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Research Article

Drug Resistance Testing in HIV Infected Individuals on Treatment and Naive: Implications on Treatment Outcome

Abstract

Background: The Government of Kenya started offering ART in the public sector since 2003. Despite the dramatic reduction in AIDS related morbidity and mortality, the emergence and spread of drug resistance (DR) threatens to negatively impact on treatment regimens and compromise efforts to control the epidemic. Therefore, there is a need for information on the situation of DR Mutations (DRMS) and their implications on treatment.

Objectives: To evaluate DRMS and their implications on treatment in HIV infected individuals attending Moi Teaching and Referral Hospital (MTRH) clinics.

Method: In 2009, we consecutively collected plasma samples from two groups of HIV infected individuals, antiretroviral (ARV) naive and ARV experienced for more than 12 months and failing therapy according to world health organisation (WHO) guidelines. We performed genotypic DR using well established in-house Sanger sequencing methods. We then followed up the patients and compared the DRMS in relation to their drug regimens at the time of sample collection and16 months later.

Results: We successfully extracted and sequenced 75 samples. Median age was 36.7 years. Out of 41 drug naive individuals only 3 had DRMS. Out of the 34 ARV experienced, 29 had DRMS to nucleoside reverse transcriptase inhibitor (NRTI), and 31 to non NRTI (NNRTI). After 16 months from sample collection date, 20/31(64%) ARV experienced patients with DRMS had not been changed therapy and only 5/20(25%) were susceptible to primary ARV while 12/14 changed were susceptible to new ARV.

Conclusion: The information obtained in our study can serve as an indicator of ARV program efficiency in patients still on treatment, those who are to start treatment and those who are to be changed therapy due to failure. DR testing would be necessary before initiating and /or changing ART in order to achieve optimal clinical outcome.

Abbreviations

AIDS: Acquired immunodeficiency syndrome; AMPATH: Academic Model Providing Access to Health care partnership clinics; ARDR: Antiretroviral Drug resistance; ART: Antiretroviral treatment; ARV: Antiretroviral; AZT: Zidovudine; CD4: Cluster of differentiation; DNA: Deoxyribonucleic acid; DR: Drug resistance; DRMs: Drug resistance mutations; EFV: Efavirenz; HAART: Highly active antiretroviral therapy; HIV: Human immunodeficiency virus; MTRH: Moi Teaching and Referral Hospital; NASCOP: National AIDS control program; NNRTI: Nucleoside reverse transcriptase; NRTI: Nucleotide reverse transcriptase; NVP: Nevirapine; PCR: Polymerase chain reaction; PI: Protease Inhibitors; PR: Protease; RT: Reverse transcriptase; SD:Standard deviation; 3TC: Lamivudine; TDF: Tenofovir; TDR: Transmitted Drug resistance mutations;

Introduction

Most data concerning Human Immunodeficiency Virus (HIV) non-B subtypes remain controversial. Highly Active Antiretroviral Therapy (HAART) has radically changed the clinical outcome of HIV, leading to decreased mortality and morbidity. Development of HIV drug resistance is inevitable in patients on ART. Increase in antiretroviral therapy (ART) in resource-limited settings (RLS) will successfully reduce HIV-related morbidity and mortality [1]. The increase in ART coverage is expected to lead to an increase in drug-resistant strains among experienced patients. Improved access to alternative combinations of antiretroviral drugs in sub-Saharan Africa is warranted [2].

As the rollout of ART in Kenya is on the rise, there is a need to monitor the patients on ART [3]. The use of Cluster of Differentiation (CD4) and viral load measurements is important in monitoring HIV patients both immunologically and virologically. Though virological and immunological monitoring is important, there is a need to provide HIVDR testing services for patients who are starting therapy and those who are suspected to be failing treatment before they are switched to a different regimen [4]. A public-health approach based on standardized, affordable drug regimens and limited laboratory monitoring is crucial in scale up efforts. The ever-expanding rollout of antiretroviral therapy in RLS without routine virological monitoring

has been accompanied with development of drug resistance that has resulted in limited treatment success. A survey performed in Kampala showed a prevalence of transmitted drug resistance at 8.6% [5].

