

The Challenge of HIV-1 Antiretroviral Resistance in Africa in the Era of HAART

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Abstract

Antiretroviral therapy programs in Africa are currently providing treatment for almost two million people. The long-term success of large scale antiretroviral therapy programs in sub-Saharan Africa remains uncertain because of the limited information currently available on rates of virologic failure and selection for drug-resistant variants in the different HIV subtypes. This article provides a comprehensive review of the published literature on the prevalence of primary and secondary HIV drug resistance with different subtypes and in various settings across sub-Saharan Africa. (AIDS Rev. 2009;11:59-70)

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Key words

Antiretroviral therapy. ART. Resistance. Africa. Subtypes. Phenotypic. Genotypic.

Introduction

The Joint United Nations Program on HIV/AIDS (UNAIDS) recently estimated that in sub-Saharan Africa there are 22 million individuals infected with HIV and seven million in urgent need of treatment¹. In the last few years there has been a rapid rollout of antiretroviral therapy (ART) in the resource-limited settings of the world² that has provided treatment for 2.1 million people in Africa alone¹. The ART regimens available and most commonly prescribed for initial first-line therapy in sub-Saharan Africa include either zidovudine (AZT) or stavudine (d4T) plus lamivudine (3TC) with one of the nonnucleoside reverse transcriptase inhibitors (NNRTI), either nevirapine (NVP) or efavirenz (EFV)². Less than

10% of individuals on ART are currently receiving protease inhibitor (PI) regimens.

There is growing concern about the potential for significant levels of drug resistance with expanded access to ART due to (i) the lack of routine virologic monitoring, such that patients are more likely to remain on a failing regimen for a prolonged period, allowing accumulation of drug resistance mutations^{3,4}, and (ii) routine use of single-dose NVP for prevention of mother to child transmission (PMTCT) of HIV, which may compromise the effectiveness of first-line NNRTI-based regimens because it is associated with a significant rate of NNRTI-related mutations⁵. The ultimate impact of widespread resistance, however, remains largely hypothetical and requires documentation. In fact, some early evidence argues that ART can be feasibly administered in resource-limited settings without a laboratory infrastructure with relative safety and substantial effectiveness⁶.

The HIV-1 main group M is comprised of at least 10 subtypes (A, B, C, D, AE, F, G, H, J, K)⁷. The majority of HIV-1 infections in Africa are caused by the A, C, and D subtypes. Subtype C, for example, is responsible for 90% of infections in the Horn of East Africa, 73% in Djibouti, 70% in Tanzania and 92% in Southern Africa⁸.

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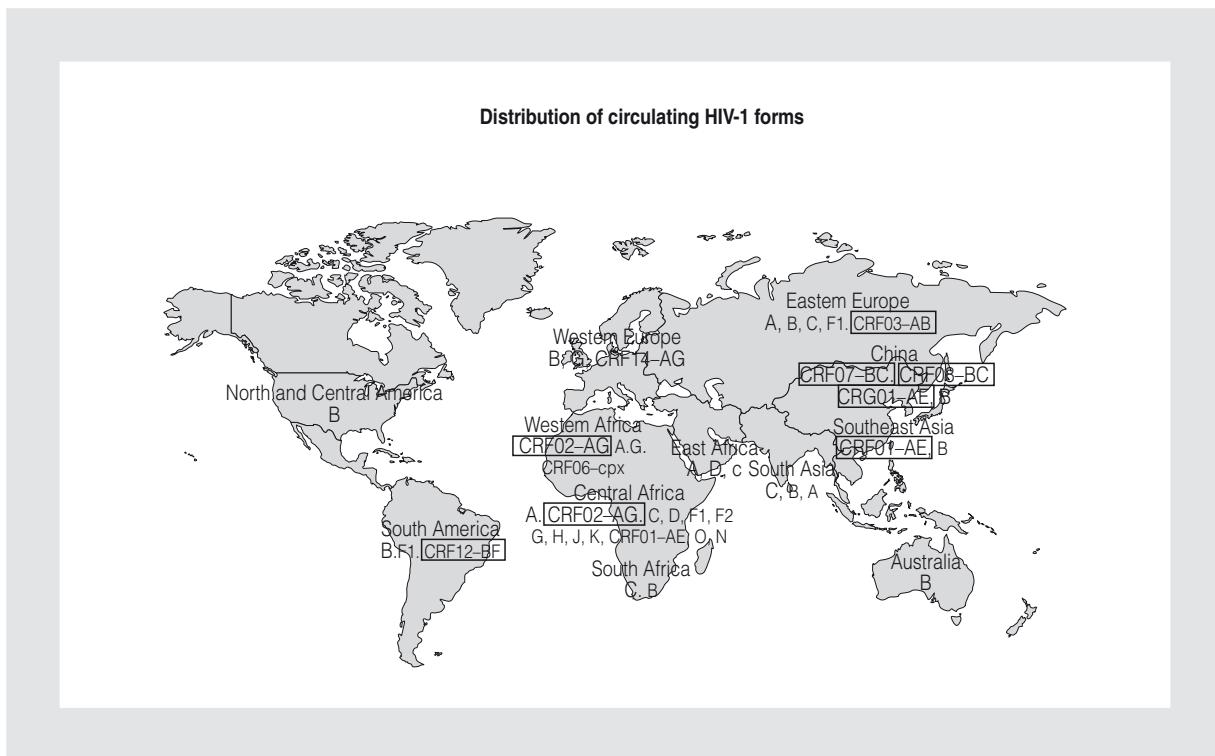


