Brazil, the distribution of HIV-1 subtypes is very heterogeneous among different regions, with many subtypes, such as B, F1, C, A, D and several recombinant forms such as CRF02_AG, CRF_BF and CRF_BC [36,52,56,57], previously described. In our study, the subtype B (B^{PR}/B^{RT}) was identified as the most prevalent, followed by subtype F1 ($F1^{PR}/F1^{RT}$) and the recombinant subtype BF1 ($B^{PR}/F1^{RT}$) or ($F1^{PR}/B^{RT}$). These results are similar to those described in preceding studies in this region [20,51], where the subtype B was the most prevalent (80-88%), followed by sub-subtype F1 (4.2-9.3%) and recombinant BF (3.2-4.0%). Our results resemble those found in studies performed in other Northern states like Amazonas [51,58], Tocantins [34] and Amapá [18,20]. The subtype B, F1 and recombinant BF were also described in these states. Conversely, other recombinant subtypes (D, C, A1, and the recombinant forms CRF_BC and CRF02_AG) which were commonly described in other investigations in Northern Brazil were not found in our study [18,20,34,51,52,58], possibly because of the reduced sample. A recent study of genetic variability of circulating HIV-1 in Northern Brazil [59], which included the States of Acre, Amazonas, Pará, Rondônia and Roraima, showed some BF recombinant subtypes that haven´t been described in Southern Brazil. These samples can represent new CRF_BF subtypes undescribed in this country. The current study presents some limitations, especially due to the sample size, and also due to the proviral DNA used, since this could present hypermutation mediated by APOBEC3GIF, a defense mechanism of host responsible for the mutations induction of type G→A in the viral genome during the reverse transcription [60]. However, some studies have showed similarities in the results about mutation resistance obtained from viral RNA and proviral DNA, making the former use higher in developing countries [61,62].

Conclusion

In conclusion, this study has found a moderate TDR level in newly-diagnosed and antiretroviral-naïve PLHA in Belém, Brazil. It suggests that some patients will not show satisfactory response to conventional treatment.

Application of research: This reinforces the necessity of continuous epidemiologic surveillance and monitoring the resistance levels of TDR in this city,

especially because of the large use of ART and the increasing number of PLHA in Brazil.

Research Category: Drug resistance, Genotypic mutations

Abbreviations: HIV: human immunodeficiency virus, AIDS: acquired immune deficiency syndrome, ART: antiretroviral therapy, PLHA: people living with HIV/AIDS, TDR: transmitted drug resistance, NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: nonnucleoside reverse transcriptase inhibitors; PI: protease inhibitor; CASA-DIA: Centro de Atendimento em Doenças Infecciosas Adquiridas, PR: protease region; RT: reverse transcriptase region, ML: Maximum-likelihood, BI: Bayesian Inference, aLRT: approximate likelihood ratio test, MSM: men who have sex with men, Hetero: heterosexual.

Acknowledgement / Funding: Authors are thankful to the Secretaria Municipal de Saúde (SESMA) and CASA-DIA. The publication of this article was supported by Public Notice PAPQ, PROPESP/FADESP of the Federal University of Pará.

***Principle Investigator or Chairperson of research: Dr Luiz Fernando Almeida Machado**
University: Federal University of Pará
Research project name or number: MSc Thesis in Biology of Infectious and Parasitic Agents Graduate Program at the Federal University of Pará.

Author Contributions: SDFI and LFAM conceived and designed the study, analyzed, interpreted data, and wrote the paper. MKST, MES, DLAP and MBS collected samples, participant recruitment and performed experiments of molecular biology. SDFI, VNA, RNMF supervised analyzed the resistance data. TCTB and FBF performed phylogenetic analysis. LFAM and ACRV reviewed the manuscript.

Author statement: All authors read, reviewed, agreed and approved the final manuscript
Conflict of Interest: No conflict of interest.

Ethical approval: This study was approved by Ethics and Research Committee of Federal University of Pará (CAAE number: 55698216.9.0000.0018). Written informed consent was obtained from all participants prior to enrollment.
Ethical Committee Approval Number: CAAE number: 55698216.9.0000.0018

