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Current Resistance of HIV-1 Strains Isolated in Volunteer Blood Donors in Gabon

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Abstract

Detection of drug resistance mutations (DRMs) and HIV-1 subtypes ensures effective therapeutic management for HIV-infected individuals. In Gabon, data on DRMs are very little available in the population of people living with HIV and also among voluntary HIV-positive blood donors. This study aimed to study subtypes and DRMs in HIV-1-positive volunteer blood donors in Gabon. A cross-sectional study was carried out at the National Blood Transfusion Center of Gabon. A purposive sampling method was used to collect 128 HIV-1 seropositive blood samples. Viral RNA was extracted on real-time PCR (Abbott 2000®), and sequencing was performed on ABI 3500 (Hitachi®). SPSS version 21.0 software was used for statistical analysis. Of the 128 seropositive volunteer donors included, men and the 29–39-age group were more representative at 78.9% and 49.2%, respectively. Eighty-two samples were sequenced. The majority strains identified were subtype A, subtype F, subtype G, CRF02_AG, and CRF45_cpx. The resistance mutations identified were K103N, L210W, E138G, V179D, V179T, and M46L. The prevalence of resistant subtypes was 25.6%. CRF02_AG strains exhibited high-level resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), including efavirenz and nevirapine. The study identified major DRMs in reverse transcriptase and protease that confer high-level resistance to most NNRTIs, nucleoside reverse transcriptase inhibitors, and protease inhibitors. CRF02_AG was more predominant, and the frequency of resistant subtypes was high. However, these data will contribute to the therapeutic choice during the initiation of antiretroviral treatment in treatment-naive patients in Gabon.

Keywords: HIV-1, subtypes, drug resistance mutations, volunteer donors, Gabon

Introduction

The Antiretroviral (ARV) therapy in sub-Saharan Africa encounters certain obstacles related in particular to the detection of drug-resistant strains of HIV, which contributes enormously to improving the therapeutic care of people living with HIV (PLHIV). Some sub-Saharan countries, such as Gabon, have not yet integrated the search for drug resistance into the treatment of PLHIV.[1,2] The World Health Organization (WHO) strongly recommends testing for transmitted drug resistance in PLHIV.[3] Study of HIV-1 strains and drug resistance mutations (DRMs) in PLHIV who have never received antiretroviral treatment (ART) is essential to improve decision-making regarding the management of PLHIV i resource-based countries, which is limited.[4] Volunteer donors come to give blood voluntarily in blood banks without financial compensation and without family ties, because their concern is to save lives. Volunteer blood donors diagnosed with HIV are a donor population that is more likely to have recent exposure to HIV.[5]

Indeed, the identification of DRMs in people who have not yet received ART also contributes to strengthening

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epidemiological data. It will help raise awareness among people responsible for caring for PLHIV by improving their decision-making when faced with a newly infected person who has never received ART.[6,7] Compliance with ARV intake must be optimal and therapeutic education is very necessary to ensure the effectiveness of the treatment. Therefore, DRMs can emerge and be transmitted to new people who are not on ART. In addition, these HIV-1 subtypes circulate in the population of PLHIV. They can also be found in the general population, that is, in people who are not infected and who can be infected in the absence of any measures to prevent the transmission of HIV.[8,9]

HIV-1 resistance mutations constitute a barrier that prevents drugs from producing the desired effect for better therapeutic efficacy. Resistance mutations impact drug activity. Resistant strains of HIV-1 are responsible for new infections. In Africa, genotyping is not systematic in newly infected people.[10–12]

It is important to underline that the early detection of DRMs contributes enormously to the surveillance of resistant strains and also to the improvement of treatment. Currently, new HIV infections are no longer caused solely by wild strains but also by resistant strains acquired from PLHIV on ART.[13] This contributes to a significant circulation of resistant strains in the population because resistance is no longer only due to contact between the virus and the ARV, but it can be acquired by transmission of the resistant virus to an uninfected person.[14,15] It then becomes necessary in Gabon to include the search for mutations and drug-resistant subtypes in the therapeutic management of PLHIV. This will allow the control of resistant strains circulating in the population and better care for PLHIV.[16] Volunteer blood donors provide a population base that is young and more likely to have been recently exposed to HIV.[17]

This study aimed to study subtypes and DRMs in HIV-1-positive volunteer blood donors in Gabon.

Materials and Methods

Study design and setting

A cross-sectional study was carried out at the National Blood Transfusion Center (NBTC) of Gabon from June to October 2020. The NBTC is the largest blood bank in Gabon providing its services in terms of labile blood products (LBP) to almost all the health structures in the city of Libreville. The NBTC ensures the application of blood transfusion procedures and the quality of LBPs in the country. The analytical methodology was carried out according to the sample analysis flowchart (Fig. 1). All donors eligible for the study criteria (aged 18–55 and having donated blood only at the NBTC, volunteer donors diagnosed as HIV positive and naive to ART) were included.

