Population dynamics of HIV drug resistance during treatment scale-up in Uganda: a population-based longitudinal study

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1

1   **Abstract**
2   *Background*
3   Clinical studies have reported rising pre-treatment HIV drug resistance during antiretroviral
4   treatment (ART) scale-up in Africa, but representative data are limited. We estimated
5   population-level drug resistance trends during ART expansion in Uganda
6
7   *Methods*
8   We analyzed data from the population-based open Rakai Community Cohort Study conducted at
9   agrarian, trading, and fishing communities in southern Uganda between 2012 and 2019.
10 Consenting participants aged 15-49 were HIV tested and completed questionnaires. Persons
11 living with HIV (PLHIV) provided samples for viral load quantification and virus deep-
12 sequencing. Sequence data were used to predict resistance. Population prevalence of class-
13 specific resistance and resistance-conferring substitutions were estimated using robust log-
14 Poisson regression.
15
16 *Findings*
17 Data from 93,622 participant-visits, including 4,702 deep-sequencing measurements, showed
18 that the prevalence of NNRTI resistance among pre-treatment viremic PLHIV doubled between
19 2012 and 2017 (PR:1.98, 95%CI:1.34–2.91), rising to 9.61% (7.27-12.7%). The overall
20 population prevalence of pre-treatment viremic NNRTI and NRTI resistance among all
21 participants decreased during the same period, reaching 0.25% (0.18% - 0.33%) and 0.05%
22 (0.02% - 0.10%), respectively (*p*-values for trend = 0.00015, 0.002), coincident with increasing
23 treatment coverage and viral suppression. By the final survey, population prevalence of
24 resistance contributed by treatment-experienced PLHIV exceeded that from pre-treatment
25 PLHIV, with NNRTI resistance at 0.54% (0.44%-0.66%) and NRTI resistance at 0.42% (0.33%-
26 0.53%). Overall, NNRTI and NRTI resistance was predominantly attributable to rtK103N and
27 rtM184V. While 10.52% (7.97%-13.87%) and 9.95% (6.41%-15.43%) of viremic pre-treatment
28 and treatment-experienced PLHIV harbored the inT97A mutation, no major dolutegravir
29 resistance mutations were observed.
30
31 *Interpretation*
32 Despite rising NNRTI resistance among pre-treatment PLHIV, overall population prevalence of
33 pre-treatment resistance decreased due to treatment uptake. Most NNRTI and NRTI resistance is
34 now contributed by treatment-experienced PLHIV. The high prevalence of mutations conferring
35 resistance to components of current first-line ART regimens among PLHIV with viremia is
36 potentially concerning.
37
38 *Funding*
39 National Institutes of Health and the Gates Foundation
40

41 **Research in context**
42 *Evidence before the study*
43 We searched PubMed for studies matching the keywords "hiv" "resistance" "longitudinal"
44 "cohort" "population" published since 2004 (the beginning of antiretroviral therapy (ART)
45 availability in sub-Saharan Africa) and identified 50 studies. We excluded 34 studies not based
46 in sub-Saharan Africa, five studies primarily concerned with infection with other pathogens (e.g.
47 HBV, *M. tuberculosis*), two studies concerned with insulin resistance, one sequencing-methods
48 paper, and one paper concerned with host susceptibility to HIV infection. The remaining seven
49 studies were not population-based meaning that the study population was not all persons but e.g.
50 people living with HIV enrolled in care at a given clinic. Population-based cohort are essential
51 for monitoring HIV drug resistance in both treated and untreated individuals, including those
52 people who may go undetected in clinical settings, capturing evolutionary dynamics of resistance
53 in real-world conditions.
54
55 *Added value of this study*
56 We estimated the prevalence of drug resistance over five consecutive survey rounds of a
57 population-based open-cohort study in southern Uganda between 2012 and 2019 during a period
58 of intense treatment scale-up. We show that among the entire population regardless of HIV
59 status, 0.8% and 0.5% of individuals harbor viremic resistance to non-nucleoside reverse
60 transcriptase inhibitors (NNRTIs) and nucleoside-reverse transcriptase inhibitors (NRTIs),
61 respectively, of which the majority is dual-class NNRTI/NRTI resistance. Despite a two-fold
62 increase in the prevalence of NNRTI resistance among pre-treatment viremic PLHIV, the overall
63 prevalence of pre-treatment viremic resistance in the entire population decreased by more than
64 50% due to increased treatment initiation and population viral load suppression. The majority of
65 resistance in recent survey rounds was contributed by treatment-experienced PLHIV. Among
66 treatment-experienced viremic PLHIV, we observe a substantial burden of mutations that confer
67 resistance to the NNRTI and NRTI components of dolutegravir and cabotegravir based regimens
68 e.g. rtM184V (34%) rtY181C (15%), rtG190A (12%), rtK65R (12%), and rtK101E (9.5%). The
69 integrase strand transfer inhibitor (INSTI) resistance mutation inT97A was observed in about a
70 tenth of viremic PLHIV.
71
72 These results provide the first longitudinal population-based estimates of temporal trends in the
73 prevalence of drug resistance during ART program expansion in a high-burden setting. Further,
74 they provide critical insight into the landscape of prevalent drug resistance substitutions
75 circulating in this population.
76
77 *Implications of all the available evidence*
78 Scale-up of HIV treatment has increased the prevalence of drug resistance mutations among
79 viremic people living with HIV in sub-Saharan Africa. The relatively high prevalence of NNRTI
80 resistance has prompted a recent shift to first-line regimens including dolutegravir (an INSTI) in
81 combination with NRTIs. The high prevalence of mutations conferring resistance to components
82 of current first-line regimens in our population warrants continued monitoring of treatment
83 failures and the prevalence of drug resistance in high burden settings.
84
85

Introduction

Antiretroviral therapy (ART) suppresses human immunodeficiency virus (HIV) replication in persons living with HIV (PLHIV),[1] which slows disease progression[2] and prevents viral transmission.[3] With increased uptake of ART as well as other interventions such as voluntary medical male circumcision, HIV incidence has fallen by nearly 40% globally since 2010.[4]

