Phylogenetic Analysis of HIV in East Africa Cross-Border Areas

Final Report

March 2018

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MEASURE Evaluation

Phylogenetic Analysis of HIV in East Africa Cross-Border Areas
Final Report

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ABBREVIATIONS

ART	antiretroviral therapy
BEAST	Bayesian Evolutionary Analysis by Sampling Trees
FSW	female sex worker
MCMC	Markov Chain Monte Carlo
MRC	Medical Research Council
MSM	men who have sex with men
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
PEPFAR	United States President's Emergency Plan for AIDS Relief
PCR	polymerase chain reaction
PI	protease inhibitor
PLACE	Priorities for Local AIDS Control Effort
SDRM	single drug resistance mutation
UNAIDS	Joint United Nations Programme on HIV/AIDS
USAID	United States Agency for International Development
UVRI	Uganda Virus Research Institute

EXECUTIVE SUMMARY

In 2015, 50–80% of roughly 8 million individuals living with HIV in East Africa received antiretroviral therapy (ART) to achieve HIV suppression and reduce transmission (Roser, 2017). These individuals are at risk of acquired drug resistance resulting from suboptimal ART regimens and/or medication nonadherence, which can cause the virus to mutate. Consequently, drug resistance can be transmitted to ART-naive individuals from treated patients and untreated carriers of drug-resistance mutations. Individuals with these mutations often experience virologic failure—failure to achieve viral suppression—after treatment. Viral failure in HIV-infected individuals has serious negative consequences for the patient's future morbidity, mortality, and probability of transmitting the virus to their contacts (Price, et al., 2011a; Oyugi, et al., 2007; Ssemwanga, et al., 2015; Onsongo, et al., 2016).

Resistance mutations are projected to increase in prevalence over time, as ART is prescribed earlier and more frequently (Roser, 2017). Settings where patients are prescribed suboptimal regimens or fail to adhere to a prescribed regimen also entail increased risk of such mutations (Oyugi, et al., 2007). Though previous studies have examined drug resistance in East Africa, many of these analyses have been restricted to smaller samples of individuals at clinical centers in central urban areas and convenience sampling methods (Price, et al., 2011a; Ssemwanga, et al., 2015; Onsongo, et al., 2016).

MEASURE Evaluation, funded by the United States Agency for International Development (USAID) and the United States President's Emergency Plan for AIDS Relief (PEPFAR), conducted the East Africa Cross-Border Integrated Health Study in 2016 (MEASURE Evaluation, 2017) with the aim of describing the health status and behaviors of mobile and vulnerable populations living in or traveling through 14 cross-border sites in the East African countries of Kenya, Rwanda, Tanzania, and Uganda. HIV status was a key focus of the study. Mobile and vulnerable populations of interest were young women, female sex workers (FSWs), fisherfolk, truck drivers, men who have sex with men (MSM), people who inject drugs, and workers at places (e.g., bars, pubs, or hotels) where people socialize at cross-border sites.

This work aligns with USAID's goal to control the HIV/AIDS epidemic and with PEPFAR's aim to "do the right things in the right places at the right times." It also supports efforts to achieve the global 90-90-90 targets.[1]

Objectives of the Study

The following are the objectives of the Phylogenic Analysis of HIV in East African Cross-Border Areas study:

- Identify possible HIV transmission clusters—groups of individuals with closely related viral sequences—in East Africa cross-border locations
- Characterize drug resistance mutations in mobile and vulnerable populations in East Africa cross-border locations
- Examine the relationship between the presence of drug resistance mutations and characteristics of the sample, including vulnerable population status and relevant demographic information (e.g., age, sex, and occupation)

Methods

Over the course of data collection, the study team interviewed 11,567 participants sampled from public places about their health behaviors and access to health services through a venue-based survey. Participants in the survey were offered an HIV test and, if results were positive, asked to provide dried

[1] By 2020, 90 percent of those with HIV will have been diagnosed, 90 percent of those diagnosed will be on antiretroviral therapy (ART), and 90 percent of those in treatment will be virally suppressed (http://www.unaids.org/en/resources/documents/2017/90-90-90).

blood spots for viral load testing. Participants who provided dried blood spots also consented to storage of their dried blood spots, in a biobank without identifying information, and future research using the samples.

To examine genetically linked HIV infections and ART resistance in cross-border areas, the HIV-1 *pol* genes of 125 samples with unsuppressed HIV were subsequently sequenced. Phylogenetic analyses were conducted to identify related sequences, and the samples were also analyzed for ART resistance. This report presents the results of these analyses.

Key Findings and Recommendations

Clusters of genetically-related sequences were found.

The phylogenetic analysis highlighted some potential clustering of sequences, suggesting that those individuals may have contracted HIV from related sources. We cannot definitively assign the identified related groups to transmission clusters, because this study did not collect information on the complete network of sexual partnerships, contained a relatively small number of samples, and did not collect information on suspected or known sexual contacts. However, the evidence of related groups suggests that transmission clusters may exist in this population and underscores the value of further investigation to identify the extent of these clusters, the burden of their contribution to HIV transmission in East Africa, and opportunities to stem transmitted drug resistance.

Identified clusters contained female sex workers, young women, fisherfolk, and workers at public venues such as bars and pubs. In addition, over half of identified clusters spanned international borders.

The distribution of HIV subtypes among all sequenced individuals and those identified in clusters were comparable to the distribution identified in previous data analysis (Department of Health and Human Services, Los Alamos National Laboratory, National Institutes of Health, 2015). Vulnerable populations identified in clusters were FSWs, young women, and fisherfolk, as well as workers at venues. This information is useful for understanding potential routes of HIV transmission at these cross-border locations and can direct efforts to focus interventions in high-risk areas. Notably, over half of potential transmission clusters identified spanned international borders, providing evidence for the importance of cross-border movement in the regional HIV epidemic. Owing to the small number of samples sequenced, there were many transmission clusters we failed to identify in cross-border areas, and some of the possible transmission clusters we identified were likely incomplete. However, results provide insight into how infections are likely related in this population.

Drug resistance mutations are sufficiently prevalent in this population to complicate viral suppression.

The overall prevalence of single drug resistance mutations in this sample (11.7%) sits within the range identified in other studies investigating single drug resistance mutations in other populations in East Africa. The most common single drug resistance mutation was K103N (prevalence 6.6%), which is a non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation known to be the most prevalent in East Africa (Price, et al, 2011a; Onsongo, et al., 2016).

As expected drug resistance was more common to NNRTIs and nucleoside reverse transcriptase inhibitor (NRTIs), which are components of the most common first line regimens used throughout East Africa, than among protease inhibitor (PIs), which are typically reserved for second line therapy.

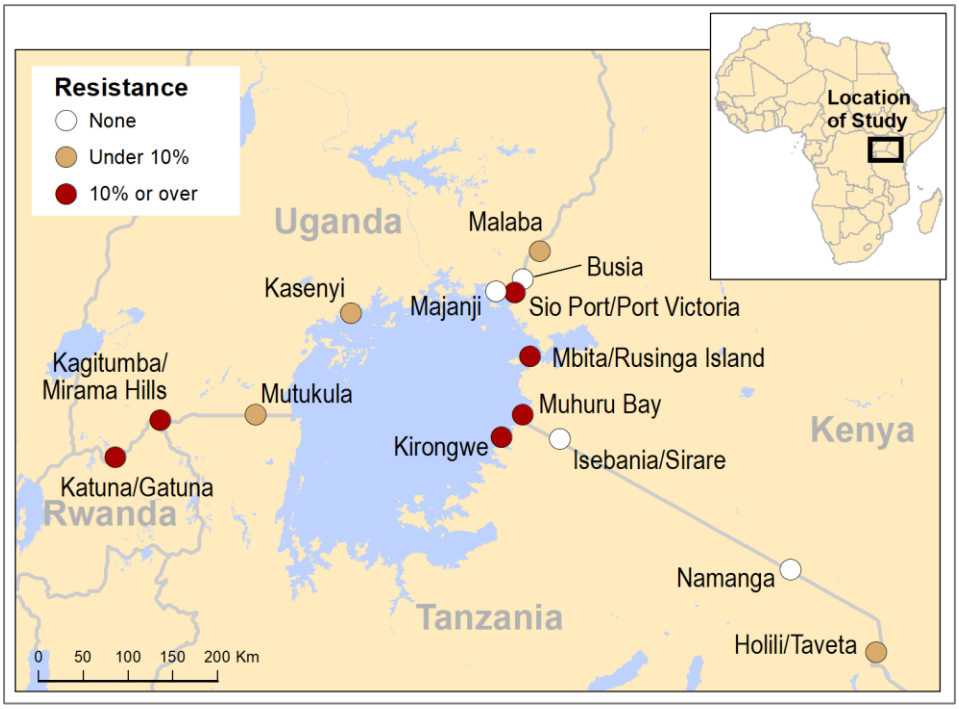
Many of the participants reported never receiving care or treatment for HIV (108 out of the 125 participants with available sequences). Among participants previously on ART, we cannot distinguish

