Low Rates of Transmitted Drug Resistance Among Newly Identified HIV-1 Seroconverters in Rural Rakai, Uganda

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Abstract

We investigated the rate of transmitted drug resistance (TDR) among HIV-1 seroconverters identified from the Rakai Community Cohort Study (RCCS) survey, a population-based cohort in Rakai District, Uganda. Participants aged 15–49 are interviewed at study visits approximately every 12–18 months and provided a serological sample. Antiretroviral therapy (ART) has been provided free of charge since 2004. RCCS participants with documented negative HIV-1 serology between January 2011 and August 2012 and confirmed seroconversion between November 2012 and October 2013 were included in this analysis. Serum was genotyped for HIV drug resistance mutations in reverse transcriptase and protease genes. Mutations were classified according to the 2009 World Health Organization surveillance of transmitted HIV-1 drug resistance update. Seventy-five (75) seroconverters were identified and genotyped. The mean age was 28 years (range 18–49) and the majority were male, $n=44$ (58%). The HIV-1 subtype frequencies were A=19 (25%), D=44 (59%), C=4 (5%), A/D recombinant=5 (7%), and C/D recombinant=3 (4%). The majority (72/75, 96%) of individuals were infected with wild-type virus with no evidence of TDR. Two individuals had a single non-nucleoside reverse transcriptase inhibitor mutation each, K101E and K103N, and one had a single protease inhibitor mutation, M46I. No mutations were identified involving nucleoside reverse transcriptase inhibitors. In conclusion, almost 10 years after the introduction of ART in rural Uganda, rates of TDR remain low. Ongoing surveillance for TDR remains an important public health priority and should be conducted among known seroconverters to estimate TDR.

Keywords: HIV, antiretroviral therapy, drug resistance

Introduction

ACCESS TO COMBINATION antiretroviral therapy (ART) over the past decade has transformed the prognosis of HIV-1-infected individuals in sub-Saharan Africa and is associated with drastically reduced adult mortality.^{1,2} In Uganda, by 2014, the number of HIV patients receiving ART reached 750,896 out of the projected 1.5 million people living with HIV. Moreover, a total of 125,705 ART-naïve HIV-

positive persons initiated ART in 2014 alone.³ Despite the success of expanding access, limitations in laboratory monitoring systems,⁴ drug stock-outs,^{5,6} lack of adherence,^{7,8} inadequate health workforce,⁹ and other issues have raised concerns regarding the potential spread of HIV-1 drug-resistant strains. A range of transmitted drug resistance (TDR) rates in Uganda have recently been reported from an average of 3% (2002–2004) in Mbarara (western Uganda)¹⁰ to an average of 7% (2005–2010)¹⁰ and 8.6% (2007)¹¹ in

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Kampala (central Uganda). Many TDR surveillance studies are conducted among putatively newly diagnosed HIV positives rather than documented seroconverters, and thus rely on self-report of past HIV testing and past use of ART. This potentially misclassifies acquired drug resistance as TDR due to possible underreporting of prior ART exposure.

Testing for HIV status at consecutive survey rounds in a longitudinal cohort accurately identifies the timing of HIV-1 seroconversion and allows for more precise identification of TDR. We report the prevalence of TDR among seroconverters identified in a longitudinal community cohort in Rakai, Uganda, between two consecutive survey rounds conducted between November 2012 and October 2013.

Materials and Methods

The Rakai Community Cohort Study (RCCS) is a well-characterized, population-based HIV surveillance cohort in Rakai District, Uganda. Methods for the RCCS have been described in detail elsewhere.¹² In brief, the RCCS includes all consenting persons aged 15–49 residing in 46 of the 720 villages in Rakai District, southwestern Uganda. This open cohort enrolls age-eligible individuals and in-migrants; participants exit RCCS surveillance by loss to follow-up, aging >50 years, out-migrating, or death. Consenting and assenting participants are administered a detailed questionnaire in the local language (Luganda), by same-sex interviewers at study visits occurring approximately every 12–18 months, and provided a serological sample at each visit. ART has been provided free of charge since 2004. RCCS participants with documented negative HIV-1 serology between January 2011 and August 2012 and documented seroconversion at the subsequent cohort survey round conducted between November 2012 and October 2013 were included in this analysis. Descriptive statistics were used to characterize the population and summarize results. Institutional review board approvals were obtained from the Uganda Virus Research Institute's Research and Ethics Committee, Uganda National Council for Science and Technology, and Western IRB (Olympia, WA).

Laboratory procedures for HIV-1 drug resistance testing

HIV-1 drug resistance testing was performed in the Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) laboratory in Uganda, which is World Health Organization (WHO) accredited for HIV-1 genotyping and HIV drug resistance testing. This laboratory is the national and regional reference laboratory in Uganda conforming to the WHO Virology Quality Assurance standards.

Whole blood was processed to obtain serum for HIV-1 RNA extraction. RNA was extracted from 140 μ l of serum using the QIAamp Viral RNA extraction Mini-kit (Qiagen, Hilden, Germany). Genotypic analysis was performed through nested polymerase chain reaction of protease (codons 1–99) and the amino terminus of reverse transcriptase (codons 1–242). Amplified products were sequenced at the MRC/UVRI laboratories with Applied Biosystems 3130 and 3500 genetic analyzers (Applied Biosystems, Foster City, CA).

