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Antiretroviral Drug Resistance Surveillance among Treatment-Naive Human Immunodeficiency Virus Type 1-Infected Individuals in Angola: Evidence for Low Level of Transmitted Drug Resistance▽†

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The prevalence of transmitted human immunodeficiency virus type 1 drug resistance in Angola in 2001 in 196 untreated patients was investigated. All subtypes were detected, along with unclassifiable and complex recombinant strains. Numerous new polymorphisms were identified in the reverse transcriptase and protease. Two (1.6%) unrelated patients harbored nucleoside reverse transcriptase inhibitor- and nonnucleoside reverse transcriptase inhibitor-resistant viruses (mutations: M41L, D67N, M184V, L210W, T215Y or T215F, and K103N). Continued surveillance of drug resistance is required for maximization of ART efficacy in Angola.

The AIDS epidemic in Angola, a South-Western African country, is caused by human immunodeficiency virus type 1 (HIV-1) strains exhibiting high levels of genetic diversity (2, 3). By the end of 2007, the HIV/AIDS prevalence in the adult population was 2.1% (1.7% to 2.5%) (21).

Antiretroviral therapy (ART) has been available in Angola since 1996 for those who could buy antiretroviral drugs in the black market or abroad. In January 2008, the estimated number of people on ART in Angola was 11,240, 25% of the estimated number of adults in need of treatment (21). According to a recent retrospective study, the most frequently used triple therapy regimens include lamivudine-zidovudine-nevirapine and lamivudine-stavudine-nevirapine (8). In this study, drug resistance mutations, mostly M184V and K103N, were detected in 78% of the patients undergoing therapy for ≥6 months. This high rate of resistance suggests that some of the patients might have initially been infected with drug-resistant strains. However, there are no data regarding transmitted drug resistance in Angola. Hence, this work was set up to investigate, for the first time, the prevalence of transmitted HIV-1 drug resistance in several provinces of Angola.

Blood samples were collected in 2001 from 196 HIV-positive, drug-naive subjects living in Benguela (4 patients; 2%), Cabinda (26 patients; 13.3%), Cuanza Norte (1 patient; 0.5%), Cuanza Sul (1 patient; 0.5%), Luanda (150 patients; 76.5%), Lunda Norte (4 patients; 2%), Malange (2 patients; 1%), Uíge (1 patient; 0.5%), and Zaire (3 patients; 1.5%). The main criterion for patient inclusion in the study was no known exposure to antiretroviral drugs. The epidemiological, clinical, and virological characterizations of the patients are given in Table 1. The study was reviewed and approved by the Ethics Committees of the participating institutions.

Plasma viral load was determined with the Abbott RealTime HIV-1 assay (Abbott Laboratories) (11). *Pol* (protease [PR] and/or reverse transcriptase [RT]) gene sequences were obtained using an in-house method described elsewhere (3). DNA sequences were obtained with a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) and a model 3100 Avant genetic analyzer (Applied Biosystems). These sequences have previously been assigned GenBank accession numbers EU031840 to EU031891 and EU068199 to EU068462 (3). Viruses were genotyped using phylogenetic analyses, as described previously (3). Resistance mutation analysis was performed using the Stanford genotypic resistance interpretation algorithm (http://hivdb.stanford.edu/pages/asi/). Mutations specifically associated with transmitted HIV drug resistance were selected from two recently published lists (17, 18).

The amplification and sequencing of the PR and/or RT regions were completed successfully for 152 (77.6%) patients (Table 1). Phylogenetic analysis revealed that 89 (58.6%) viral isolates had concordant subtype classification in the PR and RT regions; 61 (40.1%) isolates were recombinants composed of different subtypes in these two regions, and 2 (1.3%) isolates were unclassifiable (Table 1). The following pure subtypes and sub-subtypes were identified: A1 (13 isolates; 8.6%), A2 (9 isolates; 5.9%), A3 (1 isolate; 0.6%), C (19 isolates; 12.5%), D (8 isolates; 5.3%), F1 (15 isolates; 9.9%), G (10 isolates; 6.6%), H (9 isolates; 5.9%) and J (5 isolates; 3.3%). The most prevalent recombinant strains were CRF02_AG (5 patients; 3.3%), G/H (4 patients; 2.6%), and U/H (4 patients; 2.6%).