acquired ART resistance from transmitted resistance. However, among these presumed ART-naive individuals, we may tentatively assume that drug resistance was transmitted, rather than acquired. Under this assumption, the prevalence of transmitted drug resistance was 12 percent in this population, slightly higher than the 7 percent prevalence previously reported for East African populations (Ssemwanga, et al., 2015). The difference in the estimated prevalence of transmitted drug resistance between this and previous studies could be because of distinct features of cross-border mobile and vulnerable populations. Women were less likely than men to have ART resistance, and fisherfolk were more likely to have ART resistance than non-fisherfolk. Women who had exchanged sex for cash were less likely to have ART resistance than other women, but men who had paid cash for sex were more likely to have ART resistance than other men. Higher educational attainment was associated with higher probability of having a resistant infection. Participants recruited at hotels, outside venues, and at lake sites had a higher probability of resistant infection than participants recruited at other types of venues.

Study sites bordering Rwanda and those in Kenya and Tanzania along Lake Victoria had the highest prevalence of ART resistant HIV infection.

The distribution of ART resistance for each cross-border area is shown in Figure E1. Among the study sites, areas bordering Rwanda and areas along Lake Victoria on the Kenya and Tanzania side of the lake had higher prevalence of ART resistant HIV infection. However, site-level prevalence estimates were imprecise and should be interpreted with caution.

Figure 1. Prevalence of ART resistance among people with unsuppressed HIV by cross-border area in the East Africa Cross-Border Integrated Health Study, 2016



Opportunities to adapt the HIV care and treatment system to disrupt transmission networks and reduce ART drug resistance exist in East Africa cross-border areas.

Harmonization of first- and second-line treatment regimens across countries, unification of patient monitoring systems, cross-border tracing of those lost to follow-up, and index tracing, as well as collaborative partnerships between local health facilities on each side of an international border offer

options to disrupt transmission networks and reduce antiretroviral therapy drug resistance. Pursuit of these opportunities, in concert with other prevention strategies, will be important steps in ending the HIV epidemic in East Africa.

Conclusion

This study suggests the potential for the presence of HIV transmission networks within East Africa cross-border communities, across international borders, and along transportation corridors. Intervening to disrupt these routes of HIV transmission will require both traditional combination HIV prevention approaches as well as emerging "treatment as prevention" strategies to reduce the likelihood of HIV transmission from people living with HIV to their HIV-uninfected partners. However, drug resistant strains of HIV were more common than expected in this population, which may complicate efforts to reduce transmission through treatment of those already infected. Such drug resistance may be transmitted from the source of the HIV infection to the recently infected individual or acquired over time after ART initiation. Both types of drug resistance impede viral suppression, thus increasing the probability of onward transmission and threatening achievement of the Joint United Nations Programme on HIV/AIDS (UNAIDS) 90-90-90 targets. Transmitted drug resistance, sometimes identified through routine resistance mutation monitoring at ART initiation, may require initiation of specific ART regimens to which the virus is susceptible. Acquired drug resistance is typically suggested by virologic failure after remaining faithful to a first-line treatment regimen. Evidence of transmitted or acquired drug resistance after ART initiation requires timely switching from first- to second-line regimens.

Addressing both transmitted and acquired drug resistance requires successful integration of many facets of HIV care and treatment. First, resistant infections must be identified, either through routine monitoring at ART initiation or as diagnosis of virologic failure. Such diagnoses may be complicated by poor continuity of care among highly mobile populations. Second, appropriate treatment regimens must be prescribed and maintained. Patients on second-line regimens may face challenges in obtaining necessary drugs if they switch health facilities without official transfer documents or seek HIV care and treatment outside their home countries. Finally, patients receiving non-first-line treatment regimens must be fully adherent to avoid accumulating additional resistance mutations, which could then be transmitted, along with HIV infection, to their previously HIV-uninfected partners.

INTRODUCTION

MEASURE Evaluation, led by the University of North Carolina at Chapel Hill, conducted the East Africa Cross-Border Integrated Health Study in 2016, with the aim of describing the health status and behaviors of mobile and vulnerable populations living in or traveling through 14 cross-border sites in the East African countries of Kenya, Rwanda, Tanzania, and Uganda. HIV status was a key focus of the study. Mobile and vulnerable populations of interest were the following:

- Women, especially young women ages 15 to 24
- Female sex workers, defined as women who reported exchanging sex for money in the past 12 months
- Fisherfolk
- Workers at public places (e.g., bars, pubs, and hotels) where people socialize at cross-border sites
- Truck drivers
- Men who have sex with men, defined as men who had sex with at least one man in the past 12 months
- People who inject drugs

Cross-border sites were traditional land border posts—such as Busia, Uganda and Busia, Kenya—as well as "lake border" sites. Lake border sites were defined as fishing communities where fisherfolk from different countries in the region were known to intermingle.

Over the course of data collection, the study team conducted quantitative and qualitative interviews at 23 health facilities, constructed a cohort of 3,646 people first enrolled in HIV care and treatment at a cross-border site in 2014, and interviewed 11,567 participants sampled from public places about their health behaviors and access to health services through a venue-based survey. Participants in the survey were offered an HIV test and, if they tested HIV-positive, asked to provide dried blood spots for viral load testing. The full report the East Africa Cross-Border Integrated Health Study is available at https://www.measureevaluation.org/resources/publications/tr-17-188.

Participants who provided dried blood spots also consented to storage of their dried blood spots, without identifying information in a biobank, and future research using the samples. To examine genetically linked HIV infections and ART resistance in cross-border areas, the HIV-1 *pol* genes of 125 samples with unsuppressed HIV were subsequently sequenced. Phylogenetic analyses were conducted to identify related sequences and the samples were also analyzed for ART resistance. This report presents the results of these analyses.

Background

In 2015, 50–80 percent of roughly 8 million individuals living with HIV in East Africa received ART to achieve HIV suppression and reduce transmission (Roser, 2017). These individuals are at risk of acquired drug resistance owing to suboptimal ART regimens or medication nonadherence, which cause the virus to mutate. Consequently, drug resistance can be transmitted to ART-naive individuals from treated patients and untreated carriers of drug resistance mutations. Individuals with these mutations often experience virologic failure—failure to achieve viral suppression—after treatment. Viral failure in HIV-infected individuals has serious negative consequences for both the patient's future morbidity, mortality, and probability of transmitting the virus to their contacts (Price, et al., 2011a; Oyugi, et al., 2007; Ssemwanga, et al., 2015; Onsongo, et al., 2016).

Prevalence of resistance mutations are projected to increase over time as ART is prescribed earlier and more frequently (Price, et al., 2011a). The risk of such mutations is also increased in settings where patients are prescribed suboptimal regimens or fail to adhere to a prescribed regimen (Oyugi, et al., 2007). Though previous studies have examined drug resistance in East Africa, many of these analyses have been

restricted to smaller samples of individuals at clinical centers in central urban areas and convenience sampling methods (Price, et al., 2011a; Ssemwanga, et al., 2015; Onsongo, et al., 2016).

Objectives

This study had the following objectives:

1) Conduct a phylogenetic analysis of study sequences to identify possible transmission clusters (groups of individuals with closely related viral sequences, where relatedness is defined by an average genetic distance threshold).
2) Characterize WHO surveillance-recommended drug resistance mutations in mobile and vulnerable populations across HIV drug classes. The main characterization will be prevalence of drug resistance mutations at each site, which are expected to be 7–12 percent of the population and will be conducted using Stanford University's HIV Drug Resistance Database (Rhee, et al., 2003).
3) Examine the relationship between the presence of drug resistance mutations and characteristics of the sample, including vulnerable population status and relevant demographic information (e.g., age, sex, and occupation).

