

Sequence Note

First Observation of HIV Type 1 Drug Resistance Mutations in Algeria

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Abstract

This study demonstrates for the first time HIV-1 resistance mutations to all classes of antiretroviral drugs available in Algeria (NRTIs, NNRTIs, PIs) in treated patients at failure. Moreover, it is shown that mutations to NRTIs and PIs can be observed in untreated patients in this country where there is high HIV-1 diversity.

Introduction

ACCORDING TO THE ALGERIAN AUTHORITIES, 3747 people were living with HIV in Algeria in 2007.¹ Unprotected sex is the main mode of HIV transmission. Working in the sex industry and injecting drug abuse are significant factors of risk and are contributing to the induction and the expansion of the infection among the population, particularly in the southern part of the country where the prevalence of HIV among pregnant women can reach 1% in some antenatal clinics.

HIV/AIDS care was launched in 1996 with the opening of six care centers through the infectious diseases facilities located in the hospitals of the cities of Algiers, Oran, Setif, Constantine, Annaba, and Tamanrasset.

Antiretroviral drugs (ARVs) were introduced in 1998; since that time patients have been treated for free. Available drugs are four nucleoside reverse transcriptase inhibitors (NRTIs) (AZT, 3TC, d4T, and ddI), two nonnucleoside reverse transcriptase inhibitors (NNRTIs) (EFV and NVP starting in 2007), and three protease inhibitors (PIs) (IDV, RTV, and LPV/RTV). Recommendations have been elaborated and distributed through a national guide of care. First-line therapy generally includes two NRTIs plus one unboosted PI (IDV until 2007).

In a previous work, focusing on the molecular characterization of HIV-1 in Algeria,² we have shown that the pattern of subtypes and circulating recombinant forms (CRFs) is

complicated, particularly in the southern part of the country.

In the present work, we have summarized the sequence data of reverse transcriptase (RT) and protease (Prot) from untreated and treated patients with the aim of identifying drug resistance mutations in this population infected with highly diverse viruses.

Materials and Methods

We have studied 218 samples collected between 2001 and 2007 from either untreated or antiretroviral (ARV)-treated patients under clinical failure of therapy.

Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and plasma was separated and stored at -80°C . The samples were thawed and RNA extraction was carried out using a QIAamp Viral RNA Mini Kit (Qiagen). The viral RNA was used in reverse transcription polymerase chain reaction (RT-PCR) of RT and Prot genes, using two sets of primers in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) thermal cyclor. The outer and inner primers for RT are described in a previous publication.² The fragments obtained were sequenced on both strands using the CEQ DTCS Quick Start kit on an automated sequencer Beckman CEQ 2000 DNA Analyzer System as previously described. The derived nucleotide sequences of the RT and Prot regions were aligned by the Clustal W 1.74 alignment program with known reference strains of M and N pooled from

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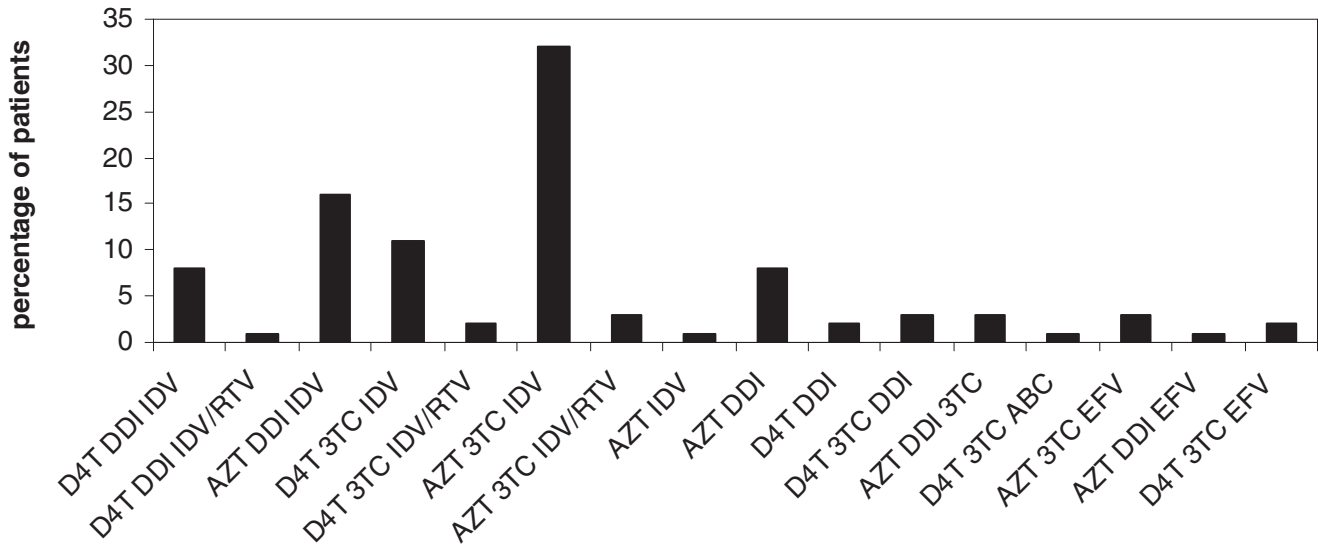