In Kenya, availability of ART is increasing. As ART use increases there is mounting evidence suggesting that DR will increase over time [6]. A recent cross-sectional study to determine treatment failure and drug resistance mutations among adults receiving first-line (3TC_d4T/AZT_NVP/EFV) and second-line (3TC/AZT/LPV/r) in Nairobi, Kenya, concluded that the detected accumulated resistance strains due to emergence of HIV drug resistance will continue to be a big challenge [7]. Another study carried out to evaluate treatment success and development of ART drug resistance at the Coast Province General Hospital, Mombasa, Kenya, revealed a high rate of treatment success after short term ART in patients treated at a public provincial hospital detected minority complex drug resistance profiles that were predictive of resistance to currently used second-line NRTIs and NNRTIs regimens [1]. In this article we present detailed data on DRMS from patients who had not started ARV and those who were failing with their implications on therapy. Identifying the relevant DRMS among non-B subtypes will be important for monitoring the evolution and transmission of drug resistance, determination of initial treatment strategies for persons infected with HIV non-subtype B [4]. According to International AIDS Society recommendations (IAS), evaluating susceptibility patterns among non-clade B persons should be a high priority because these viruses are by far the most prevalent world-wide. It is believed that surveillance will maximize the utility of first-line therapy and help minimize the cost of providing ART thereby sustaining current antiretroviral drug programs. The HIVDR testing is important as it gives the clinician accurate information of the most appropriate drug options. With ART scale up, there has been a need for monitoring for development of HIV drug resistance at a population level. Therefore, there is a need for country information on the situation of antiretroviral drug resistance (ARDR) to inform on policy guidelines.

Materials and Methods

Setting

The study was conducted at Moi Teaching and Referral Hospital (MTRH), AMPATH (Academic Model Providing Access To Health care partnership clinics), Eldoret, Kenya clinics.

The region includes the expansive Rift Valley, Western and Nyanza provinces, a cumulative population of about 15 million. The hospital is located in Eldoret Town in Uasin-Gishu (UG) County, which forms part of the UG Plateau West of the Great Rift Valley, at an altitude of 2118m above the sea level, latitude 00°30'52"N and longitude 035°17'52"E [2].

Study subjects

The study was conducted on isolates from patients who were known HIV positive attending the study site and met the selection criteria. During September 2009 and October 2011, patients receiving ARV therapy for at least 12 months and were suspected to be failing according to WHO guidelines were consecutively enrolled. After informed consent was obtained, a standardized questionnaire was administered to assess demographic, epidemiologic, clinical, and treatment information. ART-naive patients were also enrolled during the same period at the same study clinics. Samples from patients who had no history of exposure to ARV drugs and ARV drug naïve status according to a medical chart review and personal interview were collected consecutively.

Study design

The study conducted was a hospital-based prospective utilizing isolates from HIV positive patients who met the selection criteria. The study clinics provided ART according to the national guidelines for ART scale-up as recommended by WHO surveillance and monitoring surveys.

Data Collection Tools and Procedures

Laboratory procedures

Sample collection: Remnant blood samples collected for CD4 analysis from ARV naïve were centrifuged and plasma was collected. Remnant samples collected for viral load analysis from ARV experienced patients suspected to be failing therapy clinically were also centrifuged and plasma were collected and stored at −80°C.

HIV DNA extraction: HIV-1 nucleic acid was extracted from 400 µl of plasma using the Nuclisens Easy Mag system (Biomerieux, Canada) following manufacturer's instructions.

Reverse Transcription and polymerase chain reaction: HIV-1 protease (PR) and reverse transcriptase (RT) were bidirectional sequenced with an in-house protocol (8). Briefly, viral RNA was reverse transcribed and amplified according to the manufacturer's directions using the QIAGEN one-step RT-PCR kit (QIAGEN, Canada). The primers used were GaGp1-PR-out.for with a sequence of TGA ARG AIT GYA CTG ARA GRC AGG CTA AT and RT-new-out. Rev of CCT CIT TYT TGC ATA YTT YCC TGT T with nested primers GaGp6-PR-in.for YTC AGA RCA GRC CRG ARC CAA CAG C and RT-new-in.rev GGY TCT TGR TAA ATT TGR TAT GTC CA. All reactions were carried out using standard conditions using GeneAmp PCR System 9700 (ABI) thermocycler.

PCR product purification and sequencing

The PCR products were purified using Multi Screen Separations System as previously described [9] and diluted to 15 ng/ml for DNA sequencing. Amplicons were sequenced using ABI Prism Big Dye 3.1 Cycle Sequencing System (Applied Bio systems, USA) following manufacturer's instructions.