Viral resistance to ART threatens the clinical and public health impact of treatment scale-up[5,6]. Drug resistance can be acquired when an individual infected with a susceptible virus develops resistance following treatment. This is more common when treatment adherence is intermittent,[7] but can occur despite high adherence.[8] Throughout sub-Saharan Africa, the epicenter of the global HIV epidemic[4], the majority of patients who remain viremic despite being on treatment with first-line regimens harbor resistance to at least one component of that regimen[1]. Viral genomes with resistance-conferring mutations can be transmitted to HIV seronegative individuals, increasing the risk of first-line treatment failure approximately five-fold.[9]

Through 2018, preferred first-line HIV ART regimens relied on a combination of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitor (NNRTI)[10]. During the scale-up of ART, an increase in the prevalence of NNRTI resistance among pre-treatment PLHIV has been observed globally[11]. A systematic meta-regression estimated a 1.3% annual increase in NNRTI resistance in eastern Africa, reaching 10% by 2016. These findings have been corroborated by recent cross-sectional studies and World Health Organization (WHO) surveys[1,12,13] and prompted a shift in recommendations to dolutegravir (DTG, an integrase strand transfer inhibitor [INSTI]) given in combination with NRTIs (e.g. Tenofovir disoproxil fumarate [TDF] and Lamivudine [3TC]) for first-line ART. Further, long-lasting injectable INSTIs (e.g. cabotegravir [CAB]) in combination with an NNRTI (rilpivirine [RPV]) are currently being rolled out throughout sub-Saharan Africa[14].

The vast majority of data on the prevalence of HIV ART resistance in sub-Saharan Africa is derived from the population of PLHIV who report to healthcare clinics or hospitals[1,11–13]. Clinic-based studies are subject to biases in the population of PLHIV who are engaged and retained in care, which is not universal[15]. Specifically in eastern and southern Africa, only 95% of women and 91% of men are aware of their HIV status and of those only 92% and 86% are on ART, respectively [4]. Further, clinic-based studies are able to estimate only the prevalence of ART resistance among PLHIV and not among the general population. The latter is the epidemiologically relevant parameter to inform the risk of exposure to HIV with ART resistance among seronegative individuals[16].

General population-based studies, in which all individuals, regardless of HIV serostatus, are recruited to participate, can address these shortcomings. This design also allows for the accurate estimation of the overall prevalence of resistance and the relative contributions from different groups, such as pre-treatment and treatment-experienced PLHIV. A recent cross-sectional population-based study of drug resistance in KwaZulu-Natal, South Africa found very low (<1%) levels of resistance to INSTIs prior to DTG roll-out, but observed rtM184V (3TC resistance), rtK65R (TDF resistance), and rtK70E (TDF resistance) in 32.6%, 12.0%, and 6.2% of treatment-experienced PLHIV. Further, the rtE138A mutation, which confers resistance to RPV, was observed in in 6.5% and 7.9% of ART-experienced and -naïve PLHIV, respectively,

4

132 in this setting[17]. Cross-sectional studies, however, are unable to assess temporal trends in the
133 prevalence of resistance among PLHIV and the general population and do not capture the overall
134 decrease in the prevalence of viremic HIV during ART scale-up. Conversely, longitudinal
135 population-based cohort designs enable more precise monitoring of resistance evolution and a
136 dynamic evaluation of the risks posed to current and future ART regimens. This study design is
137 particularly useful in the context of rapidly changing population sizes of pre-treatment and
138 treatment-experienced viremic PLHIV as has been observed globally in recent decades during
139 expansion of treatment and prevention programs[15].
140
141 Here, we analyzed HIV deep-sequence data collected from 3,407 PLHIV as part of a general
142 population-based open cohort study in southern Uganda spanning a nine-year period of intense
143 ART scale-up and declines in HIV incidence[18–20]. Our validated deep-sequencing protocol[21]
144 allows for the identification and quantification of drug resistance mutations present in a minority
145 of the viral population within a given PLHIV, which can be selected for upon treatment initiation
146 but are missed by consensus sequencing methods[22–24]. We estimated the prevalence of NNRTI,
147 NRTI, and protease inhibitor (PI) resistance among the entire study population and among
148 PLHIV as well as the temporal dynamics of viral resistance-conferring mutations among pre-
149 treatment and treatment-experienced PLHIV.
150
151 **Methods**
152 *Study design and participant selection*
153 The Rakai Community Cohort Study (RCCS) is an open population-based census and cohort
154 study conducted at approximately 18-24 month intervals (appendix pp 2-3) in agrarian (HIV
155 prevalence 9-26%[25]), semi-urban trading (11-21%[25]), and Lake Victoria fishing (38-43%[25])
156 communities in southern Uganda.[20] At each survey round, households in participating
157 communities are censused and residents aged 15-49 capable of providing informed written
158 consent (or assent if under 18) are invited to participate. Consenting participants are administered
159 a structured questionnaire that obtains sociodemographic, behavioral, and health information,
160 including self-reported past and current ART use. Voluntary HIV testing of participants is
161 conducted using a rapid test algorithm[26] and venous blood samples are taken for viral
162 quantification and sequencing.
163
164 The RCCS is administered by the Rakai Health Sciences Program (RHSP) and has received
165 ethical approval from the Uganda Virus Research Institute's Research and Ethics Committee
166 (HS540), the Uganda National Council for Science and Technology (GC/127/08/12/137), and the
167 Johns Hopkins School of Medicine (IRB00217467). Participants provided written informed
168 consent at each survey round.
169
170 We used survey data from 19 RCCS surveys conducted between November 5, 1994 and
171 November 4, 2020, and HIV viral load and sequence data from five rounds conducted between
172 August 10, 2011 and November 4, 2020. Participants with serologically confirmed HIV infection
173 were considered pre-treatment during a given round if they reported never having taken ARTs at
174 that round and all prior rounds in which they participated. PLHIV were considered treatment-
175 experienced during a given round if they reported using ART at that round or any earlier rounds.
176 Recommended first-line ART regimens in RCCS communities are presented in appendix p 4.
177 Herein, study rounds were referred to by the year of the median interview date (appendix p 2).

