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Publication date
2013

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Citation for published version (APA):
Hamers, R. L. (2013). *Drug-resistant HIV-1 in sub-Saharan Africa: clinical and public health studies*. [Thesis, fully internal, Universiteit van Amsterdam].

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Chapter 6

HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study

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Lancet Infectious Diseases 2011;11(10):750-759

ABSTRACT

Background

There are few data on the epidemiology of primary HIV-1 drug resistance after the roll-out of antiretroviral treatment (ART) in sub-Saharan Africa. We aimed to assess the prevalence of primary resistance in six African countries after ART roll-out and if wider use of ART in sub-Saharan Africa is associated with rising prevalence of drug resistance.

Methods

We did a cross-sectional study in antiretroviral-naive adults infected with HIV-1 who had not started first-line ART, recruited between 2007 and 2009 from 11 regions in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. We did population-based sequencing of the *pol* gene on plasma specimens with greater than 1000 copies per mL of HIV RNA. We identified drug-resistance mutations with the WHO list for transmitted resistance. The prevalence of sequences containing at least one drug-resistance mutation was calculated accounting for the sampling weights of the sites. We assessed the risk factors of resistance with multilevel logistic regression with random coefficients.

Findings

2436 (94.1%) of 2590 participants had a pretreatment genotypic resistance result. 1486 participants (57.4%) were women, 1575 (60.8%) had WHO clinical stage 3 or 4 disease, and the median CD4 count was 133 cells per µL (IQR 62–204). Overall sample-weighted drug-resistance prevalence was 5.6% (139 of 2436; 95% CI 4.6–6.7), ranging from 1.1% (two of 176; 0.0–2.7) in Pretoria, South Africa, to 12.3% (22 of 179; 7.5–17.1) in Kampala, Uganda. The pooled prevalence for all three Ugandan sites was 11.6% (66 of 570; 8.9–14.2), compared with 3.5% (73 of 1866; 2.5–4.5) for all other sites. Drug class-specific resistance prevalence was 2.5% (54 of 2436; 1.8–3.2) for nucleoside reverse-transcriptase inhibitors (NRTIs), 3.3% (83 of 2436; 2.5–4.2) for non-NRTIs (NNRTIs), 1.3% (31 of 2436; 0.8–1.8) for protease inhibitors, and 1.2% (25 of 2436; 0.7–1.7) for dual-class resistance to NRTIs and NNRTIs. The most common drug-resistance mutations were K103N (43 [1.8%] of 2436), thymidine analogue mutations (33 [1.6%] of 2436), M184V (25 [1.2%] of 2436), and Y181C/I (19 [0.7%] of 2436). The odds ratio for drug resistance associated with each additional year since the start of the ART roll-out in a region was 1.38 (95% CI 1.13–1.68; p=0.001).

Interpretation

The higher prevalence of primary drug resistance in Uganda than in other African countries is probably related to the earlier start of ART roll-out in Uganda. Resistance surveillance and prevention should be prioritised in settings where ART programmes are scaled up.

Funding

Ministry of Foreign Affairs of the Netherlands.

INTRODUCTION

Sub-Saharan Africa has the highest prevalence of HIV-1 worldwide and access to combination antiretroviral treatment (ART) has expanded in recent years to reach millions of infected people, although access is not universal [1]. The use of standardised and affordable first-line combinations of antiretroviral drugs through a public health approach, including two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), has been crucial to allow the scale-up of ART [2]. However, concern has been raised about the public health implications of the emergence of resistance to antiretroviral drugs [3]. Mutations in the HIV genome that confer drug resistance, acquired during ART failure, might limit the response to subsequent lines of treatment. The threat of increased onward transmission of drug-resistant strains to newly infected people—primary drug resistance—has the potential to compromise the effectiveness of first-line ART regimens [4-7].

In developed countries, the wider use of ART has been associated with an initial increase [5,7,8] and subsequent stabilisation [9,10] of levels of primary resistance to NRTIs and NNRTIs. In Europe [9] and the USA [10] an estimated 9–15% of antiretroviral-naive people harbour viruses with at least one drug-resistance mutation, and therefore pretreatment resistance testing is recommended to guide individual therapy choices [11,12].

In developing countries, the use of less potent and less tolerable ART regimens, restricted access to virological monitoring, and inconsistent drug supply could accelerate the emergence of resistance [3,13]. Few data exist on the epidemiology of primary resistance after the scale-up of ART in sub-Saharan Africa, and the ability to compare previous studies has been limited by differences in study populations, time periods, and definitions of drug resistance. The prevalence of primary resistance has been estimated to be low (<5%) in surveys of individuals newly diagnosed with HIV in several African countries [14] and by mathematical modelling [15]. However, recent reports have suggested an increase in primary resistance in east and southern Africa, in parallel with the widespread distribution of ART [16,17]. Furthermore, the extent to which the genetic diversity in HIV-1 subtypes and recombinants in Africa might affect the emergence of resistance is controversial [18].

To establish the extent of HIV-1 drug resistance in sub-Saharan Africa, the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort was started in 2007 [19]. The aim of the present study was to establish the prevalence, distribution, and risk factors of primary drug-resistance in antiretroviral-naive individuals infected with HIV-1 in six African countries. We specifically sought to assess if the wider use of ART in sub-Saharan Africa is associated with an increasing prevalence of drug resistance in pretreatment populations.

METHODS

Participants

PASER-M is a multicentre, prospective cohort of individuals infected with HIV-1 receiving first-line or second-line ART in routine circumstances at 13 clinical sites situated in 11 regions, mainly major cities or urban areas, in six African countries (three sites in South Africa, three in Uganda, three in Zambia, two in Kenya, one in Nigeria, and one in Zimbabwe). The median site-specific enrolment period was 12 months (range 6–18 months) between March, 2007, and September, 2009. Cohort and site characteristics have been profiled elsewhere [19]. Our study focused on the epidemiology of primary drug-resistance per region. For the three collaborating sites situated in Lusaka, Zambia, we previously reported similar primary resistance prevalence and patterns [20]; therefore, for the purpose of our analysis we deemed it appropriate to group them into one region.

For our cross-sectional baseline analysis, we included PASER-M study participants if they were aged 18 years or older, infected with HIV-1, and eligible to start first-line ART in accordance with national guidelines—ie, advanced immunodeficiency (CD4 cell count <200 cells per μL) or advanced HIV disease (WHO clinical stage 3 or 4) [21]. We excluded individuals who reported previous use of antiretroviral drugs for treatment or prophylaxis. We reassessed, with a standard questionnaire, the antiretroviral drug histories of all individuals who were identified as harbouring HIV-1 with at least one detectable drug-resistance mutation, to minimise possible bias from the misclassification of individuals with undisclosed previous exposure to antiretroviral drugs as drug-naive. Our other exclusion criteria were pregnancy at study screening, or, in Nigeria, HIV-2 co-infection.

Participants provided written informed consent at enrolment. Participants were sequentially enrolled during a site-specific enrolment period of a maximum of 18 months. The study protocol was approved by the appropriate national and local research ethics committees at the collaborating sites and the Academic Medical Centre of the University of Amsterdam, Netherlands.

Procedures

Medical staff at each site extracted routine clinical and laboratory data recorded in medical records into standard case-report forms, which were double-entered into a central web-based database. For recorded values of CD4 cell counts, the most recent measurement before the date of enrolment was defined as the pre-treatment count.

To assess the possible population-level effect of ART programmes on the prevalence of primary resistance, we assessed the time that elapsed since the initial roll-out for each region as a proxy measure for the amount of circulating drug-resistant HIV-1 variants in the general population. We calculated the ecological time variable for each participant as the number of years elapsed between start of ART rollout in each region (estimated as July 1 of the calendar year) and the date of sampling of patients. We obtained information on the calendar year of start of ART roll-out in each region from UN General Assembly special session country reports[22] and the respective site study teams (calendar year range 2000–04).

EDTA (edetic acid)-anticoagulated plasma specimens were collected before the start of ART, and stored for later assessment of HIV RNA viral-load and genotypic drug-resistance testing. We did population-based sequencing of HIV-1 protease and codons 1–300 of reverse transcriptase on all specimens in which viral load was greater than 1000 copies per mL. Virological testing was done at two reference laboratories in South Africa (serving the sites in Kenya, Nigeria, South Africa, Zambia, and Zimbabwe) and Uganda (serving the sites in Uganda). Viral-loads were established with NucliSens EasyQ real-time assay (version 2.0; bioMérieux, Lyon, France) in South Africa or COBAS Ampliprep/COBAS Taqman assay (Roche, Branchburg, NJ, USA) in Uganda. Genotyping was done with in-house sequencing methods with an ABI Prism 3730 Analyzer (Applied Biosystems, Foster City, CA, USA) in South Africa [23] or BC CEQ 8000 Analyzer (Beckman Coulter Inc, Fullerton, CA, USA) in Uganda [24]. Sequences were manually edited with Sequencher (version 4.8; Genecodes, Ann Arbor, MI, USA) in South Africa or BioEdit (version 7.0.9.0) in Uganda. Both laboratories participated in external quality assessment schemes for genotypic drug-resistance testing [25]. The quality of the sequences was verified with ViroScore Suite (version 8.4; ABL SA, France). Drug-resistance mutations were identified on the basis of the 2009 WHO list for surveillance of transmitted resistance [26], with the Stanford Calibrated Population Resistance Analysis Tool [27]. We judged that sequences with genetic mixtures of wild-type and mutant sequences at amino-acid sites that code for drug-resistance mutations contained a resistant strain. HIV-1 subtypes were inferred from the *pol* sequences with the REGA algorithm [28] and confirmed with the STAR algorithm [29]. To predict the effect of the identified drug-resistance mutations on drug susceptibility, we used the Stanford drug-susceptibility algorithm (version 6.0.9) [26] to

classify sequences as susceptible (Stanford level 1 or 2), low-level resistance (Stanford level 3), intermediate-level resistance (Stanford level 4), or high-level resistance (Stanford level 5) to the drug classes and specific drugs. All sequences have been deposited in GenBank (webappendix panel).

