Transmitted HIV Type 1 Drug Resistance Among Individuals with Recent HIV Infection in East and Southern Africa

Matt A. Price,¹ Carole L. Wallis,² Shabir Lakhi,³ Etienne Karita,³ Anatoli Kamali,⁴ Omu Anzala,⁵ Eduard J. Sanders,^{6,7} Linda-Gail Bekker,⁸ Rogers Twesigye,⁴ Eric Hunter,^{3,9} Pontiano Kaleebu,^{1,4} Kayitesi Kayitenkore,³ Susan Allen,^{3,10} Eugene Ruzagira,⁴ Mary Mwangome,⁶ Gaudensia Mutua,⁵ Pauli N. Amornkul,¹ Gwynn Stevens,¹ Sergei L.K. Pond,¹¹ Malinda Schaefer,⁹ Mary A. Papathanasopoulos,² Wendy Stevens,² Jill Gilmour,¹ and the IAVI Early Infection Cohort Study Group

Abstract

To characterize WHO-defined transmitted HIV drug resistance mutation (TDRM) data from recently HIV-infected African volunteers, we sequenced HIV (*pol*) and evaluated for TDRM the earliest available specimens from ARV-naïve volunteers diagnosed within 1 year of their estimated date of infection at eight research centers in sub-Saharan Africa. TDRMs were detected in 19/408 (5%) volunteers. The prevalence of TDRMs varied by research center, from 5/26 (19%) in Entebbe, 6/78 (8%) in Kigali, 2/49 (4%) in Kilifi, to 3/106 (3%) in Lusaka. One of five volunteers from Cape Town (20%) had TDRMs. Despite small numbers, our data suggest an increase in DRMs by year of infection in Zambia ($p = 0.004$). The prevalence observed in Entebbe was high across the entire study. ARV history data from 12 (63%) HIV-infected sexual partners were available; 3 reported ARV use prior to transmission. Among four partners with sequence data available, transmission linkage was confirmed and two had the same TDRMs as the newly infected volunteer (both K103N). As ARV therapy continues to increase in availability throughout Africa, monitoring incident virus strains for the presence of TDRMs should be a priority. Early HIV infection cohorts provide an excellent and important platform to monitor the development of TDRMs to inform treatment guidelines, drug choices, and strategies for secondary prevention of TDRM transmission.

Introduction

AS ANTIRETROVIRAL (ARV) THERAPY is becoming more available throughout Africa, the prevalence of HIV-1 drug resistance mutations is also likely to increase. Viral resistance to these drugs can develop through selective pressure when taking ARVs, can in rare cases arise spontaneously as

polymorphisms, or can be acquired through infection with an already resistant virus. The latter is often referred to as transmitted drug resistance.^{1,2} Transmitted drug resistance may have a substantial impact in areas in which options for first-line therapy are limited, as it increases the risk of virologic failure.³ Studies in the United States and Europe have shown an increased incidence in transmitted drug resistance

¹International AIDS Vaccine Initiative, San Francisco, California; Amsterdam, The Netherlands; Nairobi, Kenya; and New Delhi, India.

²Department of Molecular Medicine and Haematology, University of the Witwatersrand, Johannesburg, South Africa.

³Rwanda Zambia HIV Research Group, Lusaka, Zambia; Ndola, Zambia; Kigali, Rwanda.

⁴MRC/UVRI Uganda Research Unit on AIDS, Masaka and Entebbe, Uganda.

⁵Kenya AIDS Vaccine Initiative, Nairobi, Kenya.

⁶Centre for Geographic Medicine-Coast, Kenya Medical Research Institute, Kilifi, Kenya.

⁷Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Headington, United Kingdom.

⁸Desmond Tutu HIV Foundation, Cape Town, South Africa.

⁹Emory Vaccine Research Center, Emory University, Atlanta, Georgia.

¹⁰Emory School of Public Health, Emory University, Atlanta, Georgia.

¹¹Department of Medicine, University of California San Diego, California.

mutations (TDRMs) with time after the introduction of ARV therapy.⁴⁻⁶ This has led to recommendations for drug resistance testing as soon as HIV-1 infection is detected to guide first-line drug choices.^{7,8} Published reports have suggested that TDRMs in ARV-naïve Africans has been uncommon⁹⁻¹⁴; however, much higher rates of drug resistance in Africa have been reported among patients with a history of ARV use.¹⁵ Recently, the WHO has published recommendations for surveillance in countries scaling up ARV therapy programs,^{16,17} and results from Africa using these recommendations have shown a low (<5%) prevalence of TDRMs.^{16,17}

ARV resistance mutations have been reported to persist for 2 years or longer in the absence of drug pressure.¹⁸ Some have suggested that TDRMs may also be acquired from an ARV-naïve individual who was infected with a TDRM virus from another individual (secondary transmission).¹⁹ Because the prevalence of transmitted resistance mutations can influence policy and guide treatment choices in the absence of individual TDRM testing, TDRM surveillance is important,³ particularly in sub-Saharan Africa where first-line ARV therapy options are limited and second- or third-line regimens are not always available.²⁰ To this end, the World Health Organization has recommended adopting a consensus list of surveillance TDRMs that was updated in 2009 and excludes polymorphisms^{1,21} and has provided guidelines for surveillance in developing countries.¹⁶

Since 2006, a prospective cohort of HIV-1-infected volunteers identified and enrolled within 12 months of their estimated date of infection continues at eight research centers in five sub-Saharan African countries. We present the infecting HIV-1 subtype, TDRM data, and, where available, ARV use in the suspected transmitting partner.

