Research Square

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Alarming rate of transmitted drug resistance mutations and genetic diversity in newly HIV-1-infected patients in Benin

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Abstract

Background

Seventeen years after the start of the IBAARV (Beninese initiative for access to antiretrovirals), transmitted drug resistance mutations in ARV naïve patients and HIV-1 genetic diversity were investigated in Benin.

Methods

353 plasma samples were collected between October and December 2017 in nineteen facilities care in Benin from HIV-1 positive and ARV naive individuals. Pol (protease + partial RT) region was amplified and sequenced in 248 samples.

Results

Drug resistance mutations were detected in (27/248; 10.9%) according to the WHO SDRM 2009 list, with predominance of mutations directed to NNRTIs drugs (24/248; 10%).

Phylogenetic and recombination analyses showed a predominance of CRF02_AG strains (165/248; 66.5%) and a high genetic diversity with five other variants and 39 URFs (15.7%) which contained portions of strains that co-circulate in Benin. Eight recent transmission chains revealed active ongoing transmission of HIV-1 strains among ARV naïve patients.

Conclusions

Our study showed a high primary drug resistance rate and a complex genetic diversity. Regular monitoring of primary drug resistance is required to adapt HIV-1 treatment strategies and adoption of new WHO recommendations in Benin.

Background

In 2019, about 37.9 million people were living with HIV that remains a major global public health problem [1]. Seven hundred seventy thousand people died from AIDS related illnesses and 1.7 million people became newly infected with HIV despite the expansion of antiretroviral treatment (ART) programs in 2019 [1]. An undesired consequence of antiretroviral therapy expansion is the selection of mutations [2, 3] allowing viruses to become resistant to treatment. Factors that contribute to this occurrence in Sub-Saharan African are inappropriate use of drugs, changing medication frequently, interruptions in treatment due to financial difficulties. Transmission of these resistant viruses to therapy-naïve individuals could jeopardize the clinical benefits associated with ART [4] and effectiveness of first-line ARV treatment. This leads increasing the need of second-line regimens which are available in many Sub-Saharan Africa countries because the cost of second-line antiretroviral therapy limitation strategies have been reviewed [5]. Several studies reported variable rates of transmitted drug resistance ranging between

2% and 10.8%. In West Africa, rates of 8.3% and 10.8% were reported in Niger [6] and Togo [7] while rates of 4.2% and 8.2% were reported in Morocco [8] and Cameroon [9]. The rate of 3.9% was reported in Benin in 2012 [10]. To maximize the long-term effectiveness of first-line ART and ensure the sustainability of ART programs, it is essential to reduce the further spread of HIV drug resistance mutations. Therefore, WHO recommends that HIV treatment scale-up should always be accompanied by a robust assessment of drug resistance emergence and transmission (https://www.who.int/hiv/topics/drugresistance/protocols/en/). One of the five keys of WHO HIV drug resistance monitoring and surveillance strategy is the Surveillance of Transmitted HIVDR in recently infected populations.

Benin is a small country of 11 340 504 habitants located in West Africa. It is bordered by Nigeria in the East, Togo in the West, Niger in the North, Burkina Faso in North West and the Atlantic Ocean in the south. HIV prevalence was 1.2% in the general population in 2019 and 44231 infected individuals were under antiretroviral (ARV) treatment in 2019. In this study we report 7 years after the first study [10] and after 17 years of ARV circulation in Benin, HIV drug-resistance mutations and analyze whether the pattern of HIV-1 variants that circulate in Benin is stable over time in ARV naive HIV-1 infected individuals.

Materials And Method

Study population

A total of 353 samples from antiretroviral-naïve HIV-1 infected individuals were studied. Samples were collected from October 2017 to December 2017 as recommended by WHO on nineteen facilities care covering all the country. The infected individuals were newly diagnosed with HIV-1 infection, and never exposed to antiretroviral therapy.

Blood Sampling and Processing

For all patients, blood samples were collected on EDTA tubes and RNA was extracted from plasma by using the QIAmp Viral RNA kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions.

