Chapter 4
HIV-1 Diversity in Brazil

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Core Message

Genetic variation in human immunodeficiency virus (HIV)-1 poses significant public-health and clinical challenges. In Brazil, several subtypes cocirculate which are diversely distributed in different geographical regions. The continuous molecular evolution of these subtypes, together with the introduction of new subtypes as well as intermixing of HIV-1 variants, is inevitable and may have significant implications for diagnosis of infection, quantification of viral loads, drug-resistant pathways, transmissibility, response to therapy, and challenges to development of vaccine. Thus, understanding the genetic diversity and geographical distribution of HIV is significantly important for planning effective intervention measure to prevent infection, either through educational programs, therapy interventions, or vaccine strategies.

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4.1 Introduction

Acquired immune deficiency syndrome (AIDS) was first recognized as a cluster of symptoms when an increasing number of young homosexual men in New York City and San Francisco died from opportunistic infections and rare malignancies. Eventually, similar symptoms were found among intravenous drug users, hemophiliacs, and other recipients of blood transfusions. In 1984, the human immunodeficiency virus (HIV) was isolated and subsequently assigned as the causative agent of AIDS. Since then, HIV/AIDS has become one of the most devastating epidemics in humans in recent history. At the end of 2014, the WHO global health report showed that about 36.9 million people were living with HIV and tens of millions of people had died of AIDS-related causes since the beginning of the epidemic (http://www.who.int/gho/hiv/en/). Over 12 million children have been orphaned by AIDS and about 1600 babies acquire HIV from their infected mothers everyday [1]. In the past three decades, the HIV pandemic has caused a great burden on global wealth and health, especially in sub-Saharan Africa where the highest rate of HIV infection has been recorded (http://www.unaids.org/).

As the prospects of an effective vaccine and curative treatments remain uncertain, HIV/AIDS will continue to be a significant threat to public health in the coming years. As of 2014, the Joint United Nations Program on HIV/AIDS (UNAIDS) reported an estimated 87,000 new infections in Latin America, bringing the number of people living with HIV to an estimated 2 million (http://files.kff.org/attachment/fact-sheet-the-global-hivaids-epidemic). The Caribbean has been hardest hit by the epidemic and has the second highest HIV prevalence rate in adult subjects in the world after sub-Saharan Africa. Six countries in Latin America and the Caribbean have generalized epidemics, with Haiti having the region's highest prevalence rate (1.9%), and Brazil the greatest number of people living with the disease (approximately 730,000–1,000,000) and home to roughly half of all new HIV infections in the region (http://www.unaids.org/sites/default/files/media_asset/MDG6Report_en.pdf).

The immense genetic variability of HIV-1 viruses is considered the key factor that frustrates efforts to halt the virus epidemic and poses a serious challenge to the development and efficacy of vaccines. Like other human positive-sense RNA viruses, HIV has a high mutation rate as a result of the error-prone nature of the reverse transcriptase (3×10^{-5} mutations per nucleotide per replication cycle) [2, 3]. This high rate of mutation, coupled with the increased replication capacity of the virus (10.3×10^9 particles per day) [4], allows for the accumulation and fixation of a variety of advantageous genetic changes in a virus population, which are selected for by the host immune response and can resist newly evolving host defenses.

Recombination is another potential evolutionary source that significantly contributes to the genetic diversification of HIV by successfully repairing defective viral genes and by producing new viruses [5]. The most recent common ancestors (MRCA) of HIV-1 groups M and O were estimated to have existed around 1920 [6, 7] and became a worldwide public health threat 60 years later. To date, HIV-1

