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Pretreatment and Acquired Antiretroviral Drug Resistance Among Persons Living With HIV in Four African Countries

Trevor A. Crowell,[1,2] Brook Danboise,[1] Ajay Parikh,[1,2] Allahna Esber,[1,2] Nicole Dear,[1,2] Peter Coakley,[1,2] Alex Kasembeli,[1,3] Jonah Maswai,[1,3] Samoel Khamadi,[1,4] Emmanuel Bahemana,[1,4] Michael Iroezindu,[1,5] Francis Kiweewa,[6] John Owuoth,[1,7] Joanna Freeman,[1,2] Linda L. Jagodzinski,[1] Jennifer A. Malia,[1] Leigh Ann Eller,[1,2] Sodsai Tovanabutra,[1,2] Sheila A. Peel,[1] Julie A. Ake,[1] and Christina S. Polyak[1,2]; for the AFRICOS Study Group

[1]US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA, [2]Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, Maryland, USA, [3]HJF Medical Research International, Kericho, Kenya, [4]HJF Medical Research International, Mbeya, Tanzania, [5]HJF Medical Research International, Abuja, Nigeria, [6]Makerere University Walter Reed Project, Kampala, Uganda, and [7]HJF Medical Research International, Kisumu, Kenya

Background. Emerging HIV drug resistance (HIVDR) could jeopardize the success of standardized HIV management protocols in resource-limited settings. We characterized HIVDR among antiretroviral therapy (ART)-naive and experienced participants in the African Cohort Study (AFRICOS).

Methods. From January 2013 to April 2019, adults with HIV-1 RNA >1000 copies/mL underwent ART history review and HIVDR testing upon enrollment at 12 clinics in Uganda, Kenya, Tanzania, and Nigeria. We calculated resistance scores for specific drugs and tallied major mutations to non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), and protease inhibitors (PIs) using Stanford HIVDB 8.8 and SmartGene IDNS software. For ART-naive participants, World Health Organization surveillance drug resistance mutations (SDRMs) were noted.

Results. HIVDR testing was performed on 972 participants with median age 35.7 (interquartile range [IQR] 29.7–42.7) years and median CD4 295 (IQR 148–478) cells/mm^3. Among 801 ART-naive participants, the prevalence of SDRMs was 11.0%, NNRTI mutations 8.2%, NRTI mutations 4.7%, and PI mutations 0.4%. Among 171 viremic ART-experienced participants, NNRTI mutation prevalence was 83.6%, NRTI 67.8%, and PI 1.8%. There were 90 ART-experienced participants with resistance to both efavirenz and lamivudine, 33 (36.7%) of whom were still prescribed these drugs. There were 10 with resistance to both tenofovir and lamivudine, 8 (80.0%) of whom were prescribed these drugs.

Conclusions. Participants on failing ART regimens had a high burden of HIVDR that potentially limited the efficacy of standardized first- and second-line regimens. Management strategies that emphasize adherence counseling while delaying ART switch may promote drug resistance and should be reconsidered.

Keywords. Africa South of the Sahara; acquired immunodeficiency syndrome; drug resistance; public health surveillance.

The global expansion of access to antiretroviral therapy (ART) has driven dramatic reductions in HIV-related morbidity and mortality over the last 2 decades [1]. In sub-Saharan Africa, the region hardest hit by the HIV epidemic, over 16 million persons living with HIV (PLWH) are currently receiving ART, largely through programs supported by the US President's Emergency Plan for AIDS Relief (PEPFAR) [1, 2]. However, emergence of HIV drug resistance (HIVDR) could jeopardize the long-term success of treatment programs, leading to eventual rebound in HIV incidence and HIV-related mortality, as well as increased programmatic costs [3]. As ART access continues to improve, it is essential that routine surveillance be conducted for pretreatment and acquired HIVDR to inform national and international HIV treatment strategies [4, 5].

The massive global scale-up of ART leveraged simplified and standardized treatment protocols to facilitate care delivery in resource-limited settings such as sub-Saharan Africa [6]. While HIV-1 genotype resistance testing is routine in resource-rich settings prior to ART initiation or upon viral failure [7], this is rarely available in sub-Saharan Africa and is generally not supported by PEPFAR-funded programs [8]. Instead, ART selection is based on empiric national standards for first- and second-line regimens, a strategy that can promote further development and transmission of resistant viruses in populations with a high burden of unrecognized HIVDR [9].

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Correspondence: T. A. Crowell, US Military HIV Research Program, 6720A Rockledge Drive, Suite 400 Bethesda, MD 20817 (tcrowell@hivresearch.org).

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In many African countries, PLWH who fail first-line regimens are required to complete a series of ART adherence counseling sessions with documentation of sustained viremia before switching to second-line therapy [10–13]. These strategies minimize unnecessary and costly regimen changes when poor ART adherence is the major driver of viremia. However, when underlying HIVDR drives viral failure, these strategies may delay medically necessary regimen changes and promote the development of further resistance [14, 15].

We evaluated HIVDR among ART-naive and ART-experienced PLWH attending PEPFAR-supported clinics in four African countries.

METHODS

Study Population

The ongoing African Cohort Study (AFRICOS) is an observational study that enrolls adults living with and at risk for HIV aged 18 years and older at 12 PEPFAR-supported clinical care sites in Uganda, Kenya, Tanzania, and Nigeria, as previously described [16]. The sites are all hospital-based with staff and facilities to provide primary care, HIV counseling and testing, and ART adherence support with on-site clinical pharmacies. At enrollment and every 6 months thereafter, participants with HIV undergo medical history-taking, physical examination, and laboratory assessments that include CD4 and HIV-1 RNA assessments. Extensive medical record review at each visit includes extraction of any past or ongoing ART exposure. Participants who were enrolled between 23 January 2013 and 1 April 2019 with HIV-1 RNA >1000 copies/mL were eligible for HIVDR testing and inclusion in these analyses.

HIV Diagnosis and Monitoring

HIV was diagnosed according to national testing guidelines in each country, including algorithms based on HIV rapid tests and/or immunoassays. CD4 count was enumerated using standard clinical flow cytometric methods at each site. HIV-1 RNA was measured via nucleic acid amplification methods on one of several testing platforms with lower limit of quantification 20–48 copies/mL, including the Cobas® Ampliprep/Cobas® TaqMan HIV-1 Test, v2.0 (Roche Diagnostics), High Pure/COBAS® TaqMan® HIV-1 Test v2.0 (Roche Diagnostics,), COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 Test (Roche Diagnostics), or Real Time HIV-1 Viral Load assay (Abbott). All testing was performed according to package inserts.

HIV Genotyping and Subtyping

Plasma samples from participants with HIV-1 RNA >1000 copies/mL at enrollment underwent sequencing of the *Pol* region using a laboratory-validated modification to the ViroSeq HIV-1 Genotyping System v2.0 (Abbott Molecular). HIV-1 subtype was assigned using the NCBI HIV Subtyping Tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/), NCBI HIV-1 Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/), BioAfrica REGA HIV-1 automated subtyping v2.0 (http://www.bioafrica.net/subtypetool/html/subtypinghiv.html), and Jumping Profile HMM-HIV (http://jphmm.gobics.de/submission_hiv.html). Results from the 4 tools were compared to achieve a consensus assignment. Sequences with discordant subtyping assignments underwent advanced analysis prior to final assignment.

Sequences were evaluated for major mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), and protease inhibitors (PIs), using the SmartGene Integrated Database Network System (SmartGene) to access mutation lists and drug resistance scoring algorithms from the Stanford HIV Drug Resistance Database Version 8.8.0 (Stanford University). Participants who had never received ART, taken prophylactic ART only, received single-agent ART for prevention of mother-to-child transmission (PMTCT), or who started ART within 28 days of genotyping were considered ART-naive and evaluated for World Health Organization (WHO) surveillance drug resistance mutations (SDRMs) [17].