References

[1] Brasil. Ministério da Saúde - Secretaria de Vigilância em Saúde - Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais. Boletim Epidemiológico: HIV Aids, 2017; ano V, n. 1.
[2] Hassan A.S., Nabwera H.M., Mwaringa S.M., Obonyo C.A., Sanders E.J., Rinke de Wit T.F., *et al*. (2014) *AIDS Res Ther*, 11,9 .
[3] Asahchop E.L., Wainberg M.A., Sloan R.D., Tremblay C.L. (2012) *Antimicrob Agents Chemother*, 56, 5000-5008.
[4] WHO. (2012) *World Health Organization. The HIV drug resistance report.Geneva* (http://www.who.int/hiv/pub/drugresistance/report2012/en/).
[5] Myatt M., Bennett D.E. (2008) *Antivir Ther*, 13(Suppl 2), 37-48.
[6] Sucupira M.C., Caseiro M.M., Alves K., Tescarollo G., Janini L.M., Sabino E.C., *et al*. (2007) *AIDS Patient Care STDS*, 21, 116-128.
[7] Sprinz E., Netto E.M., Patelli M., Lima J.S., Lima M.P., Furtado J.J., *et al*. (2009) *AIDS Res Hum Retroviruses* 2009, 25, 861-867.
[8] Ferreira J.L,. Rodrigues R., Lança A.M., de Almeida V.C., Rocha S.Q., Ragazzo T.G., *et al*. (2013) *Adv Virol*, 2013, 878237.
[9] Guimarães P.M., Ferreira J.L., Coelho L.P., Cavalcanti Je.S., Lopes G.I., Matsuda E.M., *et al*. (2015) *AIDS Res Hum Retroviruses*, 31, 1219-1224.
[10] Delatorre E., Silva-de-Jesus C., Couto-Fernandez J.C., Pilotto J.H., Morgado M.G. (2017) *AIDS Res Hum Retroviruses*, 33, 68-73.
[11] Moura M.E., Reis M.N., Lima Y.A., Eulálio K.D., Cardoso L.P., Stefani M.M. (2015) *AIDS Res Hum Retroviruses*, 31, 250-254.
[12] Moura M.E., da Guarda Reis M.N., Lima Y.A., Eulálio K.D., Cardoso L.P., Stefani M.M. (2015) *J Med Virol*, 87, 798-806.
[13] Cavalcanti A.M., Brito A.M., Salustiano D.M., Lima K.O., Silva S.P., Diaz R.S., *et al*. (2012) *Mem Inst Oswaldo Cruz*, 107, 450-457.
[14] da Silveira A.A., Cardoso L.P., Francisco R.B., de Araújo Stefani M.M. (2012) *AIDS Res Hum Retroviruses*, 28, 304-7.
[15] da Costa Z.B., de Lima Y.A., Martelli C.M., Stefani M.M. (2013*) AIDS Patient Care STDS*, 27(8), 439-441.
[16] Soares E.A., Santos R.P., Pellegrini J.A., Sprinz E., Tanuri A., and Soares M.A. (2003*) J Acquir Immune Defic Syndr*, 34, 520–526.
[17] Andrade S.D., Sabidó M., Monteiro W.M., Benzaken A.S., Tanuri A. (2017) *J Antimicrob Chemother*, 72, 1774-1783.
[18] Dos Anjos Silva L., Divino F., da Silva Rêgo M.O., Lima Lopes I.G., Nóbrega Costa C.M., da Silva Pereira F.C, *et al*. (2016) *AIDS Res Hum Retroviruses*, 32, 373-376.
[19] Corado A.L., Bello G., Leão R.A., Granja F., Naveca F.G. (2017) *PLoS One*, 12, e0173894.
[20] Machado L.F., Ishak M.O., Vallinoto A.C., Lemos J.A., Azevedo V.N., Moreira M.R., *et al*. (2009) *AIDS Res Hum Retroviruses*, 25, 961-966.
[21] Janini L.M., Pieniazek D., Peralta J.M., Schechter M., Tanuri A., Vicente A.C., *et al*. (1996) *Virus Genes*, 13, 69-81.
[22] Stefani M.M., Pereira G.A., Lins J.A., Alcantara K.C., Silveira A.A., Viegas A.A., *et al*. (2007) *J Clin Virol*, 39, 205-209.
[23] Hall T.A. (1999) *Nucleic Acids Symp*, 41, 95–98.
[24] de Oliveira T., Deforche K., Cassol S., Salminen M., Paraskevis D., Seebregts C., *et al*. (2005) *Bioinformatics*, 21, 3797-800.
[25] Alcantara L.C., Cassol S., Libin P., Deforche K., Pybus O.G., Van Ranst M., *et al*. (2009) *Nucleic Acids Res, 37(Web Server issue)*, W634-42.
[26] Stamatakis A. (2006) *Bioinformatics*, 22, 2688-2690.
[27] Ronquist F., Huelsenbeck J.P. (2003) *Bioinformatics*, 19, 1572-4.
[28] Darriba D., Taboada G.L., Doallo R., Posada D. (2012) *Nat Methods*, 9, 772.
[29] Guindon S., Gascuel O. (2003) *Syst Biol*, 52, 696-704.
[30] Rambaut A., Suchard M.A., Xie D., Drummond A.J. (2014) Tracer v1.6, Available from http://tree.bio.ed.ac.uk/software/tracer/.
[31] Lole K.S., Bollinger R.C., Paranjape R.S., Gadkari D., Kulkarni S.S., Novak N.G., et al. (1999) *J Virol*, 73, 152-160.
[32] Siepel A.C., Halpern A.L., Macken C., Korber B.T. (1995) *AIDS Res Hum Retroviruses,* 11, 1413-1416.
[33] Arruda E., Simões L., Sucupira C., Medeiros M., Diaz R.S., Lima A. (2011) *AIDS Res Hum Retroviruses*, 27, 153-156.
[34] Carvalho B.C., Cardoso L.P., Damasceno S., Stefani M.M. (2011) *AIDS Res Hum Retroviruses*, 27, 1081-1087.
[35] Gräf T., Passaes C.P., Ferreira L.G., Grisard E.C., Morgado M.G., Bello G., *et al*. (2011) *J Clin Virol*, 51, 186-191.
[36] de Medeiros R.M., Junqueira D.M., Matte M.C., Barcellos N.T., Chies J.A., Matos Almeida S.E. (2011) *J Med Virol*, 83, 1682-1688.
[37] Sanabani S.S., Pastena E.R., Neto W.K., Martinez V.P., Sabino E.C. (2010) *Virol J*, 7, 74.
[38] Pfrimer I.A., Bizinoto M.C., Brandão N.A., Camargo M., Sucupira M.C., Janini L.M., *et al*. (2013) *AIDS Res Hum Retroviruses*, 29, 205-206.
[39] Guimarães P.M., Ferreira J.L., Coelho L.P., Cavalcanti Je.S., Lopes G.I., Matsuda E.M., *et al*. (2015) *AIDS Res Hum Retroviruses*, 31,1219-1224.
[40] Moura M.E., da Guarda Reis M.N., Lima Y.A., Eulálio K.D., Cardoso L.P., Stefani M.M. (2015) *J Med Virol*, 87, 798-806.
[41] Boucher C.A., Cammack N., Schipper P., Schuurman R., Rouse P., Wainberg M.A., *et al*. (1993) *Antimicrob Agents Chemother*, 37(10), 2231-2234.