Data analysis

All the data generated in this study was saved in Microsoft Excel worksheets with a detailed database established to capture all the necessary information. Generated sequences were edited using Bio Edit v 7.0.5B. Aligned fasta files were uploaded to Stanford HIV Drug resistance (http://hivdb6.stanford.edu/asi/deployed/hiv_central.pl?program=hivdb&action=showSequenceForm). Phylogenetic relationships of newly derived viral sequences for comparisons with those of previously reported HIV group M from the Los

Alamos database by CLUSTAL W profile alignment was utilized. To improve the accuracy of HIV-1 subtyping, the genotyping tool (http://www.ncbi.nih.gov/projects/genotyping/formpage.cgi) was used and the REGA sub typing tool (http://dbpartners.stanford.edu/RegaSubtyping/) was utilized as needed. Drug resistance mutation and subtype data collected from the Stanford HIV database sequence analysis program were manually input into appropriate excel spreadsheet file, verified and corrections made as needed. Categorical variables were presented in form of frequency tables while continuous variables were mainly summarized using means together with standard deviation and median.

To test significance of skewed continuous variables, Wilcoxon rank sum test was employed. Chi-square test was used to compare the association between categorical variables. Fisher's exact test was also used to compare categorical variables where some cells had expected value of less than 5. Level of significance was set at $p < 0.05$, with a 95 % confidence interval. All analyses were done using STATA version 11.0.

Ethical considerations

The study was approved by the Institutional Research and Ethics Committee (IREC) of the Moi University School of Medicine (MUSOM) and MTRH Review Board (IREC):(IREC/2010/06) and AMPATH (RES/STUD/17/2010).

Results

Socio-demographic characteristics

Among the 264 individuals who met the selection criteria, 128 were declared to be ART naïve and 136 had been on ARV for more than 12 months and were failing therapy clinically. Mean age was 37.01 years (SD=12.50). Majority were female 44(60.02%) (Table 1).

Polymerase Chain Reaction and Sequencing outcomes: One hundred and ten (110) samples were successfully amplified. Out of these, 75 samples were successful sequenced and analysed for the presence of drug resistance mutations. Out of 75, ARV experienced individuals failing therapy were 34 and most patients, 25 (73.5%) received 3TC + d4T/AZT + EFV/NVP as first-line treatment. Patients who reported treatment interruption or switch were 9(26.5%). Switch concerned mainly replacement of d4T or AZT by TDF or ABC and only 3(8.8%) had been switched to protease inhibitor (PI) regimens (Tables 1-3).

Drug resistance mutations in ARV naive

Drug resistance mutations were identified in 3/41 (7.3%) of patients and per drug class the values were as follows: 1 for PI and 2 for NRTI and 1 for NNRTI. In 1 patient, multiple mutations against 2 drug classes were seen, suggesting that they were probably not naïve (Table 4). Approximately 3/28(10.7%) of female subjects had DRMS. None of their male counterparts had mutations. There was no statistical difference when male vs. female respondents were compared (p=0.235).

Drug resistance mutations in ARV experienced

Among the ARV experienced patients who were failing therapy according to WHO guidelines, 34 samples which were successfully extracted and sequenced were studied. Mean age was 35.85years (SD=14.06). Majority were male 18(52.9%). Out of 34 samples, 27 had DRMS to nucleoside reverse transcriptase inhibitor (NRTI), 30 had DRMS to non-nucleoside reverse transcriptase inhibitor (NNRTI), and 2 had DRMS to NNRTI only (Table 5).

ARV therapy sixteen months after sample collection

Sixteen months after sample collection, 20/34 ARV experienced patients failing therapy were still on the same ARVs. Only 4/20(20%) of these patients were susceptible to the ARVs they were taking from DRMS analysis. Twelve out of the fourteen who were changed

Table 1: Socio-demographic and clinical characteristics among study subjects.

Characteristic	N, (%)
Male	31 (39.8)
Female	44 (60.2)
Median age, (IQR)	36.29 (28.80-45.81)
Mean age (sd)	37.01 (12.50)
Median CD4 count, (IQR)	287 (119.0-430.0)
Median viral load, (IQR)	10,970 (1,676.0-59,901.0)

N; number, (%); percentage, IQR; interquartile range, sd; standard deviation, CD4; cluster of differentiation.

Table 2: Patient Regimen.

Regimen	N, (%)
d4T-3TC-NVP	12 (29.4)
d4T-3TC-EFV	2 (5.9)
AZT-3TC-NVP	11 (26.5)
TDF-3TC-EFV	2 (5.9)
TDF-3TC-NVP	3 (8.8)
Alluvia	3 (8.8)
ABC-3TC-EFV	1 (14.7)

EFV; Efavirenz; 3TC; Lamivudine; NVP; Nevirapine, AZT; zidovudine, d4T; Stavudine, TDF; Tenofovir, ABC;Abacavir, LPV/r; Lopinavir, ALUVIA; Lopinavir /Ritonavir.

