Temporal trends of transmitted HIV drug resistance in a multinational seroconversion cohort

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Abstract

Background

The rate of transmitted drug resistance (TDR) may increase with wider use of antiretroviral therapy and can contribute to therapeutic failure. We analysed time trends in TDR among HIV seroconverters.

Methods

Using CASCADE data of individuals with well estimated dates of HIV seroconversion, we examined HIV nucleotide sequences collected prior to antiretroviral therapy use from 1996–2012. All samples were taken within 12 months of testing HIV positive. Using logistic regression, we examined the association between TDR and year of seroconversion, adjusting for confounders.

Results

Of 4717 individuals seroconverting between 1996 and 2012, median (IQR) age at seroconversion was 33 (27, 39) years. The majority (3839; 92%) were male, mainly exposed through MSM (3767; 80%), and infected with subtype B (3464; 73%). Overall, 515 (11%) individuals had at least one drug resistance-related mutation; 280 individuals with nucleoside reverse transcriptase, 185 with nonnucleoside reverse transcriptase, and 144 with protease inhibitor mutations. Estimated TDR prevalence was 19.4% (8.2, 36.0) in 1996, significantly decreasing to 8.5% (5.9, 11.9) in 2012 [odds ratio (OR; 95% confidence interval (CI)) = 0.92 (0.90, 0.95) per year increase]. Individuals exposed through sex between men and women were significantly less likely to have been infected with a drug-resistant strain [OR (95% CI) = 0.59 (0.41, 0.87) compared with MSM], and there was marginal evidence that sampling during acute infection was associated with higher odds of resistance [OR (95% CI) = 1.20 (0.97, 1.7), P = 0.093] compared with later sampling.

Conclusion

TDR has decreased over calendar time although a significant proportion of new infections still carry resistance-related mutations.

Introduction

Combination antiretroviral therapy (cART) is effective at suppressing plasma HIV RNA to undetectable levels [1] thereby improving patient prognosis [2,3] and reducing the risk of onward transmission of HIV when viral suppression is achieved [4]. However, poor adherence [5–9] can lead to the development of mutations [10] which are associated with HIV drug resistance and subsequent cART failure. Individuals failing treatment have worse health outcomes [11–13], are less likely to benefit from newer drugs, and can pass drug resistant strains of HIV to others [14]. Given this concern, international guidelines recommend that newly diagnosed individuals are tested for evidence of resistance to optimize the selection of first-line cART regimes [15,16].

Recent data from cART-naive seroprevalent cohorts suggest the prevalence of TDR has either stabilized [17,18] or decreased from 2002 to 2009 [19–21]. Given that timing of HIV infection is not known for individuals in seroprevalent cohorts; however, estimated TDR rates may reflect historical trends but not necessarily trends among those recently infected. Furthermore, because of the reversion of a number of mutations to wild-type over time in the absence of cART [22], analysis of TDR rates among seroprevalent cohorts may under-estimate actual TDR prevalence. Trends of TDR among HIV seroconverters are unclear with some studies showing increased TDR between 1987 and 2003 [23] or stability between 1996 and 2007 [24].

Temporal trends of transmitted drug resistance (TDR) among individuals recently infected need to be monitored as new drugs and classes are introduced to inform clinical decision making. We aim to describe the temporal trends of TDR among recently infected individuals using CASCADE data of HIV seroconverters, and to identify predictors of TDR.

Methods

Study Population

We used pooled data from the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) 2014 data release on HIV-1 seroconverters in EuroCoord (www.EuroCoord.net), which has been described in detail elsewhere [25]. Briefly, CASCADE is a cohort collaboration of 31 772 HIV-1 seroconverters from 16 countries across Europe (95%), Australia (1%), Canada (1%), and Sub-Saharan Africa (3%). Date of HIV seroconversion was estimated most commonly (87%) as the midpoint between the last documented negative and the first documented positive HIV antibody test dates with an interval of less than 3 years between the two dates. The remaining individuals had seroconversion dates estimated through laboratory evidence of seroconversion (PCR positivity in the absence of HIV antibodies or antigen positivity with fewer than four bands on western blot – 10%), or as the date of seroconversion illness with both an earlier documented negative and a later positive HIV test not more than 3 years apart (2%).

