

Survey of HIV-1 Drug Resistance Mutations in Recently Infected, Antiretroviral Naïve Patients from Sub Saharan Africa and South East of Asia

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ABSTRACT

Background

Universal access to antiretroviral therapy in developing countries has raised concerns that HIV drug resistance could develop and spread quickly in recently infected people. We evaluated the frequency of transmitted HIV drug resistance (HIVDR) in 3 South-east Asian (Cambodia, Thailand and Vietnam) and 2 African (Burkina Faso, Cameroon) countries.

Methods

The target population, in which a relatively high proportion of recently infected persons are likely to appear, was adapted to the characteristics of the HIV epidemic in each country. As recommended by the generic protocol for HIV-1 drug resistance developed by WHO-HIVRESNET, pregnant women aged between 15 and 24 years at first pregnancy were enrolled in Cameroon. In Burkina Faso and Thailand, pregnant women were also included but, in addition to age and number of pregnancies, CD4 count > 500/mm³ was added as marker for recent infection. In Cambodia and Vietnam, individuals attending Voluntary Counseling and Testing (VCT) centers were included based on age and CD4 counts. Using binomial sequential sampling, up to 47 consecutively collected eligible specimens are needed to classify presence of drug resistant strains (for each drug or drug class) as <5%, 5-15%, and > 15%. HIV-1 genotypic resistance tests were performed on plasma samples and phylogenetic analysis was performed based on protease and RT sequences. Resistance associated mutations were interpreted with the 2007 Stanford HIVdb algorithm, IAS USA list and ANRS algorithm.

Results

Overall, 280 drug naïve HIV infected patients were included in the 5 sites. Four individuals (1.4%; 95% CI [0.6-3.6]) harbored virus with drug resistance mutations, 1 in Cameroon (K103N), 1 in Cambodia (K101KQ+K103N+M184V) and 2 in Vietnam (G190A and M46I). Phylogenetic analysis revealed that CRF01_AE predominates (>90%) in Cambodia, Thailand and Vietnam. CRF02 (>60%) co-circulates with many other HIV-1 variants in Cameroon and with CRF06 in Burkina Faso.

Conclusions

Prevalence of HIV-1 drug resistance mutations among recently infected individuals was low in our study. However, the upper limit of the confidence intervals suggests that population size could be increased for future surveys. Population survey for antiretroviral drug resistance will provide important public data in resource-limited countries

PARTICIPATING COUNTRIES, PATIENTS AND METHODS

Five countries (2 in Africa, Burkina Faso and Cameroon) and 3 in South East of Asia (Cambodia, Thailand and Vietnam) participated to the study (Fig 1).

In Burkina Faso, Cameroon and Thailand, drug naïve pregnant women were included. In Cameroon, strict criteria of WHO HIVDR-TS generic method requiring that women should be in their first pregnancy and age between 15 and 24 were followed. In Burkina Faso, CD4 count greater to 500 cells was used as a criterion of recent infection. In Cambodia and Vietnam, drug naïve recently infected, based on CD4 and/or the questionnaire, adults were included. At baseline, clinical data including age, history of antiretroviral treatment (ART) were recorded. CD4 cells counts were determined by flow cytometry and plasma viral load quantified by standard or in house methods.

Genotypic drug resistance study was performed on viral RNA extracted from plasma by using in house methods. The whole protease gene and at least the first 250 codons of the RT gene were sequenced.

Presence of drug resistance was inferred from the latest versions of ANRS (October 2007), Stanford HIVdb, REGAV7.1.1 algorithms, together with the IAS USA (October 2007) list. Numeric data were statistically compared by Kruskal-Wallis non parametric test. Dunn's multiple comparison test was performed when the overall p-value by Kruskal-Wallis was less than .05.



Figure 1: location of the 5 participating countries (Burkina Faso and Cameroon in Africa; Cambodia, Thailand and Vietnam in South-east of Asia).