Statistical Analyses

Wilcoxon rank-sum and Pearson's Chi-squared tests were used to compare continuous and categorical variables, respectively, across groups of interest. The prevalence of specific drug resistance mutations and categories of drug resistance mutations were calculated by dividing the number of participants with one or more mutations by the total number of participants genotyped. The prevalence of anticipated resistance to specific drugs based on scoring algorithms was calculated similarly. Robust Poisson regression was used to estimate prevalence ratios and 95% confidence intervals for prespecified factors potentially associated with drug resistance in separate analyses for ART-naive and ART-experienced participants [18]. To test for temporal associations with HIVDR prevalence, a variable for study period dichotomized around the midpoint was included in the models. The outcomes of the models were any WHO SDRM and any major NNRTI, NRTI, or PI mutation, respectively. All analyses were performed using Stata 15.0 (StataCorp LP).

Ethical Assurance

The study was approved by institutional review boards of the Walter Reed Army Institute of Research Silver Spring, MD, USA; Makerere University School of Public Health, Kampala, Uganda; Kenya Medical Research Institute, Nairobi, Kenya; Tanzania National Institute of Medical Research, Mbeya, Tanzania; and Nigerian Ministry of Defence, Abuja, Nigeria. All participants provided written informed consent prior to enrollment.

RESULTS

Study Population

A total of 2839 PLWH were enrolled, including 1496 (52.7%) from Kenya, 535 (18.8%) from Tanzania, 512 (18.0%) from Uganda, and 296 (10.4%) from Nigeria. There were 1756 (61.8%) ART-experienced participants, among whom 206 (11.7%) had HIV RNA > 1000 copies/mL and 171 (83.0%) underwent retrospective HIVDR testing. Among 1083 (38.1%) ART-naive participants, there were 896 (82.3%) with HIV-1 RNA > 1000 copies/mL and 801 (89.4%) underwent retrospective HIVDR testing.

The 972 participants with HIVDR testing results had a median age of 35.7 (interquartile range [IQR] 29.7–42.7) years, median CD4 of 295 (IQR 148–478) cells/mm^3, and median HIV-1 RNA of 47 705 (IQR 11 910–163 353) copies/mL. The 801 ART-naive participants differed from the 171 ART-experienced participants by age, country, and other characteristics (Table 1). HIV-1 RNA at genotype testing was higher in ART-naive as compared to ART-experienced participants (median 53 292 [IQR 13 805–183 872] vs 26 903 [7417–81 199] copies/mL, $P < .001$).

HIV Subtypes

The distribution of HIV-1 subtypes based on *Pol* region sequencing varied substantially by country (Figure 1). Subtype A comprised the majority of infections in Uganda and Kenya (51.2 and 64.0%, respectively), while subtype C was most common in Tanzania (54.1%), and subtypes G and CRF02_AG together dominated the viruses observed in Nigeria (77.4%).

Pre-treatment Drug Resistance Mutations

Among 801 ART-naive participants, 88 (11.0%) had WHO SDRMs. WHO SDRM prevalence was lowest in Kenya (7.5%), followed by Tanzania (7.8%), then Uganda (13.7%), and highest in Nigeria (16.7%, $P < .001$; Table 2). The single most common mutation in each country was the major NNRTI resistance mutation K103N, which was observed in 3.9% of ART-naive participants in Tanzania, 4.7% in Kenya, 6.5% in Uganda, and 6.9% in Nigeria ($P = .569$). Only 3 participants had major PI resistance mutations.

Between 2013–2015 and 2016–2019, WHO SDRM prevalence in the cohort increased from 8.8% to 16.2% ($P = .002$), driven largely by increased pretreatment drug resistance in Uganda (8.0% to 24.3%, $P < .001$; Figure 2). Similar cohort-wide increases were observed in major NNRTI resistance mutations (6.5% vs 12.4%, $P = .006$) and major NRTI resistance mutations (3.4% vs 7.3%, $P = .015$), though PI resistance remained relatively rare in both eras (0.2% vs 0.8%, $P = .153$).

WHO SDRMs also varied by HIV subtype, with prevalence of 5.3% in subtype CRF02_AG, 7.3% C, 7.7% G, 9.3% A, 10.7% D, and 17.9% in other subtypes combined ($P = .037$).

Based on scoring algorithms from the Stanford HIV Drug Resistance Database, high-level resistance to at least one drug from any class would be expected in 8.4% of participants, including high-level resistance to nevirapine in 7.7% of ART-naive participants and to efavirenz in 6.2% (Figure 3A). Evidence of high-level resistance to newer generation NNRTIs, NRTIs, and PIs was observed in fewer than 3.0% of participants across all 4 countries. Seven (0.9%) ART-naive participants had evidence of high-level resistance to both efavirenz and lamivudine and one (0.1%) to both tenofovir and lamivudine.

Drug Resistance Mutations in ART-Experienced Participants

Among 171 ART-experienced participants with HIV-1 RNA > 1000 copies/mL, major NNRTI resistance mutations were observed in 143 (83.6%), NRTI resistance mutations in 116 (67.8%), and PI resistance mutations in 3 (1.8%; Table 3). While mutations conferring resistance to NNRTIs were the most common group of mutations in each country, the NRTI resistance mutation M184V/I was the most common individual mutation in Uganda (50.0%), Kenya (69.2%), and Tanzania (64.5%). K103N was the most common individual mutation in Nigeria (61.5%). Of 17 participants with a history of exposure to PI-containing regimens, 3 (17.6%) had PI resistance mutations.

Anticipated resistance to specific drugs among the 171 viremic ART-experienced participants included high-level resistance to efavirenz in 68.8%, lamivudine and emtricitabine in 65.3%, and tenofovir in 7.7% (Figure 3B). Among the 13 ART-experienced participants with high-level resistance to tenofovir, 10 (76.9%) had the K65R mutation, and 4 (30.8%) had 2 or more thymidine analogue mutations. High-level resistance to tenofovir was numerically more common in participants with subtype C virus as compared to non-C subtypes, but the difference was not statistically significant (11.5% vs 6.9%, $P = .411$). High-level resistance to drugs from 2 or more classes was observed in 114 (66.7%) ART-experienced participants, including 90 (52.6%) with high-level resistance to both efavirenz and lamivudine, of whom 33 (36.7%) were still prescribed regimens that included both drugs. There were 10 ART-experienced participants with high-level resistance to both tenofovir and lamivudine, including 8 (80.0%) who were still prescribed both drugs.

Factors Associated With Drug Resistance Mutations

After adjusting for potentially confounding factors using multivariable robust Poisson regression, the prevalence of WHO SDRMs was significantly higher among ART-naive participants in Nigeria as compared to other countries and among participants evaluated during the latter half of the enrollment period (Table 4). Decreased prevalence of WHO SDRMs was observed with increasing education level. The prevalence of major resistance mutations among viremic ART-experienced participants did not vary significantly by any of the factors evaluated.