FIG. 1. Different combinations of antiretroviral drugs in patients treated in the study.

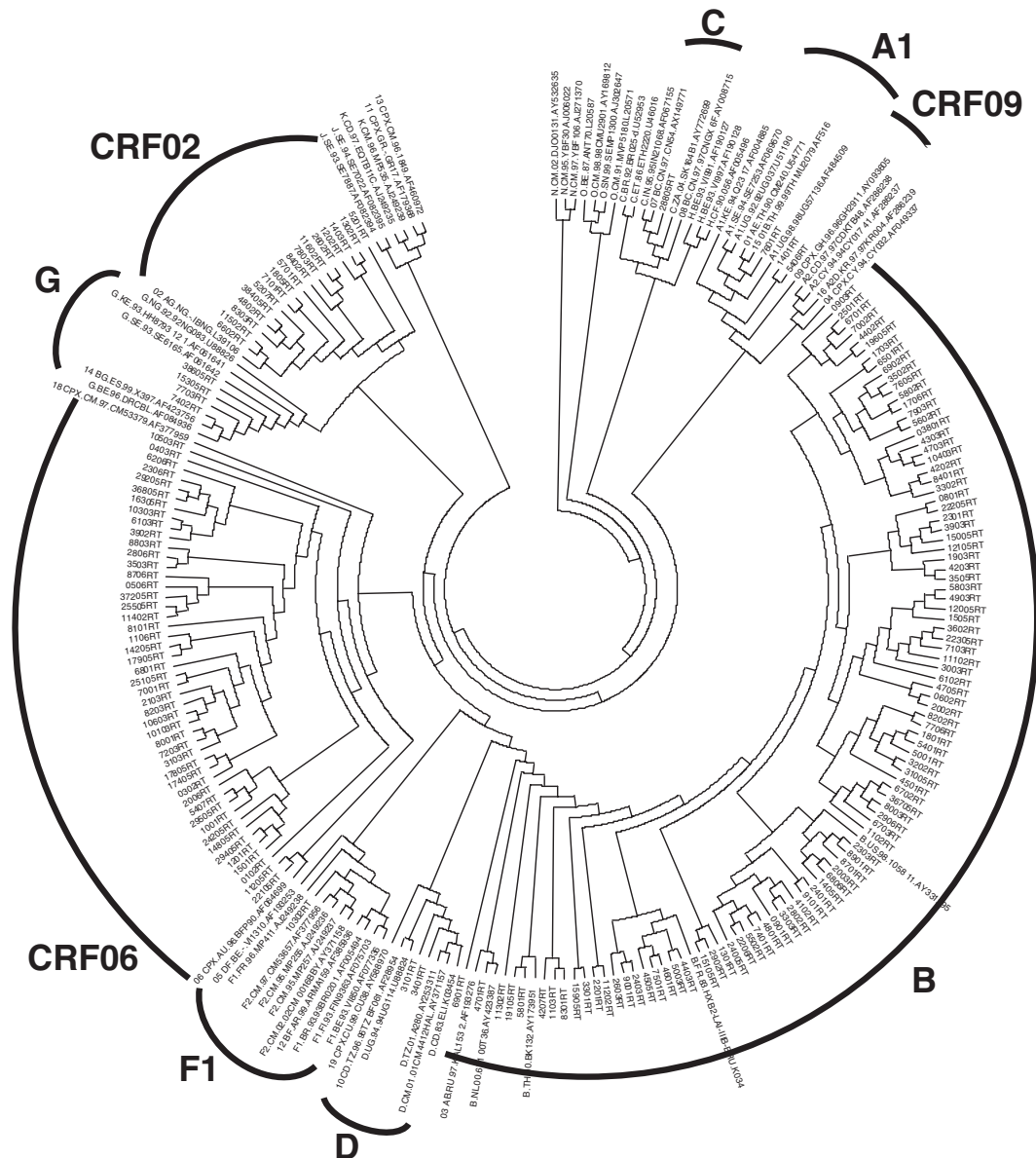


FIG. 2. Phylogenetic tree of reverse transcriptase (RT) sequences from the Algerian HIV-1 isolates. Neighbor-joining analysis used Clustal W and Mega4 software.