Figure 1. Map of the world showing the various HIV subtypes prevalent in different regions. Image reproduced from AIDS Rev. 2003;5:205-13.

In general, the evidence suggests that the regimens available in Africa select for similar drug-resistant mutations as for the HIV-1 subtype B⁹. However, there is still limited information on the HIV-1 resistance patterns in non-B subtypes, and subtype differences may influence reverse transcriptase (RT) genotypic diversity and, therefore, the emergence of drug resistance^{10,11}.

In this report, we review the prevalence and patterns of phenotypic and genotypic resistance to the currently available HIV drugs in different subtypes and different regions of sub-Saharan Africa over time. Our approach was to search the published literature from PubMed from 1995 to 2007 using the following key words: antiretroviral therapy, resistance, and Africa. We identified 127 articles, of which 57 were determined to be relevant. For each of the studies, we abstracted core data items: country of study population with virologic failure, number of patients studied, most prevalent or tested HIV-1 subtypes, frequency of primary phenotypic/genotypic resistance and resistance patterns, natural polymorphisms, and frequency of secondary phenotypic/genotypic resistance and resistance patterns. The objective was to describe resistance patterns by subtype in different regions of sub-Saharan Africa during the ART rollout.

Antiretroviral therapy has been beneficial in Africa

Despite early concerns that the widespread availability of ART would result in significant rates of treatment failure, most ART programs in Africa have so far reported rates of virologic and immunologic responses comparable to those observed in the developed world⁶. Long-term theoretical models for ART have shown substantial declines in mortality rates, HIV incidence rates, and ultimately HIV prevalence. If high rates of ART were also accompanied by reductions in risky behavior, then it is estimated that the HIV incidence would also fall significantly¹². In a review of treatment outcomes from different sites in Africa, Akileswaran⁶ reported that the median proportion of patients who achieved undetectable viral loads by the end of the study period was 73%. Good health outcomes and high levels of treatment adherence were comparable to those of industrialized countries^{6,13}.

At the Infectious Diseases Institute (IDI) in Kampala, Uganda, a center of excellence for HIV care, less than 150 patients were on ART before May 2004. In August 2004, with the massive expansion of available ART, more than 10,000 patients had registered for care within less than a year. With the support of The U.S. President's Emergency Plan for AIDS Relief (PEPFAR), the

Global Fund and the World Bank Multi-country AIDS program, by the end of 2007 the clinic had provided ART to 6,451 patients. Analysis on a subgroup of patients who were participants in a more closely monitored research cohort showed that 556 (82%) of 676 who completed 12 months on ART had achieved viral suppression to < 400 copies/ml¹⁴.

At the same time, there has also been more than a 50% reduction in vertical transmission of HIV from mother to infant at the time of delivery through the administration of single-dose NVP. The effectiveness of single-dose NVP when used in successive pregnancies is an area of active research, with preliminary evidence suggesting that viral resistance selected by prior exposure to single-dose NVP may wane with time¹⁵. Other treatment programs have also examined the efficacy of regimens other than NVP, and found comparable results with AZT^{16,17}. There were no mutations associated with a short course of AZT in a randomized study of 240 patients on AZT vs. placebo in Cote d'Ivoire, and no adverse HIV-1 virologic consequences for either mother or baby^{16,17}. Comparable results between AZT and NVP have also been reported from Uganda¹⁸.

HIV-1 genes are highly polymorphic in African subtypes

Genetic differences between subtypes do not initially impact antiretroviral therapy clinical outcome, but could subsequently predispose to development of resistance

The distribution of HIV subtypes varies widely in different parts of the world, with A, C, and D predominant in Africa, and subtype B in the western world. Studies have reported on the highly polymorphic nature of the non-B subtypes, such that some wild-type viruses harbor mutations on sites that are otherwise associated with resistance in B subtypes.

