High Prevalence of HIV-1 Subtypes B and C Circulating Recombinant Forms (CRF_BC) in Pretoria, South Africa: A Comparison with other African Countries

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ABSTRACT

Background: HIV-1 is a widespread virus affecting more than 30 million people worldwide, mostly located in sub-Saharan Africa. It is of clinical importance to be able to classify HIV-1 into subtypes because not only is it helpful when designing anti-retroviral treatments (ARTs) but also in studying the geographical spread of different strains of the virus. This study sets out to evaluate the frequency of certain subtypes in different parts of Africa, specifically in South Africa, Cameroon, and Guinea Bissau.

Methods: A total of 1297 samples were collected from patients infected with HIV-1. The viral RNA extraction was performed using plasma specimens on the Abbott m2000 platform. Subtypes were determined by amplification of the *env* gp41 immunodominant region (IDR) and *pol* integrase (*pol* IN) region with specific primers for each of the regions. The HIV-1 sequences from both regions were then blasted against available sequence databases. To further explore the prevalence of CRF_BC strains in South Africa, sequences with this subtype collected in Pretoria and the closest viral sequences obtained from the NCBI-NIH database were aligned by Clustal W along with sequences from China and Myanmar. Neighbor-joining trees were then constructed using the Jukes-Cantor method to compute evolutionary distances.

Results: Similar patterns were observed in Cameroon and Guinea Bissau with subtype CRF02_AG being the most predominant with a prevalence of 73.35% and 68.59% respectively, followed by subtype A with a prevalence of 4.52% and 26.92% respectively. In contrast, samples from South Africa showed the most common subtype to be circulating forms of BC strains with an occurrence of 84.53%. Sequences from South Africa were further compared using phylogenetic trees. This analysis showed a separation with a strong bootstrap between CRF_BC strains found in South Africa and subtype C and CRF_BC strains from Myanmar and China. Furthermore, the recombinant form B/C is more closely related to sequences classified as subtype C rather than those classified as subtype B. For the *pol* IN region, the tree illustrates that recombinant B/C strains show more homology to subtypes C found particularly in the KwaZulu-Natal province in South Africa.

Conclusions: Subtype C is the most prevalent subtype commonly found in South Africa, however an increase of CRF_BC has been observed in the last five years. In agreement with previous studies carried out in South Africa, the crossover between subtypes B and C are yielding unique CRF_BC strains that are unlike the ones found in China and Myanmar. The increase of these recombinant forms could be a consequence of the rising tourism and migration to Africa. However, further studies within this particular region of South Africa could reveal the factors involved in the sudden increase of BC recombinant strains in HIV-1 infected people.

METHODS & PROCEDURES

Extraction

RNA was extracted using the Abbott Molecular m2000 sample preparation instrument

PCR

- *env* gp41 IDR: cDNA and PCR primers: JH35F and JH38R [1,6]
- *pol* IN region
  - ❖ cDNA and PCR primers: Poli5_OF and Poli8_OR [6]
  - ❖Nested PCR and sequencing primers: Poli6_IF and Poli7_IR [6]

Gel Electrophoresis

The PCR products (DNA) were visualized and quantified in 1.2% agarose gel using a Bio-Rad electrophoresis chamber

Sequencing

- The PCR products were sent to Macrogen Corp and to Roswell Park Cancer Institute for purification and sequencing.
- *Env* gp41 and *pol* IN sequences were blasted against the NIH and Los Alamos databases. Additionally, *pol* IN sequences were blasted against the Stanford University HIV Drug resistance database and the Geno2pheno resistance database .

METHODS & PROCEDURES CONT.

Following the classification of the specimens into subtypes, they were further categorized based on the country of origin of the sample. These results are shown in Figures 1, 2, and 3 below. Furthermore, neighbor-joining trees were drawn by MEGA software [5] using sequences from databases that were the most closely related as well as sequences from China and Myanmar. The tree for *pol* IN sequences can be found in Figure 4.

RESULTS

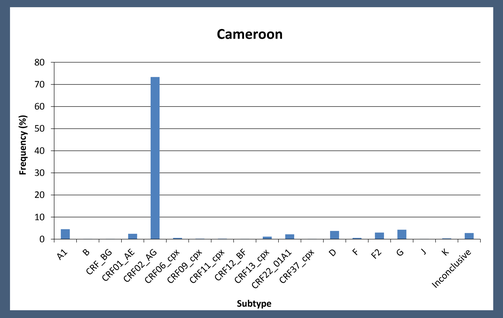

Figure 1. Bar graph representing the frequency of each subtype from the 863 samples collected in Cameroon.