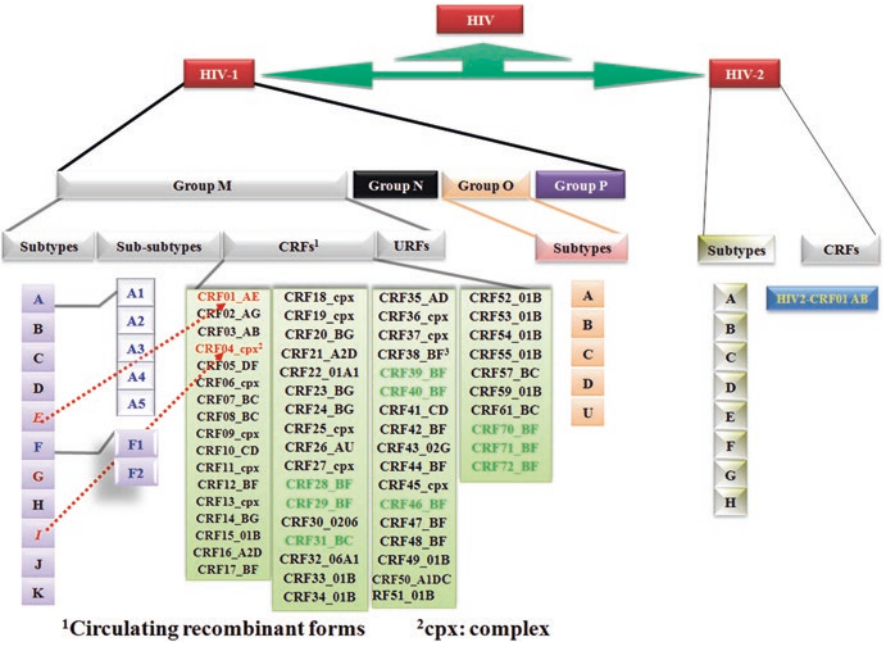

Fig. 4.1 Classification of HIV-1 and HIV-2 variants: The vast majority of HIV-1 strain belong to group M which has further been classified into subtypes, sub-subtypes, circulating recombinant forms (CRFs), and unique recombinant forms (URFs). In contrast, HIV-2 has been classified into eight different groups. Among these, only group A and B are epidemic. The full-length genome sequencing of subtype E and I determined that they are actually CRF01_AE and CRF04_cpx, respectively. The CRFs written in green color have been identified to circulate in Brazil

viruses are classified into four phylogenetic groups: M, O, N, and P, which most likely reflect four independent events of cross-species transmission from chimpanzees [8, 9], as shown in Fig. 4.1. The M group (for main), responsible for the majority of viral infections worldwide, is further subdivided into nine subtypes (A–D, F–H, J, and K), among which subtypes A and F have been further classified into two sub-subtypes [8]. Moreover, early sequencing studies have provided evidence of a recombination between genomes of different HIV subtypes [10, 11]. Such interclade recombinant strains are consistently reported from regions where two or more clades are predominant. Recombinant strains from at least three unlinked epidemiological sources, which exhibit identical mosaic patterns, have been classified separately as circulating recombinant forms (CRFs) [12, 13]. Currently, there are more than 70 defined CRFs http://www.hiv.lanl.gov that are epidemiologically important as subtypes. In addition to the known CRFs, a large number of unique recombinant viruses, which are called unique recombinant forms (URFs), have been characterized worldwide [14].

Together, CRFs and URFs are estimated to account for 18% of incident infections in the global HIV-1 pandemic [15]. It is likely that novel subtypes and CRFs

will be isolated in the future as recombination and mutation continue to occur. HIV-1 subtypes, CRFs, and URFs show considerably different patterns of distribution in different geographical regions [15, 16]. This diversity has an impact not only on diagnosis, replication, development of mutations, and therapy response, but also on the ability of distinct viruses to recombine during coinfection [17].

Available epidemiological data indicates that subtype B is the major clade in the Americas, Western Europe, Japan, and Australia [15, 18]. Subtype A is the main genetic form in Russia and the former Soviet Union countries, but it is also commonly observed in several African countries such as the Democratic Republic of Congo (DRC), Kenya, and Tanzania [19]. Subtype C is prevalent in South Africa, Somalia, Djibouti, Ethiopia, as well as in India, while subtype D is the dominant form in other regions, such as Uganda, Sudan, and Libya. In some geographic areas, recombinants are the most widely distributed forms, as is the case with CRF01_AE in Southeast Asian countries, CRF02_AG in West African countries, and CRF07_BC and CRF08_BC in China [20]. Subtypes A1, B, C, and the CRF02_AG are the most prevalent HIV-1 group M genetic clades and are responsible for the infection in more than 75% of AIDS cases worldwide. Molecular epidemiological studies have, thus, made significant contributions to our understanding of the molecular diversity of HIV-1, enabling the detection of emerging HIV-1 subtypes, and improving the tracking of the epidemic worldwide. Of note, the transmission of the CRFs and URFs further complicates efforts toward the development of effective vaccines due to existing diversity [21].