Statistical methods

Because our original study design was a prospective cohort, we estimated a sample size of at least 190 individuals per site on the basis of the predicted virological outcome after 24 months of ART, accounting for attrition [19]. Assuming 95% (n=181) of individuals to be antiretroviral-naive before the start of ART [20] and a prevalence of primary resistance of 5% [16] the statistical power was 87% to discriminate the prevalence of resistance to within 4% with a 95% CI of 2.6–9.5, with a two-sided significance level of 5%. We compared categorical data with the χ^2 test and continuous data with the Kruskal Wallis test or one-way ANOVA, where appropriate. We calculated the prevalence of sequences containing at least one drug-resistance mutation accounting for the sampling weights of the sites, and further specified for each drug class (ie, NRTI, NNRTI, and protease inhibitors). We expressed prevalence estimates with a 95% CI based on the normal approximation to the binomial distribution.

We used multilevel analysis with random coefficients to assess the effects of explanatory variables, at the levels of individuals and sites (while accounting for the possible interdependence of observations clustered within sites) on two outcomes: any resistance and NNRTI-resistance. We assessed all variables separately and entered those associated ($p<0.1$) with the outcomes stepwise into the multivariate model. We assessed biologically plausible interactions in the multivariate model. Co-variables investigated were age, sex, WHO clinical stage, CD4 cell count (fitted as a continuous variable), HIV RNA load ($\log_{10}$ transformed, fitted as a continuous variable), HIV-1 subtype, HIV exposure category, patients' performance status, clinical site administration, calendar year of sampling, and the time since start of ART roll-out in the region (fitted as a continuous variable). We expressed our results as odds ratios (ORs) with 95% CI and two-sided p values, with $p<0.05$ being statistically significant. We did all analyses with Stata version 10.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Viral load measurements before treatment were available for 2574 (99.0%) of 2601 participants included in our study, of whom 2478 (96.3%) had a value of greater than 1000 copies per mL (figure 1). Upon reassessment of the antiretroviral drug histories of the 150 participants (6.1%) who harboured virus with at least one drug-resistance mutation, 96 were confirmed antiretroviral-naive, 11 reported previous antiretroviral drug use (five ART, one post-exposure prophylaxis, and six prophylaxis for prevention of mother-child transmission), and 43 had an unconfirmed previous antiretroviral drug status because they had died or were lost to follow-up. After exclusion of all participants with previous

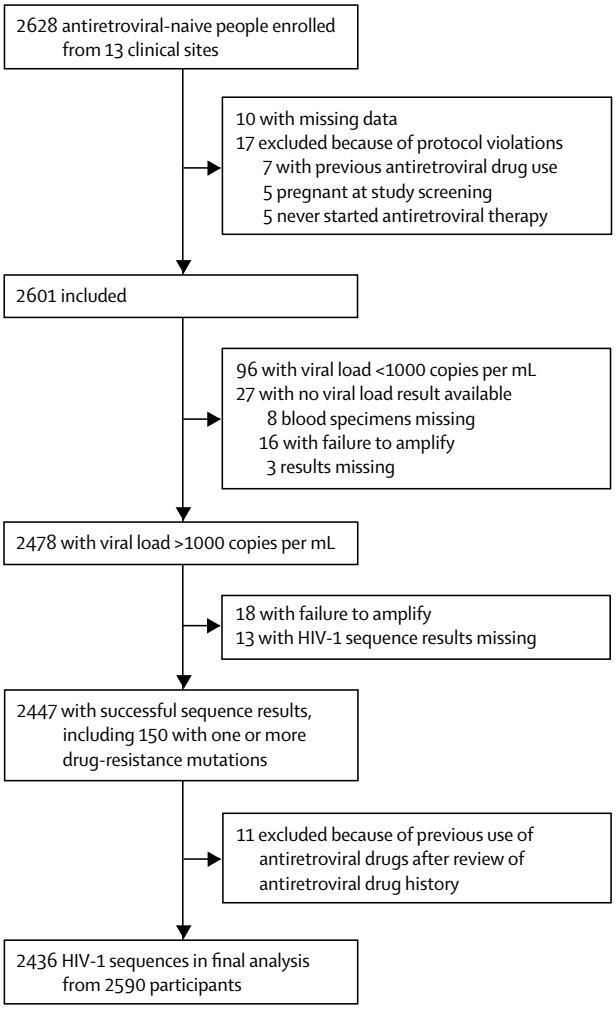

Figure 1. Study profile

antiretroviral exposure, the final analysis included 2590 participants, and a pretreatment sequence result was available for 2436 (94.1%; figure 1).

The median number of participants per site was 213 (range 182–552) and 57.4% were women (table 1). Mean age was 38.0 years (SD 9.0), and women were younger than men (36.2 years [SD 8.6] *vs* 40.3 years [SD 9.0]; p<0.0001). HIV acquisition was predominantly through heterosexual contact (66.5%) or unknown (32.8%). Nearly all participants (2570; 99.2%) were native residents in their countries. Advanced HIV disease (WHO stage 3 or 4) was present in 60.8% of participants (table 1). Initial first-line ART regimens were almost exclusively NNRTI-based (2582; 99.7%), with dual-NRTI-backbones consisting of zidovudine (962; 37.2%), tenofovir (866; 33.5%), stavudine (688; 26.6%), or abacavir (66; 2.6%) combined with either lamivudine (1740; 67.4%) or emtricitabine (842; 32.6%). The remaining patients were prescribed regimens based on protease inhibitors (six; 0.2%) or triple NRTIs (two; 0.1%). The median time elapsed since the start of ART roll-out in the region was 4.7 years (IQR 4.0–6.0; range 2.8–8.0).

HIV-1 subtype C was most commonly identified followed (in descending order) by A, D, A/G recombinant, G, and other subtypes and recombinants (table 1); we classed six as other subtypes (0.3%) and 44 as other recombinants (1.8%). Subtype C was predominant in the regions of South Africa (98.0%), Zambia (97.7%), and Zimbabwe (99.5%); A and D were most common in Uganda (57.5% and 38.4%, respectively) and Kenya (67.1% and 12.6%, respectively); and A/G (59.1%) and G (29.6%) in Nigeria (table 1).

The overall prevalence of resistance was 5.6% (95% CI 4.6–6.7%), which included 2.5% (1.8–3.2) resistance associated with NRTIs, 3.3% (2.5–4.2) associated with NNRTIs, and 1.3% (0.8–1.8) associated with protease inhibitors (figure 2, table 2, and webappendix table 1). Of the 139 sequences with at least one drug-resistance mutation, resistance was confined to a single drug-class for 112 sequences (80.6%), and 104 sequences (74.8%) had a single mutation. Dual-class resistance to NRTIs and NNRTIs was uncommon (25 sequences; sample-weighted proportion 1.2%, 95% CI 0.7–1.7), and triple-class resistance was rare (two sequences; 0.1%, 0.0–0.3). The most common drug-resistance mutations for NNRTIs were K103N, Y181C/I, and G190A/S; for NRTIs, the most common mutations were thymidine analogue mutations (TAMs) and M184V. Of the TAMs, M41L was identified most, then T215F/Y, K70R, and D67N and K219E (table 2). The K65R mutation, associated with cross-resistance to the NRTI class, was noted in one participant from Kampala. In the protease gene a high frequency of various naturally occurring polymorphisms was recorded, but few significant drug-resistance mutations, the most common being M46I/L and L90M (table 2). Of the 139 sequences with at least one drug-resistance mutation, 84 (59.2%, 95% CI 50.0–68.3) had high-level resistance (Stanford