The various subtypes (A, C, D, F2, G, H, J, CRFO2_AG) that were studied in Cameroon and Uganda harbored virtually no primary mutations when compared to HIV-1 subtype C in Botswana and South Africa¹⁹⁻²³. Minor mutations were seen in Ghana²⁴, but in a UK study of patients infected with various African subtypes, multiple polymorphisms were detected²⁵. A total of 133 polymorphisms were identified in the pol gene (37 in protease and 96 in RT), with a mean of 9.0 in protease and 22.3 in the RT gene per patient. Neither subtype nor any single polymorphism had an impact on treatment

outcome, and all the studied non-B subtypes were fully sensitive to ART²⁵.

Although many of the naturally occurring polymorphisms in C and A subtypes are not associated with phenotypic drug resistance on their own, there is evidence that they may amplify the effects of drug-resistant mutations such as V82F/I84V²⁶. In a study from Ethiopia, the RT sequences of subtype C isolates had a KVEQ-specific motif of silent amino acid mutations at sites 65, 106, 138, and 161, respectively. These mutations were associated with broad phenotypic cross-resistance, particularly against NVP and delavirdine²⁷. These drug-resistant variants were more rapidly selected at lower drug doses in culture with subtype C than with subtype B wild-type isolates. In subtype C, there were similar mutations in RT as observed with subtype B (e.g. K103N, V106A, V108I, and Y181C) as well as previously unseen mutations, i.e. V106M and S98I, which were selected rapidly by NVP and/or EFV together with a multidrug resistance mutation A62V²⁷. As a result, it is now understood that ART may drive the evolution of resistance differently in the various HIV subtypes²⁶⁻²⁹.

Primary mutations associated with nucleoside reverse transcriptase inhibitor resistance

Some of the primary nucleoside reverse transcriptase inhibitor (NRTI) mutations found in HIV subtypes from Africa are T69N/T, A62V, and M184V in various subtypes in Cameroon, M41L and M41L+T69S in Burkina Faso and Cameroon³⁰, and K65R in subtype C in Djibouti³¹. Other mutations have been found at positions 118, 211, and 214 in subtype C in Malawi³² and positions 179, 211, and 214 in multiple subtypes, but mainly subtype A, in Uganda³³. In one Ethiopian subtype C isolate, the mutation K70R associated with resistance to AZT and tenofovir, was susceptible phenotypically²⁷. The D123N plus I135V mutations were observed in CRF02_AG isolates from Cote d'Ivoire, which showed resistance to abacavir. Substitutions at positions 20, 36, 63, and 82 were also associated with some degree of resistance, with a potentially crucial role of the V82I substitution in resistance to atazanavir³⁴.

Primary mutations associated with nonnucleoside reverse transcriptase inhibitor resistance

With the exception of one CRF01_AE isolate with an I135T substitution that exhibited a decreased susceptibility to

Table 1. Primary resistance mutations and natural polymorphisms prior to antiretroviral therapy in different subtypes

Country	Prevalent HIV-1 subtypes	Time period	Patients studied (n)	1° Phenotypic resistance	Primary genotypic mutations associated with resistance			Unique patterns/remarks
					NNRTI	PI	NNRTI	
Burkina Faso and Cameroon ³⁰	CRF02-AG CRF06_0px	2001-2003	199 (with resistance: 97 Burkina Faso, 102 Cameroon), 53 blood donors	—	M41L (n = 1) M41L+T69S (n = 1) T69NT (n = 1) M184V (n = 1) A62V (n = 1)	V106A/V (n = 1) V108I (n = 4) P236L (n = 1)	L33F (n = 2)	
Uganda ³³	A (n = 17) D (n = 10)	1993-1994, blood donors	27	—	T139M (n = 1) G141E (n = 1) V179I (n = 15) R21/N/K/S (n = 23) L214F (n = 25)	— K20/R (n = 4) L33F/N (n = 2) M36I (n = 23) K45/R (n = 1) D60E (n = 3) L63V/P (n = 7) H69K/Y (n = 20) V77.1 (n = 20) V77.1 (n = 2) V82I (n = 1)	Natural polymorphisms/ subtype motifs Subtype A 35D, 57K, 69K, 89M	
Malawi ³²	C	1996-2001	21	—	None	None	193I (n = 21) M36I (n = 19) L63P (n = 8) K20R (n = 6) V77I (n = 3) L10I (n = 2)	
Djibouti ³¹	C (n = 21) CRF_02 (n = 5), A (n = 2), D (n = 2).	2002	30/47	—	K219E (n = 2) K219Q (n = 1) D67N (n = 1) K70R (n = 1)	K101E (n = 3) K103T (n = 2) L100I (n = 1) G190V (n = 1)	M36I (n = 20) R41K (n = 19) K20/R (n = 9) L101V (n = 4) E35D (n = 1) L63P (n = 1) A71V (n = 1)	The mutations R41K and E35D were not included in the IAS list of March/April 2005.
Ghana ²⁴	CRF02-AG (n = 39)	2001-2002	39	Less susceptible to the PI NFV = LPV > IDV > SQV > RTV > APV.			K20I (n = 39) M36I (n = 39) L10V (n = 5) L63S/F/P (n = 8)	I13V, R41K, H69K, and L89M were common silent mutations present in all.
Ethiopia ²⁷	C	1994-1995	G190A high-level phenotypic resistance to NVP. K70R no phenotypic resistance to AZT.				K65, V106, E138, Q161	

NNRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor; NVP: nevirapine; NFV: nevirapine; LPV: lopinavir; SQV: saquinavir; RTV: ritonavir; APV: amprenavir; IDV: indinavir; L63S/F/P: lopinavir/ritonavir/amprrenavir; AZT: Zidovudine

NNRTI, other isolates (CRF01_AE, CRF02_AG and C) from Cote d'Ivoire were all sensitive to NNRTI³⁴. Major resistance mutations (V106A/V and V108I) were found in eight of 97 treatment-naïve individuals from Burkina Faso and Cameroon infected with various subtypes³⁰. However, an Ethiopian subtype C isolate that naturally harbored the G190A mutation had high-level resistance to NVP²⁷. Primary mutations found elsewhere include: K103N in one strain in the Democratic Republic of Congo (DRC)³⁵, P236L and V108I in Cameroon³⁰, K101E, K103T in subtype C, L100I and G190V in CRF02_AG in Djibouti³¹.

Primary mutations associated with protease inhibitor resistance

High numbers of polymorphisms in the protease (PR) coding region before ART have been reported from many studies. In a study from Rwanda, these included substitutions in secondary PR resistance sites in the following subtypes: PR 35D, 36I, and 37N were always present within subtype A, and PR 93L in subtype C strains. The polymorphisms 10I/V, 20R, 33F, and 77V were frequently found (11, 6, 1, and 1 of 34 respectively) in subtype A, and PR 36I was highly prevalent in subtype C strains. The A/C recombinant displayed substitutions known to be related to resistance (PR 10, 33, 36, and RT 118)³⁶. In Uganda, frequent polymorphisms were detected at positions 36 and 69. Most of the subtype A isolates had the amino acids DKKM at positions 35, 57, 69, and 89, whereas most subtype D sequences had the amino acids ERHL at these positions³³. In subtype C isolates from Malawi, minor mutations were found at positions 10, 20, 36, 63, 77, and 93³². Other mutations found were M461/L and L33F in various subtypes from Burkina Faso and Cameroon³⁰ and N88D from at least one strain of subtype C and D in Djibouti³¹. Minor mutations L90M and M46L were also identified in the protease genes of recombinant subtypes involving 2-5 subtypes from treatment-naïve HIV-infected individuals in the Democratic Republic of Congo³⁵. Mutations associated with resistance to atazanavir were frequently seen in subtype C isolates and occasionally in CRF02_AG³⁴.

Development of resistance secondary to antiretroviral therapy in Africa in different subtypes

Table 2 summarizes data on phenotypic resistance and corresponding genotypic mutations among patients

with non-B subtypes after ART initiation from various countries across sub-Saharan Africa. Overall, the resistance patterns were similar to those with subtype B infections in North America and Europe. This therefore supports similar treatment approaches to those used for subtype B infections³⁷.

The observed rapid emergence of drug resistance with the initial availability and use of ART prior to the global rollout was associated with poor adherence to treatment regimens, in part related to treatment costs⁴³, and poor treatment guidelines involving dual therapy^{39,41}. There were also some subtype-specific factors. For example, there was a higher frequency of resistance with subtype D (21/33) than subtype A (7/25) infected individuals in Uganda⁴⁵. In a study from Zimbabwe, 81% (17/21) of subtype C patients rapidly developed drug resistance mutations within about two months of ART initiation. Mutations at 15 RT and 11 PR positions were more common in subtype C than subtype B isolates³⁸.