The Joint United Nations Programme on HIV/AIDS has generated ambitious goals around increasing treatment in Africa to end the AIDS epidemic by 2030, but they have not explicitly defined the implication of drug resistance on these goal (Grabowski, 2014). As ART use increases in Africa to prevent and control the HIV epidemic, monitoring the presence of drug resistance is critical to protecting infected individuals, ensuring viral suppression, and maintaining successes in controlling the epidemic. The presence of related sequences or possible transmission clusters suggests a predictable structure to HIV transmission networks that could be intervened upon in these populations. Understanding the extent of drug resistance in cross-border areas will contribute to a more comprehensive picture of drug resistance in East Africa, and—more importantly—may help anticipate treatment failure, focus efforts to initiate individuals on additional or alternative ART, and examine adherence issues in specific communities.

METHODS

This study was nested within the East Africa Cross-Border Integrated Health Study (2016). Full details on study sites and participant recruitment for the East Africa Cross-Border Integrated Health Study are provided below.

Study Sites

Criteria for the selection of study sites were presence of cross-border movement and trade, high HIV/STI prevalence, gaps in health services, presence of mobile and vulnerable populations including migrants, and recognition by the East Africa Community and partner states as a priority underserved cross-border area. In addition, criteria for lake sites were a high dependence on fishing for livelihood and the presence of beach management units. The study sites are listed below and shown in Figure 1.

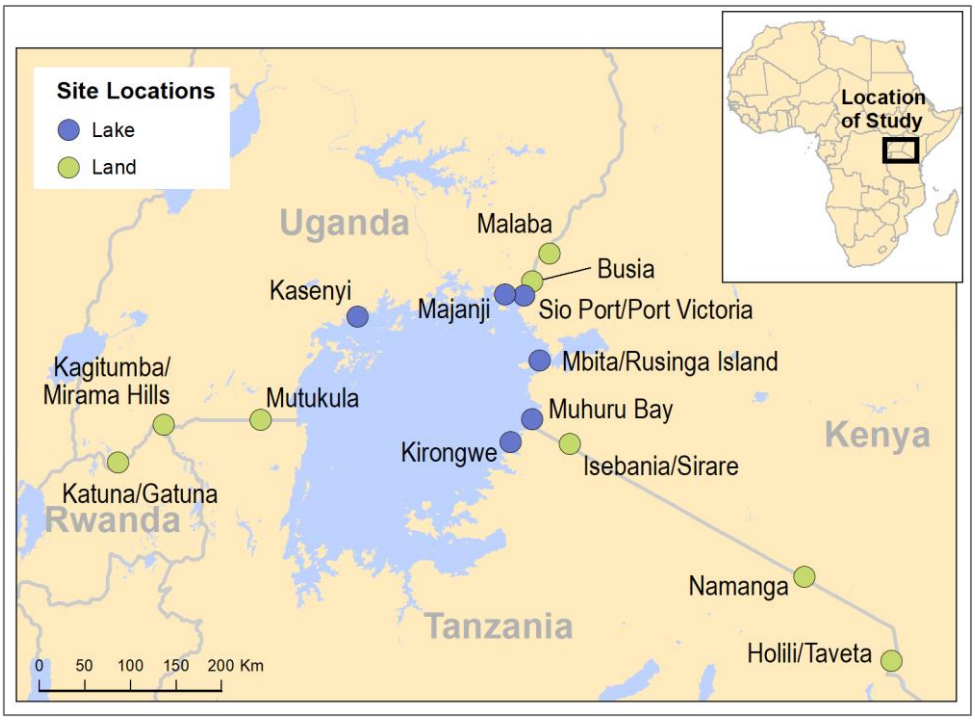
Land sites

- Malaba, Kenya; Malaba, Uganda
- Busia, Kenya; Busia, Uganda
- Katuna, Uganda; Gatuna, Rwanda
- Holili, Tanzania; Taveta, Kenya
- Isebania, Kenya; Sirari, Tanzania
- Mutukula, Tanzania; Mutukula, Uganda
- Namanga, Kenya; Namanga, Tanzania
- Kagitumba, Rwanda; Mirama Hills, Uganda

Lake sites

- Sio Port/Port Victoria, Kenya
- Majanji, Uganda
- Muhuru Bay, Kenya
- Kirongwe, Tanzania
- Mbita and Rusinga Island, Kenya
- Kasenyi, Uganda

Figure 2. Cross-border study sites



Recruitment of Participants Using PLACE

The Priorities for Local AIDS Control Efforts (PLACE) method[2] was employed by the East Africa Cross-Border Integrated Health Study. This method identifies public places or events known as "spots" (e.g., hotels, bars, or markets) where populations of interest socialize and meet new sexual partners. These spots are potential intervention areas where the individuals most likely to transmit HIV can be accessed. Spots are identified by community members ("community informants") and a knowledgeable person ("spot informant") at a sample of spots is interviewed about the spot. Next, spot patrons and workers are interviewed about their sexual and health-seeking behaviors at a sample of spots. Patrons and workers are also tested for HIV at this time using an HIV rapid test. Dried blood spots are collected from HIV-positive participants for viral load testing. These three data collection steps of PLACE are described below in the context of this study.

PLACE Step 1: Community Informant Interviews

The data collection team interviewed up to 200 community informants at each of the cross-border study sites to identify spots where members of the populations of interest socialize and meet new sexual partners. Community informants are men and women knowledgeable about the movement and behavior of people in the area. Community informants were taxi drivers, food stand sellers, transport workers, truck drivers, and fisherfolk. The categories relevant to each site were determined in collaboration with local officials.

Community informants were recruited through purposive sampling, and research assistants were given targets for how many community informants from each category to interview. A minimal amount of information about the informant was collected in addition to the list of spots identified by the informant as places where mobile and vulnerable populations socialize and meet new sex partners.

[2] The PLACE protocol can be found at https://www.measureevaluation.org/resources/tools/hiv-aids/place.

PLACE Step 2: Spot Verification Interviews

For each cross-border site, a list of unique spots was generated from the community informant interviews. The list of spots was then sorted based on reported presence of FSWs, MSM, people who inject drugs, sex onsite, and the number of community informants reporting the spot. The sorted spots were assigned to priority categories for sampling (mandatory, high, medium, and lower priority) to facilitate oversampling of spots where community informants suggested that populations of interest were more likely to be found. At sites where community informants listed a total of 100 spots or fewer, all spots across all priority categories were visited for verification and spot informant interviews. At sites where more than 100 spots were listed, stratified random sampling was used to sample 100 spots for verification, with spots in the higher priority categories having a higher probability of selection.

At each of the cross-border sites, research assistants visited sampled spots to verify their existence and gather information about the spot, such as the type of people who visit the spot, whether people meet new sexual partners at the spot, and if any HIV prevention activities occur at the spot. Spot informants were purposively selected based on their knowledge of the specific spot in question. Often the spot informant was the owner or manager of the spot. The geographic coordinates of the spot were also collected during the interview using GPS.

PLACE Step 3: Patron and Worker Interviews

Following spot verification and spot informant interviews, all spots that were found and operational were eligible to be sampled for patron and worker interviews, unless management refused to participate.

Forty spots across each site were sampled for patron/worker interviews. These 40 spots were distributed across the priority categories described above, again with spots in the higher priority categories having higher probabilities of selection. For each spot, an overall sampling probability was calculated using the known sampling fractions in each of the stratified random sampling stages.

As most spots were small, all patrons and workers were approached to participate in interviews. If more patrons or workers were present at a given venue than could feasibly be interviewed in the time allotted to research assistants, a systematic sample of patrons and a systematic sample of workers were approached and asked to participate. This process involved interval sampling, such that a large "X" was conceptually drawn through the venue and respondents were selected by using predetermined points in the physical space of the site along the X. An interval number, i, was calculated by counting the number along the X and dividing by the target number of interviews. The goal was to match the strategy to the layout of the venue so that a representative sample of patrons and workers was selected.