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TABLE 1. Comparison of demographic, immunologic, and virologic characteristics between HIV infected patients analyzed in this study

Variable[a]	Value for sample group			P^b
	Total	Unsequenced	Sequenced	
No. of patients (%)	196	44 (22.4)	152 (77.6)	
Mean age (yr) (SD)	32 (10.9)	27 (9.7)	34 (10.8)	**0.0005****
No. of patients by gender (%)				
Male	77 (39.3)	22 (50)	58 (38)	0.2926#
Female	104 (53.1)	21 (48)	83 (55)	
Unknown	15 (7.6)	1 (2)	11 (7)	
No. of patients by transmission route (%)				
Sexual	142 (72.4)	30 (68.2)	112 (73.7)	0.5656#
Heterosexual	135 (68.9)	29 (65.9)	106 (69.7)	0.7120#
MSM	1 (0.5)		1 (0.7)	
Bisexual	6 (3)	1 (2.3)	5 (3.3)	1.0000#
Parenteral	7 (3.5)	5 (11.4)	2 (1.4)	**0.0069**#
Blood transfusion	4 (2)	3 (6.8)	1 (0.7)	**0.0360**#
IDU	3 (1.5)	2 (4.6)	1 (0.7)	0.1271#
Vertical	8 (4.1)	4 (9)	4 (2.6)	0.0773#
Unknown	39 (20)	5 (11.4)	34 (22.3)	0.1345#
CDC clinical stage				
A	38 (19.4)	9 (20.5)	29 (19.1)	0.8307#
B	69 (35.2)	18 (41)	51 (33.6)	0.3758#
C	42 (21.4)	12 (27.1)	30 (19.7)	0.3005#
Unknown	47 (24)	5 (11.4)	42 (27.6)	**0.0276**#
Mean log no. of HIV RNA copies/ml (SD)	5.4 (0.59)	5.5 (0.49)	5.4 (0.67)	0.8268#
No. of isolates (%)				
Pure subtype			89 (58.6)	
Recombinant*			61 (40.1)	
Untypeable			2 (1.3%)	

[a] MSM, men who have sex with men; IDU, intravenous drug user; *, different subtype classifications in PR and RT.
[b] P values are based on comparison of unsequenced and sequenced samples. Bold indicates statistical significance ($P < 0.05$). **, Mann-Whitney U test; #, Fisher's exact test.

Plasma viral load could be determined for all 35 specimens that were tested. Sequences were obtained for 21 (60%) of these patients, and these sequences belonged to the following subtypes: A1 (one isolate), A2 (one isolate), C (two isolates), D (one isolate), F1 (five isolates), G (one isolate), H (four isolates), J (one isolate), A1/J (one isolate), A1/CRF02_AG (one isolate), G/A1 (one isolate), G/H (one isolate), and CRF05_DF/D (one isolate). These results provide definitive evidence that the Abbott RealTime HIV-1 assay is particularly well suited for viral load quantification in countries with highly complex and divergent HIV strains (11). Consistent with the lack of ART, viral load was high in most patients (mean, 5.4 log copies/ml; standard deviation [SD], 0.59). There was a positive correlation between age of patient and viral load (Spearman $r = 0.4543$; $P = 0.0386$).

The minor protease inhibitor (PI) resistance mutations L10I, L10V, V11I, and T74P were detected in some Angolan isolates (see Table S2 in the supplemental material). L10I and L10V, associated with resistance to most of the PIs when present with other mutations (4, 16), were found in 17.7% of the isolates. This is higher than the frequencies previously described for untreated patients (5 to 10%) (http://hivdb.stanford.edu/pages/asi/). V11I, associated with resistance to darunavir (7, 16), was detected in 4.3% of the Angolan patients, all associated with subtype A. This frequency is significantly higher than that found in sequences of the same subtype available in the Stanford database. T74P was found in one patient (0.7%). T74P is one of 11 darunavir minor resistance mutations (7) and was considered a major tipranavir resistance mutation in the RESIST study (4). These results suggest that some Angolan isolates might have a low genetic barrier for resistance to some PIs.