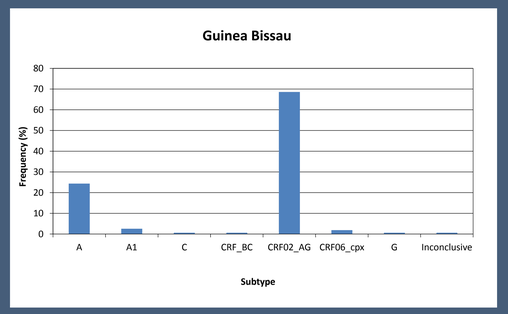

Figure 2. Bar graph representing the frequency of each subtype from the 156 samples collected in Guinea Bissau.

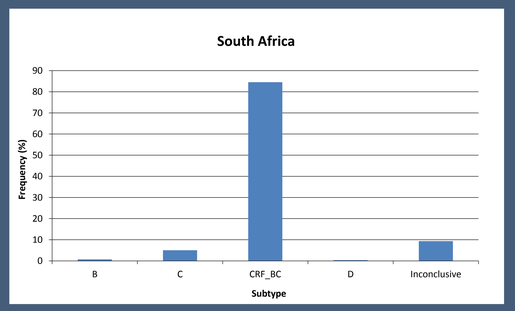

Figure 3. Bar graph representing the frequency of each subtype from the 278 samples collected in South Africa.

As can be observed from Figures 1 and 2, specimens from Cameroon and Guinea Bissau follow similar patterns. In both cases the most predominant subtype is CRF02_AG with a prevalence of 73.35% in Cameroon and 68.59% in Guinea Bissau, followed by subtype A with a prevalence of 4.52% and 26.92% respectively. In contrast, as can be seen in Figure 3, the most common subtype in specimens from South Africa are circulating forms of BC strains with an occurrence of 84.53%.

RESULTS CONT.

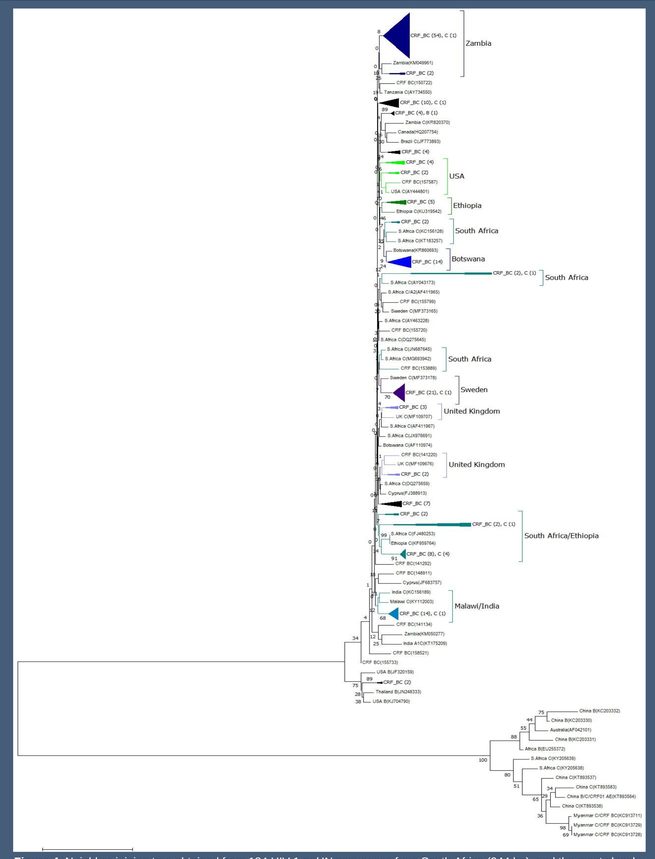

Figure 4. Neighbor-joining tree obtained from 184 HIV-1 *pol* IN sequences from South Africa (944 bp), and the most closely related sequences available on the NIH database. The numbers at each node represent the percent bootstrap support for 1,000 replicates. The evolutionary distances were computed using the Jukes-Cantor method [4] and are in the units of the number of base substitutions per site. Samples are identified by numbers next to each branch. Bold triangles represent closely related sequences that have been grouped together.

Figure 4 clearly shows a strong separation between CRF_BC strains found in South Africa and subtype C and CRF_BC strains from Myanmar and China. However as can be seen in some of the highlighted groups, some sequences from South Africa were associated more closely to sequences found in other countries. Furthermore, specimens classified as B/C recombinant forms are more closely related to sequences classified as subtype C rather than those classified as subtype B, which can be seen in the grouped sequences.

CONCLUSIONS

In agreement with previous studies carried out in South Africa, the crossover between subtypes B and C are yielding unique CRF_BC strains that are unlike the ones found in China and Myanmar, and which have been on the rise for the last five years. The increase of these unique recombinant forms could be a consequence of the rising tourism and migration to Africa [3], as well as due to the circulation and introduction of varying subtype C strains [2]. Further studies within this particular region of South Africa could reveal the factors involved in the sudden increase of BC recombinant strains found in HIV-1 infected people.

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