Table 1. Baseline characteristics

Country		Zambia		South Africa			Uganda		Kenya		Zimbabwe	Nigeria
	Total (n=2590)	Lusaka* (n=555)	Pretoria (n=182)	Johannesburg (n=195)	White River (n=215)	Kampala (n=188)	Fort Portal (n=195)	Mbale (n=220)	Mombasa (n=210)	Nairobi (n=213)	Harare (n=213)	Lagos (n=204)
Site type		PFP, NGO, FBO	PFP	Public	NGO	Public	Public	Public	Public	FBO	NGO	Public
Year of start of ART rollout		2003	2004	2004	2004	2000	2002	2001	2003	2003	2004	2002
Enrolment period (month/year)		03/07-09/08	05/07-07/08	09/07-11/08	11/07-06/08	01/08-06/08	01/08-10/08	02/08-09/08	10/07-07/08	02/08-06/09	09/08-09/09	09/08-07/09
Sex												
Female	1486 (57.4)	300 (54.1)	93 (51.1)	145 (74.4)	127 (58.5)	100 (53.2)	107 (54.9)	116 (52.7)	126 (60.0)	114 (53.5)	137 (63.9)	124 (60.8)
Male	1104 (42.6)	255 (46.0)	89 (48.9)	50 (25.6)	90 (41.5)	88 (46.8)	88 (44.1)	104 (47.3)	84 (40.0)	99 (46.5)	77 (36.2)	80 (39.2)
Mean age, yrs (SD)	38.0 (9.0)	38.0 (9.1)	38.7 (8.5)	36.6 (7.4)	39.0 (10.1)	36.5 (8.9)	37.7 (10.3)	38.7 (9.6)	37.4 (8.6)	38.8 (8.7)	38.9 (8.8)	37.1 (8.5)
WHO clinical stage												
1 or 2	1015 (39.2)	251 (45.2)	71 (39.0)	125 (64.1)	57 (26.5)	70 (37.2)	70 (35.9)	44 (20.0)	79 (37.6)	63 (29.6)	100 (47.0)	85 (41.7)
3 or 4	1575 (60.8)	304 (54.8)	111 (61.0)	70 (35.9)	158 (73.5)	118 (62.8)	125 (64.1)	176 (80.0)	131 (62.4)	150 (70.4)	113 (53.1)	119 (58.3)
Median CD4 count, cells/μL (IQR) †	133 (62-204)	131 (63.5-198.5)	140 (50.5-229.5)	94.5 (28.8-160.3)	94.5 (35-154)	130.5 (49.3-211.8)	177 (94.5-259.5)	114 (50-178)	130.5 (63.5-194)	165 (106-224)	186 (112.5-259.5)	134 (68-200)
Mean HIV-RNA, $\log_{10}$ c/ml (SD) ‡	4.90 (1.0)	4.95 (0.9)	4.58 (1.0)	4.60 (1.0)	4.89 (0.8)	5.37 (0.8)	5.20 (0.9)	5.50 (0.8)	4.67 (0.9)	4.54 (0.9)	4.56 (1.2)	4.96 (1.0)

Table 1 (continued)

Country		Zambia		South Africa			Uganda			Kenya		Zimbabwe	Nigeria
	Total (n=2590)	Lusaka* (n=555)	Pretoria (n=182)	Johannesburg (n=195)	White River (n=215)	Kampala (n=188)	Fort Portal (n=195)	Mbale (n=220)	Mombasa (n=210)	Nairobi (n=213)	Harare (n=213)	Lagos (n=204)
HIV-1 subtypes §												
A	609 (25.0)	3 (0.6)	1 (0.6	1 (0.6)	0	100 (55.9)	102 (56.0)	126 (60.3)	131 (64.2)	140 (70.0)	0	5 (2.7)
C	1323 (54.3)	513 (97.7)	170 (96.6)	173 (97.2)	207 (100.0)	7 (3.9)	5 (2.8)	4 (1.9)	25 (12.3)	28 (14.0)	189 (99.5)	2 (1.1)
D	275 (11.3)	1 (0.2)	2 (1.1)	0	0	70 (39.1)	73 (40.1)	76 (36.4)	28 (13.7)	23 (11.5)	0	2 (1.1)
G	64 (2.6)	3 (0.6)	0	0	0	1 (0.6)	0	0	2 (1.0)	2 (1.0)	1 (0.5)	55 (29.6)
A/G	115 (4.7)	3 (0.6)	0	1 (0.6)	0	0	1 (0.6)	0	0	0	0	110 (59.1)
Other	50 (2.1)	2 (0.4)	3 (1.7)	3 (1.7)	0	1 (0.6)	1 (0.6)	3 (1.4)	18 (8.8)	7 (3.5)	0	12 (6.5)

Data are number of participants (%) unless otherwise stated. NGO, non-governmental organization; FBO, faith-based organization; PFP, private for profit; ART, antiretroviral therapy. *Combines participants from three clinical sites in Lusaka. †Data for n=2580. ‡Data for n=2563. §Data for n=2436.

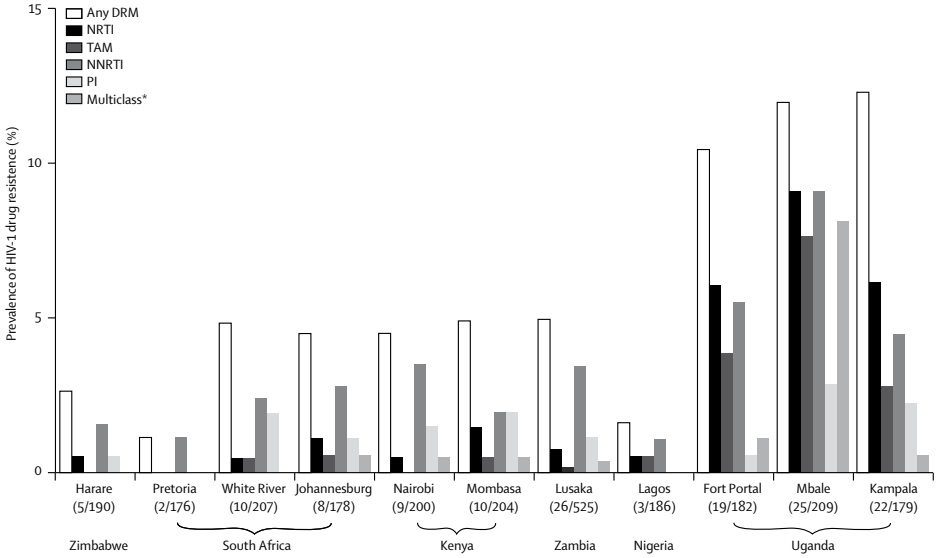

Figure 2. Prevalence of HIV-1 primary drug-resistance in antiretroviral-naive individuals in the PASER-M cohort, by region and drug class

People with at least one drug-resistance mutation shown as proportion of all people by region and drug class. Regions are clustered by country and sorted by descending calendar year of rollout of antiretroviral therapy. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor; TAM, thymidine analogue mutation. * Multiclass, resistance to at least two drug classes

5), 25 (17.4%, 10.5–24.2) had intermediate-level resistance (Stanford 4), and 30 (23.5%, 15.5–31.4) had low-level resistance (Stanford 3). 84 sequences (60.1%, 95% CI 50.9–69.3) showed loss of susceptibility to NNRTIs (nevirapine 60.1%, efavirenz 59.3%), 51 (41.4%, 32.1–50.7) to NRTIs (lamivudine or emtricitabine 23.7%, zidovudine 28.2%, stavudine 29.2%, tenofovir 13.6%), and 13 (6.1%, 2.4–11.4) to ritonavir-boosted protease inhibitors.

The prevalence of primary drug resistance varied substantially between regions, ranging from 1.1% (95% CI 0.0–2.7) in Pretoria, South Africa, to 12.3% (7.5–17.1) in Kampala, Uganda (figure 2 and webappendix table 1). The pooled prevalence for all three Ugandan sites—situated in east, west, and central Uganda—was 11.6% (66 of 570; 95% CI 8.9–14.2), compared with 3.5% (73 of 1866; 95% CI 2.5–4.5) for all other sites. In Mbale, east Uganda, an unexpectedly high proportion of participants harboured the M184V (17 of 209; 8.1%, 95% CI 4.4–11.8) and T215F/Y mutations (11 of 209; 5.3%, 2.2–8.3), whereas these mutations were uncommon at all other sites (webappendix table 2). The exclusion of the 17 Mbale participants who harboured M184V would reduce the overall resistance prevalence to 4.8% (122 of 2419; 95% CI 3.8–5.7), the prevalence of M184V to 0.3% (eight of 2419; 0.1–0.5) and of T215F/Y to 0.0% (none of 2419; 0.0–0.3), and the Mbale site-specific overall resistance prevalence would decrease from 12.0% (25 of 209; 7.6–16.4) to 4.2% (eight of 192; 1.4–7.0; webappendix table 2).

Table 2. Frequencies of primary drug-resistance mutations.