Serological analysis

A blood sample was taken in an ethylenediaminetetraacetic tube for serological assay of HIV markers from each volunteer donor. The enzyme-linked immunosorbent assay test was used for the detection of anti-HIV 1/2 antibodies and p24 antigen. The analysis was performed according to the manufacturer's protocol.

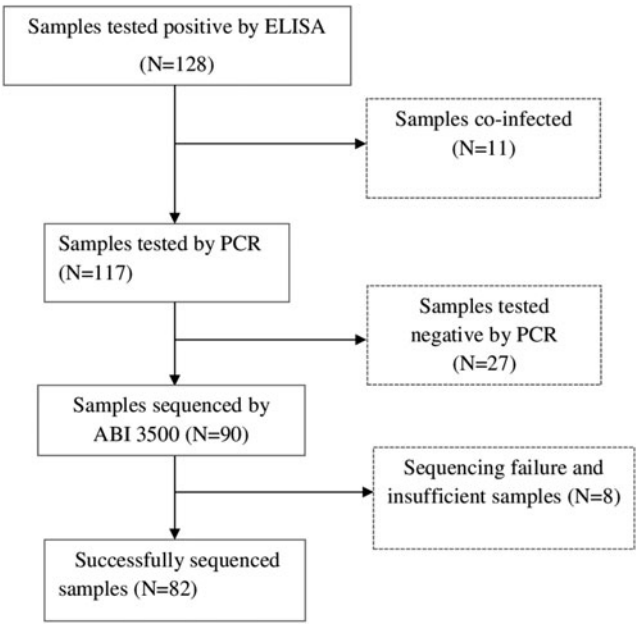

FIG. 1. Flowchart of sample analysis. ELISA, enzyme-linked immunosorbent assay.

Molecular analysis

RNA extraction and viral load quantification by real-time Abbott m2000. The real-time PCR (Abbott) automated system was used for viral RNA extraction. HIV-1 RNA extraction was performed in plasma samples using the Abbott™ Sample Preparation Kit. The preparation of the samples had made it possible to extract, concentrate, and purify the target RNA molecules to make them accessible for amplification. The extract (HIV-1 RNA) was first transcribed into complementary DNA (cDNA) by reverse transcriptase (RT) activity (thermostable rth DNA polymerase) followed by real-time nuclide (cDNA) amplification with detection fluorescent obtained using the Abbott real-time HIV-1 amplification kit. Amplified cDNA strands were quantitated using the stored standard curve, and results were automatically reported on the Abbott m2000rt workstation.

Sequencing. Viral RNA was isolated and purified using the PureLink™ Viral RNA Kit. An real-time polymerase chain reaction (RT-PCR) protocol followed by nested PCR protocols was used to amplify the genes of interest using the AMV RT-PCR Kit 2.0. The amplified nested PCR products were run on an agarose gel and visualized under ultraviolet rays transillumination to determine the correct size products. Amplified products were purified by the ExOSAPiT method and subjected to Sanger sequencing by the Big Dye terminator method in the ABI 3500 Genetic Analyzer. Sequences were analyzed in Seqscape and ReCALL and submitted to Stanford HIV resistance database drugs, REGA and COMET, for analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 21.0 for descriptive data analysis. Descriptive data were presented as frequencies and percentages to express the prevalence

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of resistant strains. Excel software was used to graph the resistance profile of the HIV-1 strains in the study.

Results

Sociodemographic data and frequencies of resistant HIV-1 subtypes in voluntary donors

A total of 128 seropositive volunteer donors were included in the study. The study was composed of 78.9% men and 21.1% women. The most representative age group was that of 29–39 years with 49.2%. Regular donors were more representative with 68% (Table 1). This study allowed the determination of the frequency and the characterization of the strains of HIV-1 circulating in the donors. Eighty-two samples were successfully sequenced. The majority strains identified were subtype A, subtype F, subtype G, CRF02_AG, and CRF45_cpx. The prevalence of drug-resistant strains was 25.6% (21/82) (Table 2).

DRMs observed in RT and protease and resistance profile of HIV-1 subtypes

During the study, 128 seropositive volunteer donors were included. Eighty-two samples were confirmed positive by real-time PCR (Abbott) and were successfully sequenced. DRM types were identified during the study, namely: K103N, E138G, L210W, V179D, V179T, and M46L (Table 2). The algorithm used for the interpretation of HIV-1 resistance mutations was the Stanford HIV database algorithm. The resistance interpretation score according to the Stanford HIV database algorithm ranged from 0 to 70 (Score ≥60: high-level resistance, Score between 20 and 59: intermediate resistance, and Score <20: high sensitivity). The study revealed that the A and CRF02_AG subtypes had a high-level resistance to nevirapine and efavirenz. The A and CRF45_cpx subtypes exhibited low-level resistance to etravirine and rilpivirine. Subtype G also had low-level resistance to protease inhibitors (PIs) (Fig. 2).