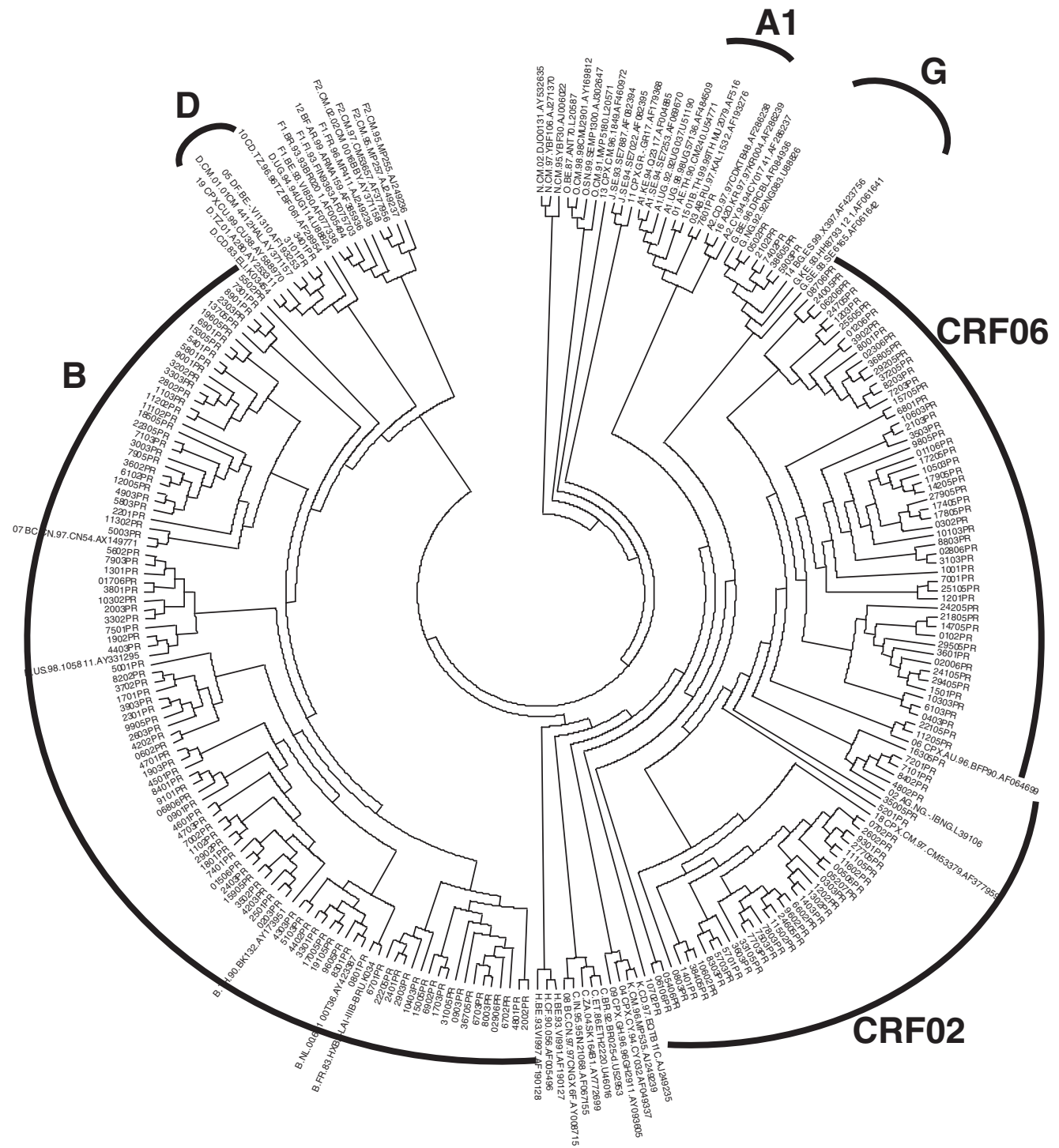


FIG. 3. Phylogenetic tree of protease (Prot) sequences from the Algerian HIV-1 isolates. Neighbor-joining analysis used Clustal W and Mega4 software.

the HIV-1 gene databank (<http://hiv-web-lanl.gov/>). Phylogenetic trees were inferred using the neighbor-joining method from matrix distances calculated after gapstipping of alignments, with a Kimura two-parameter algorithm. The mutations involved in antiretroviral resistance were recorded according to the algorithm of the Agence Nationale de Recherche sur le SIDA (ANRS/France) (<http://hivfrenchresistance.org>, October 2007). Other drug resis-

tance databases and organizations are <http://hivdb.stanford.edu/>, [http://www.iasusa.org/resistance mutations/index.html](http://www.iasusa.org/resistance%20mutations/index.html), [http://www.iasusa.org/resistance mutations/mutations figures.pdf](http://www.iasusa.org/resistance%20mutations/mutations%20figures.pdf), and [http://resdb.lanl.gov/Resist DB/default.htm](http://resdb.lanl.gov/Resist%20DB/default.htm). GenBank accession numbers for the sequences reported in this study are AY828587 to AY828703 and EU743685 to EU743739 for RT plus AY828704 to AY828838 and EU743623 to EU743684 for Prot.

TABLE 1. AMINO ACID POLYMORPHISMS AND RESISTANCE MUTATIONS (ACCORDING TO THE FRENCH ANRS ALGORITHM) IN RT GENE^a

		AA positions																			
		V 21	E28	K 30	K 32	V 35	E36	T 39	M41	E44	K49	V 60	K 65	D 67	T 69	K70	L74	R83	V90	A98	L100