RESULTS

1.1. Study population

Country	n	Female	Male
Burkina Faso	51	51	0
Cambodia	58	46	12
Cameroon	52	52	0
Thailand	56	56	0
Vietnam	63	34	29

1.2. Study Population: socio anthropologic data

Figure 2: Age at inclusion (years)

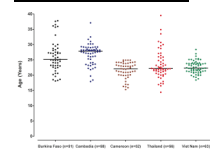
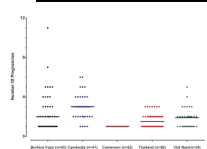


Figure 3: Number of Pregnancies



Legend to figures 2 and 3: age and number of pregnancies were recorded on a questionnaire following the obtention of an informed consent to participate to the study. The figures represents age (figure 2) and number of pregnancies, including the index case (figure 3). Each dot represents one individual. The horizontal bar crossing each column of dots represents the median value.

Table 2: Awareness of HIV Status (months)

Country	n	Median	IQR*
Burkina Faso	50	2.3	1.2-4.0
Cambodia	55	13.0	7.6-33.2
Cameroon	52	ND	ND
Thailand	56	0.9	0.0-2.2
Vietnam	62	1.8	0.1-5.8

*: Inter Quartile Range

1.3. Study population: clinical Data

Figure 4: CD4 counts at inclusion

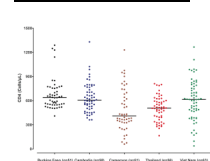
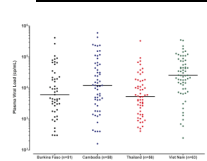


Figure 5: Plasma viral load at inclusion



Legend to Figure 4: CD4 T-lymphocytes cells were quantified by flow cytometry on blood samples collected before delivery. Each dot represents one individual. The horizontal bars represent median value of CD4 for the considered series.

Legend to Figure 5: Plasma viral load was quantified with a generic ANRS Real Time PCR method. Each dot represents one individual and the horizontal bars are the median values of each series of data.

2. Genetic Diversity of the sequenced viral isolates

Figure 6A: Genetic diversity in South East Asia

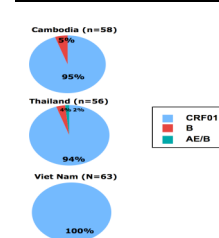
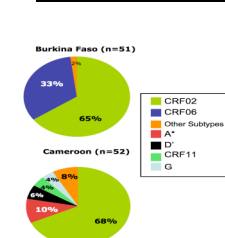


Figure 6B: Genetic diversity in Africa



Legend to Figures 6A & 6B: Viral subtypes were determined by phylogenetic analysis of reverse transcriptase sequences by using subtypes reference alignments from Los Alamos databank, together with our own sequences. Sequences alignment was performed through CLUSTAL2.0. Phylogenies were constructed by NJ. If necessary, a SimPlot was performed to identified recombinant strains.

3. HIV drug resistance mutations in the studied population

Table 3: Prevalence of HIV-1 drug resistance mutations

Country	Number of Sequences (RT and PR)	Number of samples with resistant viruses (%)	95% Confidence Interval
Burkina Faso	51	0 (0)	0.0-7.0
Cambodia	58	1 (1.7)	0.3-9.2
Cameroon	52	1 (1.9)	0.3-10.2
Thailand	56	0 (0)	0.0-6.4
Vietnam	63	2 (3.2)	0.9-10.9

Table 3: Characteristics of drug resistant samples

Sample ID	Country	Gender	Age	CD4 (cells/μL)	VL (Log cp/mL)	HIV-1 subtype	Scored mutations
KC12134-12 (RT)	Cambodia	F	28	568	2.6	AE	K101KQ, K103N, M184V
CYN0632 (RT)	Cameroon	F	20	525	ND	CRF02	K103N
R26 (RT)	Vietnam	F	24	868	3.82	AE	G190A
R40 (PR)	Vietnam	F	22	981	5.35	AE	M46I

DISCUSSION

The aim of this study was to evaluate the prevalence of HIV-1 drug resistance mutations (DRM) in ARV naïve, recently infected patients from Africa and South East of Asia. We have shown here that HIV-1 DRM is low: 4 samples out of 280 (1.4%; 95% CI 0.6-3.6) analyzed bore mutations conferring resistance to one or more drugs. This percentage is consistent with what is expected in countries where ARV are relatively recently introduced.