Materials and Methods

Study populations

Eight clinical research centers in Africa (Kigali, Rwanda; Lusaka, and Copperbelt, Zambia; Masaka and Entebbe, Uganda; Nairobi and Kilifi, Kenya; and Cape Town, South Africa) participate in a collaborative network established for HIV preventive clinical trials. Current activities include HIV-incidence studies in at-risk volunteers, primarily sex workers, HIV-discordant cohabitating couples, and men who report sex with men. These male and female volunteers are typically followed monthly or quarterly with risk reduction counseling, clinical evaluation, and HIV-1 testing at each study visit. Volunteers who were diagnosed with HIV-1 infection were invited to enroll in the Early Infection Cohort (EIC) study. Volunteers who are not followed in HIV-incidence studies, but have documentation of a previous HIV-negative test result (e.g., regular attendees at neighboring voluntary counseling and testing (VCT) clinics or women in antenatal care) were also eligible to enroll. Written informed consent for the EIC was obtained prior to conducting any study procedures. Date of HIV infection was estimated as the midpoint between the last HIV antibody-negative test result and the first antibody-positive test result. Volunteers who were identified as HIV infected by p24 antigen ELISA (HIV-1 p24 antigen assay; Vironostika) or polymerase chain reaction (PCR) assay (Amplicor Monitor v1.5, Roche) while they were still HIV-1 antibody negative have an estimated date of infection (EDI) of 14 days prior to the positive p24 or PCR assay. p24 positive

volunteers were followed closely to confirm diagnosis with eventual HIV antibody seroconversion and HIV-1 PCR. Volunteers who did not seroconvert after p24 antigen positivity were removed from the EIC study. Volunteers provided blood and demographic data, received clinical evaluations and additional counseling, and were invited to return for regular follow-up visits. When possible, suspected transmitting partners were invited to enroll and provided blood for viral load and sequencing. Partners were administered a questionnaire that included demographics, medical history, and report of any current or previous medications with a focus on ARV drugs.

Ethical considerations

This study was approved by the Kenya Medical Research Institute Ethics Committee, Kenyatta National Hospital Ethics and Research Committee, National Ethics Committee of Rwanda, the Uganda Virus Research Institute Science and Ethics Committee, the Uganda National Council for Science and Technology, the University of Cape Town Health Science Research and Ethics Committee, the University of Zambia Biomedical Research Ethics Committee, and the Emory University School of Public Health Ethics Committee. All EC/IRBs are registered with the U.S. Office of Human Research Protection.

Genotyping

The earliest available specimens collected after the EDI were shipped to Contract Laboratory Services in South Africa for TDRM testing. All study volunteers report no ARV usage at this point in the study; these samples were frequently from the same visit at which HIV was first detected. In the event of a test failure or other problem, the next available specimen was tested for TDRMs. HIV RNA was extracted from 200 μ l of plasma using the Total Nucleic Acid Isolation Kit on the MagNaPure LC analyzer (Roche, Germany) and amplified using primers designed to obtain an amplicon of approximately 1.7 kb in length.²² The amplicon encompasses the entire protease (PR) and partial reverse transcriptase (RT) regions of the *pol* gene. The 1.7-kb amplicon was sequenced using five primers and the ABI BigDye Terminator kit (v3.1; Applied Biosystems, Foster City, CA). Sequences were run on the ABI Prism 3100-*Avant* Genetic Analyzer (Applied Biosystems, Foster City, CA), assembled, and manually edited using Sequencher v4.7 (Genecodes, Ann Arbor, MI).