Viral Load and nested PCR amplification

Plasma HIV-1 RNA viral load (VL) was performed using Cobas® TaqMan® 96/Cobas® Ampliprep® (CAP/CAP-CTM) HIV-1 quantitative assay (Roche Molecular Diagnostics, Basel, Switzerland) according to manufacturer's instructions.

The Nested PCR was performed on the entire protease gene and at least the first 240 amino acids encoding the reverse transcriptase (RT) as previously described [8], generating a fragment of 1017 base pairs (bp). After amplification testing, 248 individuals were eligible for HIV-1 drug resistance genotyping.

HIV drug Resistance Testing

Genotypic resistance testing was performed on plasma samples using the consensus technique of the Agence Nationale de Recherches sur le SIDA et les Hepatites Virales (ANRS, Paris, France), as previously described (http://www.hivfrenchresistance.org, [11, 12]. PCR products were purified (Qiagen) and directly sequenced on AB 3500 Genetic Analyzer using Big Dye Terminator v3.1 (Applied Biosystems, Courtaboeuf, France). The generated sequences were edited online using RECall (beta v3.05) - Web based sequence analysis (https://pssm.cfenet.ubc.ca/account/login).

Phylogenetic Analyses

The newly obtained sequences were aligned with reference sequences representing the overall genetic diversity of HIV-1 in West and Central Africa (available from Los Alamos HIV sequence database: http://hiv-web.lanl.gov/), by using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/) and G-Blocks to eliminate poorly aligned positions and divergent regions (molevol.cmima.csic.es/castresana/Gblocks_server). Phylogenetic tree reconstruction was done by the maximum likelihood method with the GTR+I+G model as implemented in Seaview v4.4.2[13]. In addition, bootscanning analysis was done to explore any eventual mosaic structure for each strain with Simplot software and confirmed by phylogenetic analysis of the corresponding sub-segments in case of mosaic viruses[14]. Finally, recent transmission clusters were ascertained using the statistical robustness of ML topologies based on high bootstrap values (98%) with 1000 resamplings and short branch lengths following criteria previously defined [15].

The newly generated sequences were submitted to Genbank database under accession numbers: MT022598 to MT022684, MT022685 to MT022845 and MT022846 to MT023006.

Analysis of drug-resistance mutations

Newly nucleotide sequences of partial *pol* (protease and/or RT) were translated into amino acids and then inspected to identify mutations associated to drug resistance. Amino acid sequences were analyzed to determine the presence of mutations in *protease* and *rt* genes at positions known to be associated with drug resistance by using the latest (2009) version of the WHO list of mutations for surveillance studies [16].

Results

Characteristics of the 248 Study Patients

The median age was 38 years [IQR: 18-82] with 64.1% women and 35.9% men. Nearly 90% of patients were over 25 years of age and nearly 46% were married and living with a partner. Some patients (n=23) were key populations members, 11 of whom were female sex workers (FSW) (blood samples were collected for 4 FSW) and 12 of whom were men who have sex with men (MSM) (blood samples were collected for 8 MSM). Also some patients (n=20) were coming from neighbouring countries such as Togo, Nigeria, Niger, Côte d'Ivoire, Burkina Faso, Ghana, Guinea, Gabon and Cameroon. The median viral load was $5.12\log_{10}$ [IQR : $2.20\text{-}7\log_{10}$].

HIV drug-resistance mutations in antiretroviral naive patients in Benin

Newly nucleotide sequences translated into amino acid sequences were inspected to identify drug-resistance mutations [16] (http://cpr.stanford.edu/cpr/). Drug resistance mutations were detected in 27 of 248 (10.9%) strains (Table 1).

Mutations associated with NNRTIs, NRTIs, PIs and (NNRTIs + NRTIs) represented 10% (24/248), 6% (16/248), 1% (2/248) and 2% (5/248) respectively.