	N	Proportion (95% CI) of sequences carrying DRMs (n=139)	Proportion (95% CI) of all sequences (n=2436)
Any DRM	139	100.0	5.6 (4.6, 6.7)
NRTI			
Any	54	43.7 (34.4, 53.0)	2.5 (1.8, 3.2)
Any TAM*	33	28.0 (19.6, 36.3)	1.6 (1.0, 2.1)
≥2 TAMs	13	10.9 (5.0, 16.7)	0.6 (0.3, 1.0)
M41L	16	14.0 (7.5, 20.5)	0.8 (0.4, 1.2)
K65R	1	0.7 (0.0, 2.0)	0.04 (0.0, 0.1)
D67E/G/N	12	9.9 (4.4, 15.4)	0.6 (0.2, 0.9)
K70R	11	8.5 (3.4, 13.7)	0.5 (0.2, 0.8)
M184V†	25	21.4 (13.7, 29.2)	1.2 (0.7, 1.7)
T215F/Y‡	14	12.8 (6.4, 19.1)	0.7 (0.3, 1.1)
K219E/Q	8	6.2 (1.7, 10.7)	0.4 (0.09, 0.6)
NNRTI			
Any	83	59.3 (50.1, 68.5)	3.3 (2.5, 4.2)
≥2	16	9.8 (4.2, 15.3)	0.6 (0.2, 0.9)
K101E	11	8.3 (3.0, 13.6)	0.5 (0.2, 0.8)
K103N/S	46	31.8 (23.1, 40.4)	1.8 (1.2, 2.4)
Y181C/I	19	12.3 (6.1, 18.6)	0.7 (0.3, 1.1)
G190A/S	17	12.1 (6.0, 18.2)	0.7 (0.3, 1.0)
PI			
Any	31	22.4 (14.6, 30.2)	1.3 (0.8, 1.8)
M46I/L	9	6.8 (2.2, 11.3)	0.4 (0.1, 0.6)
L90M	7	3.1 (0.0, 6.3)	0.2 (0.0, 0.4)

Proportions and CIs calculated accounting for the sampling weights of the sites. The table lists all drug-resistance mutations (DRMs) from the 2009 WHO DRM list, identified in 0.3% or greater of all sequences. DRMs identified in less than 0.3% of sequences were T69D, K70E, L74I/V, V75M, F77L, Y115F, L100I, V106M, Y188C/L, L210W, P225H in reverse-transcriptase and L23I, L24I, D30N, I50L/V, I54T/V, N83D, I85V, N88D in protease. NRTI, nucleoside reverse transcriptase inhibitor; TAM, thymidine analogue mutation; NNRTI, non-NRTI; PI=protease inhibitor. *M41L, D67N, K70R, L210W, T215Y/F, K219Q/E. †Exclusion of Mbale site reduces M184V to a frequency of 8. ‡Frequency of individual mutations at codon 215: F 4; I 3; S 1; and Y 10.

For additional quality control, we sent the 17 electropherograms from Mbale, which contained several drug-resistance mutations, to the South African laboratory for repeat analysis by an experienced laboratory technician from whom the first result was masked. The inter-observer result was concordant for 95 drug-resistance mutation pairs and discordant for four pairs of wild-type and drug-resistance mutation mixtures (reverse transcriptase positions 41, 67, 190, and 210), yielding no evidence for a significant difference (McNemar $p>0.1$). Additionally, viral phylogenetic trees for each site showed that each sequence was from a different individual and there was no evidence of laboratory

carry-over contamination. The previous antiretroviral-drug status was unconfirmed for only two Mbale participants, because they had died.

In multivariate analyses, the OR for drug resistance associated with each additional year since the start of the ART roll-out in a region was 1.38 (95% CI 1.13–1.68; p=0.001; table 3). In four sensitivity analyses, done by including the 11 participants who disclosed previous antiretroviral drug use, excluding Mbale, excluding all participants harbouring M184V, and excluding Mbale and all participants harbouring M184V, the strength of the association with time since start of ART roll-out did not significantly change (range OR 1.26–1.41 for each additional year). For NNRTI-resistance, the OR for each additional year since start of ART roll-out was 1.35 (1.01–1.81; p=0.041).

Table 3. Demographic and clinical factors associated with primary HIV-1 drug resistance.

	Number of sequences	**Prevalence of HIVDR**	**Univariate analysis***			**Multivariate analysis†**		
	N	N (%)	OR	95% CI	p-value	OR	95% CI	p-value
Total	2436	139						
Sex								
Female	1388	70 (5.0)	Reference					
Male	1048	69 (6.6)	1.33	0.94, 1.87	0.106			
Age (years)	37.9 (9.04)‡	38.3 (9.39)‡	1.01§	0.99, 1.02	0.591			
WHO clinical stage								
I	355	14 (3.9)	Reference					
II	582	38 (6.5)	1.70	0.91, 3.19	0.097			
III	1087	63 (5.8)	1.50	0.83, 2.71	0.180			
IV	412	24 (5.8)	1.51	0.77, 2.96	0.234			
CD4 count (cells/µl)	131 (61-202)¶	122 (44-181)¶	0.85 ‖	0.70, 1.04	0.108			
HIV RNA (log10 c/ml)	5.01 (0.81)‡	5.16 (0.78)‡	1.03**††	0.82, 1.30	0.777††			
Probable mode of infection								
Heterosexual contact	1620	103 (6.4)	Reference					
Other exposures‡‡	17	1 (5.9)	0.92	0.12, 7.01	0.936			
Unknown	799	35 (4.4)	0.67	0.46, 1.00	0.050			
HIV-1 subtype								
A	609	43 (7.1)	Reference			Reference		
C	1232	54 (4.1)	0.56	0.37, 0.85	0.006	1.08	0.59, 1.95	0.808
D	275	35 (12.7)	1.92	1.20, 3.07	0.007	1.61	0.99, 2.60	0.053
G	64	2 (3.1)	0.42	0.10, 1.79	0.244	0.48	0.11, 2.12	0.330

Table 3 (continued)

	Number of sequences	Prevalence of HIVDR	Univariate analysis*			Multivariate analysis†		
	N	N (%)	OR	95% CI	p-value	OR	95% CI	p-value
A/G	115	1 (0.9)	0.12	0.02, 0.85	0.034	0.13	0.02, 0.98	0.048
Other	50	4 (8.0)	1.14	0.39, 3.33	0.804	1.78	0.59, 5.37	0.308
Performance status§§								
Optimum	1240	51 (4.11)	Reference			Reference		
Reduced	974	82 (8.4)	2.14	1.50, 3.07	<0.001	1.68	1.12, 2.53	0.012
Sector								
Non-government	1298	52 (4.0)	Reference					
Public	1138	87 (7.6)	1.98	1.39, 2.82	<0.001			
Calendar year of sampling								
2007	581	28 (4.8)	Reference			Reference		
2008	1511	99 (6.6)	1.38	0.90, 2.13		0.64	0.36, 1.13	0.121
2009	344	12 (3.5)	0.71	0.36, 1.42		0.56	0.26, 1.21	0.143
Time since ART rollout in region (years)	4.68 (3.98-5.97)¶	5.65 (4.17-7.14)¶	1.38§	1.22, 1.56	<0.001	1.38§	1.13, 1.68	0.001

*Univariate logistic regression. †Multilevel multivariate logistic regression analysis. ‡Mean (SD). §OR per year. ¶Median (IQR). ||OR per 100 cells per µL CD4 count increase (nine values missing). **OR per 1 $\log_{10}$ copies per mL HIV-1 RNA increase. ††Adjusted for HIV-1 RNA assay. ‡‡Includes recipients of blood products, homosexual contact, and perinatal transmission. §§WHO performance scale (222 values missing). HIVDR, HIV-1 drug resistance; OR, odds ratio; ART, combination antiretroviral therapy.

Additionally, the association of any resistance with subtype was of marginal significance, with risk increased for D and reduced for A/G, compared with subtype A (table 3). Risk of resistance was increased for individuals who had a reduced performance status (table 3). Any resistance and NNRTI-resistance were not associated with sex, age, type of exposure, viral load, and site administration, including no apparent association with CD4 cell count and WHO stage—both markers of duration of infection. We did not identify significant interactions between region, calendar year of sampling, subtype, and time since ART roll-out.

DISCUSSION

Prevalence of primary drug resistance in antiretroviral-naive individuals is substantially higher in Uganda, where antiretroviral drugs were first available, compared with other African countries. Resistance is mostly confined to a single drug-class, most commonly NNRTIs. The spectrum of the major drug-resistance mutations in non-B subtype infected

Africans is largely similar to those known from studies in people infected with subtype B in developed countries [5,7-10]. The risk of primary resistance in a region rose by 38% for each additional year that elapsed since the start of the local ART roll-out. We confirmed the validity of this association in sensitivity analyses that eliminated possible effects of a single site (Mbale) and undisclosed previous antiretroviral-drug exposure (M184V mutation). The strengths of our study were its large international sample of patients, representing pretreatment populations in routine clinical practice, and the use of standardised data collection and measurement methods, which allowed for comparison across sites.