The REGA HIV-1 subtyping tool on the Stanford database was used to designate the HIV-1 subtype of each volunteer's sample (<http://hivdb.stanford.edu/>). Samples that could not be assigned a subtype using REGA were subjected to extensive phylogenetic analysis. A multiple alignment was created with the 2008 HIV-1 reference subtype sequences available through the Los Alamos HIV database (<http://www.hiv.lanl.gov>) using CLUSTAL X version 2.0.²³ Neighbor-joining trees were constructed with MEGA version 4,²⁴ using the Kimura two-parameter method. Recombinants were further evaluated with the Recombinant Identification program (RIP 3.0) and jumping profile Hidden Markov Model²⁵ (jpHMM-HIV; <http://www.hiv.lanl.gov>). In addition, matched *env* was PCR amplified, sequenced, and extensively analyzed²⁶ for further clarification of selected samples. When sequence data were available, epidemiological linkage for suspected

TABLE 1. STUDY POPULATION CHARACTERISTICS^a

Characteristic	Country, location, source population(s)											
	Kenya			Uganda			Rwanda			Zambia		
	Nairobi, Mtraapa, Kilifi			Entebbe			Kigali			Lusaka, Copperbelt		
Total	Urban and periurban			Urban			Urban			Urban and periurban		
	MSM, SW, SW clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients	DC, VCT clients
	N	%	N	%	N	%	N	%	N	%	N	%
Number enrolled	468		76		32		82		196		7	
Not amplified	49	10.5	10	13.2	5	15.6	2	2.4	23	11.8	2	28.6
Amplification failed	11	2.4	2	2.6	1	3.1	2	2.7	4	2.0	0	0
Amplified	408	87.2	64	84.2	26	81.3	78	95.1	169	86.2	5	71.4
Gender ^b												
Female	159	39.0	10	15.6	17	65.4	33	42.3	74	43.8	5	100.0
Male	249	61.0	54	84.4	9	34.6	45	57.7	95	56.2	0	—
Age at infection ^b												
Mean		31.5		25.2		29.3		33.7		33.1		23.4
Median		30		24		27.5		32.5		33		21
Range (min, max)		16, 59		19, 36		18, 58		18, 53		18, 59		16, 37
TDRM detected ^b	19	4.7	2	3.1	5	19.2	1	1.5	4	2.4	1	20.0
HIV-1 subtype ^b												
A1	139	34.1	42	65.6	12	46.2	22	33.3	63	80.8	0	—
B	2	0.5	1	1.6	0	—	1	1.5	0	—	0	—
C	195	47.8	9	14.1	1	3.9	3	4.6	9	11.5	168	100.0
D	54	13.2	7	10.9	11	42.3	34	51.5	2	2.6	0	—
Recombinant	16	3.9	4	6.3	1	3.9	6	9.1	4	5.1	1	0.6
Missing	2	0.5	1	1.6	1	3.9	0	—	0	—	0	—

^aMSM, men who report sex with men; SW, sex workers (male and female); DC, HIV discordant, cohabitating couples. Other risk factors include report of multiple partners, sexually transmitted disease in the past 3 months, and/or inconsistent condom use with a partner of unknown HIV status; TDRM, surveillance drug resistance mutations.^{1,21}

^bAmong volunteers with amplified specimen, *pol* region.

transmitted partners was confirmed or rejected as previously described²⁷ using amplicons derived from gp41.

Data analysis

Data on ARV program initiation were obtained from the respective study teams. The sequence data obtained for each sample were submitted to the Stanford and ViroScore databases to identify known HIV-1 drug resistance mutations associated with decreased efficiency of the PR and RT inhibitors. In this report, we present transmitted (i.e., acquired from donor) drug resistance mutations that are recommended for surveillance.^{1,21} The presence of TDRMs was compared by the year a study volunteer became HIV infected, the HIV-1 subtype, viral load, volunteer gender, and clinical research center. A Chi-squared test and nonparametric test for trend were conducted for significance; results are shown with their corresponding *p*-values.

Results

From February 2006 through September 2009, 468 volunteers were enrolled in the EIC (Table 1). A total of 408/468 (87.4%) volunteer samples were successfully amplified, 11/419 (2.6%) failed to amplify, and 49 (10.3%) were not analyzed, typically due to insufficient sample or shipping difficulties. The proportion of samples that failed to amplify or samples that were not analyzed did not vary across study site, volunteer age at infection, or volunteer gender (data not shown). Amplified volunteers were primarily (381/408, 93.4%) originally HIV incidence study cohort volunteers, with the exception of volunteers from Entebbe, with only 10/26 (38.5%) enrolled from the local HIV incidence study. The *pol* subtyping results are summarized in Table 1, and reflect the expected circulating HIV-1 subtypes for each region. The majority of samples were classified as “pure” subtypes in *pol* with only 16 (3.9%) recombinants reported.