Table 3: Differences between Patient characteristics between the two study groups.

	ARV Naive		ARV Experienced	
Sex	F	M	F	M
Age	28	13	16	18
Mean Age	36	40	36	35
Mean Viral loads	-	-	50,669	34,527
Mean CD4 counts	405	218	178	287
HIV Subtype A	16	8	11	14
B	2	1	1	1
C	3	1	0	0
D	7	3	4	2
G	0	0	0	1

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therapy were susceptible to the new drugs and 2 patients had their ARVs changed into other drugs that they were already resistant to (KE12-027, KE12043) (Table 6).

Discussion

The results present depiction of the importance of HIV DR testing in a resource limited setting and their implications on treatment in both ARV naive and ARV experienced failing therapy before starting or changing therapy. However, we noted a low rate of amplification which may have been due to integrity of sample storage and transportation. Sequences obtained from 35 samples that were successfully amplified did not meet the integrity of good sequences for final drug resistance analysis.

Drug resistance mutations in ARV naive

In our study, drug resistant mutants were detected in three patients who were ARV naïve according to chart review. The prevalence of DRMs among drug naive populations revealed in our

Table 4: DRMS in ARV Naive Patients.

PID	AGE	GENDER	NRTI drms	NNRTI drms	PI drms
KE12-086	30	FEMALE	M184I	NONE	I50V
KE12-112	36	FEMALE	T215I	NONE	NONE
KE12-128	66	FEMALE	NONE	K103N	NONE

NRTIs; nucleoside reverse transcriptase inhibitors, drms; drug resistant mutations NNRTIs; non-nucleoside reverse transcriptase inhibitors, Pi; protease inhibitors.

Table 5: DRMS and level of resistance to baseline ARVs.

PID	NRTI	NNRTI	PI	Level of RS to baseline ARV
KE12-006	M41L,K70R,M184V, T215SY	Y181C, G190S	None	HL RS to TDF,3TC
KE12-007	K65R, M184V	Y181C	None	HL RS to NVP,3TC
KE12-009	None	K103N	None	susceptible
KE12-016	None	None	None	susceptible
KE12-018	F116Y, Q151M, M184V	K103N,Y181I,P225H	None	susceptible
KE12-023	K70R, M184V,T215FIS	K103N, P225H	None	HL RS to EFV,3TC
KE12-027	M184V	K103N, Y188L	None	HLRS to NVP,3TC
KE12-028	M184V	G190A	None	HLRS to NVP,3TC
KE12-030	M184V, T215SY	K103N	None	HLRS to NVP,3TC
KE12-031	M184V,T215Y,K219Q	K103N, M230L	None	HLRS to NVP,3TC
KE12-034	None	None	None	susceptible
KE12-035	K70R, M184V	Y181C	None	HLRS to NVP,3TC
KE12-036	M184V	G190A	None	HLRS to NVP,3TC
KE12-037	M184V	K103N	None	HLRS to NVP,3TC
KE12-039	D67N,M184V,L210W,T215Y,K219Q	Y181V	None	HLRS to NVP,3TC
KE12-040	M184V, T215Y	Y181C	None	HLRS to NVP,3TC
KE12-043	M184V, T215F	G190A	None	HLRS to NVP,3TC
KE12-052	D67N, K70R, M184V, K219Q	K103N	None	HLRS to NVP,3TC
KE12-058	M184V, T215Y	Y181C	None	HLRS to NVP,3TC
KE12-059	K70R, M184V, K219Q	Y181C	None	susceptible
KE12-060	K65R, D67N,Y115F, F116Y, K219E	G190E	None	HLRS to EFV,ABC
KE12-081	K70R, M184V, K219Q	K103N	None	HLRS to NVP,EFV
KE12-093	None	None	None	susceptible
KE12-245	M41L,K70R,V75M,M184V,L210W,T215F	G190A	None	HLRS to NVP,3TC
KE12-246	M184V	K103S, G190A	None	HLRS to NVP,3TC
KE12-250	None	K103N	None	HL RS to NVP
KE12-252	D67N	K103N	None	HL RS to NVP
KE12-282	D67N, M184V, T215I	Y181C, G190A	None	HLRS to NVP,3TC
KE12-300	M184V	K103N	None	HLRS to NVP,3TC
KE12-307	M184V	G190A	None	HLRS to NVP,3TC
KE12-309	M184V	K103N, V106M	None	HLRS to NVP,EFV
KE12-316	D67N, K70R, M184V, K219Q	K101E, G190A	None	HLRS to NVP,3TC
KE12-324	K70R, M184V	Y181C	None	susceptible
KE12-326	M184V	G190A	None	HLRS to NVP,3TC