DISCUSSION

The 2017 WHO guidelines recommended changing empiric first-line NNRTI-based ART to an integrase inhibitor-based

Table 1. Characteristics of ART-Naive and ART-Experienced AFRICOS Participants With HIV-1 RNA > 1000 Copies/mL at the Time of Genotypic Testing for Drug Resistance Mutations

Characteristic	All (n = 972)	ART-Naive (n = 801)	ART-Experienced (n = 171)	*P*
Age (years)				
<30	252 (25.9%)	224 (28.0%)	28 (16.4%)	**.005**
30–39	402 (41.4%)	326 (40.7%)	76 (44.4%)	
40+	318 (32.7%)	251 (31.3%)	67 (39.2%)	
Sex				
Male	396 (40.7%)	324 (40.4%)	72 (42.1%)	.69
Female	576 (59.3%)	477 (59.6%)	99 (57.9%)	
Country				
Uganda	301 (31.0%)	291 (36.3%)	10 (5.8%)	**<.001**
Kenya	358 (36.8%)	254 (31.7%)	104 (60.8%)	
Tanzania	185 (19.0%)	154 (19.2%)	31 (18.1%)	
Nigeria	128 (13.2%)	102 (12.7%)	26 (15.2%)	
Year				
2013	84 (8.6%)	62 (7.7%)	22 (12.9%)	**<.001**
2014	280 (28.8%)	209 (26.1%)	71 (41.5%)	
2015	331 (34.1%)	296 (37.0%)	35 (20.5%)	
2016	188 (19.3%)	160 (20.0%)	28 (16.4%)	
2017	66 (6.8%)	55 (6.9%)	11 (6.4%)	
2018	21 (2.2%)	17 (2.1%)	4 (2.3%)	
2019	2 (0.2%)	2 (0.2%)	0 (0.0%)	
Education				
Less than Primary	339 (34.9%)	292 (36.5%)	47 (27.5%)	**.023**
Primary School	389 (40.1%)	320 (40.0%)	69 (40.4%)	
Secondary School and Above	243 (25.0%)	188 (23.5%)	55 (32.2%)	
CD4 Count (cells/mm^3)				
<200	325 (33.4%)	251 (31.3%)	74 (43.3%)	**<.001**
200–349	237 (24.4%)	182 (22.7%)	55 (32.2%)	
350–499	179 (18.4%)	155 (19.4%)	24 (14.0%)	
500+	231 (23.8%)	213 (26.6%)	18 (10.5%)	
HIV-1 RNA (copies/mL)				
1001–10 000	219 (22.5%)	168 (21.0%)	51 (29.8%)	**<.001**
10 001–100 000	415 (42.7%)	334 (41.7%)	81 (47.4%)	
100 001+	338 (34.8%)	299 (37.3%)	39 (22.8%)	
Years Since HIV Diagnosis				
<1	677 (69.7%)	655 (81.9%)	22 (12.9%)	**<.001**
1–5	156 (16.1%)	94 (11.8%)	62 (36.3%)	
>5	138 (14.2%)	51 (6.4%)	87 (50.9%)	
Prior Exposure to NVP for PMTCT				
No	945 (97.5%)	781 (97.5%)	167 (97.7%)	.90
Yes	24 (2.5%)	20 (2.5%)	4 (2.3%)	
Prior Exposure to EFV				
No	891 (91.7%)	801 (100.0%)	90 (52.6%)	**<.001**
Yes	81 (8.3%)	-	81 (47.4%)	
Prior Exposure to TDF				
No	878 (90.3%)	801 (100.0%)	77 (45.0%)	**<.001**
Yes	94 (9.7%)	-	94 (55.0%)	
Last Known ART Regimen				
EFV/TDF/3TC	-	-	56 (32.7%)	-
NVP/AZT/3TC	-	-	57 (33.3%)	
NVP/TDF/3TC	-	-	25 (15.2%)	
LPV/r/TDF/3TC	-	-	16 (9.4%)	
EFV/AZT/3TC	-	-	9 (4.2%)	
EFV/TDF/FTC	-	-	5 (3.0%)	
ABC/EFV/3TC	-	-	2 (1.2%)	
LPV/r/3TC/AZT	-	-	1 (0.6%)	

All data are presented as n (%).

P values were calculated using Pearson's Chi-squared test.

Statistically significant *P* values (<.05) are in **bold**.

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, azidothymidine (zidovudine); EFV, efavirenz; FTC, emtricitabine; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; PMTCT, prevention of mother-to-child transmission of HIV; TDF, tenofovir.

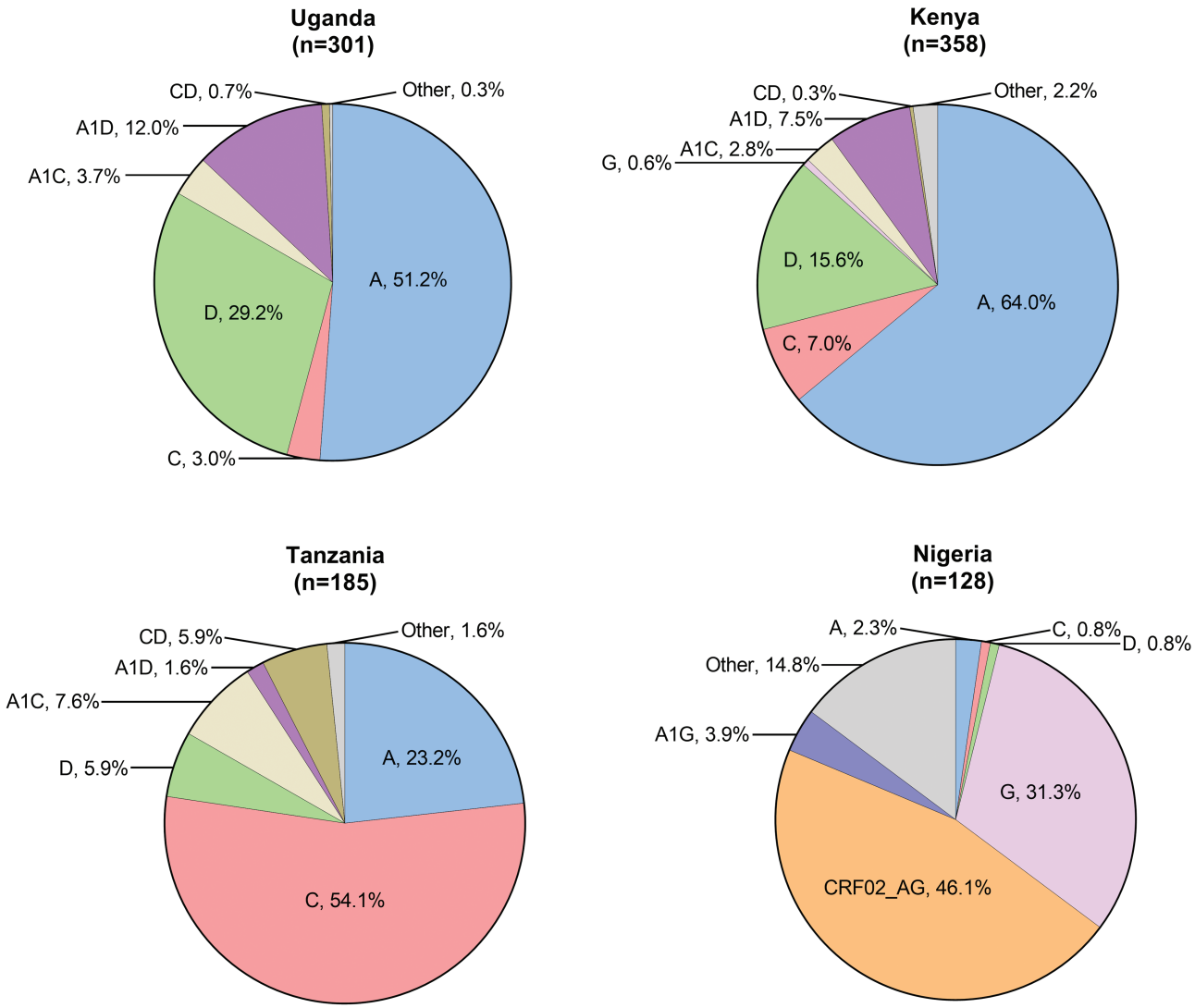

Figure 1. HIV-1 subtypes by country. Plasma samples from participants with HIV-1 RNA > 1000 copies/mL at enrollment underwent sequencing of the *Pol* region with HIV-1 subtype assignment using 4 different tools to achieve a consensus. "Other" subtypes include mixed and recombinant forms.

regimen when the prevalence of transmitted HIVDR exceeded 10% [19]. In our study, we found that the prevalence of pretreatment HIVDR surpassed this threshold in both Uganda and Nigeria. While pretreatment HIVDR less than 10% was observed in Kenya and Tanzania, this may represent an underestimation of transmitted resistance in chronically infected PLWH due to a tendency for overgrowth of more fit and drug-sensitive viral quasi-species over time [20, 21]. Pretreatment HIVDR prevalence was driven primarily by NNRTI resistance mutations such as K103N, which is slower to revert than some other mutations [22] and causes an over 20-fold increase in resistance to efavirenz [23, 24]. Updated WHO guidelines support the ongoing transition to dolutegravir-based first-line ART in sub-Saharan Africa, which will mitigate the short-term population-level impact of transmitted resistance to efavirenz [25]. This transition presents an opportunity to identify and resolve programmatic gaps that promoted HIVDR development before they compromise the next generation of ART regimens [26].