The successfully amplified samples were drawn a median of 68 days (range: 11–1692 days) post EDI. We observed 19 volunteers (4.7%) with TDRMs.¹ Due to inadequate early sample, or failure of an earlier sample to amplify, 24 (5.9%) of the amplified samples were from >1 year postinfection. The distribution of TDRMs did not vary significantly by the timing of the sample tested [18/384 (4.7%) drawn before 1 year vs. 1/24 (4.2%) drawn after 1 year, *p* = 0.91], but did vary significantly across sites (*p* < 0.001). Two volunteers had more than one TDRM, including one volunteer infected in 2009 who had TDRMs to two classes of ARVs, both nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, Table 2). Another volunteer had both the TDRM I85V mutation and V118I, a polymorphism that can confer resistance to the NRTI class of drugs. A third volunteer had the K103N mutation as well as T74S, a polymorphic mutation to the protease inhibitor class of ARVs (Table 2). Because both V118I and T74S are polymorphic in multiple subtypes they are not recommended for surveillance of TDRMs.¹

Although the numbers of TDRMs our study detected were small, the prevalence of TDRMs in our study appeared to increase with year of infection in Zambia (*p* = 0.004) (Table 3) and a trend was also evident in Kigali, although these did not reach statistical significance. The prevalence of TDRM appeared to decrease in Entebbe (*p* = 0.03). Only one TDRM was detected at the Copperbelt research center (Table 2); however,

the trend of increasing TDRMs over time in Lusaka remained significant if Copperbelt was considered separately (non-parametric test for trend, *p* = 0.002 and 0.15, respectively). The highest prevalence of TDRMs, which was based on small numbers but persisted over time, was observed in Entebbe. Over half (16/26, 61.5%) of the volunteers with HIV incident infections from Entebbe were recruited from a VCT clinic and while most TDRMs were observed in these volunteers, the prevalence of TDRMs was not significantly different across source populations [4/16 (25.0%) in VCT attendees vs. 1/10 (10.0%) in the HIV incidence cohort volunteers, *p* = 0.48].

The prevalence of TDRMs was significantly higher in urban study centers (Kigali, Nairobi, Lusaka, Entebbe, and Cape Town) compared to rural or periurban centers [Kilifi, Masaka, and Copperbelt; 15/230 (6.5%) vs. 4/178 (2.3%), respectively, *p* = 0.04]. Gender, infecting HIV-1 subtype, and viral load in the earliest amplifiable specimen post-EDI did not correlate significantly with the presence or absence of TDRMs (data not shown). The median age at infection was higher among volunteers with a TDRM than those without, but this did not achieve statistical significance (35 vs. 30 years at infection, respectively, *p* = 0.11).

A total of 293/408 (71.8%) volunteers reported a single partner they identified as the suspected transmitter; 153/293 (52.2%) were confirmed and 58 (19.8%) were rejected genetically. The remaining 82 (28.0%) did not have sequence data available and were identified by self-report only. Of the 19 volunteers with TDRMs, 7 (36.8%) did not identify a suspected transmitting partner, 9 (47.4%) were genetically linked to their reported partner, and 3 (15.8%) did not have genetic linkages done (Table 2). Nine of the 12 sexual partners identified (75.0%) report no history of ever taking ARV medication (Table 2). Two sexual partners (16.7%) reported taking single-dose nevirapine (NVP) as prevention of mother-to-child transmission (PMTCT) of HIV between 8 months and 4.7 years before the corresponding study volunteer's EDI; one of the partner's corresponding study volunteer was found to have K103N, which confers resistance to NVP. Another sexual partner reported starting therapy with lamivudine (3TC), zidovudine (AZT), and NVP just before the estimated time of the transmission event; this partner's corresponding study volunteer had K103N and Y181C mutations, which also confer resistance to NVP. Viral sequence data from the *pol* region were available for four partners from samples drawn approximately the same time as their corresponding volunteer's samples: two (50%) had no TDRMs detected and two had the same mutation as their respective sexual partners, K103N (Table 2).

Discussion

With increasing ARV therapy availability in sub-Saharan Africa and the recent WHO recommendations for earlier treatment,²⁸ it follows that the prevalence of TDRMs will also increase, highlighting the importance of TDRM monitoring.⁷ Our study provides evidence to support this assertion. We detected significant variability in the overall prevalence of TDRMs across study populations in our cohort with an increasing prevalence over time, particularly in Lusaka and Zambia, and a high prevalence in Entebbe and Kigali. Based on WHO guidelines for surveillance in areas expanding ARV programs,¹⁶ we observed low (<5%) prevalence at most sites,