178

179   Reporting of this study adheres to the STROBE guidance[27].

180

181   *HIV viral load quantification*

182   HIV viral load was measured on serum/plasma samples using the Abbott real-time m2000 assay

183   (Abbott Laboratories) at the Rakai health Sciences Program (Kalisizo, Uganda). Viral load

184   measurements were conducted primarily among PLHIV in fishing communities in the 2012

185   survey round and for all PLHIV in later survey rounds. Viral loads ≥ 1000 copies/mL were

186   considered viremic. Pre-treatment PLHIV in the 2012 round with missing viral load were

187   imputed (appendix pp 4-5). In the rare instance where viral load measurements were missing for

188   PLHIV in subsequent survey rounds, these observations were dropped from the analysis.

189

190   *HIV deep sequencing*

191   Full-length HIV deep sequencing was conducted through the Phylogenetics and Networks for

192   Generalized HIV Epidemics in Africa consortium (PANGEA-HIV).[28,29] As described

193   elsewhere,[19] for the 2012 and 2014 surveys sequencing on Illumina MiSeq and HiSeq platforms

194   using an amplicon-based approach[30] was attempted for participants who self-reported never

195   having been on ART and either had a missing viral load or were known to be viremic (appendix

196   p 21). All viremic participant-visits, regardless of treatment status, in the 2015 through 2019

197   survey rounds, as well as select 2012 and 2014 participant-visits, were sequenced using the

198   veSEQ-HIV protocol, which involves oligo-nucleotide bait enrichment of HIV from pooled

199   metagenomic libraries prepared without virus-specific PCR.[31] Our high-throughput

200   implementation of the veSEQ-HIV protocol incorporates quantitative positive controls consisting

201   of a serial dilution HXB2 cultured virus diluted in pooled human plasma from donors testing

202   negative for HIV, as well and negative plasma controls included with every batch of samples

203   processed. Sequencing quality was monitored from total counts of HIV reads detected in the

204   quantitative controls, their PCR duplication rates and median insert sizes. Where contaminations

205   were detected either by the presence of HIV reads in the negative control, or HXB2 reads present

206   in the samples, sequencing runs were repeated. Sequencing for the 2019 round was only

207   conducted for samples collected through May 17, 2019 (N = 171/453, 37.7% of viremic

208   participant visits). Consensus sequences were generated using shiver[32] and subtyped by

209   identifying the most similar reference sequence and using the Recombination Identification

210   Program.[33]

211

212   *Identification of drug resistance mutations*

213   A validated bioinformatic pipeline, drmSEQ, was used to identify amino acid substitutions

214   associated with reduced susceptibility to ART and predict individual drug and drug class

215   susceptibilities at the University of Oxford Nuffield Department of Medicine .[34] Paired-end reads

216   were trimmed of adapters, primers, and low-quality bases with trimmomatic[35] and then filtered to

217   remove *pol* hypermutated sequences and non-HIV *pol* sequences. Duplicate reads introduced by

218   PCR were removed using Picard MarkDuplicates[36] and unique reads were locally aligned to 142

219   HIV subtype references using blastx.[37] A manually-curated codon-restricted multiple-alignment

220   of the references was used to lookup coordinates and mutations relative to HXB2 (GenBank:

221   K03455.1). Only mutations supported by a minimum of 10 PCR-deduplicated reads and by ≥5%

222   of reads spanning the corresponding site were considered.[38] These thresholds are based on HIV

223   read counts after removal of non-unique PCR duplicate reads. Importantly, the same thresholds

224   were used in a previous validation and demonstrated comparable sensitivity to a gold standard
225   clinical assay[34]. Amino acid substitutions were scored according to the Stanford University HIV
226   Drug Resistance Database. Scores were summed to predict susceptibility to 25 HIV drugs
227   (appendix p 13).[39–41] A score ≥30 (intermediate/high-level) for a given drug was categorized as
228   resistant. Resistance was not predicted if less than half of the relevant positions for a given drug
229   had fewer than 10 reads. Samples in which there was insufficient sequencing coverage for one or
230   more drug within a class were not assigned a resistance categorization for that class. Samples
231   with resistance to at least one drug within each class were categorized as resistant to that class.
232

233   *Outcome measures*
234   The primary outcomes of this study were the prevalence of viremic PLHIV with INSTI, NNRTI,
235   NRTI, and or PI resistance among all participants, regardless of HIV serostatus, in each survey
236   round. We also estimated the population prevalence of NNRTI, NRTI, and PI resistance
237   contributed by viremic pre-treatment and treatment-experienced PLHIV and the population
238   prevalence of multi-class resistance. We further estimated the prevalence of NNRTI, NRTI, and
239   PI resistance and individual resistance-conferring viral mutations specifically among viremic
240   pre-treatment and treatment-experienced PLHIV in each survey round. Given the greater
241   prevalence of viremic HIV in fishing communities, among men, and among younger age groups
242   in the RCCS[19,20,25] we evaluated the association between these variables and resistance in
243   bivariate analyses. Stratified estimates were generated for covariates identified as significant in
244   bivariate analyses. Given the availability of sequence data, we restricted prevalence estimates
245   that include treatment-experienced PLHIV to the 2015 and 2017 survey rounds and use the 2017
246   survey as an end-point for pre-treatment PLHIV. For context, we estimated the prevalence of
247   PLHIV, viremic PLHIV (2014 and later due to missing viral load data), viremic PLHIV pre-
248   treatment, and viremic treatment-experienced PLHIV among participants in each round (2014
249   and later).
250

251   *Statistical methods*
252   Statistical analyses were conducted in R v.4.4.1.[42] Prevalence was estimated using Poisson
253   regression with a log-link and robust (sandwich) standard errors[43] which were fit with general
254   estimating equations using geepack v.1.3.11 to account for repeated measures[44]. Correlation
255   structures were chosen by minimizing the Quasi Information Criterion (QIC). We used inverse
256   probability weighting to account for missing sequence data among viremic study participants.
257   Sampling weights were calculated based on availability of a viral load measurement
258   (True/False), $\log_{10}$ copies/mL where available, community type (agrarian/fishing/trading), age
259   category ((14,24]/(24,34]/(34,49]), and sex (M/F) stratified by survey round. Emmeans v. 1.10.4
260   was used to calculate prevalence within strata[45]. 95% confidence intervals and *p*-values ($\alpha =$
261   0.05) were calculated using the Wald method. χ^2 *p*-values were calculated using the stats
262   package in R. Data analysis and visualization was done using tidyverse v.2.0.0,[46] ggplot2
263   v.3.5.1,[47] cowplot v.1.1.3,[48] patchwork v. 1.2.0[49], and ggpattern v.1.1.1[50]. Readxl v.1.4.3[51] and
264   haven v.2.5.4.9[52] were used to parse data files. See appendix pp 5-17 for detailed methods.
265