Two out of 122 (1.6%) patients harbored nucleoside RT inhibitor- and nonnucleoside RT inhibitor-resistant viruses. The nucleoside RT inhibitor resistance mutations M41L, D67N, M184V, L210W, and T215Y were detected in one patient (01AOHDC232; subtype G); T215F was detected in patient 01AOHAB83 (F1/C recombinant). The former isolate also carried the K103N mutation, which confers resistance to nevirapine and efavirenz. The origins of these isolates were Luanda (01AOHAB83) and Cabinda (01AOHDC232). These mutations are among the most common transmitted drug resistance mutations reported worldwide (9, 13, 17–19). These two isolates should not be fully sensitive to the standard first-line antiretroviral regimens currently used in Angola and most other resource-limited settings (8, 10, 23). According to the WHO criteria (5), Angola has a low prevalence of transmitted resistance that is similar to the levels for other Sub-Saharan African countries where ART is still not widely available (1, 6, 9, 12, 15, 20, 22). Nonetheless, the finding of transmitted HIV-1 drug resistance in Angola was unexpected due to the restricted availability of antiretroviral drugs until 2001. The two most likely explanations for the origin of the resistant isolates are (i) the unregulated and unmonitored use of antiretroviral drugs bought in the black market or abroad (14) and (ii) displacements of people from countries where ART is available for a longer period of time (22). In this context, it is not surprising that transmitted HIV-1 resistance was first de-

tected in Cabinda and Luanda, since the oil business as always attracted an important number of migrant workers to Cabinda and escape from the civil war, ending in 2002, has brought many war refugees to Luanda.

The level of genetic diversity among the Angolan isolates was significantly higher than that among the isolates (of the same or different subtypes) present in the Stanford Database. For instance, the frequencies of the PR polymorphisms found in Angolan isolates of subtypes A (80% of polymorphisms), C (23%), D (50%), F (61.5%), G (12.5%), and CRF02_AG (10%) were significantly different from those found in isolates from worldwide treatment-naive patients infected with the same subtypes (see Table S2 in the supplemental material) (http://hivdb.stanford.edu/pages/asi/). Likewise, the frequencies of the RT polymorphisms found in subtypes A (46%), C (31%), D (14%), F (34.8%), G (34.6%), and CRF02_AG (37.5%) were significantly different from those found in isolates from worldwide treatment-naive patients infected with the same subtypes (see Table S3 in the supplemental material).

Finally, we found some new polymorphisms in Angolan viruses that have not previously been described in the Stanford database for untreated patients. In the PR, this is the case for N37I in subtype F, T74P in subtype G, and H69A in CRF02_AG (see Table S2 in the supplemental material). In the RT, V35Q, Q174V, V245G (subtype A), D121F (subtype D), and A272S (subtype F) are all new polymorphisms (see Table S3 in the supplemental material). Elucidation of the extent to which these polymorphisms may affect ART therapy requires further studies.

In conclusion, our results show an unprecedented level of genetic diversity in the PR and RT proteins of HIV-1 isolates circulating in Angola. Future studies are needed to assess the impact of this diversity on ART and resistance development. Our results also indicate that drug-resistant HIV-1 strains were already being transmitted in 2001 in Angola. These results are particularly important in view of the recent increase in ART programs in Angola (21). Overall, the low prevalence of transmitted HIV drug resistance found here indicates that simplified first-line regimens can be successfully used in the vast majority of HIV-1 patients from Angola. Continued surveillance of drug resistance in treated and untreated populations will be important for maximizing the efficacy of ART in Angola.

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