TABLE 2. HIV-1 SUBTYPE AND TRANSMITTED DRUG RESISTANCE MUTATIONS (TDRM) BY ANTIRETROVIRAL-NAÏVE VOLUNTEERS^a

Location	Gender	Estimated year of HIV-1 infection	Risk category ^b	Days postinfection of amplified sample	Viral load of amplified sample (copies/ml)	HIV-1 subtype (pol)	HIVDRM ^c			Transmitting partner status	Partner ARV drug history prior to transmission	Partner HIVDRM
							PI	NRTI	NNRTI			
Entebbe, Uganda	Male	2006	UNK	233	73,200	A1		K103N		No partner reported	NA	NA
Kilifi, Kenya	Male	2006	MSM	42	16,000	A1		D67N		No partner reported	NA	NA
Entebbe, Uganda	Female	2006	UNK	193	73,600	A1		L210W		No partner reported	NA	NA
Masaka, Uganda	Male	2006	UNK	252	125,000	D	I85V			Self-report	No ARV use prior to transmission	NA
Entebbe, Uganda	Female	2006	Het	87	690,000	A1/D	I85V	V118I		Self-report	No ARV use prior to transmission	NA
Kigali, Rwanda	Male	2007	DC	46	139,000	D			L100I	No partner reported	NA	NA
Lusaka, Zambia	Male	2007	DC	84	5,390	C		K70R		Genetically linked	No ARV use prior to transmission	NA
Cape Town, South Africa	Female	2007	Het	508	134,000	C			K103N	No partner reported	NA	NA
Kigali, Rwanda	Female	2007	DC	73	70,400	A1			L100I	Genetically linked	No ARV use prior to transmission	None detected
Entebbe, Uganda	Male	2007	UNK	66	242,000	D			K103N	Self-report	PMTCT NVP in January 2007	NA
Kigali, Rwanda	Male	2007	DC	169	195,000	A1			K103N	Genetically linked	No ARV use prior to transmission	K103N
Kigali, Rwanda	Male	2008	DC	23	148,000	A1	M46L			Genetically linked	PMTCT NVP in June 2003	NA
Entebbe, Uganda	Female	2008	Het	144	32,500	D	M46L			No partner reported	NA	NA
Kigali, Rwanda	Male	2008	DC	28	1,020,000	A1			K103N, Y181C	Genetically linked	AZT, 3TC, NVP started in May 2008	None detected
Kigali, Rwanda	Male	2008	DC	57	17,600	C			K103N	Genetically linked	No ARV use prior to transmission	K103N
Lusaka, Zambia	Male	2009	DC	39	392,000	C	T74S		K103N	Genetically linked	No ARV use prior to transmission	NA
Lusaka, Zambia	Male	2009	DC	24	452,000	C		M184V	K103N, V108I	Genetically linked	No ARV use prior to transmission	NA
Copperbelt, Zambia	Male	2009	DC	19	73,800	C		M41L	K103R	Genetically linked	No ARV use prior to transmission	NA
Kilifi, Kenya	Male	2009	MSM	49	97,600	A1			K101E, E138A	No partner reported	NA	NA

^aARV, antiretroviral therapy before the estimated date of infection; PMTCT, prevention of mother-to-child transmission treatment, in this case single-dose NVP (AZT + NVP available as alternative in Zambia); NA, no data available; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

^bDC, heterosexual discordant couple; MSM, men who report sex with men; Het, unprotected sex with partner of unknown HIV status; UNK, unknown, volunteer does not recall or was not asked about suspected route of exposure.

^cTDRM recommended for surveillance¹ are shown in bold; other potentially significant mutations that were also found are not in bold.

TABLE 3. PREVALENCE OF TDRM BY YEAR AND REGION AND TIMING OF ARV THERAPY NATIONAL PROGRAM ROLLOUT^a

Timing of ARV rollout in country/region		Year of HIV infection															p Value ^b
		2005			2006			2007			2008			2009			
		N	DRM	%	N	DRM	%	N	DRM	%	N	DRM	%	N	DRM	%	
Total		45	0	0	118	5	4.2	115	6	5.2	81	4	4.9	49	4	8.2	0.17
Rwanda	Early 2004	17	0	0	18	0	0	20	3	15.0	20	3	15.0	3	0	0	0.081
Kenya	Late 2003	1	0	0	18	1	5.6	16	0	0	19	0	0	10	1	10.0	0.796
Masaka, Uganda	Mid-2004	6	0	0	13	1	7.7	20	0	0	13	0	0	14	0	0	0.327
Entebbe, Uganda	Mid-2004	0	—	—	6	3	50.0	6	1	16.7	11	1	9.1	0	—	—	0.032
Zambia	Late 2002	21	0	0	61	0	0	50	1	2.0	18	0	0	19	3	15.8	0.002
Cape Town	Early 2004	0	—	—	2	0	0	3	1	33.3	0	—	—	0	—	—	0.546

^aN, samples successfully amplified; DRM, number of transmitted drug resistance mutations detected; ARV, antiretroviral therapy.

^bNonparametric test for trend.

with medium (5–15%) prevalence in Rwanda in 2007–2009, and, despite small numbers of volunteers, a higher than previously reported¹⁴ prevalence in Entebbe (>15%) and more recently in Zambia (>15%). The reported prevalence of TDRMs in industrialized countries has ranged from 8% to 27% and has been shown to be increasing over time, with the introduction of ARV therapy.^{4–6,29,30} The variability of TDRM prevalence reported here by the African research center likely reflects the extent and duration of coverage of local ARV therapy programs, particularly urban versus rural/periurban localities.