266   *Role of the funding source*
267   The funders had no role in study design, collection, analysis, and interpretation of data; and no
268   role in the writing of the report and decision to publish.
269

270 **Results**

271 *Study population*

272 Between August 10, 2011 and November 4, 2020, a total of 43,361 people participated in the

273 RCCS, of whom 7,923 (18.27%) were PLHIV. Of 93,622 participant-visits about a fifth were

274 from PLHIV (table 1). Over the analysis period, the median age of study participants remained

275 stable whereas the age of PLHIV increased slightly (appendix pp 18-19). Viral load

276 measurements were available for 1,959/3,498 (56.00%) of PLHIV in the 2012 survey and

277 13,962/14,008 (99.67%) PLHIV in later survey rounds. A total of 46 participant-visits from the

278 2014-2019 surveys with missing viral loads were dropped from subsequent analyses. Among

279 participant-visits from PLHIV in 2014-2019, 26.29% were contributed by viremic PLHIV and of

280 those 79.81% were pre-treatment viremic. After imputation of missing viral loads (*Methods*),

281 56.75% of PLHIV in the 2012 survey were identified as pre-treatment viremic.

282

283 HIV seroprevalence among participants decreased from 20.38% (95% CI 19.78% - 20.99%) in

284 the 2012 survey to 17.20% (95% CI 16.68% - 17.74%) in the 2019 survey (figure 1A, appendix

285 p 20). Concurrent with an increase in the proportion of PLHIV reporting ever having been on

286 treatment from 25.04% (2012) to 85.25% (2019, appendix p 21), HIV viremia among all

287 participants decreased significantly from 8.14% (2014, 95% CI 7.75% - 8.55%) to 2.34% (2019,

288 95% CI 2.14% - 2.57%). These declines were driven by a nearly nine-fold (prevalence ratio (PR)

289 0.13, 95% CI 0.11 – 0.15) decrease in the prevalence of pre-treatment viremia among

290 participants over the study (figure 1A, appendix p 20). The prevalence of treatment-experienced

291 viremia remained stable at around 1% of participants.

292
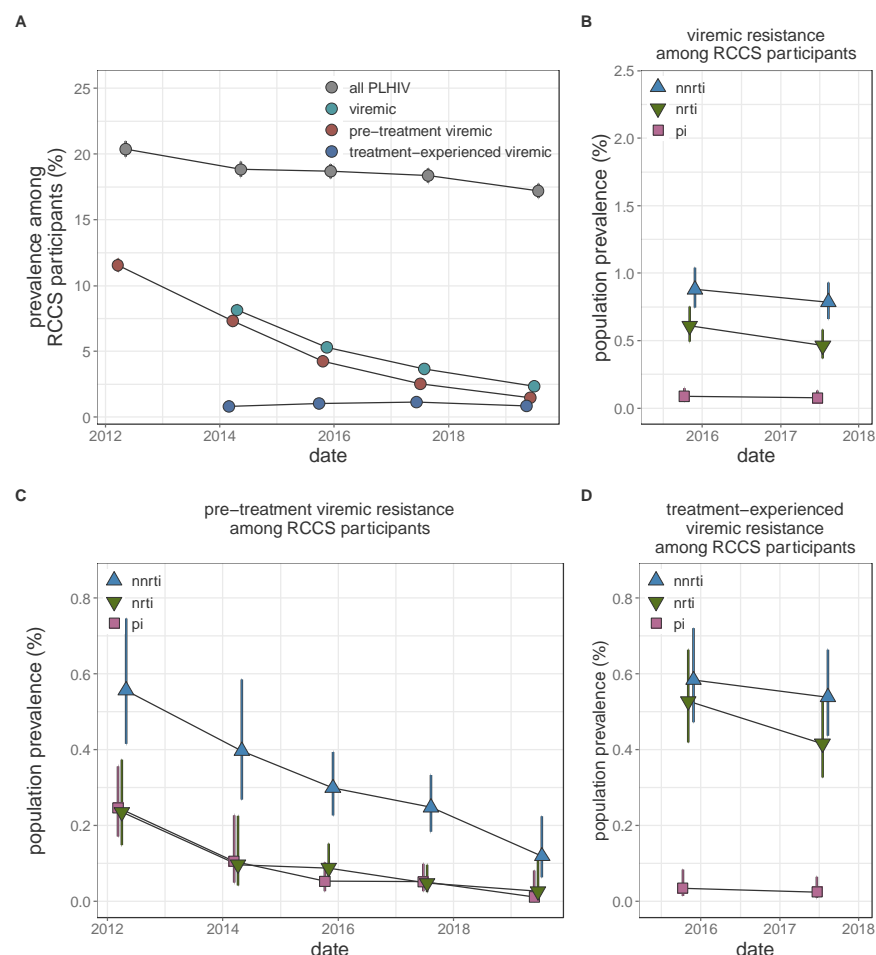
Figure 1: Longitudinal trends in HIV seroprevalence and population prevalence of viremic HIV drug resistance among Rakai Community Cohort Study participants, 2012-2019. (A) Estimated prevalence of all HIV, viremic HIV, viremic pre-treatment HIV, and viremic treatment-experienced HIV in each round. Due to missing viral load data, prevalence of viremic HIV and viremic treatment-experienced HIV were not estimated in the 2012 survey. For some estimates confidence bands do not extend beyond point. (B-D) Estimated population prevalence of all viremic (B), pre-treatment viremic (C), and treatment-experienced viremic (D) NNRTI, NRTI, and PI resistance among all study participants. Estimates were generated using Poisson regression with robust standard errors with survey round as a predictor variable. Generalized estimating equations with correlation structure selection by Quasi Information Criterion value (A: independent, B: independent, C: independent, D: exchangeable (NNRTI and PI), independent (NRTI)) were used to account for repeat participants across study rounds. Error bars indicate the Wald 95% confidence interval for the mean value. For clarity, points are jittered along the x-axis. PLHIV = people living with HIV. NNRTI = non-nucleoside reverse transcriptase inhibitors (blue upwards facing triangles). NRTI = nucleoside reverse transcriptase inhibitors (green downwards facing triangles). PI = integrase inhibitors (pink squares).