We restricted our analysis to those with documented seroconversion in the cART era (>1995) with at least one viral genetic sequence within the first year of testing positive for HIV while still being ART naive. Additionally, we restricted the analysis to those seroconverting before 1 January 2013 as to allow at least 1 year of follow-up.

Resistance and subtype analysis

Genotypic resistance data were derived from sequencing of the protease and reverse transcriptase genes performed by laboratories in the country of care using a variety of in-house and commercial resistance assays. The Stanford HIVdb algorithm 7.0 was used centrally to analyse all nucleotide sequences (http://hivdb.stanford.edu); updated on 27 February 2014) [26]. Subtype was analysed and assigned centrally using the REGA algorithm [27].

An individual was categorized as having a transmitted HIV-1 drug resistance-associated mutation if their virus contained one or more mutations from the Surveillance Drug Resistance Mutations list defined by the WHO [28]. We further derived susceptibility to antiretroviral drugs using the Stanford HIV database algorithm. Individuals were considered to have high level of resistance if the Stanford score was higher than 3. Using this algorithm, we further identified mutations associated with drugs of current first-line recommendations according to the European AIDS Clinical Society guidelines (categories A and B) [29].

Statistical methods

Proportions and their associated 95% confidence intervals (CI) were calculated using exact CIs for binomially distributed data. Linear logistic regression was used to assess the time trends of TDR as there was no statistical evidence for departures from linearity using natural cubic splines [30]. Time trend models were adjusted for sex, HIV transmission risk group, seroconversion age, and HIV diagnosis during acute HIV infection, defined as laboratory evidence of HIV seroconversion or having an HIV test interval of less than 30 days. Age at HIV seroconversion was modelled linearly as there was no evidence for departures from linearity using natural cubic splines. Owing to small numbers, we were not able to evaluate the time trends of individual mutations. Instead, we list the most common mutations over the calendar period.

In a sensitivity analysis, we restricted our analysis to include only individuals infected with subtype B as our cohort consists predominantly of subtype B (>70%), and HIV genetic diversity may influence the emergence and type of resistance mutations.

Results

Baseline Characteristics

We analysed data from 4717 seroconverters in CASCADE with at least one ART-naive nucleotide sequence available during the first year following HIV seroconversion. Median age at HIV seroconversion was 33 (IQR = 27, 39) years, and the most common HIV transmission risk group was MSM (80%) followed by sex between men and women (MSW, 15%), people who inject drugs (PWID, 3%) and unknown (n = 101, 2%). HIV subtype was mainly B (n = 3464, 73%), followed by C (n = 288, 8%), A (n = 240, 6%), and a recombinant form (n = 176, 4%), Table 1. Median (IQR) time from HIV seroconversion to sample collection was 124 (44, 256) days, and did not differ between those with and without mutations associated with HIV drug resistance (P = 0.31, data not shown). Of the 4717 seroconverters, 1222 (26%) were diagnosed with HIV during acute HIV infection, a proportion which did not differ between those with and without mutations associated with HIV drug resistance (P = 0.26, data not shown). The majority of individuals were receiving care in Germany (34%), the UK (21%), or Sweden (12%).

Transmitted Drug Resistance

Overall, 203 (4.3%; 95% CI = 3.7–4.9) individuals had one mutation and 515 (10.9%; 95% CI = 10.0–11.8) had one or more mutations associated with TDR. Among these 515 individuals, 93 (2.0%; 1.6–2.4), 98 (2.1%; 1.7–2.5), and 67 (1.4%; 1.1–1.8) had one mutation associated with nucleoside reverse transcriptase inhibitors (NRTI), non-NRTIs (NNRTI), or protease inhibitors, respectively, and 280 (5.9%; 5.2–6.6), 185 (3.9%; 3.4–4.5), and 144 (3.1%; 2.6–3.6), had one or more mutations associated with NRTI, NNRTI, or protease inhibitors, respectively.