A total of 42 drug resistance-associated mutations have been identified (Table 1). Two patients harbored each one protease mutation (I85V, n=1) selected by saquinavir (SQV) and (L90M, n=1) selected by indinavir (IDV) nelfinavir (NFV), fosamprenavir (FSP), tripanavir (TPV), saquinavir (SQV), lopinavir (LPV) and atazanavir (ATV). Analysis of the *rt* gene revealed mutations selected by thymidine analogs: (D67N, n=1), (M41L, n=1), (T215S, n=1), (D67G, n=1), (K70R, n=1), (K219Q, n=1). One patient was infected with HIV-1 containing K65R and 19% of naïve patients carried M184V (n=8). The mutations associated with NNRTI resistance were as follow: (V106M, n=1), (Y188L, n=2), (P225H, n=1), (V106A, n=1), (Y181C, n= 2), (G190A, n= 3), (K103N, n=14). Four patients harboured multi-resistant virus whose one with 7 mutations (D67G, K70R, V106M, Y181C, M184V, G190A and K219Q), the second with 4 mutations (K65R, K103N, M184I, and Y188L), the third with three mutations (M184V, V106A, G190A) and the last one with two mutations (Y181C, M184V).

The distribution of 248 HIV-1 group M variants in Benin

The phylogenetic tree analysis of the 248 *pol* sequences is presented in figure 1.

CRF02_AG predominated and represented (165/248, 66.5%) of the strains, followed in decreased order by CRF06_cpx (19/248, 7.7%), G (16/248, 6.5%), sub-subtype A3 (6/248, 2.4%), A (1/248, 0.4%), F (1/248, 0.4%). In addition, (39/248, 15.7%) samples were unique recombinants (URFs), composed with strains that predominate or co-circulate in Benin: CRF02_AG, CRF06_cpx, G and A3.

Identification of transmission clusters

A maximum likelihood phylogenetic tree with 1000 bootstraps was inferred among thirty sample sequences suspected to share links. Phylogenetic analysis evidenced eight recent transmission chains based on high bootstrap values (98%) with 1000 resamplings and very short branch lengths (<0.015). Three other older transmission clusters were supposed with branch lengths values 0.016 and 0.018 (figure 1).

Discussion

The study reports the presence of mutations in ARV-naive patients and genetic diversity of HIV-1 variants that circulate in Benin. Globally, the prevalence of transmitted drug resistance was 10,89% after 17 years of ARV circulation in the city, that is consistent with results from other African countries in which prevalence rates are also higher than 10% [7, 17-19]. But, studies from some others Sub-Saharan countries have reported rates lower than 10%, by using the same WHO standard protocol and the WHO list of resistance mutations for epidemiological surveys [20, 21]. HIV-1 drug resistance mutations were known to be one of the major factors limiting the effectiveness of ARVs. In our study, the mutations encountered were those associated with the ARVs used in first-line treatment since the start of the IBAARV (Beninese initiative for access to antiretroviral) in 2002. This first-line treatment used two NRTIs (AZT/D4T + 3TC) plus a NNRTI (NVP/EFV), NNRTI as well as non-boosted protease inhibitor (indinavir) regimens [22]. At that time, virologic monitoring was not available and patients were followed based on clinical signs and CD4 counts [23]. Moreover those who were in therapeutic failure stayed long periods of time with an ineffective treatment, leading to an accumulation of resistance mutations [24]. This accumulation of resistance mutations may compromise the effectiveness of second line drugs [25] and increases the risk of transmission of drug resistant strains to naïve patients [7]. Among naïve patients in our study, 27 already harboured at least one drug resistance mutations and the NNRTIs represent 10% while the NRTIs and PIs represent 6% and 1% respectively.

NRTIs resistance-associated mutations were present in sixteen patients. M184V was the predominant NRTI encountered which confers resistance to lamivudine (3TC). The K65R confers resistance to abacavir (ABC) and tenofovir (TDF). Thymidine-associated mutations (TAMs) were found but only one patient in our study harboured at least two TAMs conferring intermediate resistance to zidovudine (AZT). TAMs M41L, T215S have been described in Togo [7, 19] and also in Burkina Faso with D67N and K219Q [19]. K70R was observed in one case in our study was also found in Guinea-Conakry [21]. For non-adherence reasons to treatment, M184IV is quickly selected in patients under 3TC which explains its presence in high proportion in studies of transmitted resistance [20, 21]. Since the study was conducted after the time of TDF use (replacing D4T as recommended by WHO), the presence of K65R in patients could easily be explained.