Identification of resistance genotypes in deep-sequence data

Deep-sequence based identification of drug resistance mutations (DRMs) was attempted on 4,525/5,724 (79.51%) of viremic participant visits (appendix p 22). Attempted genotyping did not vary by participant age, sex, or community type of residence. The veSeq-HIV sequencing protocol was used for 44.99% of all sequenced viremic participants and the vast majority (99.37%) of those in the 2015 through 2019 survey rounds (appendix p 23). Among samples from viremic participant-visits on which deep-sequence based genotyping was attempted,

9

316   sufficient data were available to reliably genotype 4,072/4,525 (90.01%, appendix pp 24-25)
317   viruses from 3,407 PLHIV for at least one drug. Sequencing success did not depend on age,
318   community, type or sex, (*p*-values ≥ 0.37) but was more likely among samples with higher viral
319   load and those sequenced with veSeq-HIV (*p*-values = 0.0005). Among sequenced participant-
320   visits successfully genotyped for all INSTIs (*n*=2,578, appendix pp 26-32), NNRTIs (*n*=3,050),
321   NRTIs (*n*=3,009) or PIs (*n*=3,520) <1%, 12.46%, 6.75%, and 1.88% had predicted resistance,
322   respectively (appendix pp 33-34). Given the minimal INSTI resistance we did not estimate the
323   prevalence of INSTI resistance.
324
325   *Population prevalence of viremic resistance*
326   In 2017, the population prevalence of viremic NNRTI, NRTI, and PI resistance among all
327   participants, regardless of HIV serostatus, was 0.79% (95% CI 0.66% - 0.93%), 0.46% (95% CI
328   0.37% - 0.58%), and 0.08% (95% CI 0.04% - 0.13%), respectively. These levels were stable
329   compared to 2015 (figure 1B, appendix p 35). In stratified analyses, NNRTI and NRTI resistance
330   was more than three-times as common in fishing as compared to agrarian or trading communities
331   and most prevalent among people aged 25-34 years old (*p*-values ≤ 0.0001, appendix pp 36-38).
332
333   The prevalence of NNRTI and NRTI resistance contributed by pre-treatment viremic PLHIV
334   decreased 2.3-fold (PR 0.44, 95% CI 0.29 – 0.68) and 5-fold (PR 0.21, 95% CI 0.09 – 0.47)
335   between the 2012 and 2017 surveys (*p*-values < 0.0001, figure 1C, appendix p 38), concurrent
336   with the observed decline in population prevalence of pre-treatment viremia. Consequently, in
337   the 2017 survey round, treatment-experienced viremic PLHIV contributed 68.46% (95% CI
338   59.49% - 75.44%) and 89.61% (79.92% - 94.62%) of all NNRTI and NRTI resistance,
339   respectively. Specifically, the population prevalence of resistance to NNRTIs and NRTIs
340   contributed by treatment-experienced viremic PLHIV in the 2017 survey was 0.54%, 95% CI
341   0.44%-0.66% and 0.42%, 0.33%-0.53% as compared to 0.25%, 0.18%-0.32% and 0.05%,
342   0.02%-0.1% (appendix pp 39 – 40) contributed by pre-treatment viremic PLHIV.
343
344   Resistance profiles varied considerably by treatment status (figure 2 and appendix p 41). Among
345   pre-treatment viremic PLHIV with available genotype for NNRTIs, NRTI, and PIs the majority
346   with any resistance were NNRTI mono-resistant (2017 survey *n*=35, 70%). In contrast, among
347   treatment-experienced viremic PLHIV with any resistance, NNRTI/NRTI dual-class resistance
348   was the most common profile (2017 survey *n*=64, 73.56%). Among all participants, the most
349   common forms of viremic resistance were NNRTI/NRTI dual-class resistance (2017: 0.44%,
350   95% CI 0.35% - 0.56%) and NNRTI mono-resistance (2017: 0.32%, 95% CI 0.25% - 0.42%),
351   consistent with a dominant contribution from treatment-experienced viremic PLHIV.
352   Other resistance profiles were extremely rare (<0.1%).
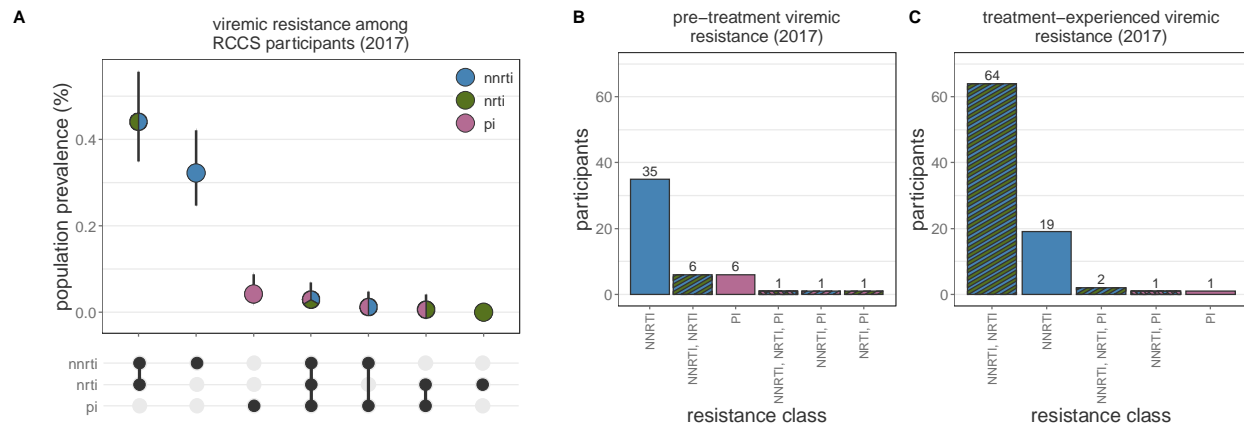
Figure 2: Patterns of multi-class resistance in Rakai Community Cohort Study, 2017. (A) Estimating population prevalence of NNRTI, NRTI, and PI mono-resistance and NNRTI/NRTI, NNRTI/PI, NRTI/PI, and NNRTI/NRTI/PI multi-class resistance among all RCCS study participants. Estimates were generated using Poisson regression with robust standard errors with survey round as a predictor variable. General estimating equations with the best fit correlation structure by QIC value (NNRTI, NRTI, PI mono-resistance and NNRTI/NRTI and NNRTI/PI multi-class resistance: independent, NRTI/PI and NNRTI/NRTI/PI: exchangeable) were used to account for repeated measures from the same participant Error bars indicate the Wald 95% confidence interval for the mean value. (B) Multi-class resistance profiles among 50 pre-treatment viremic 2017 participant-visits with genotype data for all NNRTIs, NRTIs, PIs, and resistance to at least one of these drug classes. (C) Multi-class resistance profiles among 87 treatment-experienced viremic 2017 participant-visits with genotype data for all NNRTIs, NRTIs, PIs, and resistance to at least one of these drug classes. NNRTI = non-nucleoside reverse transcriptase inhibitors. NRTI = nucleoside reverse transcriptase inhibitors. PI = integrase inhibitors.