Unplanned treatment interruptions, usually among patients who were unable to continue payment for their drugs, were associated with a high rate of virologic failure in many studies^{43,44}. In Kigali, Rwanda, 26 (43%) of 60 patients had virologic failure with a viral load > 1,000 viral copies/ml, and 11 of the 26 presented with major drug resistance mutations, mainly because of the high frequency of treatment interruptions⁴⁴. Drug resistance was observed in 58% of a patient cohort from Gabon after a mean of 17.7 months of ART drug experience, and in 21 of 128 (16.4%) patients after a median ART treatment period of 10 months in Cameroon.

Among ART-treated patients in Uganda with subtypes A and D and > 1,000 viral copies/ml, 61 of 94 (65%) patients who had phenotypic testing had evidence of phenotypic resistance, including resistance to a NRTI in 51 of 92 (55%), to NNRTI in nine of 16 (56%), and to a PI for eight of 37 (22%)³⁷. In a further study from Uganda, of the ART-resistant isolates, 68% had three or more mutations in the RT gene. Resistance mutations in protease were less prevalent, but only 34% of the patients were receiving a PI upon sample collection⁴⁵. Of note, in a study from Senegal, 39% of patients tested had resistance to drugs that they had never received⁴⁰.

Secondary mutations associated with nucleoside reverse transcriptase inhibitor resistance

In Cote d'Ivoire, 79% patients had genotypic resistance to at least one NRTI. Resistant mutations were

Table 2. Mutations found after initiation of antiretroviral therapy

Country	Prevalent HIV-1 subtypes	Time period	Number tested	Phenotypic resistance	Time on ART/ % resistance	2 ^o Genotypic resistance			Unique patterns/ remarks
						NRTI	NNRTI	PI	
Uganda ³⁷	A (n = 41) C (n = 3) D (n = 42) A/D (n = 4) D/A (n = 2) D/C (n = 1)	1999-2000	94	NRTI 51/92 (55%) NNRTI 9/16 (56%) PI 8/37 (22%)	≥ 90 days	T215Y/F (n = 22) M41L (n = 19) K70R (n = 10) D67N (n = 16) L210W (n = 9) M184V N/I (n = 74)	A98G (n = 2) K103K>N (n = 10) V108I/L (n = 1) Y181Y/C (n = 2) Y190P/H (n = 1) P236P/L (n = 2)	L10V/I/L/F (n = 10) K20V/M/T/R (n = 8) D30DN (n = 6) V32I (n = 2) M36I (n = 10) M46M/I/L (n = 3) I54V (n = 3) A71A/V (n = 1) G73T (n = 1) V77I (n = 7) V82A (n = 3) I84V (n = 1) N88/N/D (n = 3) L90L/M (n = 3)	Phenotypic and genotypic resistance patterns were similar to subtype B.
Zimbabwe ³⁸	C	2001	25	Resistant mutations (n = 21/25)	–	Not specified	None (n = 7/21) N184V (n = 11) T215Y/I/S/F (n = 8) K70R (n = 7) M41L (n = 6) D67N/G (n = 6)	Y181C (n = 2) V82I (n = 3) K20R (n = 4) I71V (n = 3) L10I (n = 2) G73S (n = 2)	Mutations at 15 reverse transcriptase and 11 protease positions more common in C than B.
Côte d'Ivoire ³⁹	A/G (89% of 86) A (10%) G (1%)	Aug 1998-April 2000	214 Analyzable sequences (n = 86/214)	Phenotypic results concordant with genotypic (n = 44/59)	At least 6 months	T215Y/F (n = 48) K70R (n = 30) M184V (n = 13) K65R (n = 4) Q151M (n = 2) V75T (n = 1) 44D (n = 1) 118RT (n = 1) D67N (n = 30) L214F (n = 38) M41L (n = 11) L210W (n = 6) F77L (n = 3) K219E/Q (n = 2) F116Y (n = 2)	G190A (n = 2) K103N (n = 1) I84V (n = 1) M36I (n = 50) L63P (n = 9) L10I/R/V (n = 7) V32I (n = 1) A71V (n = 1)	High-level resistance due to use of dual ART.	