PID; patient identification, NNRTIs; non-nucleoside reverse transcriptase inhibitors, NRTIs; nucleoside reverse transcriptase inhibitors, PI; Protease inhibitors, RS; resistant, HLRS; highly resistant, ARV; antiretroviral therapy; EFV; Efavirenz; 3TC; Lamivudine; NVP; Nevirapine, AZT; zidovudine, d4T; Stavudine, TDF; Tenofovir, ABC;Abacavir.

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Table 6: The ARV therapy, level of susceptibility and resistance.

PID	ARV on sample collection	RS to primary ARV	ARV at 16 months	RS to changed ARV
KE12-006	TDF-3TC-EFV	HL RS to TDF,3TC	Not changed	
KE12-007	TDF-3TC-NVP	HL RS to NVP,3TC	Not changed	
KE12-009	ALUVIA	susceptible	TDF,3TC,NVP	susceptible
KE12-016	TDF-3TC-NVP	susceptible	Not changed	
KE12-018	ALUVIA	susceptible	LPV,3TC,TDF,ABC	Susceptible
KE12-023	TDF-3TC-EFV	HLRS to EFV,3TC	LPV,3TC,TDF,ABC	Susceptible
KE12-027	d4T-3TC-NVP	HLRS to NVP,3TC	AZT-3TC-NVP	Resistant
KE12-028	AZT-3TC-NVP	HLRS to NVP,3TC	ALUVIA,3TC,TDF	Susceptible
KE12-030	AZT-3TC-NVP	HLRS to NVP,3TC	ALUVIA,3TC,TDF	Susceptible
KE12-031	AZT-3TC-NVP	HLRS to NVP,3TC	ALUVIA,3TC,TDF	Susceptible
KE12-034	d4T-3TC-NVP	susceptible	Not changed	
KE12-035	d4T-3TC-NVP	HLRS to NVP,3TC	ALUVIA,3TC,TDF	Susceptible
KE12-036	d4T-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-037	d4T-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-039	d4T-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-040	d4T-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-043	d4T-3TC-NVP	HLRS to NVP,3TC	TDF,3TC,NVP	Resistant
KE12-052	TDF-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-058	d4T-3TC-NVP	HLRS to NVP,3TC	ALUVIA,3TC,TDF	Susceptible
KE12-059	ALUVIA	susceptible	Not changed	
KE12-060	ABC-3TC-EFV	HLRS to EFV,ABC	Not changed	
KE12-081	d4T-3TC-EFV	HLRS to NVP,EFV	TDF,3TC,NVP	Susceptible
KE12-093	AZT-3TC-NVP	susceptible	Not changed	
KE12-245	AZT-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-246	AZT-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-250	AZT-3TC-NVP	HL RS to NVP	Not changed	
KE12-252	d4T-3TC-NVP	HL RS to NVP	ALUVIA,3TC,TDF	Susceptible
KE12-282	AZT-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-300	AZT-3TC-NVP	HLRS to NVP,3TC	Not changed	
KE12-307	AZT-3TC-NVP	HLRS to NVP,3TC	ALUVIA	Susceptible
KE12-309	d4T-3TC-EFV	HLRS to NVP,EFV	Not changed	
KE12-316	d4T-3TC-NVP	HLRS to NVP,3TC	ALUVIA	Susceptible
KE12-324	AZT-3TC-NVP	susceptible	Not changed	
KE12-326	d4T-3TC-NVP	HLRS to NVP,3TC	Not changed	

PID; patient identification, NNRTIs; non-nucleoside reverse transcriptase inhibitors, NRTIs; nucleoside reverse transcriptase inhibitors, RS; resistant, HLRS; highly resistant, ARV; antiretroviral therapy; EFV; Efavirenz; 3TC; Lamivudine; NVP; Nevirapine, AZT; zidovudine, d4T; Stavudine, TDF; Tenofovir, ABC;Abacavir, LPV/r; Lopinavir, ALUVIA; Lopinavir /Ritonavir.

study might have been the result of the transmission of drug-resistant viruses from partners infected with the resistant virus or selection as a result of undisclosed use of ART. One patient had multiple NRTI drug resistance mutations, an indication that the patient may have had previous drug exposure. Although mutations conferring NRTI resistance have previously been reported among drug naive patients, the possibility that our patients had previous unreported contact with antiretroviral drugs could not be excluded [8].