Prevalence of resistance among pre-treatment viremic PLHIV

Between the 2012 and 2017, NNRTI resistance among pre-treatment viremic PLHIV increased by a factor of 1.98 (95% CI: 1.34-2.91), reaching 9.61% (95% CI: 7.27% - 12.7%) (figure 1A and appendix pp 42). This increasing trend did not vary by sex, age, type of community of residence, or sequencing approach (appendix pp 43-44).The prevalence of NRTI and PI resistance remained stable and below 2.1% over the same time period.
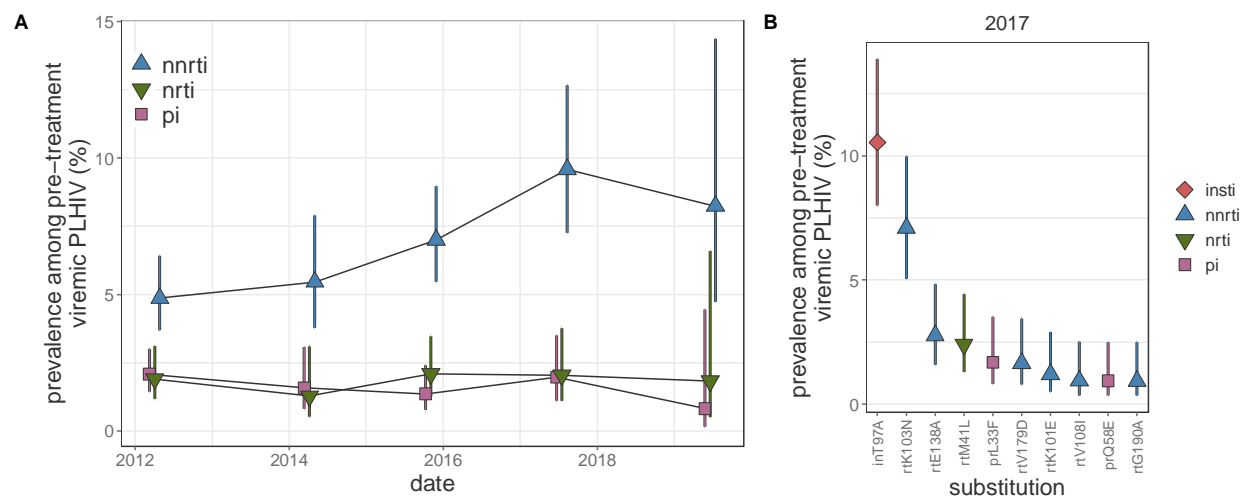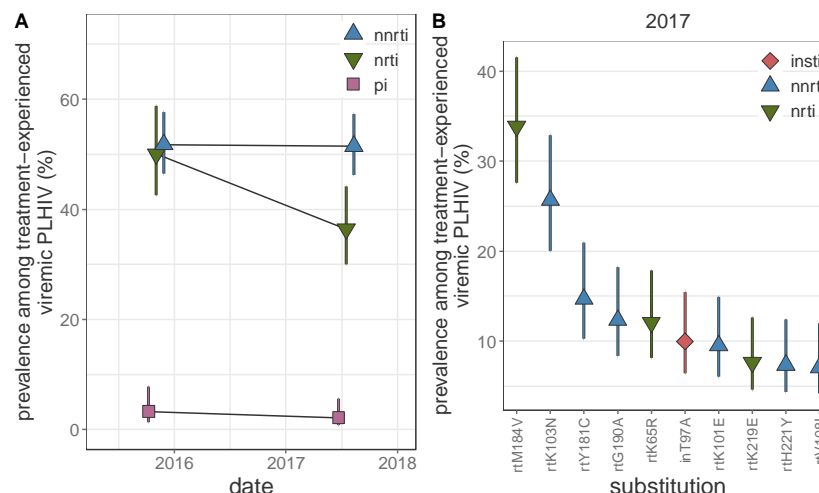

Figure 3: Longitudinal trends in HIV drug resistance among pre-treatment viremic Rakai Community Cohort Study participants, 2012-2019. (A) Estimated prevalence of NNRTI, NRTI, and PI resistance among pre-treatment viremic PLHIV. For visual clarity, points are jittered along the x-axis. (B) Prevalence in the 2017 survey of the 10 most prevalent substitutions in pre-treatment viremic PLHIV sorted by prevalence. Estimates were

11

379 generated using Poisson regression with robust standard errors with survey round as a predictor variable. General
380 estimating equations with the best fit correlation structure by QIC value (NNRTI: exchangeable, NRTI:
381 exchangeable, PI: AR1, substitutions: independent to ensure convergence) were used to account for repeated
382 measures from the same participant Error bars indicate the Wald 95% confidence interval for the mean value within
383 each category. PLHIV = people living with HIV. NNRTI = non-nucleoside reverse transcriptase inhibitors. NRTI =
384 nucleoside reverse transcriptase inhibitors. PI = integrase inhibitors.