The major mutations associated to NNRTIs were K103N (33%) encountered in fourteen patients which compromise effectiveness of NNRTIs first generation (NVP and EFV) and G190A (7%) identified in three patients which compromise nevirapine, efavirenz and etravirine [26]. These mutations associated with high-level resistance have been described in one and four patients in Togo and Conakry [7, 21]

respectively in naïve patients. The others mutations excepting Y181C and Y188L detected in two cases were V106A, P225H, and V106M, each detected in one case.

Mutations V106A, Y188L, V106M are associated with high-level resistance to NVP and EFV while Y188L and both Y188L and V106A are associated with high-level resistance to rilpivirine (RPV) and to third NNRTI generation doravirine (DOR) respectively. The Y188L and both V106M and P225H are also associated with intermediate resistance to ETR and DOR while only P225H is associated with intermediate resistance to both NVP and EFV.

Two people exhibited each one mutations associated to PIs (I85V, n=1) and (L90M, n=1). In our study, none patient harboured both mutations which conferred intermediate resistance to atazanavir.

Globally, the presence of these mutations could be explained by the large use of Triomune which contains stavudine, lamivudine and nevirapine, and the use of Efavirenz, zidovudine and Indinavir in first-line therapy in Benin.

Phylogenetic analysis evidenced eight recent and three probable older transmission chains (6.5-8.9% of the study patients), reflecting active ongoing transmission. Interestingly, 8 patients reported as being MSM and from them, two were involved into the same transmission chain, in which one patient came from Togo and the second one was from Benin. Studies on MSM in Africa also evidenced behavioral links with heterosexual networks, in such a way that the MSM population could serve as a bridge for intermixing of HIV-1 variants

between low-risk women and high-risk men. This situation was described in Dakar where the subtype C predominating in the MSM group is increasing in the general population [27, 28], confirming the existence of a dual epidemic in the country. In our study we did not find any transmission chain involving both population groups individuals, however the number of MSM patients is too low to conclude. Obviously further studies are needed in key groups in order to assess whether HIV-1 strains from MSM intermix with those from the general population in Benin or with those from other countries.

In our study, the CRF02_AG predominated with 66.5% rate. Overall, the genetic diversity in Benin matches with results found in a neighboring country, Togo [7]. The other strains (CRF06_cpx, subtype G and sub-subtype A3) have been also reported in other neighbouring African countries [6, 20, 29]. Regarding the unique recombinants (URFs), their proportion among different regions have changed over time [30]. The rate observed in our study (15.7%, 39/248) is fully concordant with the proportion from West Africa [30] and is not significantly different from that was observed in Togo (22.9%, 19/83) ($p = 0.27$) [7]. These URFs being composed of predominant or co-circulating strains, demonstrate the existence of frequent dual infections with at least two strains of HIV-1 in the country.

Conclusion

The study documents the high prevalence of transmitted drug resistance of HIV-1 in Benin, and active ongoing transmission of HIV-1 strains circulating usually in West Africa. Therefore, there is a need to regularly monitor primary drug resistance through other studies to better adapt HIV-1 treatment strategies in Benin. Getting data on HIV-1 resistance will be the root for the adoption of new WHO recommendations.