At each cross-border site, approximately 960 patron/workers participated in interviews. Selected patrons and workers received HIV pre-test counseling and a rapid HIV test if they consented (those that did not consent to an HIV test could still participate in an interview). While waiting for results, research assistants conducted an interview to gather socio-demographic information, health history, family information, sexual behavior, health-seeking behavior, and exposure to HIV prevention programs. After the interview, participants who were tested received their result and post-test counseling. Those with positive results were linked to care and offered viral load testing. Those that consented to viral load testing provided dried blood spot samples that were sent to a designated lab for viral load measurements. Viral load measurements were communicated back to the facility from which the local HIV counseling and testing staff on the study team were associated. Respondents were given a card with an ID code and facility name, so they could obtain their viral load results.

PLACE Sample Size Determination

Quantitative data from approximately 960 participants in each cross-border site were used to estimate the prevalence of primary endpoints, including HIV prevalence, the prevalence of risk behaviors, and care-seeking behaviors. With an estimated lower bound on the population HIV prevalence of 6 percent, we

expected to see a minimum of 57 cases of HIV per cross-border site, with most sites expected to have higher HIV prevalence. In addition, we expected to see more cases in each site than would have been expected under the general population prevalence, as the sampling strategy was optimized to recruit populations thought to be at high risk of HIV transmission. Given the lower bound on HIV prevalence (6%), we expected to be able to estimate the prevalence of HIV with a precision of +/- 2 percentage points. The precision of the estimates will increase as HIV prevalence increases.

Other risk behaviors and health-seeking behaviors are expected to be very common in the populations of interest, resulting in more precise estimates of the prevalence of these behaviors. Among subjects who are HIV positive, a primary outcome of interest is the difference in the proportion suppressed before and after the intervention. Assuming 6 percent HIV prevalence and 20 percent of HIV positive participants with a suppressed viral load at baseline, we had over 90 percent power to detect a 10 percent difference in the proportion suppressed.

PLACE Response Rates

Across all sites, 11,567 patrons socializing at public spots, and workers at those spots, were approached and asked to participate in the study. Overall, 11,428 (98.8%) agreed to participate in the survey and 10,549 (91.2%) agreed to an HIV test (Table 1).

Table 1. Number and proportion of people approached for patron/worker interviews at public spots who agreed to participate in a bio-behavioral survey and HIV test, 2016

	N	%
Number approached	11,567	
Agreed to participate	11,428	98.8
Agreed to HIV test	10,549	91.2

Sequencing

Samples from Uganda, Rwanda, and Tanzania were transferred directly from the field to the Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) Uganda Research Unit on AIDS, in Entebbe, for viral load testing and then stored at -80 Celsius until analysis. Samples from Kenya underwent viral load quantification at an in-country lab and then were transferred to the MRC/UVRI lab on dry ice and stored at -80 Celsius until analysis. The MRC/UVRI lab is both a national and regional World Health Organization accredited HIV drug resistance genotyping lab for both plasma and dried blood spot specimens.

Genotyping of the HIV-1 *pol* gene was conducted using a validated and modified in-house method (Parry et al., 2014; Yang, et al., 2010). Briefly, one or two dried blood spots containing 100 µl of whole blood were punched out using a single-use device from the dried blood spot card and added to a 9 ml buffer solution. Samples were incubated at room temperature under gentle rotation for two hours, and then centrifuged at 250 x g for five minutes. The supernatant was transferred to a 15-ml conical tube, and total nucleic acids were extracted using NucliSens isolation reagents according to the manufacturer's instructions. Total nucleic acids were resuspended in 50 µl of elution buffer and stored at -80 Celsius.

In-house primers were used to amplify and generate the 1,300-base-pair fragment of the 5' region of the *pol* gene by reverse transcription polymerase chain reaction followed by nested PCR using total nucleic acid extracted from dried blood spots. The PCR fragments were purified, sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Life Technologies, Foster City, CA, USA), and analyzed on an ABI Prism 3130 or 3500 Genetic Analyzer (Life Technologies). A combination of the Sequencer software (Sequencher® version 5.4.1 sequence analysis software, Gene Codes Corporation, Ann Arbor, MI, USA) and a customized RECall software program (Woods, et al., 2012) was used to edit the raw sequence data

and generate consensus sequences. Drug resistance mutations were identified using the Stanford HIVdb program (http://hivdb.stanford.edu/).

Phylogenetic Analysis

To understand the genetic relatedness between individuals sequenced, a random sample of rooted phylogenetic trees from the posterior distribution were generated using the Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) package v1.8.4. We specified a log-normal prior for the uncorrelated relaxed clock rate and a HKY85 nucleotide evolution. The HKY85 model is considered a more accurate representation of nucleotide substitutions in comparison to other models, since it incorporates multiple parameters to generate a more realistic simulation of how nucleotide sequences behave. A Bayesian Skyline coalescent tree prior was selected to be most appropriate for this dataset, using an informative prior to impose on the tree root height, as a normal distribution of mean 94 and standard deviation of five years before the most recent tip (Kingman, 1982).

Three independent runs of 100 million steps were performed with samples saved at every 10000 iterations, which was sufficient to ensure that the effective sample sizes for all the parameters were above 200. Optimization of parameters was verified using TRACER v.1.6. All resultant trees were combined using TreeAnnotator (a component of the BEAST package), which identified the maximum clade credibility tree and the optimized tree was visualized using FigTree v1.4.3. Clusters for this tree were identified using Cluster Picker v1.2.3, based on a posterior probability threshold of 0.9.

Statistical Analysis

Characteristics of the study sample were summarized using frequencies and percentages. Survey sampling weights were used to reweight the study sample to represent all people with unsuppressed HIV who socialize in public venues in the selected cross-border sites. In addition, we accounted for missing data owing to informative refusals of the HIV test and viral load testing using inverse probability weights. Population percentages were weighted to account for both the complex survey design and the missing data caused by refusals to participate in the HIV test and to provide dried blood spots.

Sampling Weights

Sampling weights were used to weight the study sample to the target populations of interest—people who work or socialize at spots at the selected cross-border sites. The weights were designed to account for variations in sampling probabilities across survey participants and spots. For example, individuals who were recruited at a spot that had a relatively low a probability of selection were up-weighted to represent additional people who could have been recruited from similar spots had we visited all spots in the cross-border site.

Sampling weights were applied to spot-level and individual data. Unweighted, spot-level data reflect the distribution of spot characteristics in a sample of operational spots at the sites. Once weights are applied, results reflect the distribution of characteristics that would have been observed if all spots in a site had been visited or if a simple random sample of spots in the site had been assessed, rather than a stratified random sample.

Sampling weights were also applied to the patron/worker data. Unweighted, patron/worker data reflect the distribution of population characteristics in a sample of male workers, female workers, male patrons, and female patrons present at a sample of spots at the time of data collection. With weights applied, the data reflect the distribution of population characteristics that would have been observed if a simple random sample of people at spots in cross-border sites has been taken, rather than a stratified random sample of people at a stratified random sample of spots.

Taken together, weighted data depict the characteristics of spots and populations at spots across the cross-border sites included in the study.

Study Sample

Of the 11,428 participants in the East Africa Cross-Border Integrated Health Study, 576 were HIV-positive. Of these patients, 224 agreed to provide dried blood spots and had any undetectable viral load, and 179 had viral load over 1000 copies/mL, which was necessary for sequencing. Sequencing information was available for 125 of these participants.

Parameters of Interest

For identification of related sequences (i.e., possible transmission clusters), the primary parameters of interest were the number of clusters generated from a phylogenetic sample of the 125 sequences analyzed in the special study. The possible transmission clusters were identified using a posterior probability of greater than 95 percent, a threshold for genetic distance suggesting significant relatedness between sequences. Once clusters were identified, descriptive analyses were conducted to compare individuals in clusters to those not in clusters.

For identification of ART resistance, the primary parameters of interest were the population prevalence of drug resistance mutations to various classes of antiretroviral drugs. These parameters were estimated as the weighted proportion of participants meeting the inclusion criteria who had the mutation of interest. Furthermore, we estimated associations between having any drug resistance mutation and participant characteristics using weighted bivariate prevalence ratios. Corresponding 95-percent confidence intervals for prevalence estimates and prevalence ratios were based on standard errors estimated using Taylor series linearization to account for the sampling design, including clustering by spot (venue).