<i>Polymorphisms of strains Subtypes</i>																					
From untreated patients	49	B	2I	1D	1E	1R	1I11T1M1AIT	1D	1A	1K	6R	8I	6R	8I	1S	1N	16K				
	33	06	23I1D1L	9D	T3	21R	30T1Q1P3D1K4A7K1S	4A7K1S	1S	4R	33I	33I	4R	33I	1N1S				1I		
	11	02		2E		10T1A	6D			3R	7I	4I	7I	4I	1I						
	4	G				1I	4T	1A	2K	2R	2R	4I	2R	4I							
	1	C					1T		1D		1I	1I		1I							
	1	09	1I		1E			1A				1I		1I							
From treated patients	49	B	1I	1V1K1E1N	1N	8I1L1LV2M	1K1D	1A	7L2D1V7R1N10I	7L2D1V7R1N10I	7R1N	10I	4N1G	2N	2R	1I1R	11K				
	13	06	1I1D1F	1D	1E	10R1I	8T2P2K1Q	1K	1L	1L	13I	13I	1N								
	7	02				5T1K	4D	2M1K1I			3I	3I									
	2	D			1E		2T				1R	2I	1N								
	1	F					1T	1L	S				1N								
	1	A	I	E	E		T					1I	1N								
From untreated patients	49	B	30K1P1Q	D123	I 135	I142	Q 151	S162	K173	Q174	D177	I178	M184	Y188	G196	T200	Q207				
	33	06	27K	2E1N	18VWT9R1MR2L	3T2V		32A	10I5M1MV1IM10T2R	24K2R2E1EK1IK	33E	19M	19M	1N	1E	30A1I	18E4D2K				
	11	02	7K	1E1N	7V1L			11A	7T3A1IL	10K1KN	11E	2M				8A1V	6E1D1DE				
	4	G				1T		1C	4T	1N1R1K	4E	2M				4A	3E1T				
	1	C							1E	1E	1E					1A	1A				
	1	09			1V				1R	1E	1E					1A	1N				
From treated patients	49	B	33K2P	231N	13T4V1R	3V2T		6C2Y	2E1T	3K1H1R	8E	3L6M	12V	1L	12E1K	11A2I	7E2K1H				
	13	06	5K1G	2N1E	10V1T1R	1G1M		11A	4I3T1IT1M1R	10K1KN	13E	6M2L	1I	1H	3E	1N1R	1MST				
	7	02	4K	1E1G	5V1T			7A	5T1A1N	6K1G	7E	3M	1V		7A	6E1K					
	2	D						2A	1R		2E	1M	1V		1E	1E1K					
	1	F	1K		1V	1V		1C	1I	1K	1E	1L				1E					
	1	A		1S	1T				1S	1K	1E				1E	1A					