Our study provided the opportunity to make direct comparisons between regions and countries, investigating the effect of the timing of introduction of ART, as a proxy for the amount of circulating drug-resistant HIV-1 strains at the population level, on the level of primary resistance. Since populations can differ in other aspects, separation of the effects of the exposure alone is difficult. However, no evidence exists of other major differences between countries in terms of virological failure rates, drug regimens used, adherence levels, or drug supply continuity, which could provide an alternative explanation for our recorded differences in the prevalence of primary resistance. One exception is South Africa, which is the only country that has included routine viral-load monitoring in its national ART programme [30]. Our findings, therefore, support the hypothesis that the widespread distribution of ART in Africa is driving the emergence of primary drug resistance; this has important implications for public health, given that options for alternative treatment regimens are restricted in most settings. A limitation in this respect is that our results cannot be extrapolated outside the range of our measured exposure levels of 3–8 years since start of ART roll-out.

Our findings in Uganda accord with two recent reports (panel) that suggest rising levels of transmitted resistance in the region [16, 17]: from 3% (2005–06) to 7% (2007–08) in 408 people recently infected with HIV in east and southern Africa [16], and from 0% (2006–07) [31] to 8·6% (2009–10) in 70 newly diagnosed people in Kampala, Uganda [17]. After the limited-scale distribution of life-saving antiretroviral drugs in and around Kampala since the mid-1990s [32] access to ART became more widely available nationally since 2000–01, which contrasts with other countries in the region, which started scaling up ART since late 2003 and 2004. Therefore, other countries might anticipate an increase in primary resistance in coming years, similar to Uganda.

Drug-resistance mutations associated with NNRTIs were most common, which is consistent with the widespread use of this drug class as part of standard first-line ART, as well as single-dose nevirapine for prevention of mother to child transmission [33], the low

Panel: Research in context

Systematic review

We searched PubMed for English-language studies on primary antiretroviral-drug resistance in sub-Saharan Africa, published in 2001–11 with the MeSH terms "viral drug resistance","HIV-1", and "sub-Saharan Africa". Of 147 search results, we identified 38 eligible studies from 23 African countries (supplementary table 3). The comparability of most early studies was limited by differences in study populations, time periods, and definitions of drug resistance. Several WHO surveys of people newly diagnosed with HIV-1 estimated transmitted resistance to be low (<5%)[14]. Two recent reports suggested rising levels of transmitted resistance: from 3% (2005–06) to 7% (2007–08) in 408 people recently infected with HIV in east and southern Africa [16], and from 0% (2006–07) [31] to 8.6% (2009–10) in 70 newly diagnosed people in Kampala, Uganda [17].

Interpretation

Our observational multicentre study in six African countries is the first study in Africa we know of to clearly show an association between the time since start of ART roll-out in a region and the prevalence of primary drug-resistance. Our findings support the hypothesis that the ART roll-out in Africa is driving the emergence of primary drug-resistance. Resistance surveillance and prevention should be prioritized in settings where ART programmes are scaled up.

genetic barrier for the development of resistance to this class [34], the high prevalence noted in treated patients [13], persistence because of restricted fitness cost [35], and other local reports of primary resistance [16, 17]. The prevalence of NNRTI-resistant strains was not higher in women than in men, despite the possibility of undisclosed previous use of maternal prophylaxis to prevent mother-to-child transmission in women. However, this does not rule out the possibility of substantial onward transmission of these strains to men, and our study design did not allow a quantification of the extent to which the use of single-dose nevirapine contributed to the levels of primary resistance. TAMs, related to the extensive use of zidovudine and stavudine as part of first-line regimens [36], were more common in Uganda than in the other countries. In addition to the early ART roll-out in Uganda, we speculate that the restricted use of non-potent mono and dual regimens of thymidine analogues in Uganda before potent ART became available [32], might have contributed to the circulation of TAMs, as happened in developed countries [5, 7, 8].

The validity of measuring resistance in antiretroviral-naive, chronically infected populations is debatable for two main reasons. Because our study population was probably infected on average several years earlier, the detected primary resistance patterns relate to the lower availability of antiretroviral drugs and consequently fewer circulating drug-resistant strains in the past. Although transmitted drug-resistant variants might persist for a couple of years in antiretroviral-naive individuals [35, 37], chronic infection provides the opportunity for reversion to wild-type virus or diminution to levels below detection by population-based genotyping [38]. This reversion or diminution might result in an underestimation of the prevalence of primary resistance. Second, some of the

detected drug-resistance mutations might be acquired because of undisclosed previous antiretroviral-drug exposure. Through targeted efforts, we excluded an additional 11 of 107 individuals who disclosed previously unreported exposure to ART, post-exposure prophylaxis, or drugs given for prevention of mother-to-child transmission. On the basis of this finding, we can estimate that, of the 43 individuals who had an undetermined previous antiretroviral drug status, we might have missed another four antiretroviral-exposed individuals. The possibility of undisclosed exposure to ART was highlighted in Mbale, where we recorded an unexpectedly high frequency of the M184V mutation.

Nonetheless, given the challenges of identifying individuals during recent HIV infection, we argue that there is value in surveying resistance in populations starting ART. Particularly, resistance data in pretreatment populations provide important information about the probable effectiveness of available regimens for each region. Our study population comprised mostly free-access, regular ART programmes in urban areas where massive ART programmes have been implemented in recent years. However, our sites were not necessarily representative of all people with HIV/AIDS in their respective countries and caution is warranted when extrapolating results to different subpopulations, countries, or rural areas.

Prevalence of resistance was higher in subtype D than in subtype A. Given the lack of evidence for clinically relevant differences between subtypes with regard to the genetic barrier to resistance [39] and clinically significant drug-resistance mutations [18] and that subtype is highly correlated with region, the differential risk between subtypes is most probably confounded by differences in antiretroviral-drug selective pressure between regions. Other potential determinants of resistance transmission, especially accurate data on the route, time, and source of infection, and the source's antiretroviral drug history, were not available for our analysis.

In conclusion, we showed an association between the duration of ART availability in African settings and the level of primary HIV-1 drug-resistance. The high primary resistance prevalence that we identified in Uganda, where antiretroviral drugs were first available, presents an unequivocal warning to other nearby countries. Future treatment guidelines in Africa should take into account the local levels of primary resistance. Further studies are needed to establish the cost-effectiveness of extended genotypic resistance testing in Africa. Our results provide a basis for repeated epidemiological studies to measure the population effects of HIV-treatment programmes over time.

ACKNOWLEDGMENTS

We thank the study participants, the staff at the collaborating clinical sites and laboratories, and the support staff at PharmAccess Foundation and Contract Laboratory Services. We are indebted to Nicaise Ndembi (Institute of Human Virology Nigeria), Maarten Schim van der Loeff (Public Health Service of Amsterdam), Annemarie Wensing (University Medical Centre Utrecht), and Joep Lange (Academic Medical Centre Amsterdam) for useful comments on earlier drafts of this manuscript. PASER is part of the LAASER (Linking African and Asian Societies for an Enhanced Response to HIV/AIDS) programme, a partnership of Stichting Aids Fonds, The Foundation for AIDS Research (amfAR)—TREAT Asia, PharmAccess Foundation, and International Civil Society Support.

Funding

The PharmAccess African Studies to Evaluate Resistance (PASER) is an initiative of PharmAccess Foundation, with financial support provided by the Ministry of Foreign Affairs of The Netherlands through a partnership with Stichting Aids Fonds (grant 12454).

Contributors

TFRW conceived the study and was the principal investigator. RLH, CLW, WSS, RS, MvV, and TFRW designed the study and developed the protocol. RLH, KCES, and MvV contributed to implementation. CK, MS, FC, MB, KM, MW, and AO established the cohort and supervised data collection. CLW, IN, and WSS conducted and supervised the laboratory testing. RLH conducted the sequence and statistical analyses and drafted the manuscript. FWW checked and supervised the statistical analyses. CLW, KCES, RS, FWW, and TFRW provided input to interpretation of the data and critically reviewed the paper for important intellectual content. All authors reviewed and approved the final version.

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REFERENCES

1. WHO, UNAIDS, UNICEF. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector—progress report 2010. Geneva, 2010. http://www.who.int/hiv/pub/2010progressreport/report/en/index.html (accessed Jan 19, 2011).
2. Gilks CF, Crowley S, Ekpini R, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet 2006; 368: 505–10.
3. Wainberg MA, Friedland G. Public health implications of antiretroviral therapy and HIV drug resistance. JAMA 1998; 279: 1977–83.
4. Kuritzkes DR, Lalama CM, Ribaudo HJ, et al. Preexisting resistance to nonnucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment-naive HIV-1-infected subjects. J Infect Dis 2008; 197: 867–70.
5. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med 2002; 347: 385–94.
6. Wittkop L, Gunthard HF, de Wolf F, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. Lancet Infect Dis 2011; 11: 363–71.
7. Grant RM, Hecht FM, Warmerdam M, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA 2002; 288: 181–88.
8. Wensing AM, van de Vijver DA, Angarano G, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis 2005; 192: 958–66.
9. Vercauteren J, Wensing AM, van de Vijver DA, et al. Transmission of drug-resistant HIV-1 is stabilizing in Europe. J Infect Dis 2009; 200: 1503–8.
10. Wheeler WH, Ziebell RA, Zabina H, et al. Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. AIDS 2010; 24: 1203–12.
11. EACS. Guidelines Clinical Management and Treatment of HIV Infected Adults in Europe. Paris: European AIDS Clinical Society, 2010.
12. Thompson MA, Aberg JA, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. JAMA 2010; 304: 321–33.
13. Gupta RK, Hill A, Sawyer AW, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis 2009; 9: 409–17.
14. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antivir Ther 2008; 13 (suppl 2): 25–36.
15. Blower S, Bodine E, Kahn J, McFarland W. The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models. AIDS 2005; 19: 1–14.
16. Price MA, Wallis CL, Lakhi S, et al. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS Res Hum Retroviruses 2011; 27: 5–12.
17. Ndembi N, Hamers RL, Sigaloff KC, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. AIDS 2011; 25: 905–10.
18. Kantor R, Katzenstein DA, Efron B, et al. Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: results of a global collaboration. PLoS Med 2005; 2: e112.

