



HIV Diversity and Drug Resistance in Western Kenya from Plasma and non-Plasma Genotyping

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I. Background and Objectives

HIV diversity and drug resistance are unknown in western Kenya. With increasing antiretroviral global access, resistance monitoring in diverse subtypes is essential, yet limited by infrastructure and finances.

We examined HIV diversity and drug resistance in western Kenya using plasma and non-plasma analytes as simple, lower-cost monitoring methods.

II. Methods

In 2001, in response to a significant increase in HIV prevalence and mortality at Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya, the Academic Model Providing Access to Healthcare (AMPATH) was created as a joint initiative between Moi University, MTRH, Indiana University and Brown University.

The AMPATH catchment area includes parts of Nyanza Province - population of 4.39 million (15% of Kenya's population), North Rift Valley Province - population 3.6 million (12% of Western Province - population of 3.35 million (12%). This translates to 40% of the total population of Kenya (Figure 1).

As of November 2008, AMPATH has provided comprehensive clinical services to 80,964 HIV-infected patients. Of those, 76,030 patients are actively in care, and 33,561 are receiving antiretroviral therapy.

The MTRH clinic has enrolled 20,329 adults (>18 years), 44% (9,37) of whom started antiretroviral therapy. Patients are managed according to locally developed protocols based on WHO guidelines.

In this study, adult patients attending the MTRH clinic had viral load testing and *pol* sequencing (plasma and non-plasma analytes) if they were (i) antiretroviral treatment naive, or (ii) antiretroviral treatment experienced and (i) treated for more than 6 months with WHO-recommended first-line antiretroviral therapy, including AZT or D4T + 3TC + nevirapine or efavirenz; (ii) adherent to their antiretroviral therapy; and (iii) defined as failing therapy based on a CD4 count drop >25% over 6 months.

Dried analytes were stored on-site and shipped at room temperature.

Sequence interpretation was done with hivdb.stanford.edu tools.

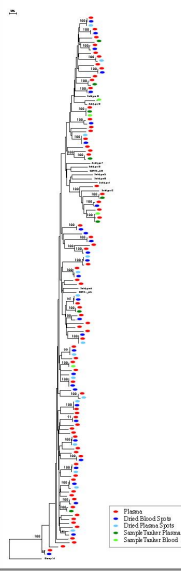
Figure 1: AMPATH Geographic Scenario



Table 1: Patient Demographic, Clinical and Laboratory Data

	Naive	Treated
# Participants	48	28
% Female	60%	68%
Age median (range)	34 (19-60)	39 (20-57)
CD4 median (range)	409 (19-1384)	170 (11-547)
VL	56,830	22,110
Median (range)	(1,813-1,940,000)	(6,015-461,600)
Progression (%)		
STC D4T Efav	25%	
STC D4T NVP	71%	
STC AZT NVP	4%	
Months on treatment Median (range)	27 (12-62)	
# sequences by analyte		
Overall	61	47
Plasma	46	23
DBS	14	16
DPS	9	6
STP	4	2
Months from sample to sequence Median (range)		
Overall	28 (6-33)	14 (5-22)
Plasma	30 (20-33)	19 (11-24)
DBS	14 (12-15)	5 (3-8)
DPS	14 (13-30)	5 (4-22)
STP	8 (7-9)	-
STP	8 (6-9)	12 (11-14)
Months from sample to shipping Median (range)		
Overall	21 (2-26)	7 (1-12)
Plasma	22 (21-24)	12 (10-16)
DBS	3 (1-4)	2 (1-2)
DPS	3 (2-4)	2 (1-2)
STP	4 (2-4)	2 (1-2)

Figure 2: Phylogenetic Tree of *pol* Sequences



III. Results

Patients (Table 1):

We analyzed 128 sequences from 76 patients, 48 naive and 28 treated. Patient demographic, clinical and laboratory data are demonstrated in Table 1. Drug exposure (Table 1): The majority of treated patients received 3TC, D4T, NVP, the most common first-line antiretroviral regimen in Kenya. The rest of the patients received 3TC, D4T, Efavir or AZT, 3TC, NVP. Length of treatment is shown in Table 1.

Sequences (Table 1 and Figure 2): 69 sequences were from plasma and 59 from non-plasma analytes. Dried samples were at room-temperature 2-4 months prior to shipping. Dried plasma/blood spots were then stored at -20°C for additional 3-11 months. Sampled patients were then stored at -20°C for 2 months prior to sequencing. Overall median time from sample to sequencing was 28 months for samples from naive patients and 14 months for samples from the treated patients. Duplicate sequences were available for 35 patients: 18 with two and 17 with three analytes. All intra-patient sequences clustered well with high bootstrap values. No hyper-mutation was identified.