Abbreviations

IBAARV: Beninese initiative for access to antiretrovirals

ARV: Antiretroviral

Pol: Polymerase

RT: Reverse transcriptase

WHO: World Health Organization

Pb: Base pairs

CD4: Cluster of differentiation 4

SDRM: Surveillance Drug Resistance Mutation

URF: Unique recombinant form

CRF: Circulating Recombinant forms

ART: Antiretroviral Treatment

HIV: Human Immunodeficience Viruse

HIVDR: Human Immunodeficience Viruse Drug Resistance

EDTA: Ethylenediaminetetraacetic

RNA: Ribonucleic Acid

TAMs: Thymidine-associated mutations

VL: Viral Load

ANRS: National Agency for Research on AIDS and Viral Hepatitis

MAFFT: Multiple sequence alignment based on fast Fourier transform

PCR: Polymerase Chain Reaction

IQR: Interquartile

NNRTI: Non Nucleoside reverse transcriptase inhibitor

NRTI: Nucleoside reverse transcriptase inhibitor

PI: Protease Inhibitor

MSM: Men having sex with Men

FSW: Female sex worker

AZT: Zidovudine

SQV: Saquinavir

IDV: Indinavir

NFV: Nelfinavir

FSP: Fosamprenavir

TPV: Tripanavir

LPV: lopinavir

ATV: atazanavir

RPV: Rilpivirine

DOR: Doravirine

ABC: Abacavir

TDF: Tenofovir

EFV: Efavirenz

NVP: Névirapine

ETR: Etravirine

A: Alanine

C: Cystéine

D: Acide aspartique

E: Acide glutamique

F: Phénylalanine

G: Glycine

H: Histidine

I: Isoleucine

K: Lysine

L: Leucine

M: Méthionine

N: Asparagine

P: Proline

Q: Glutamine

R: Arginine

S: Sérine

T: Thréonine

V: Valine

W: Tryptophane

Y: Tyrosine

Declarations

Ethical considerations

This study was approved by the Research Ethics Committee for Applied Biomedical Sciences (CER-ISBA): number 33 of august 09, 2017 and authorizing the implementation of research.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Sekpe Olga and Dagba Gbessin Hermione: Performed the viral load assays

Edmond Tchiakpe: Performed the viral load assays, genotypic drug resistance testing and interpretation, and drafted the manuscript

Vidal Nicole: Wrote the manuscript and ensured the quality control of the sequences

Harrigan Richard: Contributed of editing sequences online of RECall (beta v3.05) - Web based sequence analysis (https://pssm.cfenet.ubc.ca/account/login) software

Ahoussinou Clément: Statistical Analysis

Keke Kpemahouton Rene, Gbaguidi Eric, Tonoukouen Conrad, Bachabi Moussa, Gangbo Flore Armande: Conception of study, participated in its design and coordination

Afangnihoun Aldric: enrolled patients and collected of samples

Diop-Ndiaye Halimatou and Toure-Kane Coumba: Wrote the manuscript.

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Table

Table 1: Drug-Resistance Associated Mutations in Antiretroviral (ARV) Naïve Patients.

Samples ID	Drug Resistance Mutations Associated			HIV-1 Subtypes
	PI*	NRTI*	NNRTI*	
Ben148			K103N	URF (CRF02_AG/A3)
Ben 784			K103N	CRF02_AG
Ben 538		M184V		G
Ben 169			G190A	URF (CRF06_cpx/A)
Ben 18	I85V			CRF02_AG
Ben 226			K103N	A3
Ben 230			K103N	CRF02_AG
Ben 232		M184V		URF (CRF02_AG/A1)
Ben 255			K103N	CRF02_AG
Ben 722		D67N	K103N	CRF02_AG
Ben 607		M184V	V106A, G190A	G
Ben 96	L90M			CRF02_AG
Ben 98			K103N	CRF06_cpx
Ben 687		M41L	K103N	URF (CRF06_cpx/CRF02_AG)
Ben 420		M184V		URF (CRF06_cpx/CRF02_AG/A3)
Ben 643			Y188L	CRF02_AG
Ben 657		T215S		CRF02_AG
Ben 206			K103N, P225H	CRF02_AG
Ben 208		M184V		CRF02_AG
Ben 211			K103N	CRF02_AG
Ben 212		K65R, M184I	K103N, Y188L	CRF02_AG
Ben 213		M184V	Y181C	G
Ben 214			K103N	CRF02_AG
Ben 216		M184V		URF (CRF02_AG/U)
Ben 218			K103N	A3
Ben 390			K103N	CRF02_AG
Ben 101		D67G, K70R, M184V K219Q	V106M, Y181C, G190A	G

NRTI, Nucleoside reverse transcriptase inhibitors; NNRTI, Nonnucleoside reverse transcriptase inhibitors; PI, Protease inhibitors.