19. Hamers RL, Oyomopito R, Kityo C, et al. Cohort profile: the PharmAccess African (PASER-M) and the TREAT Asia (TASER-M) Monitoring Studies to Evaluate Resistance—HIV drug resistance in sub-Saharan Africa and the Asia-Pacific. Int J Epidemiol 2011; published online April 21. DOI:10.1093/ije/dyq192.
20. Hamers RL, Siwale M, Wallis CL, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr 2010; 55: 95–101.
21. WHO. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach, 2006 revision. Geneva: World Health Organization, 2006. http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf (accessed Jan 19, 2011).
22. UNAIDS. UN General Assembly special session country reports 2010. Geneva: Joint United Nations Programme on HIV/AIDS, 2010. http://www.unaids.org/en/regionscountries/countries/ (accessed Jan 19, 2011).
23. Wallis CL, Papathanasopoulos MA, Lakhi S, et al. Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1. J Virol Methods 2010; 163: 505–08.
24. Richard N, Juntilla M, Abraha A, et al. High prevalence of antiretroviral resistance in treated Ugandans infected with non-subtype B human immunodeficiency virus type 1. AIDS Res Hum Retroviruses 2004; 20: 355–64.
25. Land S, Cunningham P, Zhou J, et al. TREAT Asia Quality Assessment Scheme (TAQAS) to standardize the outcome of HIV genotypic resistance testing in a group of Asian laboratories. J Virol Methods 2009; 159: 185–93.
26. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One 2009; 4: e4724.
27. Gifford RJ, Liu TF, Rhee SY, et al. The calibrated population resistance tool: standardized genotypic estimation of transmitted HIV-1 drug resistance. Bioinformatics 2009; 25: 1197–98.
28. de Oliveira T, Deforche K, Cassol S, et al. An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics 2005; 21: 3797–800.
29. Myers RE, Gale CV, Harrison A, Takeuchi Y, Kellam P. A statistical model for HIV-1 sequence classification using the subtype analyser (STAR). Bioinformatics 2005; 21: 3535–40.
30. South African Department of Health. The South African antiretroviral treatment guidelines 2010. http://www.doh.gov.za/docs/factsheets/guidelines/art.pdf (accessed Jan 19, 2011).
31. Ndembi N, Lyagoba F, Nanteza B, et al. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. AIDS Res Hum Retroviruses 2008; 24: 889–95.
32. Weidle PJ, Malamba S, Mwebaze R, et al. Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance. Lancet 2002; 360: 34–40.
33. Arrive E, Newell ML, Ekouevi DK, et al. Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Int J Epidemiol 2007; 36: 1009–21.
34. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol 1994; 68: 1660–66.
35. Little SJ, Frost SD, Wong JK, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. J Virol 2008; 82: 5510–18.
36. Cozzi-Lepri A, Ruiz L, Loveday C, et al. Thymidine analogue mutation profiles: factors associated with acquiring specific profiles and their impact on the virological response to therapy. Antivir Ther 2005; 10: 791–802.

37. Novak RM, Chen L, MacArthur RD, et al. Prevalence of antiretroviral drug resistance mutations in chronically HIV-infected, treatment-naive patients: implications for routine resistance screening before initiation of antiretroviral therapy. Clin Infect Dis 2005; 40: 468–74.
38. Johnson JA, Li JF, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med 2008; 5: e158.
39. van de Vijver DA, Wensing AM, Angarano G, et al. The calculated genetic barrier for antiretroviral drug resistance substitutions is largely similar for different HIV-1 subtypes. J Acquir Immune Defic Syndr 2006; 41: 352–60.

WEBAPPENDIX

Panel. GenBank accession numbers

All HIV-1 *pol* sequences in this study have been deposited in GenBank under the following accession numbers:

HM119603, HM119605-HM119660, HM119662-HM119669, HM119671-HM119682, HM119684-HM119689, HM119691-HM119698, HM119700-HM119715, HM119717-HM119718, HM119720, HM119722-HM119725, HM119728-HM119736, HM119738-HM119760, HM119762-HM119795, HM119797-HM119851, HM119853-HM119855, HM119857-HM119869, HM119871-HM119971, HM119973-HM119982, HM119985-HM120021, HM120023-HM120054, HM120056, HM120058-HM120073, HM120075-HM120089, HM120091-HM120128, HM120130-HM120146, HM120148-HM120150, HQ993572- HQ995497

Table 1. Prevalence of primary HIV-1 drug resistance in the PASER-M cohort, by region and drug class.

	Sequences	Total HIVDR		NRTI						NNRTI		PI		NRTI+ NNRTI		NRTI+ NNRTI +PI	
				Any		TAM		M184V									
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Zambia																	
Lusaka [a]	525	26	5.0	4	0.8	1	0.2	2	0.4	18	3.4	6	1.1	2	0.4	0	0.0
South Africa																	
Pretoria	176	2	1.1	0	0.0	0	0.0	0	0.0	2	1.1	0	0.0	0	0.0	0	0.0
Johannesburg	178	8	4.5	2	1.1	1	0.6	1	0.6	5	2.8	2	1.1	1	0.6	0	0.0
White River	207	10	4.8	1	0.5	1	0.5	0	0.0	5	2.4	4	1.9	0	0.0	0	0.0
Uganda																0	0.0
Kampala	179	22	12.3	11	6.2	5	2.8	1	0.6	8	4.5	4	2.2	1	0.6	0	0.0
Fort Portal	182	19	10.4	11	6.0	7	3.9	2	1.1	10	5.5	1	0.6	2	1.1	0	0.0
Mbale	209	25	12.0	19	9.1	16	7.7	17	8.1	19	9.1	6	2.9	17	8.1	2	1.0
Mbale [b] (excluding M184V)	192	8	4.2	2	1.0	1	0.5	-	-	2	1.0	1	0.6	0	0.0	0	0.0
Kenya																0	0.0
Mombasa	204	10	4.9	3	1.5	1	0.5	1	0.5	4	2.0	4	2.0	1	0.5	0	0.0
Nairobi	200	9	4.5	1	0.5	0	0.0	1	0.5	7	3.5	3	1.5	1	0.5	0	0.0
Zimbabwe																0	0.0
Harare	190	5	2.6	1	0.5	0	0.0	0	0.0	3	1.6	1	0.5	0	0.0	0	0.0
Nigeria																0	0.0
Lagos	186	3	1.6	1	0.5	1	0.5	0	0.0	2	1.1	0	0.0	0	0.0	0	0.0
Total HIVDR [c]	2436	139	5.6	54	2.5	33	1.6	25	1.2	83	3.3	31	1.3	25	1.2	2	0.1

HIVDR, HIV-1 drug resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor; TAM, thymidine analogue mutation.

[a] Combines participants from three clinical sites in Lusaka.

[b] Resistance prevalence in Mbale site after excluding sequences carrying the M184V mutation.

[c] Overall proportions accounting for the sampling weights of the sites.

Table 2. Frequencies of primary drug-resistance mutations in PASER-M cohort, by region.