The prevalence of pretreatment HIVDR increased over time in our study, particularly in Uganda. Duration of ART availability has been strongly associated with emergence of pretreatment drug resistance at a population level in resource-limited settings [27]. Delayed HIV diagnosis, interruptions of drug supply, limited access to third-line ART regimens, and late switches from failing regimens may each contribute to the temporal trend of increasing pretreatment HIVDR [27, 28]. The country-level prevalence of pretreatment HIVDR was higher in our study than in previous reports from adults in Tanzania [29, 30] and Nigeria [31–33]. In Kenya, prior studies have shown SDRM prevalence among ART-naive participants increasing from approximately 4%–5% in the early 2000s to around 10% in

Table 2. Pretreatment Antiretroviral Drug Resistance Mutations Among Viremic ART-Naive Participants

	Uganda (n = 291)	Kenya (n = 254)	Tanzania (n = 154)	Nigeria (n = 102)	Total (n = 801)
WHO SDRMs	**40 (14%)**	**19 (7%)**	**12 (8%)**	**17 (17%)**	**88 (11%)**
Major NNRTI Resistance	**30 (10%)**	**17 (7%)**	**9 (6%)**	**10 (10%)**	**66 (8%)**
K103N	19 (7%)	12 (5%)	6 (4%)	7 (7%)	44 (5%)
Y181C/I/V	6 (2%)	2 (1%)	2 (1%)	0 (0%)	10 (1%)
G190A/S	3 (1%)	1 (0%)	1 (1%)	1 (1%)	6 (1%)
P225H	3 (1%)	0 (0%)	1 (1%)	1 (1%)	5 (1%)
K103S	3 (1%)	0 (0%)	1 (1%)	0 (0%)	4 (0%)
Y188L/C/H	1 (0%)	1 (0%)	1 (1%)	0 (0%)	3 (0%)
M230L	1 (0%)	1 (0%)	0 (0%)	0 (0%)	2 (0%)
V106A	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
L100I	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Major NRTI Resistance	**15 (5%)**	**6 (2%)**	**5 (3%)**	**10 (10%)**	**38 (5%)**
M184V/I	6 (2%)	3 (1%)	2 (1%)	2 (2%)	13 (2%)
L74V/I	6 (2%)	1 (0%)	2 (1%)	4 (4%)	13 (2%)
M41L	3 (1%)	0 (0%)	1 (1%)	5 (5%)	9 (1%)
K219Q/E	2 (1%)	2 (1%)	0 (0%)	1 (1%)	5 (1%)
D67N	2 (1%)	1 (0%)	0 (0%)	1 (1%)	4 (0%)
K65R	3 (1%)	0 (0%)	0 (0%)	0 (0%)	3 (0%)
K70R	0 (0%)	2 (1%)	0 (0%)	1 (1%)	3 (0%)
T215Y/F	0 (0%)	1 (0%)	1 (1%)	1 (1%)	3 (0%)
K70E	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Y115F	1 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
L210W	0 (0%)	1 (0%)	0 (0%)	0 (0%)	1 (0%)
Major PI Resistance	**2 (1%)**	**0 (0%)**	**1 (1%)**	**0 (0%)**	**3 (0%)**
L90M	2 (1%)	0 (0%)	0 (0%)	0 (0%)	2 (0%)
M46L	0 (0%)	0 (0%)	1 (1%)	0 (0%)	1 (0%)

All data are presented as n (%).
Abbreviations: ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; WHO SDRM, World Health Organization surveillance drug resistance mutation.

more recent years [32–35]. SDRM prevalence exceeding 10% of ART-naive participants has been previously reported in Uganda [32, 33], which is one of the countries with the longest experience with widely available ART in sub-Saharan Africa and is therefore an important sentinel setting for the region.

Viremic ART-experienced participants in our study had a high burden of HIVDR, including approximately half with high-level resistance to efavirenz, the standard first-line NNRTI in use during the study period. PLWH with viral failure driven by underlying drug resistance require timely switch to an alternative and efficacious ART regimen to prevent the development of further resistance [14, 15]. However, country-level guidelines may inadvertently encourage detrimental delays while confirming viremia and providing ART adherence counseling. Kenyan guidelines, for example, require 3 sessions of enhanced adherence counseling prior to ART switch and explicitly direct providers, "Do not change regimens until the reason/s for treatment failure have been identified and addressed, and a repeat [viral load] is > 1000 copies/ml after 3 months of good adherence" [12]. Ugandan guidelines similarly require 3 sessions of intensive adherence counseling and recommend ART regimen switch only after "Two consecutive viral loads above 1000 copies/ml, done at least 3–6 months apart, with adherence support following the 1st [viral load] test" [13]. In this study, we have documented that underlying HIVDR was observed in the majority of viremic ART-experienced participants. Public health strategies that presuppose poor adherence as the major driver of viremia should be reconsidered. Frequent HIV-1 RNA monitoring, including rapid re-testing when elevations are detected, is necessary to detect viral failure early and limit the accumulation of drug resistance mutations.

PLWH with continued exposure to a failing regimen would be expected to develop resistance to multiple drugs in their regimens, as was observed among ART-experienced participants in this study. ART-experienced PLWH in this study demonstrated a pattern of HIVDR consistent with failure of first-line therapy with NNRTI and NRTI components, as has been described previously in sub-Saharan Africa [36]. There was a particularly high burden of the NRTI resistance mutation M184V/I, which rapidly develops with viremia in the presence of lamivudine or emtricitabine (XTC) and confers resistance to these same drugs [37]. XTC is often included in second-line regimens because of evidence that continued selective pressure to maintain the mutation results in decreased viral fitness [38], viral replication [39], peripheral viral load [40, 41], and mutation accumulation [42]. However, recent studies have provided conflicting data