Discussion

This study allowed the detection of DRMs and subtypes in apparently healthy volunteer donors who tested positive for HIV-1. The objective of this study was to characterize subtypes and DRMs in HIV-1-positive volunteer donors in Gabon.

Table 1. Sociodemographic Data of Positive Volunteer Donors

Variables	n	%
Sex		
Male	101	78.9
Female	27	21.1
Age (years)		
18–28	45	35.2
29–39	63	49.2
40–50	16	12.5
51–55	4	3.1
Donation status		
Former/regular	87	68
New	41	32
Type of donor		
Unrelated volunteer	128	100

Table 2. Frequency of Resistance Mutations and HIV-1 Subtypes

	DRM		*Resistant strains*	*Nonresistant strains*
Subtypes	*RT*	*PR*	n *(%)*	n *(%)*
All			21 (25.6)	61 (74.4)
A1	*K103N*	—	5 (25)	13 (65)
	E138G	—	2 (10)	
G	—	*M46L*	2 (22.2)	7 (77.8)
F2	—	—	0	5 (100)
CRF45_cpx	*V179D*	—	1 (14.3)	
	V179T	—	1 (14.3)	
CRF02_AG	*L210W*	—	4 (9.8)	
	K103N	—	6 (14.6)	

CRF, circulating recombinant form; *D*, aspartate; DRM, drug resistance mutations; *E*, glutamate; *G*, glycine; *K*, lysine; *L*, leucine; *M*, methionine; *N*, asparagine; PR, protease; RT, reverse transcriptase; *V*, valine; *W*, tryptophan.

Indeed, the study found that men were more representative than women, 78.9% and 21.1%, respectively. The most representative age group was that of 29–39 years with 49.2%. This could be explained by a significant male participation in the blood volunteer donation process in African countries and by a participation in the blood volunteer donation process of younger people. Women are usually disqualified because of the period of menstruation, which often leads to a drop in hemoglobin level.

Studies conducted in some African countries such as Ethiopia, Eritrea, the Democratic Republic of Congo, Malawi, and Nigeria have shown that younger people and men are the major contributors to the blood donation process in Africa.[18–22] The majority of HIV-1 strains identified during the study consisted of the A, G, and F subtypes and the recombinants CRF02_AG and CRF45_cpx. The occurrence frequency of these different strains was highest for CRF02_AG, followed by A subtype, G subtype, and CRF45_cpx. The frequency of circulation of these strains of HIV-1 among blood donors could be justified by the geographical location of the country, which is one of the countries of Central Africa where almost all the different strains of HIV-1 are found. Certain activities such as tourism and travel also contribute to a significant circulation of these strains from one country to another, and from one continent to another.

Some authors have shown that the recombinant forms CRF02_AG and the A subtype were more representative and circulated more in certain countries of sub-Saharan Africa.[3,23–27] The major DRM type identified in the protease coding region consisted of M46L and the major DRM type identified in RT coding region consisted of K103N, L210W, V179T, V179D, and E138G. K103N is a nonpolymorphic mutation that confers significant reductions in susceptibility to non-nucleoside reverse transcriptase inhibitors (NNRTIs) (nevirapine and efavirenz). It is the most commonly transmitted DRM. L210W is a thymine analog mutation that confers reduced resistance to nucleoside reverse transcriptase inhibitors (NRTIs). M46L is a nonpolymorphic mutation selected by PIs. It confers significant reductions in susceptibility to PIs with the exception of darunavir. These resistance

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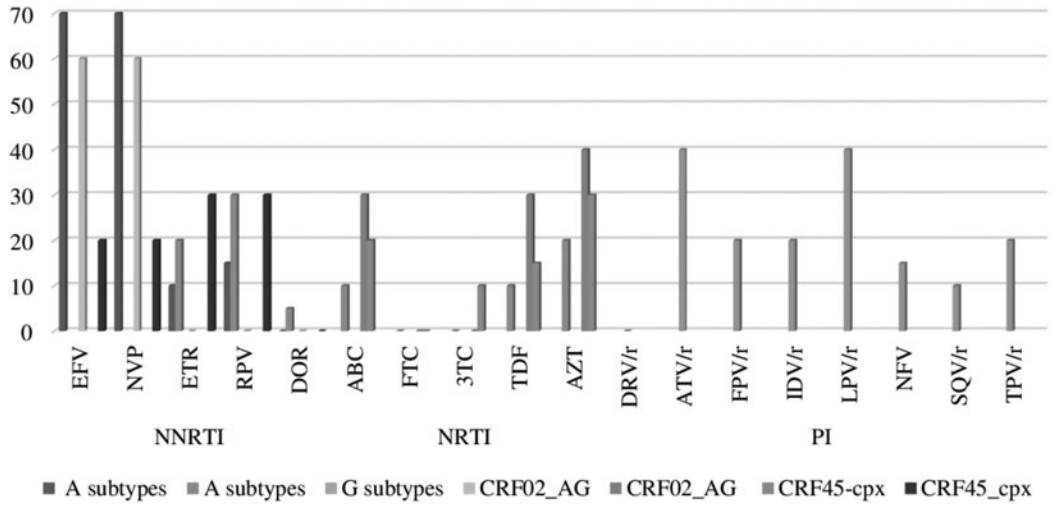