	N	Lusaka[a]	Pretoria	Johannesburg	White River	Kampala	Fort Portal	Mbale	Mombasa	Nairobi	Harare	Lagos
Total no. sequences	2436	525	176	178	207	179	182	209	204	200	190	186
Total no. DRMs	245	37	2	14	12	25	26	97	12	11	5	4
NRTI - Total	111	5	0	6	1	13	14	65	4	1	1	1
M41L	16				1	3	4	7				1
K65R	1					1						
D67E/G/N	3/1/8			1/0/1		1/0/1		0/1/6	1/0/0			
T69D	4	1				1		1			1	
K70E/R	1/11	0/1		0/1		0/1	0/2	1/6				
L74I/V	3/1					0/1		2/0	1/0			
V75M	3					1		2				
F77L	1						1					
Y115F	1						1					
M184V	25	2		1		1	2	17	1	1		
L210W	6						1	5				
T215F/I/S/Y	4/3/1/10			1/0/0/0		0/1/1/0	0/2/0/1	3/0/0/8	0/0/0/1			
K219E/Q	3/5	1/0		1/0				1/5				
NNRTI - Total	**102**	**26**	**2**	**6**	**7**	**8**	**11**	**25**	**4**	**7**	**3**	**3**
L100I	3	2							1			
K101E/P	11/0	2/0		1/0		3/0		3/0		1/0		1/0
K103N/S	43/3	7/3	1/0	3/0	5/0	2/0	8/0	9/0	3/0	3/0	1/0	1/0
V106A/M	0/2	0/1								0/1		
Y181C/I/V	18/1/0	6/0/0	1/0/0	1/0/0		2/0/0	1/0/0	4/1/0		2/0/0		1/0/0
Y188C/L	1/1	1/0									0/1	
G190A/S	16/1	3/1		1/0	1/0		2/0	8/0			1/0	
P225H	2				1	1						
PI - Total	**32**	**6**	**0**	**2**	**4**	**4**	**1**	**7**	**4**	**3**	**1**	**0**
L23I	1							1				
L24I	2					2						
D30N	1							1				
M46I/L	4/5			0/1	4/0			0/1	0/3			
I50L/V	2/0	1/0						1/0				
I54T/V	1/1					0/1		1/0				
N83D	1					1						
I85V	6	1						1	1	2	1	
N88D	1						1					
L90M	7	4		1				1		1		

DRM, drug-resistance mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor.

[a] Combines participants from three clinical sites in Lusaka.

Table 3. Systematic review of studies that measured the prevalence of primary drug resistance in HIV-1 infected persons in sub-Saharan Africa.

Angola								
Castelbranco *et al.* (1)	35	2008-2009	Newly diagnosed	WHO	5.7%	1	2	0
Botswana								
Bussmann *et al.* (2)	71	2001	Drug-naïve	n/a	0.0%	0	0	0
Bussmann *et al.* (3)	71	2007	Newly diagnosed	WHO	0.0%	0	0	0
Burkino Faso								
Vergne *et al.* (4)	97	2003	Drug-naïve	IAS-USA	8.2%	3	4	2
Tebit *et al.* (5)	104	2004-2006	Drug-naïve	IAS-USA	12.5%	11	6	0
Ayouba *et al.* (6)	51	2005	Newly diagnosed	WHO	0.0%	0	0	0
Burundi								
Vidal *et al.* (7)	101	2002	Drug-naïve	IAS-USA	1.0%	0	1	0
Cameroon								
Vergne *et al.* (4)	102	2001-2002	Drug-naïve	IAS-USA	7.8%	3	2	3
Soares *et al.* (8)	59	2002-2005	Drug-naïve	WHO	5.1%	1	0	3
Ndembi *et al.* (9)	75	2004	Newly diagnosed	IAS-USA	14.7%	6	7	2
Aghokeng *et al.* (10)	47	2006-2007	Newly diagnosed	WHO	6.4%	1	2	0
Aghokeng *et al.* (10)	44	2006-2007	Newly diagnosed	WHO	6.8%	2	1	0
Central African Republic								
Marechal *et al.* (11)	117	2005	Drug-naïve	IAS-USA	0.0%	0	0	0
Chad								
Aghokeng *et al.* (10)	34	2006-2007	Newly diagnosed	WHO	0.0%	0	0	0
Congo DR								
Vidal *et al.* (12)	70	2002	Drug-naïve	IAS-USA	4.3%	0	1	2
Cote d'Ivoire								
Toni *et al.* (13)	107	2001-2002	Drug-naïve	IAS-USA	5.6%	1	4	1
Toni *et al.* (14)	100	2002-2006	Newly diagnosed	IAS-USA	6.0%	3	3	1
Ayouba *et al.* (6)	48	2007	Newly diagnosed	WHO	0.0%	0	0	0
Djibouti								
Maslin *et al.* (15)	47	2002	Drug-naïve	ANRS algorithm	21.2%	1	7	3
Equatorial Guinea								
Djoko *et al.* (16)	41	2008	Drug-naïve	WHO	4.9%	1	0	1
Ethiopia								
Kassu *et al.* (17)	92	2003	Drug-naïve	IAS-USA	3.3%	1	2	0
Kenya								
Price *et al.* (18)	64	2006-2009	Recently infected	WHO	3.1%	1	1	0
Lihana *et al.* (19)	53	2005	Drug-naïve	IAS-USA	7.5%	5	6	0
Malawi								
Kamoto *et al.* (20)	34	2006	Newly diagnosed	WHO	0.0%	0	0	0

Table 3 (continued)

Mali								
Derache *et al.* (21)	98	2005	Drug-naïve	IAS-USA	2.0%	1	1	0
Haidara *et al.* (22)	101	2007-2008	Drug-naïve	Virco algorithm	9.9%	9	6	0
Mozambique								
Abreu *et al.* (23)	75	2002	Drug-naïve	IAS-USA	0.0%	0	0	0
Bartolo *et al.* (24)	68	2002-2004	Drug-naïve	IAS-USA	5.9%	12	1	0
Bellochi *et al.* (25)	58	2003	Drug-naïve	IAS-USA	0.0%	0	0	0
Rwanda								
Price *et al.* (18)	78	2006-2009	Recently infected	WHO	7.7%	0	6	1
Senegal								
Ndiop-Ndiaye *et al.* (26)	96	1998-2001	Drug-naïve	WHO	4.2%	4	0	1
Ndiop-Ndiaye *et al.* (26)	104	2003-2007	Drug-naïve	WHO	1.9%	0	0	2
Ayouba *et al.* (6)	48	2007	Newly diagnosed	WHO	0.0%	0	0	0
South Africa								
Gordon *et al.* (27)	72	2001-2002	Drug-naïve	n/a	2.8%	0	3	0
Bessong *et al.* (28)	35	2001-2004	Drug-naïve	IAS-USA	1.0%	1	0	0
Pillay V *et al.* (29)	65	2002	Drug-naïve	WHO	0.0%	0	0	0
Jacobs *et al.* (30)	140	2002-2004	Drug-naïve	IAS-USA	2.1%	0	3	0
Pillay V *et al.* (29)	48	2004	Drug-naïve	WHO	4.2%	2	0	0
Huang *et al.* (31)	425	2006	Drug-naïve	IAS-USA	2.3%	3	7	2
Tanzania								
Somi *et al.* (32)	39	2005-2006	Newly diagnosed	WHO	0.0%	0	0	0
Togo								
Yaotse *et al.* (33)	75	2006-2007	Drug-naïve	WHO	10.7%	6	1	1
Uganda								
Eshleman *et al.* (34)	104	1998-2003	Recently infected	Geneseq	5.8%	3	2	3
Ndembi *et al.* (35)	37	2006-2007	Newly diagnosed	WHO	0.0%	0	0	0
Ndembi *et al.* (36)	70	2009-2010	Newly diagnosed	WHO	8.6%	3	2	1
Price *et al.* (18)	89	2006-2009	Recently infected	WHO	6.7%	1	2	3
Zambia								
Gonzalez *et al.* (37)	30	1998-2002	Newly diagnosed	WHO	0.0%	0	0	0
Gonzalez *et al.* (37)	86	2005	Newly diagnosed	WHO	2.3%	1	1	0
Price *et al.* (18)	169	2006-2009	Recently infected	WHO	2.4%	3	2	0
Hamers *et al.* (38)	523	2007-2008	Drug-naïve	WHO	5.2%	5	19	6

Studies are sorted by country and by ascending calendar year of sampling.
WHO, World Health Organization mutation list; IAS-USA, International Antiviral Society-USA mutation list; ANRS, Agence Nationale de Recherches sur le Sida et les hépatites virales; NRTI, nucleoside reverse transcriptase inhibitor. NNRTI, non-NRTI; PI, protease inhibitor.