CHARACTERISTICS OF THE SEQUENCED UNSUPPRESSED HIV POPULATION AT SPOTS IN CROSS-BORDER SITES

Of these 125 participants whose dried blood spots were sequenced, 47.6 percent were female, 54.4 percent were over age 30, 79.4 percent were employed, and 11.6 percent worked in a job related to the fishing industry (Table 2). The sample consisted of 19 women who received cash for sex in the past 12 months and 18 men who paid cash for sex during the same period. Most respondents were recruited in a bar, pub, or restaurant, and 47.9 percent were recruited from a venue in which people had sex onsite. In total, 13.4 percent reported ever receiving HIV care or treatment.

Table 2. Characteristics of 125 people living with unsuppressed HIV found at public spots in 14 cross-border sites selected for the East Africa Cross-Border Integrated Health Study, 2016

Characteristic	Overall (N=125)	
	n	%
Sex		
Male	64	52.4
Female	61	47.6
Age		
15–19	10	9.8
20–29	46	35.9
30–39	46	34.1
40 +	23	20.3
Education		
Less than primary	25	22.2
Completed primary or more	99	77.8
Employed	102	79.4
Currently married or living with a partner	67	55.8
Fisherfolk	18	11.6
Received cash for sex in past 12 months	19	15.9
Paid cash for sex in past 12 months	18	11.4
Reported ever receiving HIV care and treatment	15	13.4
Type of respondent		
Worker at venues	31	18.3
Patron at venues	94	81.7
Time spent away from primary residence in past year		
2 weeks or less	71	54.5
More than 2 weeks but less than 1 month	21	15.5
More than 1 month but not more than 3 months	11	11
More than 3 months	18	12.8
Recruitment into study		
Recruited in a land border site	77	72.1
Recruited in a lake border site	48	27.9
Visited more than 1 venue on day of recruitment	56	54.4
Recruited at a venue where people have sex onsite	68	47.9

Characteristic	Overall (N=125)	
	n	%
Type of venue where recruited		
Bar/pub/restaurant	76	60.3
Hotel/guest house/lodge	22	15.7
Nightclub/disco/brothel	4	2.8
Commercial venue [a]	7	9.6
Outside venue [b]	7	5.1
Other	8	6.5
Country where recruited		
Kenya	29	21.7
Rwanda	2	0.8
Tanzania	30	30.2
Uganda	64	47.3

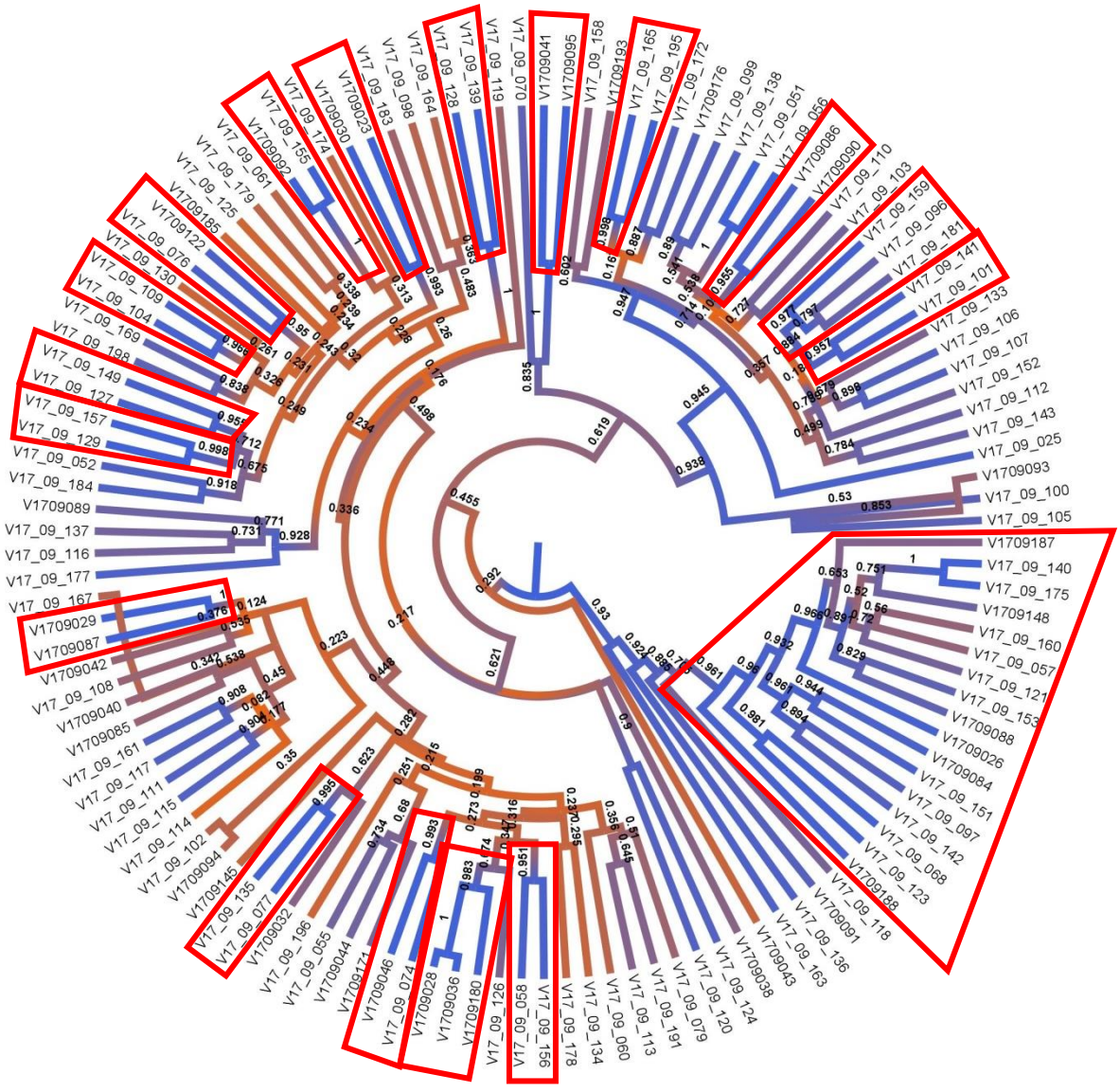
[a] Commercial venues were markets, hair salons, shops, cinemas, recreation and game centres, and schools.
[b] Outdoor venues were beaches, parks, construction sites, and streets.

RESULTS OF PHYLOGENETIC ANALYSES

Clusters Identified

All 125 sequences were examined in the phylogenetic analysis (Figure 2). Out of the entire sample, 18 clusters were identified for 52 individuals, with the largest cluster containing 16 people and the smallest containing two (n=15). HIV has a major group that consists of 11 main subtypes that are generally geographically specific. Among individuals who were identified in clusters and had subtype information available, 21 were subtype A, two were subtype A2, 14 were subtype C, and nine were subtype D.

Figure 3. Radial phylogenetic tree of 125 people living with unsuppressed HIV found at public spots in cross-border sites selected for the East Africa Cross-Border Integrated Health Study (2016), with tips labeled by sequence ID and posterior probabilities and possible transmission clusters outlined in red



Bluish tones indicate higher posterior probabilities of clustering and orange tones indicate lower posterior-probability of clustering (threshold is 0.95 to identify clade). Possible transmission clusters (i.e., related sequences) are outlined in red.

Mobile and Vulnerable Populations in Clusters

Table 3 shows that many individuals in clusters were members of cross-border mobile and vulnerable populations. Most were either young women (n=11) or fisherfolk (n=8). Six individuals in clusters were FSWs. No truck drivers, people who inject drugs, or MSM living with HIV and identified in the study were included in a cluster, but note that few of these groups tested positive for HIV in the study. In addition, though not traditionally considered a vulnerable population, 13 workers at bars, hotels, restaurants, and other public spots were included in the clusters identified.