			R 211	G213	L214	T215	K219	P225	M230	V245
From untreated patients	49	B	12K 1Q 1G	2E	32F	1F 1Y	1E	1R	1I	5E 2M 2Q
	33	06	28K 1IK 1E 1KQ		31F		1N			1D 1S 17Q 2H
	11	02	4K 1KN		11F	1D				8Q 2K
	4	G	3K		4F					1Q
	1	09	1K		1F					
From treated patients	49	B	20K 2Q		41F	6Y 4F	3E 2Q			10E 2M 2K
	13	06	9K		13F	11FS				1N
	7	02	3K		5F	1N				5Q 1H
	2	D	1K		2F	1Y				2Q
	1	F	1K		1F					1H
1	A			1F						1Q

^aThe gray columns concern positions of resistance mutations; the amino acids associated with resistance are in bold characters.

TABLE 2. AMINO ACID POLYMORPHISMS AND RESISTANCE MUTATIONS (ACCORDING TO THE FRENCH ANRS ALGORITHM) IN PROT GENE^a

		AA positions																	
		Number of strains		Subtypes															
		L10	V11	T12	I13	K14	I15	G16	L19	K20	L24	V32	L33	E35	M36	N37	R41	K43	M46
From untreated patients	65	B	5V 4I 1F	4A 7P 2S 2N 1E 1EQ 1S 1A	22V	20R	14V	6E	13I 1V 1T 1IT 3P	3I 2R 1M 23I	1V 1F	15D	11I	14S 2E 3D 1T 1DGS 1S 1D 1T	21K 1R				
	23	02	1I	1S 1A	21V 2A	19R	2V	8E	3P	23I	10D	23I	23I	1S 1D 1T	23K 1R				
	45	06	4I 2V	26S 5A 1P	43V 1A 1AV	19R 1T 1L 1G 1S	27V 5L 11F	16E	85 7I 5P 1S 1Y	45I	14D 2Q	45I	45I	3D 2T 3S	41K 2R				
	1 1 4	A D G			4V	3R		1E		4I				1I 1D 2Q 2D	1K 1S 1D				
From treated patients	34	B	7I 1IV	2S 1S 1E 1A	7V	4R	3V	1R 1E	5I 1T 1Q 1MV	1R 1M 1I	1V	7D 1G	5I	3S 3D 2A 2E 1P 1C	3K 2R			2I 1L	
	12	06	3V 1I	5S 2K 1A	12V	5R 1E	6V 2L	7E	1M 1P 1T 1I 1S	12I	3D	12I	12I	1T 1D	10K 2R				
	8 1 1 1	02 A D G	1I		8V 1V 1V 1V	5R	1L 1V	2E	2P	8I			4D	8I 1I	7K 1K 1K 1K			1I	
	1	G		1I	1V	1V	1V	1V	1I	1I			1I	1I	1I				
From untreated patients	65	B	7V 1M	32P 7S 3T 1Q 1AT 3PS 1ST 1PT	13V 2L 1M	1E	6K 3Y 1R 1Q 1H	6T 4V	12T 2E 5V 1MT 1EKU	18I 1I	1Y	1V 1N	184	1V 1N	6M 1M			4L 1M 1LV	
	23 45	02 02	4P 2S 1A 4S 3P 2V	3M 1L 19M 1V 1Q	2Y 1H 3K 1Q	22K 3E 7R	1V	2S	1V 1S	1I		1K 15 20M 2I 41M	1N	1K 15 20M 2I 41M	2I 1L				
	1 1 4	A D G	1V	1P 1P	1V 4E	1K 4K	1V	1S	1V 1S	1I		1I	1I	1I	4M			1M	
	34	B	7V	20P 3S 1AS 1PS 1T 1H	11V	1S 1D 1Y	4K 4N 3V 2T	3V 2T 1M 1T	3V 2T 1M 1T	3F 12I 2A	1V 2V	1S	1V 2V	1V	1S 4M 1M			9L	
From treated patients	12	06	2S 1Q 1P 1I 1AFSV 1A 1P	4M 1L 2L 1M	11K 1KQ 8K 1K 1G	1V	1V	1L 1M 1V	1I 1M 1V	1I 1M	1A	1I	1I	12M 7M 1I 1M					
	8 1 1 1	02 A D G			1V 1M	1V 1M	1V	1V	1V	1V					1M				