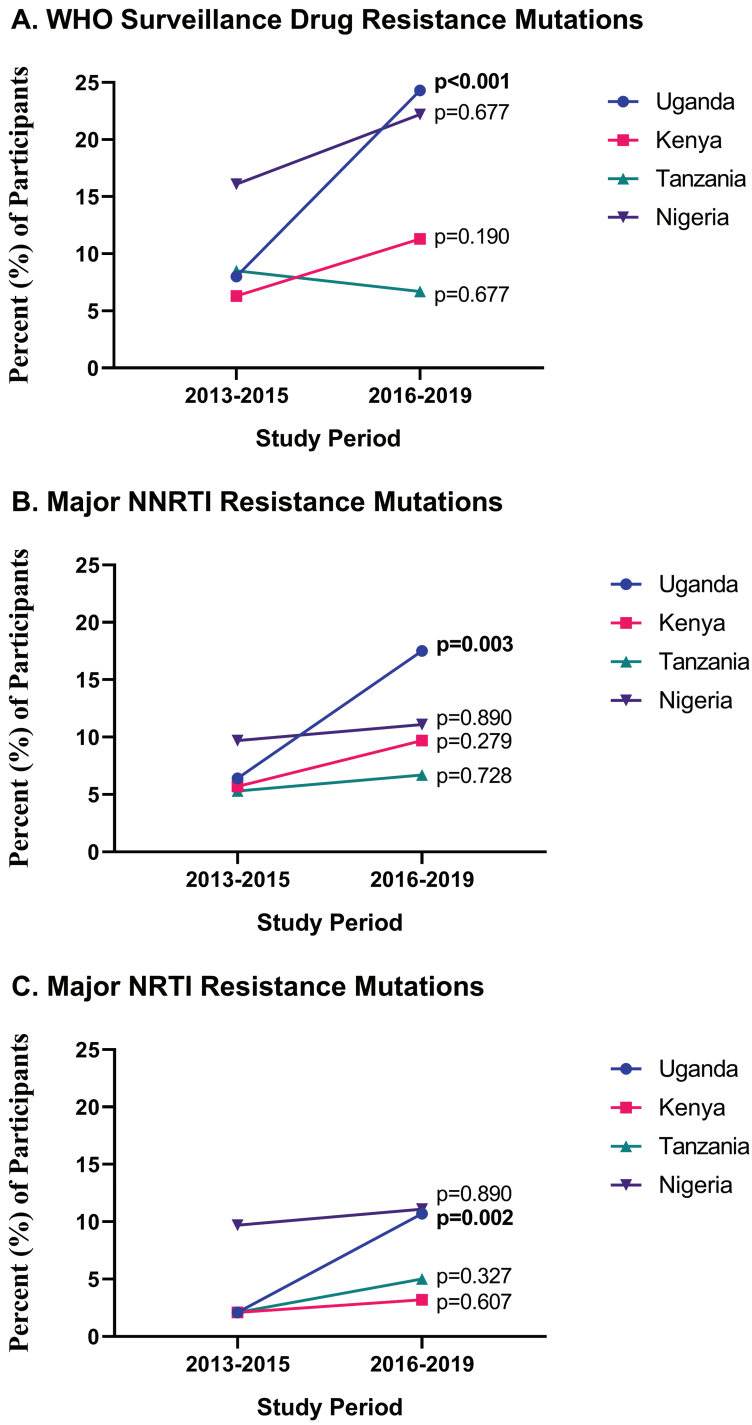

Figure 2. Temporal trends in the prevalence of pretreatment antiretroviral drug resistance mutations among viremic ART-naive participants, by country. To evaluate temporal trends in HIVDR prevalence, the study period was dichotomized around the midpoint. The prevalence of specific World Health Organization surveillance drug resistance mutations (Panel *A*), major NNRTI mutations (Panel *B*), and major NRTI mutations (Panel *C*) was compared for participants who underwent genotyping in 2013–2015 and 2016–2019 using Pearson's Chi-squared test. Statistically significant differences between study periods ($P < .05$) are shown in **bold**. Note that in 2016–2019, 3 participants from Tanzania with SDRMs had major mutations conferring resistance to both NNRTIs and NRTIs; this pattern of dual resistance was not present in the earlier period and contributes to the numeric decline in the prevalence of WHO SDRMs despite relatively stable prevalence of NNRTI and NRTI resistance mutations in Tanzania. Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; WHO, World Health Organization.

on the potential clinical benefit of this approach [43, 44]. Some participants in this study had both the M184V/I mutation and high-level resistance to tenofovir, driven largely by the K65R mutation. While some studies suggest that protease inhibitor-based second-line regimens containing tenofovir and XTC may still be efficacious in such cases [45, 46], further research

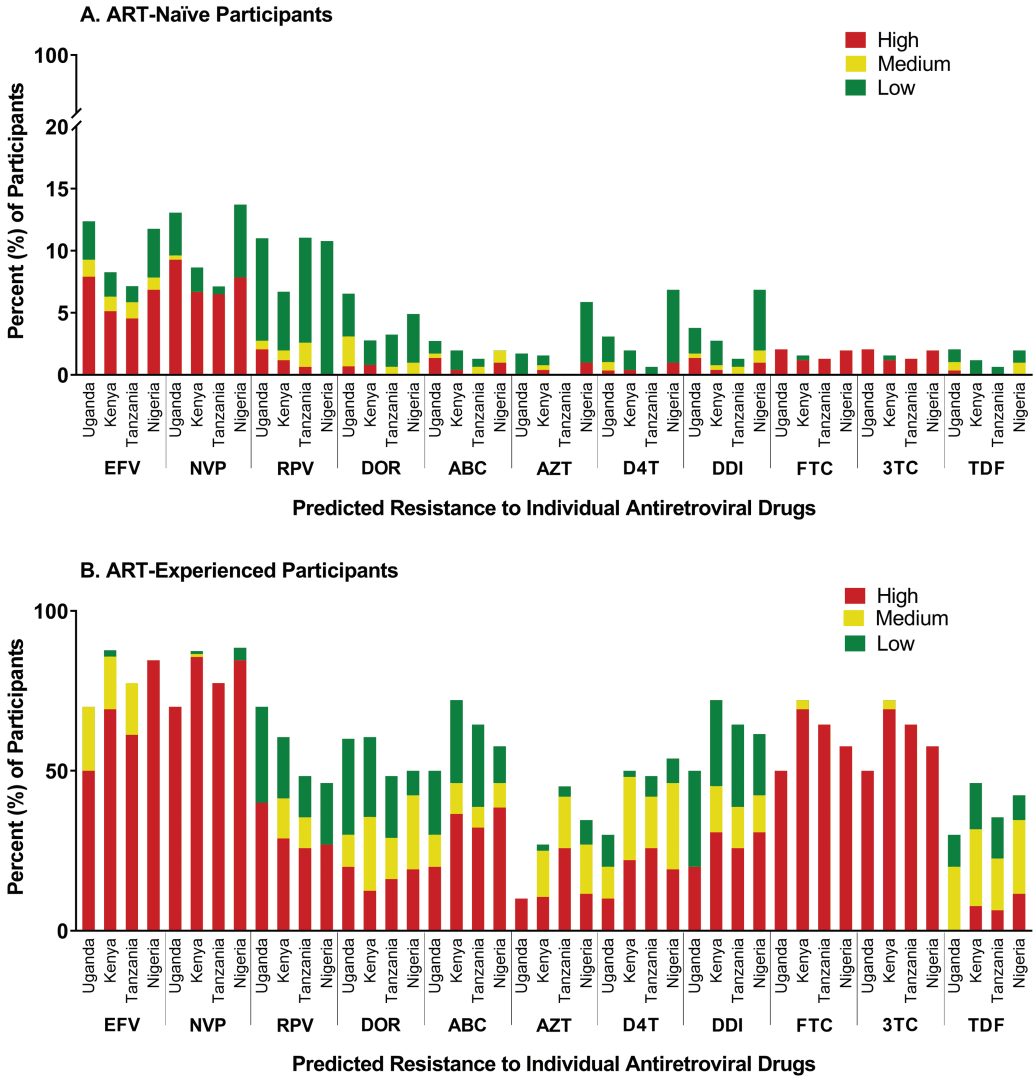

Figure 3. Predicted resistance to individual antiretroviral drugs among viremic ART-naive and ART-experienced participants. Resistance to individual antiretroviral drugs was predicted using the SmartGene Integrated Database Network System to access mutations lists and drug resistance scoring algorithms from the Stanford HIV Drug Resistance Database Version 8.8.0. Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, azidothymidine (zidovudine); D4T, stavudine; DDI, didanosine; DOR, doravirine; EFV, efavirenz; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir.

is needed to understand the potential efficacy of new integrase inhibitor-based regimens in the setting of underlying resistance to tenofovir and XTC.