Table 3. Cross-border mobile and vulnerable populations in each cluster identified among 125 people living with unsuppressed HIV found at public venues in cross-border sites selected for the East Africa Cross-Border Integrated Health Study, 2016

Cluster	Overall (n=52)								Total
	Young women*	FSW	Male fisherfolk	Female fisherfolk	Male workers	Female workers	Other men	Other women	
1	2	1	0	0	0	1	0	0	2
2	0	0	2	0	0	0	0	1	3
3	0	0	0	0	0	0	1	0	2
4	2	1	0	0	1	1	8	3	16
5	1	0	0	0	0	1	0	0	2
6	1	0	0	1	0	1	1	0	3
7	1	0	0	0	0	2	0	0	2
8	0	1	0	0	0	0	1	0	2
9	1	0	0	0	0	0	0	1	2
10	0	0	0	1	1	0	0	0	2
11	0	0	0	0	0	0	1	1	2
12	0	0	1	1	1	0	0	0	2
13	1	0	0	0	0	0	1	0	2
14	1	1	0	0	0	1	1	0	2
15	0	0	1	0	1	0	1	0	2
16	0	1	0	0	1	1	0	0	2
17	1	0	0	0	0	0	0	1	2
18	0	1	0	1	0	0	0	1	2
Overall	11	6	4	4	5	8	15	8	52

*Young women may also be FSWs, female fisherfolk, or female workers. Similarly, FSWs may also be fisherfolk or workers. For this reason, row totals may not equal the total provided in the right column.

Cross-Border Clusters

Table 4 illustrates that a little over half (10/18) of the identified clusters were cross-border clusters. That is, these clusters contained individuals recruited for the study in two or more countries. Nine of these clusters contained individuals recruited in two countries, and one contained individuals recruited in three countries.

Table 4. Composition of 18 identified clusters by country of recruitment among 125 people living with unsuppressed HIV found at public spots in cross-border sites selected for the East Africa Cross-Border Integrated Health Study, 2016

Cluster	Kenya	Tanzania	Uganda	Overall
1	1	0	1	2
2	2	1	0	3
3	1	1	0	2
4	2	10	4	16
5	0	0	2	2
6	0	2	1	3
7	0	1	1	2
8	0	1	1	2
9	1	1	0	2
10	0	0	2	2
11	2	0	0	2
12	0	0	2	2
13	1	1	0	2
14	0	0	2	2
15	0	0	2	2
16	0	0	2	2
17	1	0	1	2
18	2	0	0	2
All	13	18	21	52

Characteristics of Individuals in Clusters

Of the 52 individuals in any cluster, 56.2 percent were female, 53 percent were over age 30, 79.5 percent were employed, and 11.9 percent worked in a job related to the fishing industry (Table 5). The sample consisted of eight women who received cash for sex in the past 12 months and four men who paid cash for sex during the same period. Most respondents were recruited in a bar, pub, or restaurant, and 48.4 percent were recruited in a venue in which people had sex onsite. In total, 9.6 percent reported ever receiving HIV care or treatment.

Individuals in clusters identified in this study were less likely to report being away from home for more than two weeks and less likely to report visiting more than one venue on the day of recruitment than the total population of people with HIV eligible for this study. Clustered individuals were also more likely to be fisherfolk, less likely to be married, and less likely to have completed primary school or additional education than the general eligible population (Table 5).

Table 5. Characteristics of 52 people identified in possible transmission clusters living with unsuppressed HIV found at public spots in cross-border sites selected for the East Africa Cross-Border Integrated Health Study, 2016

Characteristic	Clustered individuals		Overall (N=125)	
	n	%	n	%
Sex				
Male	23	43.8	64	52.4
Female	29	56.2	61	47.6
Age				
15–19	5	10.8	10	9.8
20–29	19	35.2	46	35.9
30–39	20	37.5	46	34.1
40 +	8	16.5	23	20.3
Education				
Less than primary	6	10.1	25	22.2
Completed primary or more	36	73.3	99	77.8
Employed	44	79.5	102	79.4
Currently married or living with a partner	26	50.3	67	55.8
Fisherfolk	10	11.9	18	11.6
Received cash for sex in past 12 months	8	11.2	19	15.9
Paid cash for sex in past 12 months	4	9.6	18	11.4
Reported ever receiving HIV care and treatment	7	9.6	15	13.4
Type of respondent				
Workers at venues	13	13.9	31	18.3
Patrons at venues	39	86.1	94	81.7
Time spent away from primary residence in past year				
2 weeks or less	35	60.9	71	54.5
More than 2 weeks but less than 1 month	7	13.3	21	15.5
More than 1 month but not more than 3 months	4	8.1	11	11.0
More than 3 months	3	4.8	18	12.8
Recruited into Study				
Recruited in a land border site	35	76.4	77	72.1
Recruited in a lake border site	17	23.6	48	27.9
Visited more than 1 venue on day of recruitment	20	46.9	56	54.4
Recruited at venue where people have sex onsite	29	48.4	68	47.9
Type of venue where recruited				
Bar/pub/restaurant	27	52.4	76	60.3
Hotel/guest house/lodge	14	28.4	22	15.7
Nightclub/disco/brothel	2	2.7	4	2.8
Commercial venue [a]	2	4.2	7	9.6
Outside venue [b]	3	5.5	7	5.1
Other	4	6.8	8	6.5
Country where recruited				
Kenya	13	14.8	29	21.7
Rwanda	0	0.0	2	0.8
Tanzania	18	48.7	30	30.2
Uganda	21	36.6	64	47.3

[a] Commercial venues were markets, hair salons, shops, cinemas, recreation and game centres, and schools.
[b] Outdoor venues were beaches, parks, construction sites, and streets.

RESULTS OF ART RESISTANCE ANALYSIS

Among people living with HIV, achieving viral suppression is important to improve long-term health and to reduce onward transmission of the virus. Successful delivery of ART to people living with HIV is very effective in reducing viral load below detectable levels, thus preventing onward transmission (Tanser, et al., 2013; Cohen, et al., 2011). However, ART resistance can prevent viral suppression even among people receiving therapy (Price, et al., 2011b).

Resistance to antiretroviral drugs can be transmitted from person to person or can be developed through poor adherence to prescribed therapy, unnecessary regimen changes, and natural processes. Resistance may play a role in HIV transmission in cross-border sites where mobile and vulnerable populations have sporadic access to treatment, seek care in multiple countries with different first-line regimens, and face barriers to obtaining HIV care and treatment when outside their country of origin.

Cross-border areas in East Africa are frequented by many mobile and vulnerable populations, including FSWs, truck drivers, fisherfolk, people who inject drugs, young women and girls, and female hospitality workers. In this section we estimate the prevalence of resistance to antiretroviral drugs among people with unsuppressed HIV found in these cross-border areas.

Table 6 presents data for the 18 participants with HIV-1 RNA containing at least one mutation that conferred resistance to an antiretroviral medication and the number of people with that mutation who were also identified to be part of a possible transmission cluster.

Table 6. Data for 18 participants in the East Africa Cross-Border Integrated Health Study found to have single drug resistance mutations detected by oligonucleotide ligation assay, 2016

Mutant codon	Total	Number in an identified cluster
Participants with NRTI resistance mutations only	**2**	**0**
K219R	1	0
F77L	1	0
Participants with NNRTI resistance mutations only	**10**	**3**
K103N	7	3
K103NS	1	0
Y181C	1	0
Y188L + P225H	1	0
Participants with PI resistance mutations only	**1**	**1**
V82A	1	1
Participants with both NRTI + NNRTI resistance mutations	**5**	**3**
$(K65R + K70E + M184V)^{NRTI}$ + $(K103N + G190A + P225H)^{NNRTI}$	1	1
$K65R^{NRTI} + (K103N + Y181C)^{NNRTI}$	1	0
$M184V^{NRTI} + (K101E + G190A)^{NNRTI}$	1	0
$(L74I + M184V + K219R)^{NRTI} + (K103N + P225H)^{NNRTI}$	1	1
$T215S^{NRTI} + Y181C^{NNRTI}$	1	1

Overall, the prevalence of having at least one resistance mutation was 11.7 percent (95% CI: 5.0, 18.4) (Table 7). HIV-1 RNA among these participants had one or more mutations conferring resistance to NRTIs, NNRTIs, and PIs. First-line antiretroviral therapy for HIV typically includes two NRTIs and one NNRTI. Protease inhibitors are typically reserved for second-line therapy after a patient has failed first-line therapy. Given their extensive use in first-line therapy, the prevalence of resistance mutations to NRTIs and NNRTIs were higher than the prevalence of resistance to PIs. Resistance mutations to NRTIs only was seen among 1.2 percent of participants, resistance to NNRTIs only was seen among 4.9

percent of participants, and resistance to PIs only was seen among 1.8 percent of participants. Nearly 4 percent of participants had mutations conferring resistance to both NRTIs and NNRTIs. Overall, 5 percent of participants had resistance to NRTIs (either NRTIs alone or resistance mutations for both NRTIs and NNRTIs) and nearly 9 percent of participants had resistance to NNRTIs (either NNRTIs alone or resistance mutations for both NRTIs and NNRTIs). Seven of the participants identified with a resistance mutation were also identified to be in a possible transmission cluster in the phylogenetic analysis. Of participants with any NNRTI resistance mutation, two were genetically related to each other (Cluster 11) and one was in Cluster 7. Two participants with multiple NRTI and NNRTI resistance mutations were genetically related to each other (Cluster 10). The one participant with a PI resistance mutation was in Cluster 4, containing 15 other participants in this analysis.