Reference list for Table 3

1. Castelbranco EP, da Silva Souza E, Cavalcanti AM, Martins AN, de Alencar LC, Tanuri A. Frequency of primary resistance to antiretroviral drugs and genetic variability of HIV-1 among infected pregnant women recently diagnosed in Luanda-Angola. AIDS research and human retroviruses. Dec;26(12):1313-6.
2. Bussmann H, Novitsky V, Wester W, Peter T, Masupu K, Gabaitiri L, et al. HIV-1 subtype C drug-resistance background among ARV-naive adults in Botswana. AntivirChemChemother. 2005; 16(2):103-15.
3. Bussmann H, de la Hoz Gomez F, Roels TH, Wester CW, Bodika SM, Moyo S, et al. Prevalence of Transmitted HIV Drug Resistance (HIVDR) in Botswana: Lessons Learned from the HIVDR-Threshold Survey Conducted Among Women Presenting for Routine Antenatal Care as Part of the 2007 National Sentinel Survey. AIDS research and human retroviruses. Oct 29.
4. Vergne L, Diagbouga S, Kouanfack C, Aghokeng A, Butel C, Laurent C, et al. HIV-1 drug-resistance mutations among newly diagnosed patients before scaling-up programmes in Burkina Faso and Cameroon. AntivirTher. 2006;11(5):575-9.
5. Tebit DM, Sangare L, Tiba F, Saydou Y, Makamtse A, Somlare H, et al. Analysis of the diversity of the HIV-1 pol gene and drug resistance associated changes among drug-naive patients in Burkina Faso. Journal of medical virology. 2009 Oct;81(10):1691-701.
6. Ayouba A, Lien TT, Nouhin J, Vergne L, Aghokeng AF, Ngo-Giang-Huong N, et al. Low prevalence of HIV type 1 drug resistance mutations in untreated, recently infected patients from Burkina Faso, Cote d'Ivoire, Senegal, Thailand, and Vietnam: the ANRS 12134 study. AIDS research and human retroviruses. 2009 Nov;25(11):1193-6.
7. Vidal N, Niyongabo T, Nduwimana J, Butel C, Ndayiragije A, Wakana J, et al. HIV type 1 diversity and antiretroviral drug resistance mutations in Burundi. AIDS ResHumRetroviruses. 2007;23(1): 175-80.
8. Soares EA, Makamche MF, Siqueira JD, Lumngwena E, Mbuagbaw J, Kaptue L, et al. Molecular diversity and polymerase gene genotypes of HIV-1 among treatment-naive Cameroonian subjects with advanced disease. J Clin Virol. Jul;48(3):173-9.
9. Ndembi N, Abraha A, Pilch H, Ichimura H, Mbanya D, Kaptue L, et al. Molecular characterization of human immunodeficiency virus type 1 (HIV-1) and HIV-2 in Yaounde, Cameroon: evidence of major drug resistance mutations in newly diagnosed patients infected with subtypes other than subtype B. Journal of clinical microbiology. 2008 Jan;46(1):177-84.
10. Aghokeng AF, Vergne L, Mpoudi-Ngole E, Mbangue M, Deoudje N, Mokondji E, et al. Evaluation of transmitted HIV drug resistance among recently-infected antenatal clinic attendees in four Central African countries. Antivir Ther. 2009;14(3):401-11.
11. Marechal V, Jauvin V, Selekon B, Leal J, Pelembi P, Fikouma V, et al. Increasing HIV type 1 polymorphic diversity but no resistance to antiretroviral drugs in untreated patients from Central African Republic: a 2005 study. AIDS ResHumRetroviruses. 2006;22(10):1036-44.
12. Vidal N, Mulanga C, Bazepeo SE, Mwamba JK, Tshimpaka J, Kashi M, et al. HIV type 1 pol gene diversity and antiretroviral drug resistance mutations in the Democratic Republic of Congo (DRC). AIDS ResHumRetroviruses. 2006;22(2):202-6.
13. Toni TD, Recordon-Pinson P, Minga A, Ekouevi D, Bonard D, Bequet L, et al. Presence of key drug resistance mutations in isolates from untreated patients of Abidjan, Cote d'Ivoire: ANRS 1257 study. AIDS ResHumRetroviruses. 2003;19(8):713-7.

14. Toni T, Masquelier B, Minga A, Anglaret X, Danel C, Coulibaly A, et al. HIV-1 antiretroviral drug resistance in recently infected patients in Abidjan, Cote d'Ivoire: A 4-year survey, 2002-2006. AIDS research and human retroviruses. 2007 Sep;23(9):1155-60.
15. Maslin J, Rogier C, Caron M, Grandadam M, Koeck JL, Nicand E. Short communication. Antiretroviral drug resistance among drug-naive HIV-1-infected individuals in Djibouti (Horn of Africa). AntivirTher. 2005;10(7):855-9.
16. Djoko CF, Wolfe ND, Vidal N, Tamoufe U, Montavon C, LeBreton M, et al. HIV type 1 pol gene diversity and genotypic antiretroviral drug resistance mutations in Malabo, Equatorial Guinea. AIDS research and human retroviruses. Sep;26(9):1027-31.
17. Kassu A, Fujino M, Matsuda M, Nishizawa M, Ota F, Sugiura W. Molecular epidemiology of HIV type 1 in treatment-naive patients in north Ethiopia. AIDS research and human retroviruses. 2007 Apr; 23(4):564-8.
18. Price MA, Wallis CL, Lakhi S, Karita E, Kamali A, Anzala O, et al. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS research and human retroviruses 2010;27(1):5-12.
19. Lihana RW, Khamadi SA, Lubano K, Lwembe R, Kiptoo MK, Lagat N, et al. HIV type 1 subtype diversity and drug resistance among HIV type 1-infected Kenyan patients initiating antiretroviral therapy. AIDS research and human retroviruses. 2009;25(12):1211-7.
20. Kamoto K, Aberle-Grasse J. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. Antivir Ther. 2008;13 Suppl 2:83-7.
21. Derache A, Traore O, Koita V, Sylla A, Tubiana R, Simon A, et al. Genetic diversity and drug resistance mutations in HIV type 1 from untreated patients in Bamako, Mali. Antivir Ther. 2007;12(1): 123-9.
22. Haidara A, Chamberland A, Sylla M, Aboubacrine SA, Cisse M, Traore HA, et al. High level of primary drug resistance in Mali. HIV medicine 11(6):404-11.
23. Abreu CM, Brindeiro PA, Martins AN, Arruda MB, Bule E, Stakteas S, et al. Genotypic and phenotypic characterization of human immunodeficiency virus type 1 isolates circulating in pregnant women from Mozambique. Archives of virology. 2008;153(11):2013-7.
24. Bartolo I, Casanovas J, Bastos R, Rocha C, Abecasis AB, Folgosa E, et al. HIV-1 genetic diversity and transmitted drug resistance in health care settings in Maputo, Mozambique. Journal of acquired immune deficiency syndromes (1999). 2009;51(3):323-31.
25. Bellocchi MC, Forbici F, Palombi L, Gori C, Coelho E, Svicher V, et al. Subtype analysis and mutations to antiviral drugs in HIV-1-infected patients from Mozambique before initiation of antiretroviral therapy: results from the DREAM programme. J Med Virol. 2005;76(4):452-8.
26. Diop-Ndiaye H, Toure-Kane C, Leye N, Ngom-Gueye NF, Montavon C, Peeters M, et al. Antiretroviral drug resistance mutations in antiretroviral-naive patients from Senegal. AIDS research and human retroviruses 26(10):1133-8.
27. Gordon M, de OT, Bishop K, Coovadia HM, Madurai L, Engelbrecht S, et al. Molecular characteristics of human immunodeficiency virus type 1 subtype C viruses from KwaZulu-Natal, South Africa: implications for vaccine and antiretroviral control strategies. J Virol. 2003;77(4):2587-99.
28. Bessong PO, Larry OC, Cilliers T, Choge I, Phoswa M, Pillay C, et al. Characterization of human immunodeficiency virus type 1 from a previously unexplored region of South Africa with a high HIV prevalence. AIDS Res Hum Retroviruses. 2005;21(1):103-9.
29. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antivir Ther. 2008;13 Suppl 2:101-7.

30. Jacobs GB, Laten A, van Rensburg EJ, Bodem J, Weissbrich B, Rethwilm A, et al. Phylogenetic diversity and low level antiretroviral resistance mutations in HIV type 1 treatment-naive patients from Cape Town, South Africa. AIDS research and human retroviruses. 2008;24(7):1009-12.
31. Huang KH, Goedhals D, Fryer H, van Vuuren C, Katzourakis A, De Oliveira T, et al. Prevalence of HIV type-1 drug-associated mutations in pre-therapy patients in the Free State, South Africa. Antivir Ther. 2009;14(7):975-84.
32. Somi GR, Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, et al. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. Antivir Ther. 2008;13 Suppl 2:77-82.
33. Yaotse DA, Nicole V, Roch NF, Mireille PD, Eric D, Martine P. Genetic characterization of HIV-1 strains in Togo reveals a high genetic complexity and genotypic drug-resistance mutations in ARV naive patients. Infect Genet Evol. 2009;9(4):646-52.
34. Eshleman SH, Laeyendecker O, Parkin N, Huang W, Chappey C, Paquet AC, et al. Antiretroviral drug susceptibility among drug-naive adults with recent HIV infection in Rakai, Uganda. Aids 2009;23(7):845-52.
35. Ndembi N, Lyagoba F, Nanteza B, Kushemererwa G, Serwanga J, Katongole-Mbidde E, et al. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. AIDS research and human retroviruses. 2008; 24(6):889-95.
36. Ndembi N, Hamers RL, Sigaloff KC, Lyagoba F, Magambo B, Nanteza B, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. Aids 2011; 25(7):905-10.
37. Gonzalez S, Gondwe C, Tully DC, Minhas V, Shea D, Kankasa C, et al. Short communication: antiretroviral therapy resistance mutations present in the HIV type 1 subtype C pol and env regions from therapy-naive patients in Zambia. AIDS research and human retroviruses. 26(7):795-803.
38. Hamers RL, Siwale M, Wallis CL, Labib M, van Hasselt R, Stevens WS, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. Journal of acquired immune deficiency syndromes 2010;55(1):95-101.