[42] Sarafianos S.G., Das K., Clark A.D., Ding J., Boyer P.L., Hughes S.H, *et al*. (1999) *Proc Natl Acad Sci USA*, 96, 10027-10032.
[43] Diallo K., Götte M., Wainberg M.A. (2003) *Antimicrob Agents Chemother,* 47, 3377-3383.
[44] Yerly S., Rakik A., De Loes S.K., Hirschel B., Descamps D., Brun-Vézinet F., *et al*. (1998) *J Virol*, 72, 3520-3.
[45] Rhee S.Y., Gonzales M.J., Kantor R., Betts B.J., Ravela J., Shafer R.W. (2003) *Nucleic Acids Res*, 31, 298-303.
[46] Shafer R.W., Rhee S.Y., Pillay D., Miller V., Sandstrom P., Schapiro J.M., et al. (2007) AIDS, 21, 215-223.
[47] Violin M., Cozzi-Lepri A., Velleca R., Vincenti A, D'Elia S., Chiodo F., *et al*. (2004) *AIDS*, 18, 227-235.
[48] BRASIL. Ministério da Saúde. Departamento de DST, Aids e Hepatites Virais. Protocolo clínico e diretrizes terapêuticas para manejo da infecção pelo HIV em adultos. Brasília: MS, 2015.
[49] Nijhuis M., Schuurman R., de Jong D., Erickson J., Gustchina E., Albert J., *et al*. (1999) *AIDS*, 13, 2349-2359.
[50] Rhee S.Y., Liu T., Ravela J., Gonzales M.J., Shafer R.W. (2004) *Antimicrob Agents Chemother*, 48, 3122-3126.
[51] Macedo O., Ferreira L.M., Vasconcelos P.F.C., Souza R.C.M., Freitas C.A., Araújo J.R.M. (2011) *Revista Pan-Amazoônica de Saúde*, 2, 27-34.
[52] Lopes C.A., Soares M.A., Falci D.R., Sprinz E. (2015) *Biomed Res Int*, 738528.
[53] Gonsalez C.R., Alcalde R., Nishiya A., Barreto C.C., Silva F.E., de Almeida A., *et al*. (2007) *Virus Res*, 129, 87-90.
[54] Velasco-de-Castro C.A., Grinsztejn B., Veloso V.G., Bastos F.I., Pilotto J.H., Fernandes N., *et al*. (2014) *PLoS One*, 9, e87622.
[55] Corado A.L., Bello G., Leão R.A., Granja F., Naveca F.G. (2017) *PLoS* One, 12, e0173894.
[56] Gräf T., Pinto A.R. (2013) *Virology,* 435, 170-178.
[57] Librelotto C.S., Gräf T., Simon D., de Almeida S.E., Lunge V.R. (2015) Rev Soc Bras Med Trop, 48, 249-257.
[58] Cunha L.K., Kashima S., Amarante M.F., Haddad R., Rodrigues E.S., Silva K.L., *et al*. (2012) *Braz J Med Biol Res*, 45, 104-112.
[59] da Costa C.M., Costa de Oliveira C.M., Chehuan de Melo Y.F., Delatorre E., Bello G., Couto-Fernandez J.C. (2016) AIDS Res Hum Retroviruses, 32, 918-922.
[60] Cuevas J.M., Geller R., Garijo R., López-Aldeguer J., Sanjuán R. (2015) *PLoS Biol*, 13, e1002251.
[61] Derache A., Shin H.S., Balamane M., White E., Israelski D., Klausner J.D., *et* al. (2015) *PLoS One*, 10, e0117430.
[62] Novitsky V., Zahralban-Steele M., McLane M.F., Moyo S., van Widenfelt E., Gaseitsiwe S., *et al*. (2015) *J Clin Microbiol*, 53, 2581-92.