(continued)

Table 2. Mutations found after initiation of antiretroviral therapy (continued)

Country	Prevalent HIV-1 subtypes	Time period	Number tested	Phenotypic resistance	Time on ART/ % resistance	2 ^o Genotypic resistance			Unique patterns/ remarks
						NRTI	NNRTI	PI	
Senegal ⁴⁰	CRF02-AG (n = 25) A (n = 5) C (n = 5) B (n = 3) CRF06 (n = 3) G (n = 2) D (n = 1) U/K (n = 1) O (n = 2)	Aug 1998-Feb 2001	68 treatment-naive with viral rebounds. 8/68 were resistant. n = 12 treatment-experienced 5/12 resistant mutations.	—	Median 18.4 months.	TM41L (n = 2) K65R (n = 1) D67N (n = 2) K70R (n = 1) V75T (n = 1) M184V (n = 6) T215Y (n = 4) K219E/R (n = 2)	— K20I (n = 1) L63P (n = 2) V82/T (n = 2) I84V (n = 1) N88D (n = 1)	L10V (n = 1) K20I (n = 1) L63P (n = 2) V82/T (n = 2) I84V (n = 1) N88D (n = 1)	Mutations reported at baseline which were again reported at follow-up are not captured here as secondary mutations.
Gabon ⁴¹	CRF02 (n = 12) A (n = 7) G (n = 3) D (n = 2) CRF11-cpx (n = 2) B (n = 1) H (n = 1) J (n = 1) CRF01-AE (n = 1)	Nov 2000	25 Resistant mutations (n = 21/25)	13 ART-naive from sero-survey and 22 experienced.	Mean 17.7 months of treatment.	T/D69N (n = 2) V75T (n = 1) O151M (n = 1) T215Y (n = 5) M184V (n = 2) K70N/R/K/E (n = 4) V118I (n = 2) K65R (n = 1) V75A/I (n = 2) F77L (n = 1) F116Y (n = 1) R211K (n = 8) M41L (n = 4) D67N (n = 2) R221K (n = 2)	Y181C (n = 1) M36I (n = 10) I93L (n = 2) K20R (n = 2) L63P (n = 2)	L10V/I (n = 6) M36I (n = 10) I93L (n = 2) K20R (n = 2) L63P (n = 2)	Mutations reported are from the 22 experienced patients who were receiving combinations of only 2 drugs. (DDI+D4T and AZT+3TC). Rapid resistance due to inappropriate use.
Uganda, Zimbabwe DART virology group ⁴²	A (n = 6) C (n = 8) all Zimbabwe D (n = 5) D/A (n = 1)	Started in 2003	20 of 53 had successful genotype. 53 Patients with viral rebound at 24 weeks were analyzed.	—	> 1,000 copies 15% Uganda, 24% Zimbabwe.	M41L (n = 8) D67N (n = 6) K70R (n = 5) T215F/N/Y (n = 10) M184V (n = 10) K65R (n = 3)	K103 (n = 1) none	Patients received combivir/ tenofovir. NNRTI mutation was seen in one patient despite no prior drug experience.	

(continued)

Table 2. Mutations found after initiation of antiretroviral therapy (continued)

Country	Prevalent HIV-1 subtypes	Time period	Number tested	Phenotypic resistance	Time on ART/% resistance	2 ^o Genotypic resistance			Unique patterns/remarks
						NRTI	NNRTI	PI	
Uganda ⁴³	A (n = 16), D (n = 15) A/D (n = 3) AE (n = 2)	Aug-Dec 2003	46 of 137 studied had viral load.	31/36 patients showed resistance.	At least 12 weeks of treatment.	M184V/I (n = 23) K219E/Q (n = 2) A62V (n = 1) D67G/N (n = 7) K65R (n = 2) V75I (n = 1) F77L (n = 1) Y115F (n = 1) F116Y (n = 1) V118I (n = 3) Q151M (n = 1) M41L (n = 5) L210W (n = 4) T215Y/F/I/N (n = 6)	K103 (n = 14) V108I (n = 4) G190A (n = 9) Y181C (n = 5) Y188H/L (n = 3) M230L (n = 3) V106A (n = 2) K101E (n = 3) L100I (n = 1) P225H (n = 1)	L10I/V (n = 4) K20I/R (n = 4) M36I/T (n = 10) M46I/L (n = 4) I54V (n = 2) A71V (n = 1) V82A/T (n = 2) L33F/I (n = 2) L24I (n = 1) L90M (n = 1) L63P (n = 1)	Paying patients; many unplanned treatment interruptions. Thus high level resistance.
Kigali, Rwanda ⁴⁴	A (n = 11) C (n = 8) CRF15/ CRF01_AE/A (n = 3)	April-June 2002	26/60 virologic failure had viral load > 1,000 22 analyzed; 11 had major mutations.	More than 3 months.	K65R (n = 2) V179I (n = 6) D67N (n = 2) K70R (n = 2) M184V (n = 4) M41L (n = 1) T69D (n = 1) V75A/V (n = 1) T215Y (n = 1)	K103N (n = 4) K101Q (n = 1) P236L (n = 1)	I93L (n = 6) M46I (n = 1) V82F (n = 1) L90M (n = 2)	High rate treatment interruptions.	