The most frequent mutations (>5% of individuals with mutations) related to NRTIs were 41L (n = 91; 18%), 215S (n = 61; 12%), 184V (n = 34; 7%), 67N (n = 30; 6%), 210W (n = 28; 5%) 219Q (n = 27; 5%). For NNRTIs, the most common mutation was 103N (n = 119; 23%) and, for protease inhibitors these

were 90M (n = 39; 8%), 46I (n = 31; 6%), and 46L (n = 26; 5%) (Supplementary Table 1). In total, 436 (9%) individuals had mutations associated with a single class, 79 (2%) had mutations associated with two or more classes, and 15 (<1%%) had mutations associated with three classes (NRTI, NNRTI, and protease inhibitor).

We observed a significant decline in the prevalence of TDR to any class during 1996–2012, the calendar year of seroconversion; odds ratio (OR) = 0.92 (95% CI; 0.90, 0.95) per year, starting at 19.4% (8.2, 36.0) in 1996 and falling to 8.5% (5.9, 11.9) in 2012. The same decreasing trend over time was observed for transmitted NRTI resistance, OR = 0.89 (0.86, 0.91) per year, NNRTI resistance, OR = 0.96 (0.93, 1.00) per year, and protease inhibitor resistance, OR = 0.93 (0.89, 0.97) per year (Table 2, Fig. 1).

In more recent years (2007–2012), data were available on 2546 individuals, 216 [8.5% (7.4, 9.6)] of whom had a mutation associated with TDR. Among these individuals, 98 (3.8%; 3.1–4.6), 89 (3.5%; 2.8–4.2), and 62 (2.4%; 1.8–3.1), had one or more mutations associated with NRTI, NNRTI, or protease inhibitor, respectively. The most common mutations in this time period include NTRI mutations 41L (n = 34; 16%), 215S (n = 26; 12%), and 215D (n = 15; 7%); NNRTI mutation 103N (n = 56; 26%); and protease inhibitor mutations 90M (n = 19; 9%) and 46L (n = 12; 6%).

In a sensitivity analysis, restricting to those infected with subtype B, we observed the same trends of TDR decreasing over the calendar period (data not shown). Findings were also consistent across all CASCADE participating cohorts.

Drug Susceptibility

Of 4717 individuals, 296 (6.3%; 5.5–7.0) had a transmitted mutation associated with high-level resistance to a drug according to the Stanford HIV database algorithm, 190 (4.0%; 3.5–4.6) of these were associated with an agent in a recommended first-line treatment regimen with efavirenz having the highest proportion of high-level resistance, Fig. 2. In total, 102 (2.2%; 1.8–2.6), 163 (3.5%; 2.9–

4.0), and 83 (1.8%; 1.4–2.2), individuals had at least one transmitted mutation associated with high level of resistance to NRTIs, NNRTIs, and protease inhibitor, respectively. Among the 2546 individuals seroconverting more recently (2007–2012), 154 [6.0% (5.2, 7.0)] had a transmitted mutation associated with high-level resistance; 93 (3.7%; 3.0–4.5), 42 [1.6% (1.2, 2.2)], 91 [3.6% (2.9, 4.4)], 46 [1.8% (1.3, 2.4)] with high-level resistance associated with a first-line regimen, NRTIs, NNRTIs, and protease inhibitors, respectively.

During the calendar period of observation, the rate of transmitted high-level drug resistance declined; OR = 0.97 (95% CI; 0.94, 1.0005) per year, P = 0.054. A significant decreasing trend over time was observed for high-level resistance to first-line regimens, OR = 0.92 (0.87, 0.97), P less than 0.001 per year and high-level NRTI resistance, OR = 0.89 (0.85, 0.93), P less than 0.001 per year. The same trend was observed in high-level protease inhibitor resistance, OR = 0.96 (0.91, 1.01), P = 0.18 per year. There was no evidence of a decrease in high-level NNRTI resistance over calendar time, although levels have remained relatively low throughout the period of observation of our study at 3.4%.