In this chapter, we review the diversity of HIV-1 in Brazil, the largest country in South America, and the fifth largest nation in the world [22]. The first case of AIDS was recorded in the country by retrospective analysis in late 1981. At that time, Brazil was in the midst of a transition from military rule to civilian government and was facing a devastated economic and social welfare system [23]. This process of transformation would drastically affect not only the immigration pattern and spread of infection but also the overall national strategy to curb the spread of HIV/AIDS.

While the AIDS-related illnesses during the early years of the epidemic posed an unprecedented challenge to the health authorities, Brazil today is considered a model of a successful response to the epidemic, particularly for countries of low- and middle-income status. It has been reported that since the 1990s, the number of Brazilians dying from AIDS-related illnesses has fallen by 50%. The antiretroviral program currently reaches 80% of the infected population, which is similar to the coverage in wealthier, more developed nations. From 1980 to June 2014, 757,042 new AIDS cases were reported in Brazil. The HIV-1 incidence has been increasing rapidly and progressively in Brazilians exposed to HIV through heterosexual sex since the beginning of the epidemic, because of a process of feminization of the AIDS epidemic: in 1985, the male per female new AIDS case ratio was 26.7, while in 2014 it had decreased to 2.0 (http://www.aids.gov.br). The prevalence of AIDS in the adult population is 0.4%, and the incidence of new cases was 78/1000, 000 inhabitants in 2014.

Currently, the HIV-1 epidemic in Brazil is dominated by subtype B, followed, to a lesser extent, by BF1 URF subtypes, particularly in the north, northeast, west central, and southeast of the country. In contrast to these regions, subtype C is the

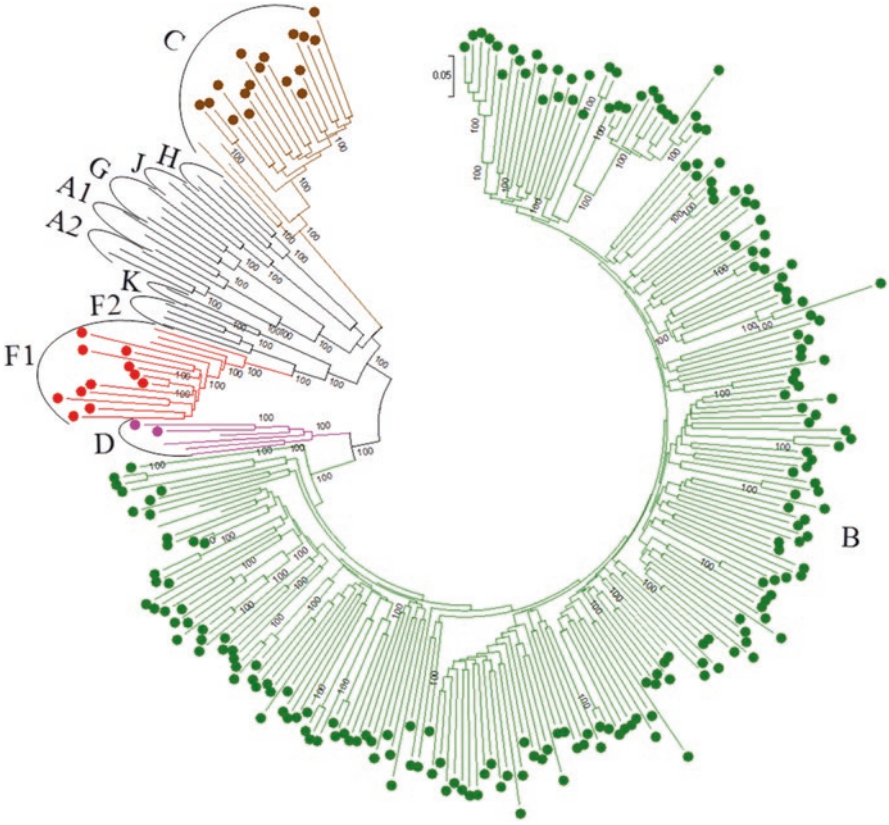

Fig. 4.2 Phylogenetic analysis of all Brazilian nonrecombinant HIV-1 near full-length (published and unpublished) genomes and 37 HIV-1 reference sequences from the Los Alamos HIV-1 database (http://hiv-web.lanl.gov) representing 11 genetic subtypes. Annotation of samples is as follows: symbol-green circle (subtype B), symbol-brown circle (subtype C), symbol-red circle (subclade F1), and symbol-pink circle (subtype D). The tree was constructed using the PHYML v.3.0 package. For clarity purposes, the trees were midpoint rooted. The approximate likelihood ratio test (aLRT) values of 100% are indicated at nodes