ART: antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor

found after six months in 78% of patients who had received AZT, and in 68.7% of those on 3TC³⁹. In a further study of phenotypic resistance, there was resistance to at least one NRTI in treatment-experienced patients in 39.7%: 42.6% to AZT, 14.7% to 3TC, and 1.5% to didanosine (DDI)⁴⁶.

In a study of patients infected with subtypes A, C, D from Uganda and Zimbabwe, M184V with or without nucleoside analog mutations (NAM) was the most common route to resistance, whereas K65R was identified less often. Eighteen of 20 genotypes from week 24 samples with a viral load > 1,000 viral copies/ml showed key resistance mutations in RT. Fourteen had M184V (10 with 1-4 additional NAM); one had three NAM only; and the remaining three had K65R. One participant with M184V had major NNRTI-associated mutations, despite no disclosed treatment with this drug class⁴².

Secondary mutations associated with nonnucleoside reverse transcriptase inhibitor resistance

The frequent use of NNRTI-based therapy, and the low genetic barrier to resistance with this class of drugs, explains why the majority of resistance mutations is seen with this drug class in Africa⁴³. The most common mutation detected among patients who take NVP as part of their therapy is K103N, which is invariably associated with cross-resistance to EFV, while Y181C is the most frequently selected mutation with users of EFV⁹.

Secondary mutations associated with protease inhibitors

Despite the presence of frequent polymorphisms prior to ART in patients infected with non-B subtypes, as already described, associated clinical PI resistance is rare. In a study conducted over a two-year period from 2000-2002 in Cameroon, the prevalence of PR mutations at the following five sites was: L10I/V (16%), K20R (8%), M36I (98%), L63P (13%), and V77I (6%). Those mutations identified were not specific to any particular subtype⁴⁷. Mutations to indinavir (M46I/L and V82A), saquinavir (G48V and L90M), ritonavir (V82A) and nelfinavir (D30N) were found in 6% of patients in another study in Cote d'Ivoire after only about six months on ART⁴⁶. Lower mutation rates have been seen in Cote d'Ivoire, with mutations in only one patient each to the drugs indinavir and ritonavir after six months of treatment³⁹.

Resistance mutations following mother to child transmission according to subtype

One or more NVP resistance mutations can be selected in women as early as seven days after single-dose NVP, and the K103N mutation may persist for six weeks to 12 months⁴⁸. This appears to vary with HIV subtype^{36,49-51}. The K103N-containing variants were rarely detected in pre-NVP samples in a study of African women in the USA. After administration of single-dose NVP, the proportion of women with K103N was lowest for subtype A (60/144; 41.7%) and highest for subtype C (44/63; 69.8%), with an intermediate prevalence for subtype D (52/94; 55.3%), with statistically significant differences for A vs. C ($p = 0.0001$), and A vs. D ($p = 0.0465$), but not C vs. D ($p = 0.09$)⁵¹. In comparative studies of children from Malawi and Uganda, mutations that confer resistance to NVP were also more frequent in infants with subtype C than with subtypes A and D (87 vs. 50%; $p = 0.016$)⁵⁰.

In Uganda, where subtypes A and D predominate, K103N and Y181C were the most common mutations detected⁵². Of 65 women with genotyping results on day 7 or after 6-8 weeks of single-dose NVP, eight had > 1 NVP resistance mutation detected seven days after single-dose NVP, 21 had NVP resistance mutations detected in one or both samples collected at either time points, and 11 had NVP resistance at both time points. Y181C was the most common NVP resistance mutation detected at seven days, and K103N at 6-8 weeks^{22,53}.

In South Africa, where subtype C is predominant, K103N resistance variants were present in almost all women at six weeks post-single-dose NVP, but this declined rapidly over time. Detectable K103N variants were found in 87.1% of RNA samples and 52.3% of DNA samples collected six weeks after single-dose NVP. This declined to 65.4%, 38.9%, and 11.3% in RNA at three, seven, and 12 months respectively⁵⁴. A novel mutation, V106M, was also present in seven out of 141 South African women (5%) infected with subtype C, six weeks after receiving NVP⁵⁵.

Important findings from a study in Cote d'Ivoire showed that short-course AZT plus 3TC with single-dose NVP followed by three days of postpartum AZT plus 3TC both prevented most peripartum HIV-1 transmission, and minimized the development of NVP resistance⁵⁶. No mutations associated with AZT resistance were identified in short-course AZT for PMTCT¹⁶.