Sequences were base called using Sequencher version 5.2.4 (Gene Codes Corp., Ann Arbor, MI), and sequence alignments were performed using BioEdit version 7.2.5¹³ and SeaView version 4.0.¹⁴ Neighbor-joining phylogenies were constructed to

determine sequence relatedness and to rule out contaminations. Drug resistance mutations (DRMs) were assigned by submission of sequences to the HIVdb program at the Stanford HIV Drug Resistance Database (<http://hivdb.stanford.edu>). Assigned DRMs were interpreted using the 2009 WHO list for epidemiological surveillance of DRMs¹⁵ alongside with the International AIDS Society 2014 update of DRMs of HIV-1.¹⁶ Sequence subtypes, including recombinants, were identified with the REGA HIV-1 subtyping tool version 3.0.¹⁷ The Recombinant Identification Program was used to confirm recombinant sequences.^{18,19} Quality assurance was done using Calibrated Population Resistance Tool, Stanford, and the Los Alamos National database for the HIV Sequence quality analysis.¹⁸ All sequence data were uploaded into the GenBank for public access.

Results

A total of 75 out of 87 (86.2%) seroconverters were successfully genotyped (Table 1). The mean age was 28 years (range 18–49). The majority were male, $n=42$ (56%), married, $n=48$ (64%), and had primary education, $n=58$ (77%). The HIV-1 subtype distribution was 59% HIV-1 subtype D, 25% subtype A, 5% subtype C, 7% subtypes A/D recombinant, and 4% subtypes C/D recombinant. The HIV-1 RNA median viral load was 4.4 $\log_{10}$ copies/ml (interquartile range = 3.9–5.0).

The majority of seroconverters were infected with wild-type virus (72/75, 96%). Three (4%) individuals had DRMs. Two individuals had a single non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation each, K101E and K103N (Table 2). One individual (1%) had a single protease inhibitor (PI) mutation, M46I. No NRTIs were identified.

Discussion

We found low rates (4%) of TDR among recently infected seroconverters in the rural population of Rakai, Uganda. This

TABLE 1. BASELINE CHARACTERISTICS AND SUBTYPE DISTRIBUTION OF NEWLY IDENTIFIED HIV-1 SEROCONVERTERS WITH GENOTYPED VIRUS

Characteristic	N (%)
Total	75 (100)
Gender	
Female, n (%)	33 (44)
Male, n (%)	42 (56)
Age	
Mean (SD)	28.4 (7.5)
Married	48 (64)
Education level	
None	6 (8)
Primary	58 (77)
Secondary	9 (12)
Subtype distribution	
A	19 (25.3)
D	44 (58.7)
AD recombinant	5 (6.7)
C	4 (5.3)
CD recombinant	3 (4.0)
HIV RNA—median $\log_{10}$ copies/ml (IQR)	4.41 (3.92–4.98)

IQR, interquartile range; SD, standard deviation.

TABLE 2. SUMMARY OF CLIENTS WITH TRANSMITTED DRUG RESISTANCE

Client	Age	Gender	Occupation	Married	Sex partners last year	Subtype	HIV RNA log ₁₀ copies/ml	Mutations
1	20	Male	Fishing	No	1	A	5.14	M46I
2	25	Female	Housekeeper	Yes	1	AD	5.04	K101E
3	29	Male	Fishing	No	10	AD	4.26	K103N

rate is lower than those recently reported from newly HIV-1 diagnosed, HIV counseling, and testing clients in Kampal, where 8.6% of clients had a TDR. Two of our participants had DRMs associated with NNRTIs and one with PIs; for each participant, drug resistance was confined to a single drug class.

Overall, variable rates of TDR have been reported in other settings, with low rates of TDR not exceeding 5% reported in Angola,^{20,21} Ethiopia,²² Botswana, Kenya, and South Africa, while high rates of TDR exceeding 6% were reported in Mombasa, Kenya,²³ Dar es Salaam, Tanzania,²⁴ fishing communities on lake Victoria, Uganda,^{25,26} and Kigali, Rwanda.²⁶ Some longitudinal studies reported increasing prevalence of TDR; in central Uganda, TDR increased from 0% in 2006/2007 to 8.6% in 2009/2010,¹¹ and the International AIDS Vaccine Initiative cohorts in east and southern Africa reported an increase of TDR in Zambia from 0% in 2005 to 17% in 2009,²⁶ while reporting a decrease in Entebbe, Uganda, from 16.7% in 2007 to 9.1% in 2008.²⁶ This variability in TDR could be due to underlying variations in ART stock-outs, which can adversely affect continuity of HIV treatment, decentralization of ART service points that affect travel distances for pill refills, and adequate staffing to attend to patients to achieve high adherence and good treatment outcomes.

The main strength of this analysis is that RCCS cohort conducts consecutive surveys to identify HIV-1 seroconverters and is the main provider of ART in this setting, which gives it a unique opportunity to avoid misclassification of acquired DR as TDR due to inaccurate self-report of ART use. A limitation of our study is that we used conventional sequencing methods to identify drug resistance, which may have underestimated the true rate due to resistance, due to minor variants, or low copy number mutations.

In conclusion, the low rates of DRMs observed in our population were reassuring, given that free ART has been offered to Rakai Health Sciences Program clients since 2004.

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Author Disclosure Statement

No competing financial interests exist.

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