second most prevalent genetic subtypes in the southern region. The distribution of these variants is based on published studies, which often used different molecular techniques including the heteroduplex mobility assay, and partial or full-length genome sequencing. Figure 4.2 shows the results of a phylogenetic tree analysis of all Brazilian HIV-1 full-length nonrecombinant genomes available in the database and other unpublished sequences from our group [24]. In this chapter, we present a detailed revision of the literature of the HIV-1 epidemic in the five main geopolitical regions. The molecular data of subtype distributions were obtained from published national studies for the last 15 years. The proportions of HIV-1 subtypes and recombinants in each region were combined in a similar way to what has been described before [15].

4.2 Molecular Epidemiology of HIV-1 Infection in Brazil

4.2.1 North Region

The north region is the largest region of Brazil, which borders six different South American countries. The cumulative number of HIV-1 cases by the end of 2014 was 41,036, resulting in an incidence rate of 122.8 per million population in 2014 according to data from the Brazilian Ministry of Health (http://www.aids.gov.br). A broad diversity of HIV-1 subtypes has been detected; however, nucleotide sequence data are still scarce in comparison to other regions of Brazil [25–29]. A molecular epidemiological survey of HIV-1 from this region revealed that the majority of the HIV-1 variant characterized between 2000 and 2011 ($n = 332$) belonged to subtype B (82.8%), and the second most prevalent strain was subtype C (5%), F1 (4.8%), BF1 recombinants (4.5%), CF1 (0.9%), and other different subtypes that circulate in small proportions (0.3% each) including CRF02_AG, as shown in Fig. 4.3.

In a recent study including 377 individuals with virologic failure, the genotypic profile indicated that the majority of the analyzed variants (90.7%) were of subtype B, followed by subclade F1 (5.7%) [30]. The same study reported the circulation of subclade A1 for the first time in the north region. The evaluation of temporal distribution of B and non-B subtypes over the 10 years failed to reveal significant differences, providing evidence of a stabilized HIV-1 epidemic in terms of diversity in this region [30]. Notably, the subtype definition of HIV-1 sequences reported in the later studies was solely based on small fragments of viral genomes, which are not sufficient to identify all recombinant viruses, and thus, it likely underestimates the complexity of these divergent strains.

4.2.2 Northeast Region

The northeast is the Brazilian region with the largest coastline in the country, which makes the region very appealing to tourists around the world. The cumulative number of reported HIV-1 patients in this region was 108,599, resulting in an incidence rate of 66 per million of population in 2014 (http://www.aids.gov.br). The molecular epidemiological data from ten published studies and others from our own laboratory (Pessoa et al. 2016) derived from the northern region of the country during 2006 and 2015 revealed the circulation of four genuine subtypes (B, F1, C, and D), four URFs (BF1, BC, DF1, and BCF1), and two novel CRF70 and 71 BF1 strains [31–40]. Overall, subtype B accounted for most of the HIV-1 infections (78%) in this region followed by a large variety of BF1 strains (11.5%) and F1 subclade (5.7%).

A recent REDS-II (Retrovirus Epidemiological Donor Study) study of HIV-1 molecular epidemiology analyzed the partial *pol* gene of 110 samples from seropositive blood donors in the state of Recife, capital of the state of Pernambuco (PE), and the principal port city of the northeast [41]. The study reported a relatively high