385

386 Among pre-treatment viremic PLHIV, the most prevalent resistance-associated mutation was
387 inT97A (figure 3B and appendix pp 46-48), an INSTI-resistance (particularly Elvitegravir[53])
388 mutation, detected in ~10% of pre-treatment viremic participants in the 2012 through 2017
389 survey rounds and in 20% (95% CI 13.89% - 28.8%) in the partial 2019 survey data. The most
390 common NNRTI-resistance mutation was rtK103N, found in 7.1% (95% CI 5.05% - 9.97%) of
391 pre-treatment viremic PLHIV in the 2017 survey, a 4.26-fold (95% CI 2.11 - 8.59) increase
392 compared to the 2012 survey. The next most prevalent NNRTI mutation, rtE138A, which is
393 associated with 2.5-fold reduced susceptibility to RPV[54], was present in only 2.77% (95% CI
394 1.59% – 4.85%) of pre-treatment viremic PLHIV and its prevalence remained stable compared to
395 the 2012 survey (*p*-value = 0.49). NRTI resistance mutations were rare compared to NNRTI
396 mutations. Genotypes associated with intermediate/high-level INSTI resistance were identified in
397 16 pre-treatment viremic participant-visits (<1%), the majority of which harbored inE92G
398 (*n*=13), which confers resistance to Elvitegravir, a drug that is not routinely used in Uganda
399 (appendix p 4). Mutations conferring intermediate/high-level resistance to DTG were not
400 observed.

401

402 *Prevalence of resistance among treatment-experienced viremic PLHIV*
403 Prevalence of NNRTI and NRTI resistance was substantially higher among treatment-
404 experienced participants as compared to pre-treatment PLHIV. In 2017, 51.49% (95 CI 46.24%-
405 57.34%) and 36.46% (95% CI 30.06%-44.22%) of treatment-experienced participants with
406 viremia harbored NNRTI and NRTI resistant viruses, respectively (figure 4A and appendix p
407 49). NRTI resistance was 1.62 (95% CI 1.03 – 2.56, appendix pp 50-51) times more common
408 among participants aged 25-34 years compared to 15-24 year-olds (*p*-value = 0.037). While
409 NNRTI resistance remained stable between the 2015 and 2017 survey rounds, NRTI resistance
410 decreased by more than quarter (prevalence ratio 0.73%, 95% CI 0.58 - 0.92, *p*-value = 0.0084).
411 Only 2.13% (95% CI 0.81%-5.63%) of treatment-experienced viremic participants in the 2017
412 survey round had viruses with PI resistance.

413

12

Figure 4: Longitudinal trends in HIV drug resistance among treatment-experienced viremic Rakai Community Cohort Study participants, 2015-2017. (A) Estimated prevalence of NNRTI, NRTI, and PI resistance among treatment-experienced viremic PLHIV. For visual clarity, points are jittered along the x-axis. (C) Prevalence of the 10 most prevalent drug resistance mutations in treatment-experienced viremic PLHIV in the 2017 survey round, sorted by prevalence. Estimates were generated using Poisson regression with robust standard errors with survey round as a predictor variable. General estimating equations with the best fit correlation structure by QIC value (NNRTI and PI: exchangeable, NRTI: independent, mutations: independent to ensure convergence) were used to account for repeated measures from the same participant Error bars indicate the Wald 95% confidence interval for the mean value within each category. PLHIV = people living with HIV. NNRTI = non-nucleoside reverse transcriptase inhibitors. NRTI = nucleoside reverse transcriptase inhibitors. PI = integrase inhibitors.

The resistance-associated mutations observed among treatment-experienced viremic participants differed considerably compared to pre-treatment viremic participants (appendix pp 46, 52). NRTI resistance among treatment-experienced viremic participants in the 2017 survey was most frequently due to the rtM184V (33.89%, 95% CI 27.61% - 41.59%), rtK65R (12.07% 95% CI 8.14% - 17.9%), and rtK219E (7.64%, 95% CI 4.6% -12.67%) substitutions, which were rarely observed among pre-treatment PLHIV. On the contrary, the most prevalent NNRTI-associated substitution among treatment-experienced viremic participants was rtK103N (25.68%, 95% CI 20.03 - 32.92%), however rtY181C (14.67%, 95% CI 10.27% - 20.95%) and rtG190A (12.34%, 95% CI 8.33% - 18.27%) were also frequently observed. inT97A was observed at a similar prevalence as among pre-treatment viremic participants (9.96%, 95% CI 6.41 -15.48%). Only four participant-visits contributed by viremic treatment-experienced PLHIV (<1%) harbored INSTI resistance mutations, which were each observed only once (inG163K, inG163R, inR263K, inS147G) and not associated with DTG resistance.

Discussion

In this study, we report on trends in HIV drug resistance from a longitudinal, population-based cohort in southern Uganda between 2012 and 2019, a period marked by the substantial expansion of ART programs. Despite a doubling in the prevalence of NNRTI resistance among pre-treatment PLHIV, we observed an overall decline in the population prevalence of pre-treatment HIV drug-resistant viremia, alongside increasing ART uptake and viral suppression among PLHIV. By the end of the analysis period, the population prevalence of NNRTI resistance was 0.78% and NRTI resistance was 0.46%, with most resistance stemming from dual-class NNRTI/NRTI resistance in treatment-experienced viremic individuals. Notably, dual-class resistance remained relatively uncommon among pre-treatment viremic PLHIV, and resistance