For such surveys, pregnant women aged between 15-24 years in their first pregnancies is the population of choice. Hence, the generic WHO HIVDR monitoring recommends this sentinel population for the threshold survey method. In the present study, we were able to include this specific population only in Yaoundé, Cameroon by screening up to 2,400 pregnant women in 7 PMTCT centres of this Capital City. In Bobo-Dioulasso, Burkina Faso, where HIV prevalence is lower than in Yaoundé (3.2% vs 9%), up to 19,000 pregnant women were screened over a period of 12 months, to finally include 51 patients recently infected but with variable number of pregnancies and sometimes aged more than 24.

The awareness of HIV status was recent and was shorter than a year for all the sites, except Cambodia where it was 13 months. The different parameters (age, CD4, viral load, number of pregnancies) were distributed significantly different between the 5 countries (p<10⁻⁴ by Kruskal-Wallis non parametric test).

As expected, the genetic diversity of viral isolates from Cameroon was high, although CRF02_AG predominates up to 68% of all the sequences. In Burkina Faso, two subtypes (CRF02 and CRF06) represents 98% of the viral isolates sequenced. Unsurprisingly, in South East Asia, CRF01_AE was predominant, representing up to 100% of sequences from Vietnam. It should be mentioned the presence of CRF01/B recombinant in Thailand, that was different from CRF15 as recombination occurred in the Pol gene, on the contrary to CRF15 which recombined in the Env gene. This recombinant strain is close to CRF33 described in Malaysia.

Drug resistance mutations analysis revealed an overall prevalence of less than 5%, which is consistent with these countries where universal access to therapy is recent. Three patients (1 from Cambodia, 1 from Cameroon and 1 from Vietnam) bore resistant viruses that were very likely transmitted from treated patients. The sample KC12134-12 from Cambodia displayed 2 major resistance mutations conferring resistance to NVP and EFV (K103N) and TIC and FIC (M184V) according to the IAS USA list, French ANRS, Stanford HIVdb and REGAV7.1.1 algorithms. This patient was very likely contaminated by her husband, an IVDU used to travel throughout the country and who died of an undisclosed disease. The sample CYN0632 presented the K103N mutation conferring resistance to EFV and NVP according to all the algorithms used. K103N also confers resistance to DLV according to Stanford HIVdb and Regav7.1.1. The sample R26 from Vietnam presented the G190A mutation that confers resistance to NVP according to all the four interpretation algorithms list. In addition, this mutation confers resistance EFV according to ANRS and IAS USA. Finally, one sample from Vietnam presented the M46I mutation in the protease gene conferring resistance to IDV or IDV boosted *rtv* according to ANRS and IAS USA, respectively, and intermediate resistance to ATV and NPV according to Stanford HIVdb. This M46I is a thought to be a polymorphism of some HIV-1 non-B subtypes.

Other mutations were observed in our samples. In Cameroon, one sample presented the ANRS mutation classified as related to NVP resistance in subtype C HIV-1 by ANRS algorithm, but not by the others. It should be mentioned that this resistance was observed in HIV-1 subtype C in India. Likewise, another sample from Cameroon presented the D67DN mutation considered as a TAM by IAS USA list.

In conclusion, the present multicenter study from 5 countries in Africa and South East Asia shows that the prevalence of HIV-1 drug resistance mutations in untreated, recently infected adults is low and consistent with ARV history of these countries.

Acknowledgements

This study was supported by the French ANRS grant to the ANRS Resistance Study Group.