Predictors of TDR

There was significant heterogeneity between HIV transmission risk group and any TDR and NRTI TDR with those exposed through MSW having a lower probability of being infected with a drug-resistant strain compared to MSM. Older individuals were more likely to have been infected with a protease inhibitor resistant strain (P = 0.003) as were females, although the evidence for females was modest [OR = 2.03 (0.92, 4.47, P = 0.08)]. Individuals diagnosed during acute HIV infection were slightly more likely to be infected with a resistant strain, OR = 1.20 (0.97, 1.47; Table 2). Of the 1222 individuals in our study diagnosed during acute HIV infection, 144 (11.8%) had at least one mutation associated with TDR compared with 10.9% of individuals with TDR diagnosed later in infection.

When we restricted to those seroconverting in more recent years (2007–2012), older age was the only significant predictor for transmitted HIV drug resistance; OR = 1.58 (1.01, 2.45; P = 0.043), 1.50 (0.94, 2.41; P = 0.092), and 1.93 (1.13, 3.29; P = 0.016) for ages 25–34, 35–44, and 45 and above, respectively, compared with those aged 15–25 years at seroconversion.

Discussion

The prevalence of TDR and high-level resistance among individuals with recent HIV infection decreased between 1996 and 2012. Our estimates provide a realistic estimation of actual TDR in those years as our study was restricted to analysing viral sequences from individuals sampled close to the time of HIV seroconversion. Our results confirm and expand findings from studies of ART-naive individuals with unknown duration of HIV infection [19,20], and consistent with European reports of TDR with unknown duration of HIV infection [31,32]. Of note, although we show clear evidence for a decline in TDR rates over time, the 8.5% TDR prevalence in the most recent years highlights a moderate but ongoing risk of being infected with drug resistant virus remains.

We detected moderate evidence of an association between TDR and sampling during acute HIV infection. This suggests that TDR may be associated with seroconversion symptoms, possibly leading to presentation to care and HIV diagnosis during acute infection. It may also simply reflect that TDR rates are underestimated if genotypic resistance testing is not performed close to seroconversion because of reversion of mutations to wild type in the absence of drug selective pressure [11]. Of note, we found a similar proportion of TDR, 11.8%, among those diagnosed during acute infection throughout our period of study. There was also a similar association between TDR and acute HIV infection [OR = 1.16 (0.84, 1.58)], although this did not reach statistical significance as fewer individuals contributed to these analyses.

We also found evidence that MSM were more likely to have been infected with resistant strains compared with PWID and MSW. This has been reported by a number of studies in high-income countries [33–36] and may be because of historical access to HIV care, where MSM have been typically more exposed to ART than other risk groups [37], particularly the use of thymidine analogues such as stavudine and zidovudine, the mutations associated with which are known to be persistent [38]. This is supported by the differentially higher rates of NRTI mutation among MSM compared with other risk groups; 6.5, 4.5, and 3.2% among MSM, PWID, and MSW, respectively. The

high prevalence of TDR among the MSM especially the last years, could also be because of the high incidence of HIV in this group in Western Europe, where in some cases transmissions may have occurred in transmission clusters of resistant strains in this population.

Our study has a number of limitations. Although we analysed data only from HIV seroconverters to assess actual TDR trends by year of infection, it is known that risk behaviour differs between seroconverters and non-seroconverter HIV-positive individuals [39,40], and that such behaviour may put them at greater risk of becoming infected with drug-resistant HIV. The prevalence of TDR, however, in our cohort was similar to that reported among other (seroprevalent) cohorts in Europe [19,20,24,41] suggesting that our time trends for TDR are generalizable to the HIV-positive population in Europe. However, our numbers outside Europe are small, so although our estimates were consistent across all CASCADE cohorts, our estimates might not be as robust and generalizable in lower income countries. It is also feasible that there were treatment misclassifications and patients with prior ART experience were included in our analysis. Research by the UK HIV Drug Resistance Database suggests that if there is more than a 4% misclassification, time trends could be distorted [42]. Being that integrase inhibitors are a new drug class, we were not able to provide temporal trend estimates for mutations associated with integrase inhibitors, as data on such mutations were limited, where only two individuals had a resistance mutation associated with integrase inhibitor raltegravir. In addition, those with genotypic tests tended to be different than those without genotypic tests, where individuals with genotype tests tended to seroconvert in later years, were more likely to be MSM, present with acute HIV infection, and have shorter HIV test intervals. Also, certain countries tended to test more for genotypic resistance (e.g. Germany and the UK) compared with other countries (e.g. France). We may, therefore, have underestimated the overall prevalence of TDR, given that the risk was higher in earlier years. It is unlikely, however, that the preferential inclusion of MSM among sequenced individuals will have affected our main finding of a decreasing TDR trend given that the proportion of MSM sequined has remained stable at about 60% over the calendar period.