It is critical to conduct surveillance for HIVDR in settings such as sub-Saharan Africa, where resources to prevent the development and transmission of HIVDR may inconsistently reach PLWH. In resource-rich settings, baseline genotypic resistance testing for HIVDR is routine. PI-based or integrase inhibitor-based therapy is recommended as first-line treatment if initiated before receipt of genotype results [47, 48], reflecting the preponderance of NNRTI resistance mutations among transmitted HIVDR [20, 49], the relatively low likelihood of acquiring PI resistance with failure of a first PI-based regimen [50, 51], and the efficacy of integrase inhibitors even in the rare setting of resistance mutations [52, 53]. The cost and relative unavailability of PI-based regimens in resource-limited settings would make their use as first-line therapy difficult. Few major PI resistance mutations were detected in this study, supporting continued empiric use of PI-based regimens as second-line therapy.

We characterized HIVDR in a large cohort of PLWH across four African countries. However, for technical and logistical reasons, genotypic testing for HIVDR could not be performed for a small subset of eligible samples. Under-reporting of ART use may have led to misclassification of some ART-experienced participants as ART-naive, although extensive medical record review likely minimized this. Because testing was conducted on samples collected at study enrollment, we do not know the

Table 3. Antiretroviral Drug Resistance Mutations Among Viremic ART-Experienced Participants

	Uganda (n = 10)	Kenya (n = 104)	Tanzania (n = 31)	Nigeria (n = 26)	Total (n = 171)
Major NNRTI Resistance	**6 (60%)**	**90 (87%)**	**24 (77%)**	**23 (88%)**	**143 (84%)**
K103N	3 (30%)	47 (45%)	13 (42%)	16 (62%)	79 (46%)
Y181C/I/V	2 (20%)	27 (26%)	9 (29%)	5 (19%)	43 (25%)
G190A/S	3 (30%)	27 (26%)	3 (10%)	2 (8%)	35 (20%)
K101E/P	1 (10%)	7 (7%)	1 (3%)	3 (12%)	12 (7%)
P225H	1 (10%)	10 (10%)	1 (3%)	0 (0%)	12 (7%)
Y188L/C/H	1 (10%)	3 (3%)	1 (3%)	2 (8%)	7 (4%)
M230L	1 (10%)	2 (2%)	1 (3%)	1 (4%)	5 (3%)
V106A	0 (0%)	2 (2%)	3 (10%)	0 (0%)	5 (3%)
V106M	1 (10%)	2 (2%)	1 (3%)	1 (4%)	5 (3%)
K103S	0 (0%)	3 (3%)	1 (3%)	0 (0%)	4 (2%)
L100I	0 (0%)	0 (0%)	1 (3%)	1 (4%)	2 (1%)
Major NRTI Resistance	**5 (50%)**	**75 (72%)**	**20 (65%)**	**16 (62%)**	**116 (68%)**
M184V/I	5 (50%)	72 (69%)	20 (65%)	15 (58%)	112 (65%)
T215Y/F	1 (10%)	17 (16%)	9 (29%)	5 (19%)	32 (19%)
K65R	1 (10%)	23 (22%)	1 (3%)	5 (19%)	30 (18%)
K219Q/E	0 (0%)	17 (16%)	6 (19%)	5 (19%)	28 (16%)
K70R	0 (0%)	16 (15%)	8 (26%)	4 (15%)	28 (16%)
D67N	0 (0%)	14 (13%)	6 (19%)	3 (12%)	23 (13%)
M41L	1 (10%)	10 (10%)	5 (16%)	5 (19%)	21 (12%)
L210W	1 (10%)	9 (9%)	3 (10%)	2 (8%)	15 (9%)
Y115F	0 (0%)	3 (3%)	0 (0%)	3 (12%)	6 (4%)
K70E	1 (10%)	3 (3%)	0 (0%)	1 (4%)	5 (3%)
L74V/I	0 (0%)	3 (3%)	1 (3%)	0 (0%)	4 (2%)
D67G	0 (0%)	0 (0%)	2 (6%)	0 (0%)	2 (1%)
Major PI Resistance	**0 (0%)**	**1 (1%)**	**1 (3%)**	**1 (4%)**	**3 (2%)**
I54V	0 (0%)	1 (1%)	1 (3%)	0 (0%)	2 (1%)
V82A	0 (0%)	1 (1%)	1 (3%)	0 (0%)	2 (1%)
I84V	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (1%)
I50V	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)
L76V	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (1%)

All data are presented as n (%).
Abbreviations: ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

duration of viremia experienced prior to HIVDR testing. Our study did not include assessment of mutations conferring resistance to integrase inhibitors, but this will be important for future research as these agents become more common in sub-Saharan Africa [54]. HIV-1 RNA > 1000 copies/mL was relatively uncommon among ART-experienced participants, resulting in small sample sizes for country-specific analyses of acquired drug resistance that may not be representative of the general population of ART-experienced PLWH in each country. Our study was conducted across a diverse network of PEPFAR-supported clinics with country-level and facility-level characteristics that may have impacted HIVDR prevalence, such as earlier access to ART in Uganda and the two more urban sites in Nigeria as compared to other sites. Our HIVDR prevalence estimates may not be generalizable to other facilities in each of the countries studied or more broadly to sub-Saharan Africa.

In conclusion, we found concerning evidence of pretreatment HIVDR in all countries evaluated that underscores the need to address programmatic gaps that promote the development and transmission of drug-resistant viruses. We found underlying HIVDR in the majority of participants failing ART, including multi-drug resistant viruses that suggest delays in medically-necessary changes to second-line ART. Our findings support the ongoing programmatic shift to integrase inhibitor-based first-line ART and the continued use of PI-based second-line ART in cases of viral failure. They also highlight how programmatic gaps and public health strategies that may promote accumulation of drug resistance mutations could compromise the efficacy of the next generation of first- and second-line ART regimens.

Notes

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Table 4. Factors Associated With Drug Resistance Mutations Among Viremic ART-Naive and ART-Experienced Participants