Many published studies of this nature from Africa were conducted prior to the increasing availability of ARVs, and, with few exceptions,³¹ do not report increases in TDRMs over time. While the results of these studies should be compared to ours with caution due to different detection assays, TDRM definitions, study populations, and time period,^{2,32} we found TDRM prevalence similar to or higher than previously published results from sub-Saharan Africa. Pregnant women represent a common population of study. In South Africa, a report of two surveys of HIV-1-infected women in their first pregnancy showed an increase in TDRMs with time, from 0% (0/65) in 2002 to 4% (2/48) in 2004.³¹ Between 2001 and 2004, no TDRMs were detected in 96 HIV-1-infected, ARV-naïve pregnant women in Cameroon.¹⁰ In another survey of pregnant women, a TDRM prevalence of 3% (1/37) in Rwanda and 5% (3/60) in Uganda was reported.⁹ None of these women reported taking ARVs at any time prior to the study.

Another report from an antenatal clinic in Entebbe, Uganda conducted in 2006–2007 found no TDRMs in 37 ARV-naïve women recently diagnosed with HIV infection.¹⁴ This latter study stands in contrast to our findings from the same city (different clinic) at approximately the same time frame, where we see 6/22 (27%) volunteers with TDRMs. Our volunteers in Entebbe represent different source populations, as 9 were male, 18 were identified as incident infections from the center-supported VCT clinic, 8 were participants in our HIV-discordant couples cohort ongoing at the same time, and all volunteers were recently infected when diagnosed; the women¹⁴ were diagnosed at an antenatal clinic, and it is not clear when they became HIV infected. Other African populations have tended to reveal modest to no TDRMs. A survey from 2002–2006 in the Ivory Coast found that 5% (5/100) of

ARV-naïve, recently HIV-1-infected blood bank donors had TDRMs.¹² TDRMs were found in one of 58 (2%) ARV-naïve patients receiving care in Mozambique in 2003.¹³ In 2001, the national sentinel surveillance in Botswana¹¹ showed no TDRMs were detected in 71 volunteers.

A strength of this study is the well-defined source populations from which the study volunteers came and the speed with which they are enrolled following HIV infection. Thousands of HIV-uninfected volunteers at risk for HIV infection have been enrolled and followed closely in the Collaborative Network's prospective HIV incidence cohort studies since 2004. Quarterly and monthly follow-up visits allowed for precise estimates of the date of HIV infection. The identification and enrollment rates of volunteers with incident HIV infection have been between 95% and 100%, allowing us to conform to the WHO surveillance guidelines of testing consecutively identified HIV-infected persons.^{16,17} Our precise estimates of the date of infection and the testing of early samples provide strong evidence that these are TDRMs, which in turn provides valuable data to guide the selection of first-line treatment therapy. Although 6% of our study population did include samples drawn >1 year after infection, the proportion of samples with TDRMs did not vary significantly by the timing of the sample tested. However, reversion to wild type does occur, and our TDRM estimates may therefore represent an underestimate.

Although some studies have shown a long-term persistence of TDRMs in humans,^{18,33,34} one study looking at TDRMs found that over half (12/20) of the individuals with TDRMs detected within 18 months of seroconversion showed evolution at the resistance position after a median follow-up duration of 15 months.³⁵ The frequency with which we saw K103N, which can confer high-level resistance to the NNRTI drugs delavirdine, efavirenz, and nevirapine, suggests that alternative therapies for PMTCT programs (which often use NVP) may need to be considered, especially if these data are borne out in future studies. Indeed, some African countries including South Africa have already introduced combination therapy to reduce the level of NNRTI drug resistance associated with single-dose NVP for PMTCT. As follow-up of the EIC continues, we will evaluate clinical events and drug treatment failures in light of these TDRMs.

This is, to our knowledge, the first report from Africa to collect data on ARV use in the sexual partner and its relation

to TDRMs in the newly HIV-infected study volunteer. We were able to collect the ARV history from the suspected and/or confirmed transmitting partners for 12 of our volunteers with TDRMs; three reported use of ARV therapy prior to transmitting HIV to their partner. Because only 3 of 12 sexual partners of volunteers with TDRMs reported prior ARV use, some of the mutations we observed may represent secondary transmissions. TDRMs are by definition not considered spontaneous polymorphisms (i.e., they occur in the absence of ARV in <1% of sequences analyzed); therefore the probability of a spontaneous TDRM remains unlikely. Resistance mutations can persist for years and be transmitted in the absence of ARV use.^{18,19}

Among the four volunteers whose sexual partners had their virus *pol* region sequenced, only two reported with the same TDRM, both K103N. All four partner-volunteer pairs were linked by HIV sequence data; however, the transmitted virus with the TDRM may represent a minor strain in the partner, and the population-based sequencing methods may not have picked up that genome for amplification. The effect TDRMs may have on transmission efficiency is unclear, especially in non-B subtypes, and represents an important future avenue for research as ARV programs expand their coverage in Africa.