FIG. 2. Resistance profile of HIV-1 subtypes. *x*-Axis: antiretrovirals; *y*-axis: drug resistance mutation score (0–70). Score ≥60: high-level resistance, Score between 20 and 59: intermediate resistance, Score <20: high sensitivity. NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

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mutations negatively impact ARV drugs by reducing or inhibiting the antiviral activity of these drugs.

All these data show that there is circulation of DRMs in the population of PLHIV. In addition, new infections can be caused by strains that are already drug resistant. This means that the spread of resistance is not always directly associated with selective drug pressure. This could be justified by the poor therapeutic compliance of PLHIV in Gabon. It should be noted that the repeated shortages of ARVs observed in recent years in Gabon could also contribute to the emergence of drug-resistant strains among PLHIV under treatment. Failure to comply with preventive measures against the transmission of the virus would also promote significant circulation of mutated strains in the general population and then in donors.

Studies carried out in certain countries have shown that there was a circulation of resistance mutations in the transfusional setting.[2,28–30] The prevalence of drug-resistant strains among donors was high (25.6%) compared with the resistance threshold set by the WHO (10%). These data show a strong circulation of resistant strains in PLHIV of Gabon. This could be explained by the fact of significant circulation of resistant strains in the general population, particularly among infected volunteer donors who are treatment-naive volunteer donors, which means that donors have inherited resistant strains from people under treatment. Other studies have revealed a high prevalence of resistant strains in treatment-naive donors in undeveloped countries.[31–34] The resistance profile of HIV-1 strains in treatment-naive volunteer donors has been characterized by high-level resistance of the CRF02_AG and A1 subtypes to certain NNRTIs, including efavirenz and nevirapine.

However, other CRF02_AG subtypes exhibited low-level resistance to certain NRTIs, including zidovudine, stavudine, and didanosine. In contrast, subtype G showed low-level resistance to almost all PIs except darunavir/r. This could also be justified by the state of poverty of populations with low financial incomes, which does not allow PLHIV under treatment to observe their daily diet imposed by the treatment, and also a supply of drugs that is not regular due to the financial means. Some authors have shown that HIV-1 strains isolated from treatment-naive volunteer donors who have never received ART were resistant to ARV drugs. There were drugs that were ineffective on strains isolated from treatment-naive donors from several blood banks in resource-limited countries.[2,35–38] It then becomes necessary to introduce drug resistance testing into the care of PLHIV in Gabon to optimize the effectiveness of the therapeutic protocol for PLHIV.

Conclusion

This study identified HIV-1 subtypes with a predominance of CRF02_AG and A1 subtypes. However, this frequency of HIV-1 subtypes is also accompanied by a circulation of major DRMs that confer high-level resistance to NNRTIs and low-level resistance to NRTIs and PIs. The study also showed that several strains of HIV-1 were potentially resistant to first-line treatment drugs in sub-Saharan Africa, such as efavirenz and nevirapine. In addition, the prevalence of resistant strains was high compared with the resistance threshold set by the WHO (10%). The detection of HIV-1 DRMs in PLHIV who have never received ARV therapy is of concern. These data should challenge decision makers in the fight against HIV not only in Gabon, but also in countries with limited resources, to introduce HIV drug resistance testing in the care of PLHIV newly diagnosed and PLHIV already on ART.

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Authors' Contributions

C.M. designed the study and wrote the article. J.F. supervised the research work. A.G.W. supervised the administrative procedures. D.M.B. supervised data collection. All the authors reviewed, read, and accepted the final article.

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Ethical Approval and Consent to Participate

The study was approved by the National Ethics Committee for Research (NECR) and the general management of the NBTC of Gabon. The number of the ethical opinion certificate was No. 0087/2019/PR/GS/NECR. The informed consent form was signed by each study participant.

Availability of Data and Material

Data generated and analyzed during the current study are not publicly available due to confidentiality and data protection reasons, but are available from the corresponding author upon reasonable request.

Application of Methods

All methods were carried out in accordance with relevant guidelines and regulations.

Author Disclosure Statement

The authors declare no conflict of interest.

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