	ART-Naive *(Any WHO SDRM)*				ART-Experienced *(Any Major Resistance Mutation)*			
	PR	95% CI	aPR	95% CI	PR	95% CI	aPR	95% CI
Age (years)								
<30	Ref		Ref		Ref		Ref	
30–39	0.77	(.50–1.21)	0.72	(.46–1.15)	1.25	(.98–1.60)	1.26	(.97–1.63)
40+	**0.56**	**(.33–.95)**	0.58	(.34–1.01)	1.15	(.89–1.49)	1.10	(.83–1.46)
Sex								
Male	Ref		Ref		Ref		Ref	
Female	1.53	(1.00–2.36)	1.38	(.89–2.14)	1.01	(.88–1.15)	0.98	(.86–1.11)
Country								
Uganda	Ref		Ref		Ref		Ref	
Kenya	**0.54**	**(.32–.92)**	0.74	(.42–1.29)	1.44	(.86–2.41)	1.45	(.86–2.44)
Tanzania	0.57	(.31–1.05)	0.74	(.38–1.48)	1.29	(.75–2.22)	1.35	(.77–2.35)
Nigeria	1.21	(.72–2.04)	**3.75**	**(1.88–7.44)**	1.47	(.87–2.50)	1.64	(.96–2.80)
Year								
2013–2015	Ref		Ref		Ref		Ref	
2016–2019	**1.84**	**(1.24–2.73)**	**2.42**	**(1.57–3.73)**	0.96	(.82–1.13)	0.94	(.80–1.10)
Education								
Less than Primary	Ref		Ref		Ref		Ref	
Primary School	**0.62**	**(.40–.96)**	**0.58**	**(.36–.95)**	0.97	(.83–1.14)	1.01	(.86–1.20)
Secondary School and Above	**0.49**	**(.28–.88)**	**0.27**	**(.14–.52)**	0.98	(.83–1.16)	0.96	(.80–1.13)
CD4 Count (cells/mm^3)								
<200	Ref		Ref		Ref		Ref	
200–349	1.09	(.65–1.83)	1.03	(.61–1.76)	0.97	(.85–1.12)	0.98	(.83–1.14)
350–499	1.12	(.65–1.90)	0.98	(.56–1.70)	0.81	(.61–1.06)	0.81	(.62–1.05)
500+	0.65	(.36–1.16)	0.59	(.31–1.09)	0.89	(.68–1.15)	0.84	(.65–1.08)
HIV-1 RNA (copies/mL)								
1001–10 000	Ref		Ref		Ref		Ref	
10 001–100 000	1.27	(.72–2.25)	1.10	(.63–1.94)	1.10	(.93–1.30)	1.08	(.92–1.28)
100 001+	1.31	(.74–2.33)	1.20	(.67–2.16)	1.08	(.89–1.31)	1.02	(.83–1.26)
Years Since HIV Diagnosis								
<1	Ref		Ref		Ref		Ref	
1–5	1.58	(.94–2.66)	**2.38**	**(1.35–4.21)**	1.02	(.79–1.33)	0.95	(.75–1.22)
>5	1.36	(.66–2.81)	1.96	(.95–4.06)	1.15	(.90–1.46)	1.10	(.86–1.40)
Prior Exposure to NVP for PMTCT								
No	Ref		Ref		Ref		Ref	
Yes	1.38	(.48–3.99)	0.76	(.26–2.24)	0.89	(.51–1.58)	0.90	(.52–1.56)
Prior Exposure to EFV								
No					Ref		Ref	
Yes					0.93	(.81–1.06)	0.90	(.78–1.05)
Prior Exposure to TDF								
No					Ref		Ref	
Yes					0.96	(.84–1.09)	0.98	(.86–1.10)

Unadjusted and adjusted Poisson regression modeling with robust variance estimators was used to estimate prevalence ratios and 95% confidence intervals for prespecified factors potentially associated with drug resistance. For ART-naive participants, the outcome of the models was any World Health Organization surveillance drug resistance mutation.
For ART-experienced participants, the outcome of the models was any major mutation conferring resistance to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, or protease inhibitors. The adjusted models included all listed variables. Statistically significant prevalence ratios (<.05) are shown in **bold**.
Abbreviations: aPR, adjusted prevalence ratio; ART, antiretroviral therapy; CI, confidence interval; EFV, efavirenz; NVP, nevirapine; PMTCT, prevention of mother-to-child transmission; PR, prevalence ratio; Ref, reference category; TDF, tenofovir; WHO SDRM, World Health Organization surveillance drug resistance mutation.

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L. Bor, E. Maera, A. Kasembeli. AIC Litein Hospital, Litein, Kericho, Kenya: J. Rotich, C. Kipkoech, W. Chepkemoi, A. Rono. Kapkatet District Hospital, Kabkatet, Kericho, Kenya: Zeddy Kesi, Janet Ngeno, Edwin Langat, Keneddy Labosso, Ken Langat, Robert Kirui. Tenwek Mission Hospital, Bomet, Bomet, Kenya: L. Rotich, M. Mabwai, E. Chelangat, J. Agutu, C. Tonui. Nandi Hills District Hospital, Nandi Hills, Kenya: E. Changwony, M. Bii, E.Chumba, J. Korir. Kapsabet District Hospital, Kapsabet, Nandi, Kenya: J. Sugut, D. Gitonga, R. Ngetich, S. Kiprotich. Kenya Medical Research Institute, Kisumu, Kenya: W. Rehema, C. Ogari, I. Ouma, O. Adimo, S. Ogai, C. Okwaro, E. Maranga, J. Ochola, K. Obambo, V. Sing'oei, L. Otieno, O. Nyapiedho, N. Sande, E. Odemba, F. Wanjiru. Walter Reed Program-Tanzania, Mbeya, Tanzania: S. Khamadi, E. Chiweka, A. Lwilla, D. Mkondoo, N. Somi, P. Kiliba, M. Mwaipopo, G. Mwaisanga, J. Muhumuza. Mbeya Zonal Referral Hospital, Mbeya, Tanzania: N. Mkingule, O. Mwasulama, A. Sanagare, P. Kishimbo. National Institute of Medical Research, Mbeya Medical Research Centre, Mbeya, Tanzania: G. David, F. Mbwayu, J. Mwamwaja, J. Likiliwike, J. Muhumuza, R. Mcharo, N. Mkingule, O. Mwasulama, B. Mtafya, C. Lueer, A. Kisinda, T. Mbena, H. Mfumbulwa, L. Mwandumbya, P. Edwin, W. Olomi. Walter Reed Program-Nigeria, Abuja, Nigeria: Y. Adamu, A. Akintunde, A. B. Tiamiyu, K. Afoke, S. Mohammed. 68 Nigerian Army Reference Hospital, Yaba, Lagos, Nigeria: N. E. Harrison, U.C. Agbaim, O. A. Adegbite, Z. Parker, G.A. Adelakun, F. O. Oni, R. O Ndbuisi, J. Elemere. Defense Headquarters Medical Center, Abuja, Nigeria: N. Azuakola, T.T. Williams, M. Ayogu, O. Enas, O. Enameguono, A. F. Odo, I. C. Ukaegbu, O. Ugwuezumba. Defense Reference Lab, Abuja, Nigeria: S. O. Odeyemi, N. C. Okeke, L. Umeji, A. Rose, H. Daniel, H. Nwando, E. I. Nicholas, T. Iyanda, C. Okolo, V. Y. Mene, B. Dogonyaro, O. Olabulo, O. Akinseli, F. Onukun, G. Knopp.