13

450   trends for NRTIs and PIs in this group remained stable throughout the ART scale-up, despite a
451   substantial burden of NRTI resistance in treatment-experienced individuals. We also observed a
452   relatively high background prevalence of the INSTI-resistance mutation inT97A. Overall, these
453   findings provide important insights into the evolving dynamics of HIV drug resistance during
454   ART scale-up in a high-burden East African population and may help guide future surveillance
455   and HIV epidemic control efforts in the region.
456
457   Consistent with previous studies, we observed an increase in the prevalence of NNRTI resistance
458   among viremic pre-treatment individuals living with HIV, supporting the recent shift to DTG-
459   based regimens[1,11,12]. However, a key finding of this population-based analysis is that, concurrent
460   with this rise in NNRTI resistance, we observed a substantial decline in the overall population
461   prevalence of pre-treatment HIV, likely driven by both increased treatment initiation and
462   declining HIV incidence[18–20]. Importantly, this decrease in pre-treatment HIV has outpaced the
463   rise in NNRTI resistance, resulting in a more than 50% reduction in the population prevalence of
464   pre-treatment HIV with NNRTI resistance over the study period. By the end of the survey
465   period, most viremic individuals with NNRTI-resistant HIV were those with prior treatment
466   experience.
467
468   We also find a lower burden of NNRTI and NRTI resistance among viremic treatment-
469   experienced PLHIV in this study as compared to clinic-based studies[1,12]. This is likely because
470   our population-based study design includes PLHIV who remain viremic because they are not
471   actively engaged in care, despite past treatment exposure. In comparison, clinic-based studies
472   may disproportionately enroll people who remain viremic due to sub-optimal adherence or are in
473   more advanced stages of disease, and thus more likely to have drug resistance, whereas
474   population-based sampling includes people lost to clinic-based care and no longer using
475   treatment altogether.
476
477   Both NNRTI and NRTI therapies remain important components of the current and future ART
478   landscape. Current first-line DTG-regimens incorporate two NRTIs (e.g. TDF and 3TC) and
479   DTG treatment failure is more likely among those with NRTI resistance[55,56]. We here observe
480   rtM184V (>1000-fold reduced susceptibility to 3TC[57]) and rtK65R (five-fold reduced
481   susceptibility to TDF[58]) in 34% and 12% of viremic treatment-experienced PLHIV. In contrast,
482   these mutations were observed only rarely among pre-treatment PLHIV. Further, we identify a
483   number of mutations associated with reduced susceptibility to the RPV (e.g. rtK101E, rtE138A,
484   rtY181C, and rtG190A), an NNRTI given in combination with CAB as part of long-lasting
485   injectable therapies, in 10-12% of viremic treatment-experienced PLHIV.
486
487   As this study pre-dates the scale-up of DTG, we do not observe major DTG resistance-conferring
488   mutations. Approximately 10% of viremic participants harbored inT97A, which is a polymorphic
489   mutation most common in subtype A and in isolation confers two-fold resistance to EVG but not
490   to other INSTIs[59]. The observed prevalence of inT97A in this study is an order of magnitude
491   higher than in a population-based cohort in South Africa[17] and about twice as prevalence as
492   globally-sampled INSTI-naïve PLHIV[59]. Further, we observe a significant increase in the
493   prevalence of inT97A among pre-treatment viremic PLHIV in the 2019 survey round. As
494   inT97A is repeatedly selected for in subjects failing DTG therapy[60] and in combination with

495   other mutations (e.g. inG140S and inQ148H) can significantly increase DTG resistance[61–63], we
496   recommend continued monitoring.

498   There are important limitations of this work. Due to unknown HIV serostatus among non-
499   participating residents of RCCS communities, we did not generalize our results beyond study
500   participants. Younger individuals, men, and residents of trading communities are less likely to
501   participate in RCCS surveys.[20] Further, only self-reported treatment status was available, which
502   may have led to the misclassification of some participants. Prior work in this cohort
503   demonstrated that 11% of self-reported ART-naïve participants had antivirals in their blood.[64]
504   Given the significant differences observed in the mutational profiles of pre-treatment as
505   compared to treatment-experienced PLHIV and the consistency of these results with estimates of
506   the fitness impact of mutations in the absence of treatment,[65] we expect minimal
507   misclassification bias. While we lack data on individual-level ART regimens, first-line therapy in
508   this setting is highly consistent across individuals. As this work is based on sequencing of viral
509   RNA, we could only identify resistance among viremic PLHIV. Consequently, our population
510   prevalence estimates are an underestimate as some PLHIV with resistance may be suppressed
511   through second-line therapy or were transiently suppressed following treatment initiation. The
512   latter may be more pronounced in recent survey rounds as treatment scale-up has increased the
513   proportion of recent treatment initiators.

515   Despite the population-based study design, viral load data and sequence data was available for
516   only a subset of participants due to budgetary and logistical constraints. We consequently restrict
517   analyses to survey rounds where sufficient data is available to generate reliable inferences and
518   use imputation to account for missing viral load data. Despite this missingness, deep-sequence
519   data was available for 4,072 participant-visits, which is considerably more than a recent
520   population-based study in South Africa (n=1,097),[17] clinic-based studies in sub-Saharan Africa
521   (n=972),[12] and WHO surveys in Uganda (n=372)[1]. Further, we utilized detailed demographic
522   data on survey participants to account for the role of potential biases in sequence data
523   availability. However, we cannot rule out potential residual biases in our estimates.

525   In summary, this study adds critical context to our understanding of the HIV epidemic in
526   southern Uganda and to the impact of treatment expansion on the population burden of HIV
527   resistance. We show that following ART scale-up, most resistance is contributed by treatment-
528   experienced PLHIV, which may inform interventions aimed at reducing transmitted HIV
529   resistance. The relatively high prevalence of NRTI and NRTI-resistance among treatment-
530   experienced PLHIV and of inT97A among all viremic PLHIV is concerning in light of the roll-
531   out of DTG+ TDF+3TC and CAB+RPV regimens in sub-Saharan Africa. Overall, these findings
532   stress the importance of continued viral sequence-based monitoring of resistance mutations
533   among PLHIV, particularly those with previous treatment exposure, during the roll-out of novel
534   HIV ART regimens.

15

Data sharing

Code to reproduce all analyses and visualizations as well as de-identified resistance and limited
patient metadata are available at https://github.com/m-a-martin/rccs_hiv_resistance_r15_r19.
Due to privacy concerns we are unable to share individual-level data on community of residence.

Declaration of interests

We declare no competing interests.

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