Drug resistance mutations in ARV experienced

From our study, we observed that due to lack of information about any existing mutations before the therapy were changed, only 4/20(20%)who had not changed therapy were susceptible to the ARVs they were taking from DRMS analysis. Twelve out the fourteen who had their therapy changed were susceptible to the new drugs and the two patients had their therapy changed into ARVs they were already

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resistant to (KE12-027, KE12043). Most harboured a mutation at position M184I/V associated with 3TC and EFV resistance. The M184V on the other hand confer high level resistance to 3TC, a key backbone to first line antiretroviral treatment regimens in Kenya. The M184I mutation has been noted to be the first to appear but is quickly replaced by the M184V since this mutation has greater ability to induce higher replicative capacity [10].

Majority of the patients we reported with drug resistance mutations, were resistant to AZT and/or d4T because they harboured either mutation in the RT gene associated with resistance to RT inhibitors: multi NRTI-69 insertion complex b which affects all NRTIS (K70R (n=2), T215YF (n=5), K219QE (n=3). The presence of 3 of the following mutations M41L, D67N, L210W, T215Y/F, and K219Q/E has been associated with resistance to didanosin [6]. The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens (nevirapine or efavirenz) in NNRTI-naive individuals, although no clinical data exist for improved response to etravirine in NNRTI experienced individuals. KE12-027 had K65R, M184V while KE12043 had both M184V and T215Y. When associated with TAMs, M184V has been reported to increase abacavir resistance [11]. Studies have shown that the presence of K70R or M184V alone does not decrease virologic response to didanosine. The presence of mutations G190A had already compromised the use of next generation NNRTI etravirine. Importantly, presence of the K65R mutation compromises also the use of second-line regimens. Previous findings suggest that implementation of programs to consider the various socioeconomic and cultural barriers that may prevent successful uptake of antiretroviral prophylaxes are important [12].

Practical implications

Patients whose physicians have access to information about any existing mutations before the therapy are changed usually have more significant decreases in the viral load than patients in whom treatment is changed without knowledge of the resistance profile. Similar studies have led to development of new NRTIs, as well as new NNRTIs and PIs with different resistances profiles [3]. The options after treatment failures if improved will thereby increase the importance of resistance testing. On the other hand, simply changing national recommendations for initial ARV therapy from an NNRTI-based regimen to a protease inhibitor (PI)-based regimen would be suboptimal because PI-based regimens are more expensive and often less tolerated than NNRTI-based regimens. From our study, practical implications show that 80% (20/34) of patients whose therapy was not changed based on immunological and virological results only had DRMS to the regimens they were still taking while 85% (12/14) of those changed therapy were susceptible to the new regimens as per DRMS Stanford report. It is worth noting that M184V mutation is also associated with reduced viral replication and this may explain the reason for not changing regimen for the individuals that harbour this mutation.

Therefore, pre-therapy genotypic resistance testing would be useful to identify which patients should receive standard first-line therapy and which should receive a PI-containing regime [12].

Limitations

Viral load data was only available for ARV experienced patients who were failing therapy clinically.

Conclusion

The outcome of our study denotes high drug resistant strains in regions with non- B subtypes. The high prevalence of DRMs among drug experienced with evidence of drug failure populations revealed in our study might have been due to lack of DR analysis before start of therapy.

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Recommendations

Drug resistance testing would be necessary before initiating and /or changing ART in order to achieve an optimal clinical outcome. Similarly, there will be a need for a continued follow-up of persons with DRVS to determine clinical impact and help guide therapies for drug naïve and experienced with evidence of drug failure populations.

Authors' contributions

WC(MTRH) conceived the study, carried out field work, sample collection; sample analysis and manuscript preparation; GK(JKUAT) and SM(MUSOM) coordinated project implementation, WE(MUSOM) coordinated sample collection and analysis; ER(MUSOM) coordinated clinical oversight, ES(KEMRI) did project implementation oversight and overall supervision; MK(KEMRI/SEKU) coordinated field work, overall supervision and manuscript preparation. All authors read and approved the manuscript.

Sequence data

The sequence data has been deposited at Gen bank under accession numbers KP877888 to KP877961 and KR003380 to KR003405.

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