Some limitations of the study exist. The highest prevalence of TDRMs was found in Entebbe (19%), where a clear source population was not well defined. Less than 7% (27/408) of all the volunteers in this study were recruited from outside our study of HIV incidence underway concurrently, and the majority of these volunteers were from Entebbe, where 62% (16/26) came from outside this incidence cohort. Limited risk data are available on these Entebbe volunteers, and while the proportion of TDRMs did not vary significantly by source population at this site, the numbers are small and a selection bias may influence our results. Our observed decrease in TDRMs by year in Entebbe was unexpected and may represent improvements in patient management or access to ARV.

Though we did not observe differences in the age, gender, and study center across samples that were not analyzed due to amplification failures, shipping difficulties, or insufficient volume, there may be unmeasured selection bias introduced by the 17% of EIC volunteers not considered in these analyses. We employed a novel method to amplify specimens that is reported to produce better results with non-subtype-B HIV²²; however, a small proportion (2%) of our samples still failed to amplify, due primarily to low viral load at all visits where specimen was available. Low viral load did occasionally impact our ability to analyze early samples as well, sometimes requiring the sequencing of samples >1 year post the estimated date of infection. Additionally, even with our cohort size, the number of volunteers with TDRMs was low, limiting both the capacity for more detailed analyses and our power to see true changes over time, if they existed.

The WHO recommends testing ≤ 47 consecutively diagnosed persons prior to categorizing local resistance as <5%, 5–15%, or >15%, and recommends a testing time frame of several months.¹⁶ Our study period is nearly 4 years, and though the WHO does not provide a minimum recommended sample size, our enrollment in Entebbe, Kenya, and Cape Town was modest. We also relied on self-report to assess ARV drug regimens. Although this likely does not present a problem with the EIC volunteers, where the specimen was typically drawn at first diagnosis, we may not have obtained complete

drug histories on the transmitting partners. Although data on this topic are limited, social desirability bias, interviewer bias, and desire to participate in a particular study all may play a role in how these data are represented by ARV patients (S. Allen and M. Hosseinipour, personal communication). Recently in our EIC two volunteers who had been HIV infected for more than 3 years saw a dramatic reduction of viral load to undetectable levels, and denied taking ARVs. These were not from visits presented in this study, and samples have been sent for ARV testing (unpublished data).

Finally, since our study populations often represent specific risk groups, geographic regions, and urban versus rural settings, generalizing these data to other populations in Africa may not be appropriate. Of note, most of our EIC volunteers had been participants in an observational research study on HIV incidence and epidemiology, and as such may have had different access to or motivations for access of care and treatment for their HIV-infected partners than other populations.

In summary, we found that TDRMs may be increasing in parts of sub-Saharan Africa since 2005, and the prevalence in these populations from Kigali, Entebbe, and more recently Zambia was considerably higher than previously reported. These data are particularly relevant now, in light of recent WHO recommendations for earlier ARV treatment initiation in developing countries,²⁸ as more national ARV therapy programs in sub-Saharan Africa are developed, and as already established programs continue to expand. Real time detection and reporting of surveillance drug resistance mutations should guide clinician and public health policy maker alike, and to this end, the WHO has recommended that sentinel surveys be conducted as part of routine public health surveillance.^{36,37} Although genotypic resistance testing is not yet routinely available across the continent, this is changing, with countries such as Burundi, Malawi, Mozambique, Nigeria, Swaziland, Tanzania, and Zambia planning or implementing TDRM surveys.³⁶ Whereas these sentinel surveys of prevalent TDRMs are helpful, early HIV infection cohorts such as this one provide an important platform from which to monitor the development of TDRMs to inform first-line treatment guidelines, national program drug choice priorities, and strategies for secondary prevention of TDRM transmission.

Acknowledgments

We gratefully acknowledge all of the volunteers who enrolled into the Early Infection Cohort study. We would also like to thank Drs. Fran Priddy and Pat Fast of IAVI for their reviews of early drafts, the Fogarty AIDS International Training in Research Program and the Emory Center for AIDS Research for support of selected authors, and Stata technical support for their help with tricky programming questions. We are grateful to Sarah Yates and the team at the Perinatal HIV Research Unit for their help in managing the data sets.

Author Disclosure Statement

No competing financial interests exist.