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References

1. Joint United Nations Programme on HIV/AIDS. Fact sheet—global AIDS update 2019. Available at: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed 6 September 2019.
2. Forsythe SS, McGreevey W, Whiteside A, et al. Twenty years of antiretroviral therapy for people living with hiv: global costs, health achievements, economic benefits. Health Aff (Millwood) **2019**; 38:1163–72.
3. Phillips AN, Stover J, Cambiano V, et al. Impact of HIV drug resistance on HIV/AIDS-associated mortality, new infections, and antiretroviral therapy program costs in Sub-Saharan Africa. J Infect Dis **2017**; 215:1362–5.
4. Jordan MR, Bennett DE, Wainberg MA, et al. Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004–2011. Clin Infect Dis **2012**; 54:S245–9.
5. 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.
6. Ford N, Ball A, Baggaley R, et al. The WHO public health approach to HIV treatment and care: looking back and looking ahead. Lancet Infect Dis **2018**; 18:e76–86.
7. Saag MS, Benson CA, Gandhi RT, et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2018 recommendations of the International Antiviral Society-USA Panel. JAMA **2018**; 320:379–96.
8. Lessells RJ, Avalos A, de Oliveira T. Implementing HIV-1 genotypic resistance testing in antiretroviral therapy programs in Africa: needs, opportunities, and challenges. AIDS Rev **2013**; 15:221–9.
9. Hosseinipour MC, Gupta RK, Van Zyl G, Eron JJ, Nachega JB. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. J Infect Dis **2013**; 207(Suppl 2):S49–56.
10. National AIDS Control Programme. National guidelines for the management of HIV and AIDS: sixth edition. Dar es Salaam, Tanzania: Ministry of Health, Community Development, Gender, Elderly and Children, **2017**.
11. National AIDS and STI's Control Programme. National guidelines for HIV prevention treatment and care. Abuja, Nigeria: Federal Ministry of Health, **2016**.
12. National AIDS and STI Control Program. Guidelines on use of antiretroviral drugs for treating and preventing HIV Infection in Kenya—2018 Edition. Nairobi, Kenya: Ministry of Health, **2018**.
13. Ministry of Health. Consolidated guidelines for prevention and treatment of HIV in Uganda. Kampala, Uganda: Ministry of Health, **2018**.
14. Shroufi A, Van Cutsem G, Cambiano V, et al. Simplifying switch to second-line antiretroviral therapy in sub-Saharan Africa: predicted effect of using a single viral load to define efavirenz-based first-line failure. AIDS **2019**; 33:1635–44.
15. Hauser A, Kusejko K, Johnson LF, et al. Bridging the gap between HIV epidemiology and antiretroviral resistance evolution: modelling the spread of resistance in South Africa. PLoS Comput Biol **2019**; 15:e1007083.
16. Ake JA, Polyak CS, Crowell TA, et al; African Cohort Study Team. Noninfectious comorbidity in the African cohort study. Clin Infect Dis **2019**; 69:639–47.
17. 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.
18. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol **2004**; 159:702–6.
19. World Health Organization. Guidelines on the public health response to pretreatment HIV drug resistance. Geneva, Switzerland: World Health Organization, **2017**.
20. Yanik EL, Napravnik S, Hurt CB, et al. Prevalence of transmitted antiretroviral drug resistance differs between acutely and chronically HIV-infected patients. J Acquir Immune Defic Syndr **2012**; 61:258–62.
21. Jain V, Sucupira MC, Bacchetti P, et al. Differential persistence of transmitted HIV-1 drug resistance mutation classes. J Infect Dis **2011**; 203:1174–81.
22. Wertheim JO, Oster AM, Johnson JA, et al. Transmission fitness of drug-resistant HIV revealed in a surveillance system transmission network. Virus Evol **2017**; 3:vex008.
23. Bacheler L, Jeffrey S, Hanna G, et al. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J Virol **2001**; 75:4999–5008.
24. Melikian GL, Rhee SY, Varghese V, et al. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. J Antimicrob Chemother **2014**; 69:12–20.
25. World Health Organization. Update of recommendations on first- and second-line antiretroviral regimens. Geneva, Switzerland: World Health Organization, **2019**.
26. World Health Organization. WHO global strategy for the surveillance and monitoring of HIV drug resistance 2012. Geneva, Switzerland: World Health Organization, **2012**.
27. Stadeli KM, Richman DD. Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. Antivir Ther **2013**; 18:115–23.
28. Gupta RK, Gregson J, Parkin N, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. Lancet Infect Dis **2018**; 18:346–55.
29. Masimba P, Kituma E, Klimkait T, et al. Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania. AIDS Res Hum Retroviruses **2013**; 29:1229–36.
30. Somi GR, Kibuka T, Diallo K, 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.
31. Crowell TA, Kijak GH, Sanders-Buell E, et al; TRUST/RV368 Study Group. Transmitted, pre-treatment and acquired antiretroviral drug resistance among men who have sex with men and transgender women living with HIV in Nigeria. Antivir Ther **2019**; 24:595–601.
32. Hamers RL, Wallis CL, Kityo C, et al; PharmAccess African Studies to Evaluate Resistance (PASER). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis **2011**; 11:750–9.
33. Boender TS, Hoenderboom BM, Sigaloff KC, et al. Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. Clin Infect Dis **2015**; 61:1749–58.

34. Chung MH, Silverman R, Beck IA, et al. Increasing HIV-1 pretreatment drug resistance among antiretroviral-naive adults initiating treatment between 2006 and 2014 in Nairobi, Kenya. AIDS **2016**; 30:1680–2.
35. Silverman RA, Beck IA, Kiptinness C, et al. Prevalence of pre-antiretroviral-treatment drug resistance by gender, age, and other factors in HIV-infected individuals initiating therapy in Kenya, 2013–2014. J Infect Dis **2017**; 216:1569–78.
36. Ssemwanga D, Lihana RW, Ugoji C, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev **2015**; 17:3–20.
37. Quercia R, Perno CF, Koteff J, et al. Twenty-five years of lamivudine: current and future use for the treatment of HIV-1 infection. J Acquir Immune Defic Syndr **2018**; 78:125–35.
38. Larder BA, Kemp SD, Harrigan PR. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science **1995**; 269:696–9.
39. Back NK, Nijhuis M, Keulen W, et al. Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. EMBO J **1996**; 15:4040–9.
40. Eron JJ, Benoit SL, Jemsek J, et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. N Engl J Med **1995**; 333:1662–9.
41. Kuritzkes DR, Quinn JB, Benoit SL, et al. Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients. AIDS **1996**; 10:975–81.
42. Wainberg MA, Drosopoulos WC, Salomon H, et al. Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science **1996**; 271:1282–5.
43. Gregson J, Rhee SY, Datir R, et al. HIV-1 viral load is elevated in individuals with reverse transcriptase mutation M184V/I during virological failure of first line antiretroviral therapy and is associated with compensatory mutation L74I. J Infect Dis **2020**; 222:1108–16.
44. Paton NI, Kityo C, Hoppe A, et al; EARNEST Trial Team. Assessment of second-line antiretroviral regimens for HIV therapy in Africa. N Engl J Med **2014**; 371:234–47.
45. Second-Line Study Group, Boyd MA, Kumarasamy N, et al. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. Lancet **2013**; 381:2091–9.
46. Hakim JG, Thompson J, Kityo C, et al; Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) Trial Team. Lopinavir plus nucleoside reverse-transcriptase inhibitors, lopinavir plus raltegravir, or lopinavir monotherapy for second-line treatment of HIV (EARNEST): 144-week follow-up results from a randomised controlled trial. Lancet Infect Dis **2018**; 18:47–57.
47. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Washington, DC: US Department of Health and Human Services, **2019**.
48. Gunthard HF, Calvez V, Paredes R, et al. Human immunodeficiency virus drug resistance: 2018 recommendations of the International Antiviral Society-USA Panel. Clin Infect Dis **2019**; 68:177–87.
49. Gagliardo C, Brozovich A, Birnbaum J, et al. A multicenter study of initiation of antiretroviral therapy and transmitted drug resistance in antiretroviral-naive adolescents and young adults with HIV in New York City. Clin Infect Dis **2014**; 58:865–72.
50. Lathouwers E, De Meyer S, Dierynck I, et al. Virological characterization of patients failing darunavir/ritonavir or lopinavir/ritonavir treatment in the ARTEMIS study: 96-week analysis. Antivir Ther **2011**; 16:99–108.
51. Soriano V, Arasteh K, Migrone H, et al; ARTEN investigators. Nevirapine versus atazanavir/ritonavir, each combined with tenofovir disoproxil fumarate/emtricitabine, in antiretroviral-naive HIV-1 patients: the ARTEN Trial. Antivir Ther **2011**; 16:339–48.
52. Hyle EP, Scott JA, Sax PE, et al. Clinical impact and cost-effectiveness of genotype testing at human immunodeficiency virus diagnosis in the United States. Clin Infect Dis **2020**; 70:1353–63.
53. Koullias Y, Sax PE, Fields NF, Walensky RP, Hyle EP. Should we be testing for baseline integrase resistance in patients newly diagnosed with human immunodeficiency virus? Clin Infect Dis **2017**; 65:1274–81.
54. Phillips AN, Venter F, Havlir D, et al. Risks and benefits of dolutegravir-based antiretroviral drug regimens in sub-Saharan Africa: a modelling study. Lancet HIV **2019**; 6:e116–27.