Subtype Distribution (Table 2)

Subtype distribution as determined by the REGA subtyping system was 64% subtype A; 6% subtype C; 17% subtype D; and 8% unique recombinant forms (AB, AC, AD and CD).

Drug Resistance (Table 2)

Among drug-naïve patients, 2/48 (4%) demonstrated drug resistance mutations in at least one analyte: one with D67N and one with L210W. Among drug-experienced patients, 28/29 (97%) had drug resistance in at least one analyte. The median number of mutations per patient was 4 (range 1-9 mutations). NRTI mutations were seen in 79% of treated patients and NNRTI mutations in 97%. 14% of patients had only NNRTI mutations and 66% had both NRTI and NNRTI mutations.

Common NRTI mutations were M184V, seen in 82% of treated patients, T215Y/F in 33%, D67N in 32% and M41L in 21%. The K65R mutation was not observed. Common NNRTI mutations were K103N/S seen in 50% of treated patients, G190A/E/S in 43% and Y181C in 18%. Y106M was seen in a common NRTI isolate for the first time.

Among 10 treated patients with >4 drug resistance mutations, most patterns were uncommon and two were absent from the sequences stored in the Stanford database (arrows in Fig 2). Both isolates were recombinants. Intermediate to high predicted levels of resistance were seen for abacavir in 41%; efavirenz in 39%; didanosine in 34%, and tenofovir in 14%.

Analyte concordance (Table 2)

Concordance of identification of mutations at resistance positions was 88% among the different analytes. Discordant mutations are circled in Table 2. Discordance was distributed evenly among plasma and non-plasma analytes.

IV. Summary and Conclusions

We present HIV diversity and drug resistance in 76 patients from western Kenya using plasma and non-plasma genotyping analytes.

Diverse HIV subtypes (A, C, D) and recombinant forms (AB, AC, AD, CD) were identified.

4% of drug-naïve patients and 97% of drug-experienced patients had drug resistance mutations.

Low-cost, concordant non-plasma sequencing is feasible, even in difficult conditions.

High rates of drug resistance and unique resistance patterns were identified and have potential detrimental effects on second line therapy in resource-limited settings.

Table 2: Selected Treatment Naïve and Treatment Experienced Patients with Multiple Analyte Genotypes

#	Subtype	NRTI	Plasma	NNRTI	PI	DBS	DPS	STP	STB	STP	PI	STB	STP	PI	STB	STP	PI	Predicted Susceptibility
1	C	L50V	None	None	None	L210V	None	None	None	None	None	None	None	None	None	None	None	S S S S
2	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S S
3	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S S
4	C	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S S
5	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S S
6	A	M41L, D67N, V181A, M184V, L210V, T215F	Y106Y, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	H H I RL
7	A	M41L, D67N, V181A, M184V, L210V, T215F, K103N/S	Y106Y, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
8	A	M41L, D67N, V181A, M184V, L210V, T215F, K103N/S	Y106Y, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
9	D	M41L, E48Q, D67N, V181A, M184V, L210V, T215F	K103N	None	None	None	None	None	None	None	None	None	None	None	None	None	None	H H I RL
10	D	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
11	A	M41L, M184V, L210V	Y106Y, V181A, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
12	A	M184V, L210V	Y106Y, V181A, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
13	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
14	A	M184V, L210V	K103N, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
15	A	D67N, M184V	K103N, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	L RL S I
16	A	ADV, M184V	K103N	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S PL
17	A	V75, M184V	Y106Y	None	None	None	None	None	None	None	None	None	None	None	None	None	None	L L L S I
18	D	M184V	K103N	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S PL
19	D	M184V	G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S L
20	A	M184V	G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S L
21	D	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S PL
22	D	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I S S PL
23	D	D67N, T602Q, K103N, M184V, T215F, K103N/S	K103N, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I S S PL
24	D	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I S S PL
25	A	D67N, K103N, V181A, M184V, L210V, T215F, K103N/S	K103N, Y106Y	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
26	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	I I I I L
27	A	M184V	K103N, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S L
28	AD	M184V	K103N, G190A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S L
29	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	RL S S L
30	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S PL
31	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S PL
32	A	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	S S S PL

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