Table 7. Prevalence of single drug resistance mutations to classes of antiretroviral drugs detected by oligonucleotide ligation assay among 125 people with HIV in the East Africa Cross-Border Integrated Health Study, 2016

Mutant codon	Overall (N=125)			ART naive (n=108)		
	n	Prevalence	95% CI	n	Prevalence	95% CI
Any resistance mutation	18	11.7	5.0, 18.4	15	12.0	4.5, 19.6
NRTI resistance mutations only	2	1.2	0.0, 3.0	1	0.5	0.0, 1.6
NNRTI resistance mutations only	10	4.9	1.0, 8.8	9	5.3	0.8, 9.7
PI resistance mutations only	1	1.8	0.0, 5.5	1	2.1	0.0, 6.4
NRTI + NNRTI resistance mutations	5	3.8	0.1, 7.5	4	4.1	0.0, 8.3
Any NRTI resistance mutation [a]	7	5.0	0.9, 9.1	5	4.6	0.3, 9.0
Any NNRTI resistance mutation [b]	15	8.6	3.2, 14.0	13	9.4	3.2, 15.5

[a] Including those with only NRTI resistance mutations and those with both NRTI and NNRTI resistance mutations
[b] Including those with only NNRTI resistance mutations and those with both NRTI and NNRTI resistance mutations

The prevalence of individual resistance mutations is shown in Table 8. The most common resistance mutation was K103N, with prevalence of 6.6 percent (95% CI: 1.4, 11.8). Results were similar when limited to participants who had never received HIV care or treatment.

Table 8. Total prevalence of individual single drug resistance mutations (occurring alone or in combination with other resistance mutations) to classes of antiretroviral drugs detected by oligonucleotide ligation assay among 125 people with HIV in the East Africa Cross-Border Integrated Health Study, 2016

Mutant codon	Overall (N=125)			ART naive (n=108)		
	n	Prevalence	95% CI	n	Prevalence	95% CI
Mutations conferring NRTI resistance						
K219R	2	0.7	0.0, 1.7	1	0.5	0.0, 1.6
F77L	1	0.8	0.0, 2.3	0	2.6	0.0, 6.6
K65R	2	2.2	0.0, 5.6	2	1.8	0.0, 5.5
K70E	1	1.6	0.0, 4.7	1	2.6	0.0, 6.5
M184V	3	2.4	0.0, 5.9	2	0.8	0.0, 2.3
L74I	1	0.2	0.0, 0.7	0	0.5	0.0, 1.6
T215S	1	0.7	0.0, 2.0	1	2.6	0.0, 6.6
Mutations conferring NNRTI resistance						
K103N	11	6.6	1.4, 11.8	9	7.0	1.0, 13.0
K103NS	1	0.4	0.0, 1.3	1	0.5	0.0, 1.5
Y181C	3	1.8	0.5, 3.2	3	2.1	0.6, 3.7
Y188L	1	0.2	0.0, 0.6	1	0.2	0.0, 0.7
P225H	3	2.0	0.0, 5.2	2	2.1	0.0, 5.8
G190A	2	2.2	0.0, 5.6	1	2.6	0.0, 6.5
K101E	1	0.6	0.0, 1.9	1	0.7	0.0, 2.2
Mutations conferring PI resistance						
V82A	1	1.8	0.0, 5.5	1	2.1	0.0, 6.4

Table 9 presents prevalence ratios describing the association between individual and spot(venue)-level characteristics and ART resistance. Prevalence ratios were imprecise, owing to the small number of participants with ART resistance, but some patterns emerged in the study sample. Women were less likely than men to have ART resistance, and fisherfolk were more likely to have ART resistance than non-fisherfolk. Women who had exchanged sex for cash were less likely to have ART resistance than other women, but men who had paid cash for sex were more likely to have ART resistance than other men. Higher educational attainment was associated with a higher probability of having a resistant infection.

Participants recruited at hotels and outside venues had a higher probability of resistant infection than participants recruited at other types of venues. Finally, participants recruited at lake sites had a higher probability of resistant infection than participants recruited at land sites.

Table 9. Prevalence ratios describing associations between key demographic variables and ART resistance mutations among people living with HIV recruited for the East Africa Cross-Border Integrated Health Study, 2016

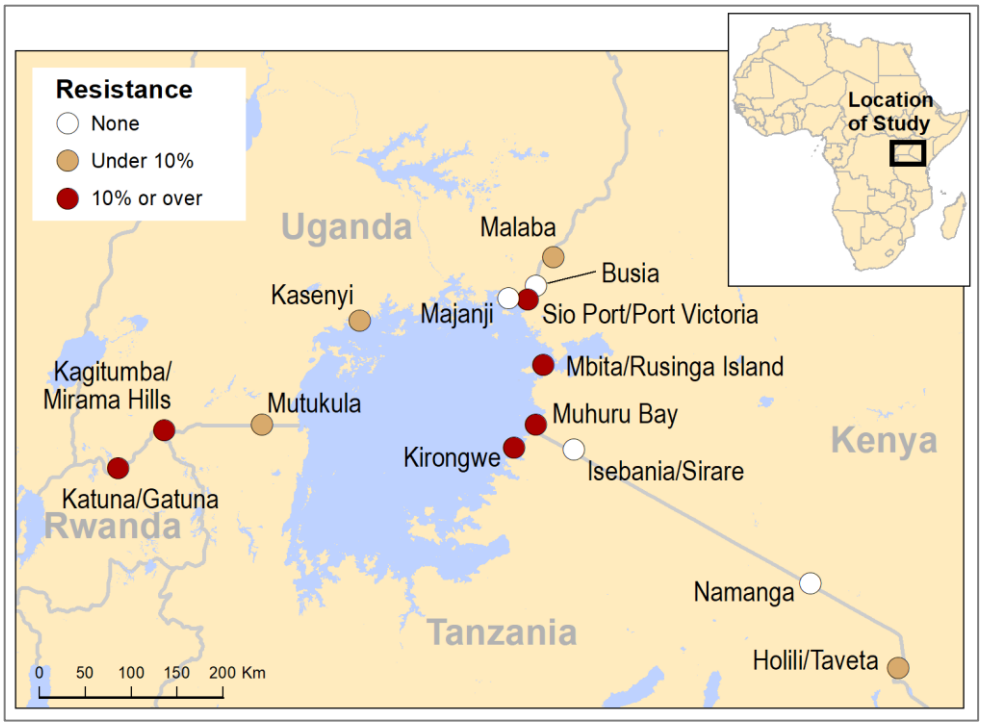
Characteristic	Number with resistance	Prevalence	Prevalence ratio	95% CI
Sex				
Female	8	8.4	0.57	0.18, 1.86
Male	10	14.7	1.00	
Age				
15–29	10	11.5	1.00	
30–39	4	7.0	0.61	0.19, 1.97
40 +	4	20.0	1.74	0.50, 6.03
Education				
Less than primary	3	4.9	0.36	0.08, 1.64
Completed primary or more	15	13.6	1.00	
Employment				
Employed	14	11.4	1.00	
Unemployed	4	12.5	1.09	0.25, 4.73
Currently married or living with a partner				
No	12	14.1	1.00	
Yes	6	9.8	0.69	0.24, 1.96
Works in job related to the fishing industry				
No	16	10.6	1.00	
Yes	2	20.0	1.89	0.52, 6.92
Received cash for sex in past 12 months				
No	17	13.3	1.00	
Yes	1	4.1	0.31	0.04, 2.37
Paid cash for sex in past 12 months				
No	15	11.2	1.00	
Yes	3	16.3	1.46	0.52, 4.11
Ever received HIV care or treatment				
No	15	12.0	1.00	
Yes	3	10.0	0.83	0.20, 3.45
Type of respondent				
Patron at venue	13	11.9	1.00	
Worker at venue	5	10.5	0.88	0.28, 2.77
Population type				
Resident	18	14.8		
Mobile	0	NA	NA	
Time spent away from primary residence in past year				
Less than 1 month	17	13.9	1.00	
1 month or more	1	2.3	0.16	0.02, 1.28
Type of site where recruited				
Land site	9	8.5	1.00	
Lake site	9	20.0	2.37	0.76, 7.37