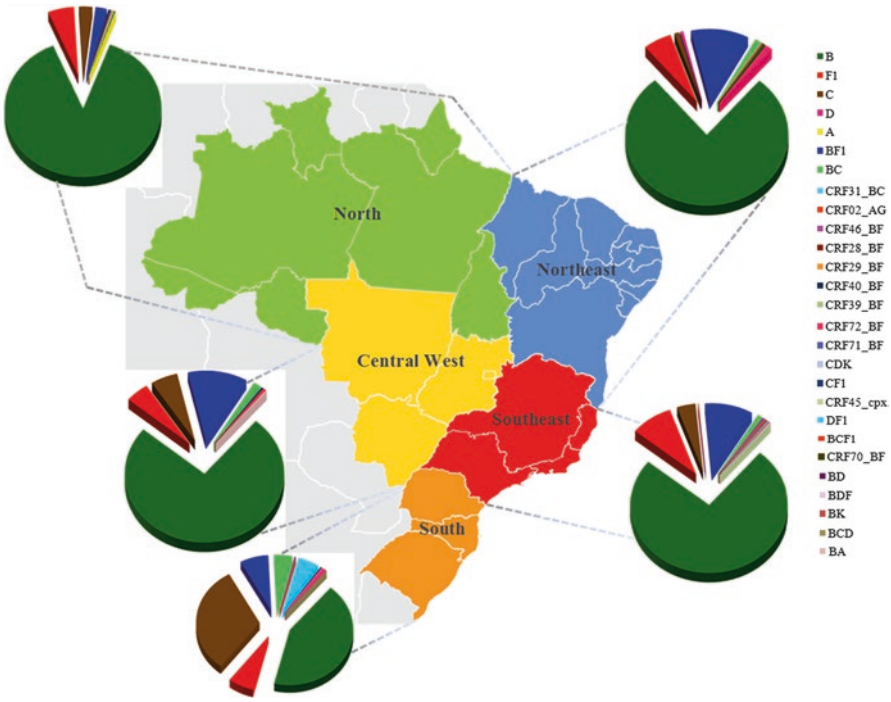

Fig. 4.3 General distribution of HIV-1 subtypes and recombinants in the five geopolitical regions of Brazil. Briefly, the subtype profile was determined by pooling data from molecular epidemiological surveys published for the last 15 years, and the resulting aggregates in each region were used to determine the overall proportions of HIV-1 subtypes and recombinants [90–113]

prevalence of subclade F1 (26 [24%] of 110) and only one case of BF1 recombinant among blood donors in this region. These findings contrast with those from the previous studies of HIV-1 NFLGs in Brazil [42–46]. Resequencing of the HIV-1 viruses from the same samples was performed to determine whether the classification of these strains extends to the NFLG and partial larger fragment [39]. The results revealed 23 of the 24 donors in whom genotyping was successful were infected with HIV-1 BF1 recombinant variants including CRF70 and 71 BF1 strains. The same study estimated the prevalence of BF1 recombinant variants at 20.4% and CRF71_BF1 at 10.2% (11/108) of HIV-1 strains circulating among blood donors in the northeast region. Again, this study showed a surprisingly low prevalence rate of 0.9% of F1 viruses, suggesting that, in previous studies, the occurrence of subclade F1 was overestimated due to partial genome sequence data. The apparently low prevalence of subclade F1 is ecological and may not be due to inherent properties of the virus itself but rather to the chance results of subtype B (a founder virus in Brazil), where it was introduced and consequently established into our HIV-1 infected population before the other subtypes were introduced [45].

4.2.3 Center-West Region

The center-west is the second largest region of Brazil by area, but in terms of population it is the least populated with 14,400,000 inhabitants. According to the current data provided by the Brazilian Ministry of Health, the reported number of HIV-1/AIDS cases by the end of 2014 was 44,112, with 1161 newly diagnosed cases during that year (http://www.aids.gov.br). In 2014, the estimated incidence rate of HIV-1 infection was 81 per million. The data from 11 studies published between 2000 and 2013 in this region showed that subtypes B and BF1 recombinants account for more than 87% of the viral subtypes circulating in this region (Fig. 4.3) [47–57]. Almost an equal prevalence was detected for subtype C (5.5%) and F1 (4.8%). Other subtypes including D, BC, CF1, BCF1, and DF1 recombinants have also been found in this region but at a low prevalence. Cardoso et al. [47] assessed the molecular diversity of HIV-1 *env*, *gag*, *protease*, and *reverse transcriptase* from 77 HIV-1 infected pregnant women. The results indicated that 66.2% of the isolates were subtype B, 6.5% subclade F1, and 3.9% and approximately 25% B and F1 virus subtypes.

4.2.4 Southeast Region

The southeast is the vital center of the country, where the largest cities and the highest population density are concentrated. It is estimated that more than 70 different nationalities compose the southeastern population of 84,400,000. According to the data from the Brazilian Ministry of Health, a cumulative total of 411,800 cases of HIV/AIDS were reported from January 1980 to 2014 (http://www.aids.gov.br) resulting in an incidence rate of approximately 68 per million of population in 2014. Of note, most studies conducted to assess the diversity of HIV-1 in Brazil have been performed using samples from the southeastern region, mainly from the states of Rio de Janeiro and São Paulo. Twenty-six studies and other HIV-1 NFLG diversity from unpublished survey (Pessoa et al. 2016) reported a circulation of five genuine subtypes (B, F1, C, D, and A), ten CRFs (CRF28_BF, CRF29_BF, CRF31_BC, CRF39_BF, CRF40_BF, CRF71_BF, CRF72_BF, CRF45_cpx), and six URFs composed with subtype B, F1, C, D, and K [42–45, 58–78].