In conclusion, we found a steady decline in TDR among individuals newly infected with HIV between 1996 (19.4% TDR) and 2012 (8.5% TDR). Although the rate of transmitted drug-resistant HIV has decreased, a not insubstantial proportion of newly infected individuals are being diagnosed with drug-resistant strains. Given that resistance testing among such individuals remains cost-effective for baseline resistance above 1% [43], testing for evidence of TDR remains justifiable.

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Table 1: Baseline characteristics for individuals in CASCADE data of HIV seroconverters between 1996 and 2012; a comparison of individuals with at least one ART-naive nucleotide sequence available within 1 year of testing positive for HIV, and the remaining individuals.

Characteristic	Individuals with ≥1 nucleotide sequence N = 4,717	Individuals without sequences n = 17,574	p-value
Seroconversion year	2007 (2004, 2010)	2004 (2000, 2008)	< 0.001
Seroconversion age	33 (27, 39)	33 (27, 39)	0.89
Males	4327 (92%)	13,911 (80%)	< 0.001
HIV Risk Group ǂ			
MSM	3767 (80%)	10,611 (60%)	< 0.001
MSW	715 (15%)	5056 (29%)	
PWID	134 (3%)	1001 (6%)	
OTH/UNK	101 (2%)	906 (5%)	
Acute HIV infection ɻ	1222 (26%)	2340 (13%)	< 0.001
HIV Test Interval (days)	179 (26, 381)	278 (108, 541)	< 0.001
Country/Continent of cohort			
Germany	1,607 (34%)	775 (4%)	< 0.001
UK	1,029 (22%)	1,061 (6%)	
Sweden	524 (11%)	299 (2%)	
Spain	400 (8%)	795 (5%)	
Africa	323 (7%)	590 (3%)	
Austria	229 (5%)	150 (1%)	
Netherlands	197 (4%)	241 (1%)	
France	183 (4%)	10,257 (60%)	
Italy	93 (2%)	2,293 (13%)	
Canada	70 (1%)	111 (1%)	
Greece	62 (1%)	192 (1%)	
Subtype			
B	3465 (73%)		
C	288 (6%)		
A	240 (5%)		
CRF01_AE	120 (3%)		
CRF02_AG	112 (2%)		
Other recombinant forms	226 (5%)		
Other/Unknown	268 (6%)		

*All numbers are N(%) or Median (interquartile range)

ǂ Abbreviations: MSM – sex between men; MSW – sex between men and women; PWID – people who inject drugs; OTH/UNK – other/unknown; UK – United Kingdom

ɻ HIV test interval < 30 days or laboratory evidence of acute HIV infection

Table 2: Predictors of transmitted HIV drug resistance for individuals with at least one ART-naive nucleotide sequence within 1 year of testing positive for HIV: CASCADE data of HIV seroconverters