Characteristic	Number with resistance	Prevalence	Prevalence ratio	95% CI
Visited more than 1 venue on day of recruitment				
No	5	4.8	1.00	
Yes	13	17.4	3.64	1.16, 11.42
Recruited at a venue where people have sex onsite				
No	8	8.1	1.00	
Yes	10	15.7	1.95	0.80, 4.72
Type of venue where recruited				
Bar/pub/restaurant	9	9.9	1.00	
Hotel/guest house/lodge	5	20.9	2.12	0.78, 5.80
Nightclub/disco/brothel	1	15.5	1.56	0.27, 9.15
Commercial venues [a]	1	7.0	0.71	0.39, 1.29
Outside venues [b]	2	27.9	2.83	2.08, 3.84
Country where recruited				
Uganda	10	11.6	1.00	
Kenya	4	15.4	1.33	0.35, 5.01
Tanzania	4	9.5	0.83	0.18. 3.83
Rwanda	0		NA	

[a] Commercial venues were markets, hair salons, shops, cinemas, recreation and game centres, and schools.
[b] Outdoor venues were beaches, parks, construction sites, and streets.

The distribution of ART resistance by cross-border area is shown in Figure 3. Among the sites selected, areas bordering Rwanda and areas along Lake Victoria on the Kenya and Tanzania side of the lake had a higher prevalence of ART-resistant HIV infection. However, site-level prevalence estimates were imprecise and should be interpreted with caution.

Figure 4. Prevalence of ART resistance among people with unsuppressed HIV by cross-border site in the East Africa Cross-Border Integrated Health Study, 2016



DISCUSSION

The analysis presented here is a novel characterization of genetic sequences at cross-border sites in East Africa, which are important mixing grounds for host and mobile populations, as well as young women, FSWs, fisherfolk, workers at spots, truck drivers, MSM, and people who inject drugs. The genetic sequences provided information on the presence of clustering and single drug resistance mutations in this subset of samples. Key findings and limitations from these analyses are summarized below.

Clusters of genetically-related sequences were found.

The phylogenetic analysis highlighted some potential clustering of sequences, suggesting that those individuals may have contracted HIV from related sources. We cannot definitively assign the identified related-groups to transmission clusters because this study did not collect information on the complete network of sexual partnerships, contained a relatively small number of samples, and did not collect information on suspected or known sexual contacts. However, the evidence of related groups suggests that transmission clusters may exist in this population and underscores the value of further investigation to identify the extent of these clusters, the burden of their contribution to HIV transmission in East Africa, and opportunities to stem transmitted drug resistance.

Identified clusters contained FSWs, young women, fisherfolk, and workers at public venues such as bars and pubs. In addition, over half of identified clusters spanned international borders.

The distribution of HIV subtypes among all sequenced individuals and those identified in clusters were comparable to the distribution identified in previous data analysis (Department of Health and Human Services, Los Alamos National Laboratory, and National Institutes of Health, 2015). Vulnerable populations identified in clusters were FSWs, young women, and fisherfolk, as well as workers at venues. This information is useful for understanding potential routes of HIV transmission at these cross-border locations and can direct efforts to focus interventions in high-risk areas. Notably, over half of potential transmission clusters identified spanned international borders, providing evidence for the importance of cross-border movement in the regional HIV epidemic. Owing to the small number of samples sequenced, there were many transmission clusters we failed to identify in cross-border areas, and some of the possible transmission clusters we identified were likely incomplete. However, results provide insight into how infections are likely related in this population.

Drug resistance mutations are sufficiently prevalent in this population to complicate viral suppression.

The overall prevalence of single drug resistance mutations in this sample (11.7%) sits within the range identified in other studies investigating single drug resistance mutations in other populations in East Africa (Appendix A). The most common single drug resistance mutation was K103N (prevalence 6.6%), which is an NNRTI resistance mutation known to be the most prevalent in East Africa (Price, et al., 2011a; Onsongo, et al., 2016). As expected, drug resistance was more common to NNRTIs and NRTIs, which are components of the most common first-line regimens used throughout East Africa, than among PIs, which are typically reserved for second-line therapy.

Many of the participants reported never receiving care or treatment for HIV (108 out of the 125 participants with available sequences). Among participants previously on ART, we cannot distinguish acquired ART resistance from transmitted resistance. However, among these presumed ART-naive individuals, we may tentatively assume that drug resistance was transmitted, rather than acquired. Under this assumption, the prevalence of transmitted drug resistance was 12 percent in this population, slightly higher than the 7 percent prevalence previously reported for East African populations (Ssemwanga, et al., 2015). The difference in the estimated prevalence of transmitted drug resistance between this and previous studies could be the result of distinct features of cross-border mobile and vulnerable populations.

CONCLUSION

This analysis characterizes the genetic profile of HIV *pol* sequences in the East African cross-border population, a group not previously analyzed in existing HIV genetic research. The genetic sequences collected in a subset of this population provided information on the existence of possible transmission clusters in this population, the variation of subtypes existing at sites, and the prevalence of single drug resistance mutations.

This study suggests the potential for the presence of HIV transmission networks within cross-border communities, across international borders, and along transportation corridors. Intervening to disrupt these routes of HIV transmission will require both traditional combination HIV prevention approaches as well as emerging "treatment as prevention" strategies to reduce the likelihood of HIV transmission from people living with HIV to their HIV-uninfected partners. However, drug resistant strains of HIV were more common than expected in this population, which may complicate efforts to reduce transmission through treatment of those already infected. Such drug resistance may be transmitted from the source of the HIV infection to the recently infected individual or acquired over time after ART initiation. Both types of drug resistance impede viral suppression, thus increasing the probability of onward transmission and threatening achievement of the UNAIDS 90-90-90 targets (18). Transmitted drug resistance, sometimes identified through routine resistance mutation monitoring at ART initiation, may require initiation of specific ART regimens to which the virus is susceptible. Acquired drug resistance is typically suggested by virologic failure after remaining faithful to a first-line treatment regimen. Evidence of transmitted or acquired drug resistance after ART initiation requires timely switching from first- to second-line regimens.

Addressing both transmitted and acquired drug resistance requires successful integration of many facets of HIV care and treatment. First, resistant infections must be identified, either through routine monitoring at ART initiation or as diagnosis of virologic failure. Such diagnoses may be complicated by poor continuity of care among highly mobile populations. Second, appropriate treatment regimens must be prescribed and maintained. Patients on second-line regimens may face challenges in obtaining the drugs needed if they switch health facilities without official transfer documents or seek HIV care and treatment outside their home countries. Finally, patients receiving non-first-line treatment regimens must be fully adherent to avoid accumulating additional resistance mutations, which could then be transmitted along with HIV infection to their previously HIV-uninfected partners.

Opportunities to adapt the HIV care and treatment system to address these threats exist in cross-border areas. Harmonization of first- and second-line treatment regimens across countries, unification of patient monitoring systems, cross-border tracing of those lost to follow-up, and index tracing, as well as collaborative partnerships between local health facilities on each side of an international border offer options to disrupt transmission networks and reduce antiretroviral drug resistance. Pursuit of these opportunities, in concert with other prevention strategies, will be important steps in ending the HIV epidemic in East Africa.

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APPENDIX A. SUMMARY OF SINGLE DRUG RESISTANCE MUTATION PREVALENCE IN PREVIOUSLY PUBLISHED LITERATURE

Study name, year	Location	Single drug resistance mutation prevalence
Ssemwanga, et al., 2015	East Africa (systemmatic review)	7.5%
Sigaloff, et al., 2012	Mombasa, Kenya	7.4%
Oyugi, et al., 2007	Kampala, Uganda	8%
Price et al., 2011a	Kenya	10%
Price et al., 2011a	Rwanda	0–15%
Price et al., 2011a	Masaka, Uganda	9.1–16.7%

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