*According to the WHO algorithm for Surveillance of Drug Resistance Mutations (SDRM), 2009

URF : Unique Recombinant forms

Ben : Benin

Figures

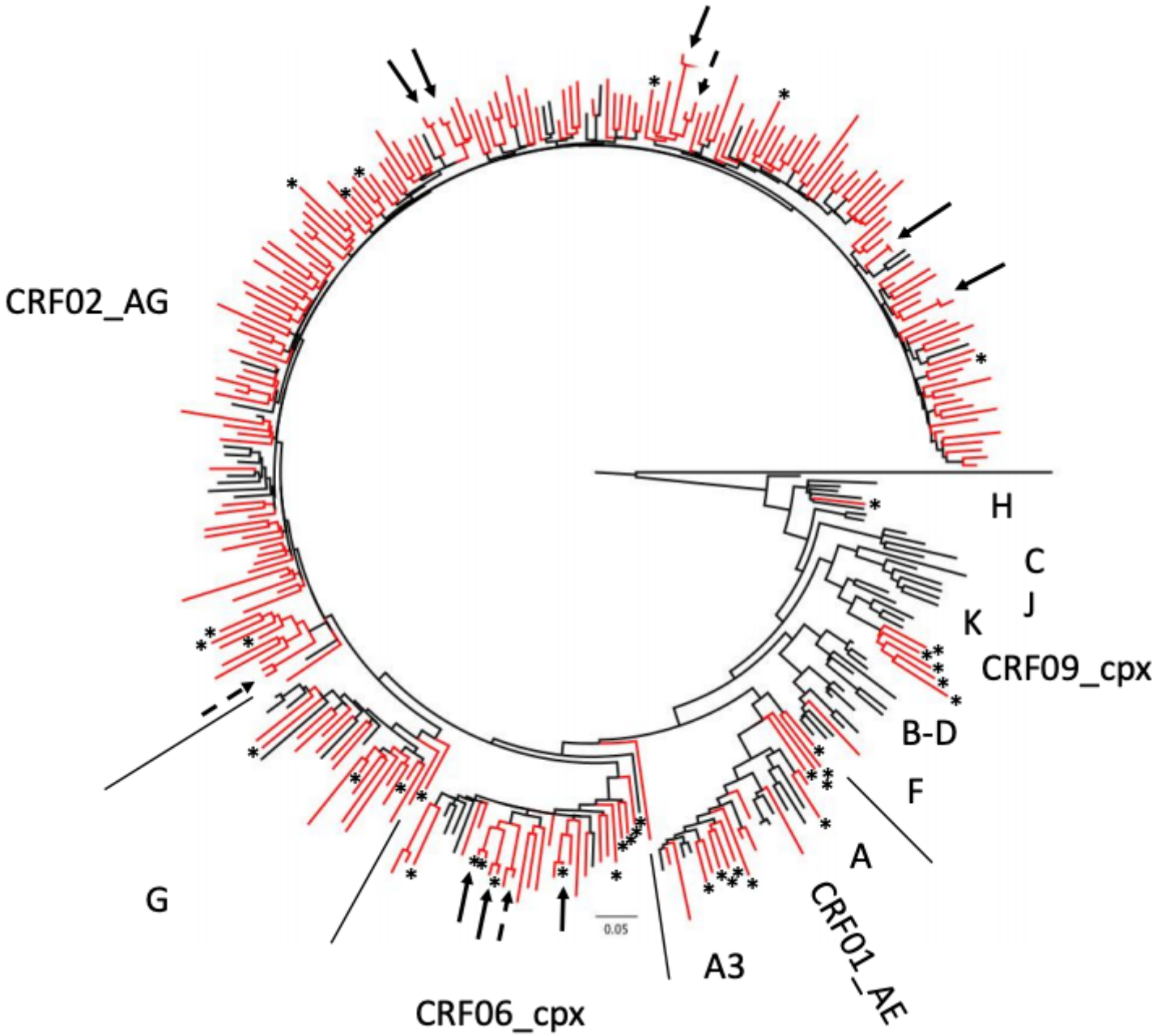

Figure 1

Maximum likelihood tree for 248 HIV-1-infected drug-naïve patients in Benin. The length of the alignment was 656 unambiguously aligned nucleotides in the reverse transcriptase region of the pol gene. Circulating recombinant forms (CRFs) and sub-subtypes not represented within the sequences genomic structure were excluded. Reference sequences are in black and patient sequences are in red. Unique

recombinants over protease and RT are shown with an asterisk. An arrow indicates the sample sequences involved into recent transmission chains (n=8), older supposed transmission chains are indicated with a broken arrow (n=3).