Discussion

Major achievements have been made in the ART roll-out in Africa, but several challenges remain. In general, the data on prevalence and patterns of drug resistance remain limited to inform practice and policy, but this is likely to change over the next few years as rates of treatment failure to first-line and then second-line therapy increases, and results from resistance testing becomes available.

The major concern is that current ART scale-up continues in a setting where viral load monitoring is limited, and patients have prolonged failure on their first-line regimens prior to switching to second-line ART. Maintenance of high rates of adherence to ART, coupled with ongoing education about behavior change and other preventative strategies, will be critical to avoiding widespread ART resistance and onward transmission of drug resistance.

Although the high levels of resistance observed in the early 2000s were of major concern, these studies were small in size, and were representative usually of the limited number of patients who were paying for their own medications, with treatment interruptions and lack of monitoring due to financial constraints, and in the setting of a lack of management guidelines or support for treatment adherence. Large-scale introduction of ART in Africa has avoided the problem of sequential mono or dual therapy that characterized the ART treatment strategies of the late 1980s and 1990s in Europe and the USA and that led to patients with multidrug resistance and limited treatment options.

A further challenge in sub-Saharan Africa has been the adoption of single-dose NVP in PMTCT, which is associated with rapid selection of viral variants resistant to NVP and other NNRTI. These observations have raised several concerns; first about the long-term viability of single-dose NVP for PMTCT of HIV-1; whether single-dose NVP will be efficacious in subsequent pregnancies; and whether treatment with NNRTI-based regimens will need to be avoided for NVP-exposed women and infants, in light of the frequent development of resistance and archiving of selected viruses that could rebound in future treatment programs⁵⁷. A number of studies show that the resistance mutations that develop after single-dose NVP decay rapidly within 12 months and repeated PMTCT therapy is useful⁵⁴, while some others have showed this decay to be slow, lasting up to 36 months⁵⁸. Combination therapies have been shown to prevent the development of resistance, and this may result in a change in treatment guidelines away from single-dose NVP for PMTCT^{59,60}.

The significance of subtype differences and their genetic polymorphisms and response to ART remains controversial. However, key findings are that multiple polymorphisms are seen in the various non-B subtypes prevalent in Africa, compared with the B subtype in North America and Europe. There is also some preliminary evidence for differences in the genetic barrier to resistance across subtypes, which may result in a differential rate of accumulation of resistance mutations across subtypes. To date, there is no consistent evidence that subtype-specific mutations are of clinical relevance. However, the possibility remains that these differences may affect the response to ART in Africa.

Major challenges face HIV healthcare providers and health delivery systems in Africa to sustain the current ART scale-up effort. Currently, first-line therapies are fairly uniform for most developing countries and may select for similar mutations and resistance across Africa. There is an urgent need to undertake surveillance programs to map out resistance patterns since the beginning of ART rollout, and to identify key areas that can be strengthened to slow the development of resistance. Although the WHO has published approaches to track emergence of HIV drug resistance and transmission in countries scaling-up HIV treatment, the clinical and laboratory parameters most helpful in deciding "when to switch" remain poorly defined. Many other issues that could affect response to ART remain understudied, including: pharmacokinetic properties in African populations, interactions with African foods and herbal medicines, the influence of diarrhea illness on absorption, and the prevalence of counterfeit drugs.

In order to delay resistance, appropriate first- and even second-line therapies need to be established based on genetic and phenotypic documentation of resistance in various parts of Africa. It is important to note that ART monitoring in the majority of Africa is done using clinical and immunologic assays, and not virologic monitoring, which is the standard of practice in the developed world. In the patients who continue to have viral replication despite ART, selection of drug-resistant viruses is inevitable and may cause major challenges to HIV treatment in Africa^{3,4}. There is an expanding population of individuals in Africa who are failing therapy and will need a change to second-line, but who are not identified in the absence of virologic monitoring, which could lead to rapid development of resistance. In addition, data from several studies have shown the poor predictive value of immunologic criteria for ART failure, and that also a significant proportion of patients failing on clinical and immunologic criteria had undetectable viral loads⁶¹⁻⁶³.

Ongoing efforts by the WHO and research groups to document the patterns of HIV drug resistance in Africa should be encouraged and supported⁶⁴. Most of the research studies to date have reported on ART treatment cohorts with considerable infrastructure support, which may not be representative of the general population. Population-based studies that could reveal a snapshot of the current resistance patterns within various regions of Africa are urgently needed.

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