The overall distribution indicates a high prevalence of subtype B (74.8%), followed by URF BF1 (9.8%), and subclade F1 (8.7%). Subtype C and BC recombinants represent 3.8% and 1% of the HIV-1 viruses circulating in this region, respectively.

The recent characterization of 233 HIV-1 NFLGs from infected blood donors in this region revealed the circulation of 13 different HIV-1 subtypes, including four pure ones that are responsible for 73% of infections, five different URF variants responsible for 24%, and four CRF strains responsible for 3% of infections [39, 59, 74, 79] (Pessoa et al. 2016). The results from the same study indicated that 67.4%

of the isolates were of subtype B, 18.9% BF1 URF, 3.9% subtype C, and 0.8% non recombinant F1 subclade and D subtype each.

In another study of 77 NFLGs and 32 partial sequences from the recently HIV-1 infected Brazilian subjects in the state of São Paulo, the phylogenetic analysis showed that subtype B is largely dominant (79.8%), followed by BF1 URF [43]. All along, previous studies suggested a consistent and continual spread of subtype B and BF1 URF variants over time in this region [43, 80, 81]. This dynamic factor together with the elevated number of dual and super-infections in this region [79, 82] may lead to the complete disappearance of subclade F1. It has been shown that the heterogeneity within the global B subtype is equally reflected within the Brazilian subtype B viruses, which seemed to have had multiple HIV-1 introductions from North America [43].

Previous studies aimed at reconstructing the past population dynamics of subtype B in Brazil via the coalescent theory have indicated that the epidemic growth of this clade started in the late 1960s, when it grew exponentially over the first two decades [83]. The element that initially contributed to and fueled the exponential growth of subtype B viruses in this region may have been primarily the transmission from acutely infected men who had sex with men and intravenous drug users [84, 85]. Based on the revision of the data from the previous studies (depicted in Fig. 4.3), it seems that there is a lack of substantial spread of subtype C and its recombinants in the southeast region. It is possible that the high prevalence of subtype B viruses and other recombinant variants saturating the HIV-1 infected population coupled with effective behavior changes may be responsible for the differential spread of these strains.

4.2.5 South Region

The south region of Brazil is the smallest area of the country, but its population of 25,800,000 is twice as large as the number of inhabitants in the north and center-west regions. The states of Rio Grande do Sul, Santa Catarina, and Paraná compose the southern region, which shares borders with Uruguay, Argentina, and Paraguay, as well as with the Brazilian states of São Paulo in the southeast and Mato Grosso do Sul in the center-west region. The epidemiological data from this region indicates a progressive increase of the AIDS-related mortality since the beginning of the 2000s and is currently considered one of the Brazilian regions with the highest AIDS incidence reaching 131.5 per million of population in 2014. The cumulative number of HIV-1 notified cases by the end of 2014 was 151,495. The southern region is also characterized by a distinct subtype profile in that the spread of HIV-1 subtype B is matched by HIV-1 subtype C [86]. These features have attracted various studies to investigate the history and dynamics of subtype C in the country, which estimated an origin between the 1960s and the 1970s [87, 88].

Several studies either based on partial or NFLG sequencing showed subtype C from different regions in Brazil to form a tight monophyletic group, indicating a

founder effect [89, 90]. The results by pooling data from 24 molecular epidemiological surveys published between 2005 and 2015 indicate a cocirculation of 14 distinct subtypes consisting of 42.8% subtype B, 31.6% subtype C, 7.2% BF1 recombinants, 6.5% subclade F1, 5.5% CRF31_BC, 4.6% BC recombinants, and 1% subtype D, as shown in Fig. 4.3.

4.3 Conclusions

This review of HIV-1 subtype diversity showed an unprecedented diverse picture of the subtype distribution in Brazil, indicating that the epidemic in this country is old and mature. Evidently, the circulation of diverse subtypes and the evolution of new recombinants with different and complex mosaics will continue. Thus, there is need for regular investigation and information updates to assist the concerned organizations in the country to plan for effective preventive measures.

Conflict of interest The authors report no conflicts of interest.

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