References

1. Bennett DE, *et al.*: Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS ONE* 2009;4(3):e4724.

2. Shafer RW, *et al.*: HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS* 2007;21(2): 215–223.
3. Daar ES and Richman DD: Confronting the emergence of drug-resistant HIV type 1: Impact of antiretroviral therapy on individual and population resistance. *AIDS Res Hum Retroviruses* 2005;21(5):343–357.
4. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *BMJ* 2001;322(7294):1087–1088.
5. Grant RM, *et al.*: Time trends in primary HIV-1 drug resistance among recently infected persons. *JAMA* 2002;288(2): 181–188.
6. Little SJ, *et al.*: Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* 2002;347(6):385–394.
7. Hirsch MS, *et al.*: Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. *Clin Infect Dis* 2008;47(2): 266–285.
8. Department of Health and Human Services: Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents, 2008.
9. Galluzzo CM, *et al.*: Antiretroviral resistance mutations in untreated pregnant women with HIV infection in Uganda and Rwanda. *AIDS Res Hum Retroviruses* 2007;23(11):1449–1451.
10. Vessiere A, *et al.*: HIV-1 pol gene polymorphism and antiretroviral resistance mutations in drug-naïve pregnant women in Yaounde, Cameroon. *J Acquir Immune Defic Syndr* 2006;42(2):256–258.
11. Bussmann H, *et al.*: HIV-1 subtype C drug-resistance background among ARV-naïve adults in Botswana. *Antivir Chem Chemother* 2005;16(2):103–115.
12. Toni, T, *et al.*: HIV-1 antiretroviral drug resistance in recently infected patients in Abidjan, Cote d'Ivoire: A 4-year survey, 2002–2006. *AIDS Res Hum Retroviruses* 2007;23(9):1155–1160.
13. Bellocchi MC, *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):452–458.
14. Ndambi N, *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(6):889–895.
15. Adjé C, *et al.*: High prevalence of genotypic and phenotypic HIV-1 drug-resistant strains among patients receiving antiretroviral therapy in Abidjan, Cote d'Ivoire. *JAIDS* 2001;26(5): 501–506.
16. Bennett DE, *et al.*: Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral Ther* 2008;13(Suppl 2):25–36.
17. Myatt M and Bennett DE: A novel sequential sampling technique for the surveillance of transmitted HIV drug resistance by cross-sectional survey for use in low resource settings. *Antiviral Ther* 2008;13(Suppl 2):37–48.
18. Brenner B, *et al.*: Persistence of multidrug-resistant HIV-1 in primary infection leading to superinfection. *AIDS* 2004;18(12): 1653–1660.
19. Brenner BG, *et al.*: High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis* 2007;195(7): 951–959.
20. Boyd MA and Cooper DA: Second-line combination antiretroviral therapy in resource-limited settings: Facing the challenges through clinical research. *AIDS* 2007;21(Suppl 4):S55–63.
21. Stanford University: Stanford University HIV Drug Resistance Database, 2009 [cited 2009 16 June]. Available from <http://hivdb.stanford.edu/pages/WHOResistanceList.html>.
22. Wallis C, *et al.*: Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1. *J Virol Methods* 2019;163(2):505–508.
23. Larkin MA, *et al.*: Clustal W and Clustal X version 2.0. *Bioinformatics* 2007;23(21):2947–2948.
24. Tamura K, *et al.*: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24(8):1596–1599.
25. Schultz AK, *et al.*: jpHMM: Improving the reliability of recombination prediction in HIV-1. *Nucleic Acids Res* 2009;37(Web Server issue):W647–651.
26. Michler K, *et al.*: Genotypic characterization and comparison of full-length envelope glycoproteins from South African HIV type 1 subtype C primary isolates that utilize CCR5 and/or CXCR4. *AIDS Res Hum Retroviruses* 2008;24(5):743–751.
27. Trask SA, *et al.*: Molecular epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia. *J Virol* 2002;76(1):397–405.
28. World Health Organization: Rapid advice: Antiretroviral therapy for HIV infection in adults and adolescents, 2009.
29. Wensing AM, *et al.*: Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: Implications for clinical management. *J Infect Dis* 2005;192(6):958–966.
30. Kim D, *et al.*: Prevalence of transmitted antiretroviral drug resistance among newly-diagnosed HIV-1-infected persons, US, 2007. In: 17th Conference on Retroviruses and Opportunistic Infections, 2010, San Francisco.
31. Pillay V, *et al.*: Antiretroviral drug resistance surveillance among drug-naïve HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. *Antivir Ther* 2008;13(Suppl 2):101–107.
32. Johnson VA, *et al.*: Update of the drug resistance mutations in HIV-1: Spring 2008. *Top HIV Med* 2008;16(1):62–68.
33. Smith DM, *et al.*: Long-term persistence of transmitted HIV drug resistance in male genital tract secretions: Implications for secondary transmission. *J Infect Dis* 2007;196(3):356–360.
34. Neifer S, *et al.*: Persistence of a sexually transmitted highly resistant HIV-1: pol quasiespecies evolution over 33 months in the absence of treatment. *AIDS* 2006;20(17):2231–2233.
35. Bezemer D, *et al.*: Evolution of transmitted HIV-1 with drug-resistance mutations in the absence of therapy: Effects on CD4+ T-cell count and HIV-1 RNA load. *Antivir Ther* 2006;11(2):173–178.
36. Jordan MR, *et al.*: World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. *Antivir Ther* 2008;13(Suppl 2):15–23.
37. Bennett DE, *et al.*: The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antivir Ther* 2008;13(Suppl 2):1–13.

Address correspondence to:

Matt A. Price

CAPS/IAVI, Suite 13105

Beale Street, 13th Floor

San Francisco, California 94114

E-mail: mprice@iavi.org