^aThe gray columns concern positions of resistance mutations; the amino acids associated with resistance are in bold characters.

Results

From the 218 samples studied, 172 RT and 193 Prot sequences were recorded. From the total RT sequences, 99 were issued from untreated patients and 73 from treated patients. From total Prot sequences, 136 were issued from untreated patients and 57 from treated patients. Drug associations are described in Fig. 1. The main association was AZT/3TC/IDV. The molecular characterization of the viruses, based on genomic sequence analysis, is presented in Figs. 2 and 3 for RT and Prot, respectively. The predominant subtype and/or CRFs were B, CRF06_cpx, and CRF02_AG; other non-B strains were G, D, A, F, C, and CRF09_cpx.

The amino acid substitutions and resistance mutations in RT and Prot are presented in Tables 1 and 2, respectively. The results are discussed below for both untreated patients and treated patients.

Untreated patients

RT drug resistance mutations M184V, T215Y/F, and K219E to NRTIs have been observed in isolates of subtype B. No resistance mutation to NNRTIs could be noted. Other substitutions are related to polymorphism and have been described elsewhere.²

In the Prot region, within a background of polymorphism, particularly at positions of secondary mutations, one major resistance mutation, L90M, was noted in a subtype B isolate.

Treated patients

M184V was the most prominent mutation and has been observed in subtype B, CRF06_cpx, and D viruses. TAMs (M41L, D67N, K70R, L210W, T215/Y/F/S/N, and K219Q/E) have been noted individually or associated in B, D, F, A, and CRF06_cpx viruses. Mutations to NNRTIs were rare; only K101E and Y188L/H could be recorded in some isolates of subtype B. In Prot sequences, some major resistance mutations were observed: M46I/L (three isolates of B subtype, one A), V82A (two B, one A), and L90M (one B).

Discussion

These results confirm our previous publication on the high diversity of HIV-1 in Algeria, the B subtype being predom-

inant followed mainly by CRF06_cpx and CRF02_AG. Drug resistance mutations have been observed in untreated patients and although this study had not been designed using WHO criteria, we can consider that transmission of viruses bearing resistance mutations to NRTIs and PIs occurs in this population; we could not observe any resistance mutation to NNRTIs and this observation can be related to the recent introduction of this class of drugs in Algeria.

Resistance mutations against NRTIs (M184V and TAMs), NNRTIs (K101E), and PIs (M46I/L, V82A, and L90M) have been observed in treated patients under failure.

This is the first observation of HIV-1 resistance mutations in Algeria; it will be important to collect more extensive data on resistance mutations in HIV-1 isolates from patients under HAART and experiencing clinical/immunological/virological failure, particularly in the context of high variability. On the other hand, it seems useful to launch surveys of resistance among untreated patients according to the WHO guidelines, particularly in pregnant women attending antenatal clinics.

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Disclosure Statement

No competing financial interests exist.

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