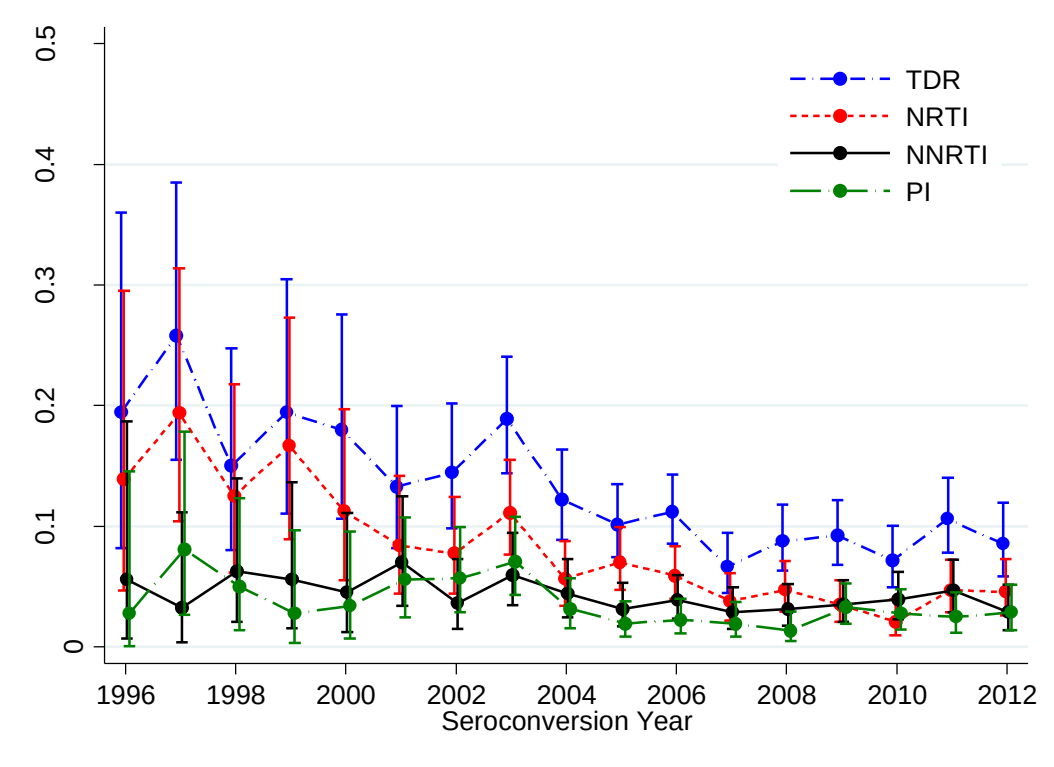
	Any TDR OR (95% CI)	p	NRTI TDR OR (95% CI)	p	NNRTI TDR OR (95% CI)	p	PI TDR OR (95% CI)	p
SC year	0.92 (0.90, 0.95)	< 0.001	0.89 (0.86, 0.91)	< 0.001	0.96 (0.93, 1.001)	0.059	0.93 (0.89, 0.97)	0.001
Sex (F vs. M)	1.19 (0.73, 1.93)	0.48	1.47 (0.72, 2.98)	0.29	0.65 (0.30, 1.37)	0.25	2.03 (0.92, 4.47)	0.08
Risk Group		0.03 ∓		0.002 ∓		0.95 ∓		0.34 ∓
MSM	1		1		1		1	
MSW	0.59 (0.41, 0.87)		0.38 (0.21, 0.70)		1.08 (0.65, 1.81)		0.56 (0.28, 1.11)	
PWID	0.62 (0.33, 1.16)		0.46 (0.19, 1.12)		0.79 (0.28, 2.20)		0.63 (0.21, 1.87)	
OTH/UNK	1.01 (0.54, 1.87)		1.23 (0.58, 2.62)		1.03 (0.37, 2.87)		1.04 (0.37, 2.96)	
Age Group		0.60 ∓		0.95 ∓		0.96 ∓		0.003 ∓
<25	1		1		1		1	
25-34	1.11 (0.84, 1.47)		0.94 (0.66, 1.35)		1.03 (0.67, 1.59)		1.34 (0.78, 2.31)	
35-45	1.02 (0.76, 1.38)		0.92 (0.63, 1.35)		0.95 (0.59, 1.52)		1.12 (0.61, 2.04)	
45+	1.22 (0.86, 1.74)		1.03 (0.65, 1.63)		0.94 (0.53, 1.66)		2.61 (1.42, 4.77)	
Acute HIV infection ↯	1.20 (0.97, 1.47)	0.093	1.10 (0.83, 1.46)	0.50	1.15 (0.83, 1.60)	0.40	1.14 (0.78, 1.67)	0.49

ŧAbbreviations: SC – seroconversion; F – females; M – males; MSM – sex between men; MSW – sex between men and women; PWID – people who inject drugs; OTH/UNK – other/unknown

↯ HIV test interval < 30 days or laboratory evidence of acute HIV infection

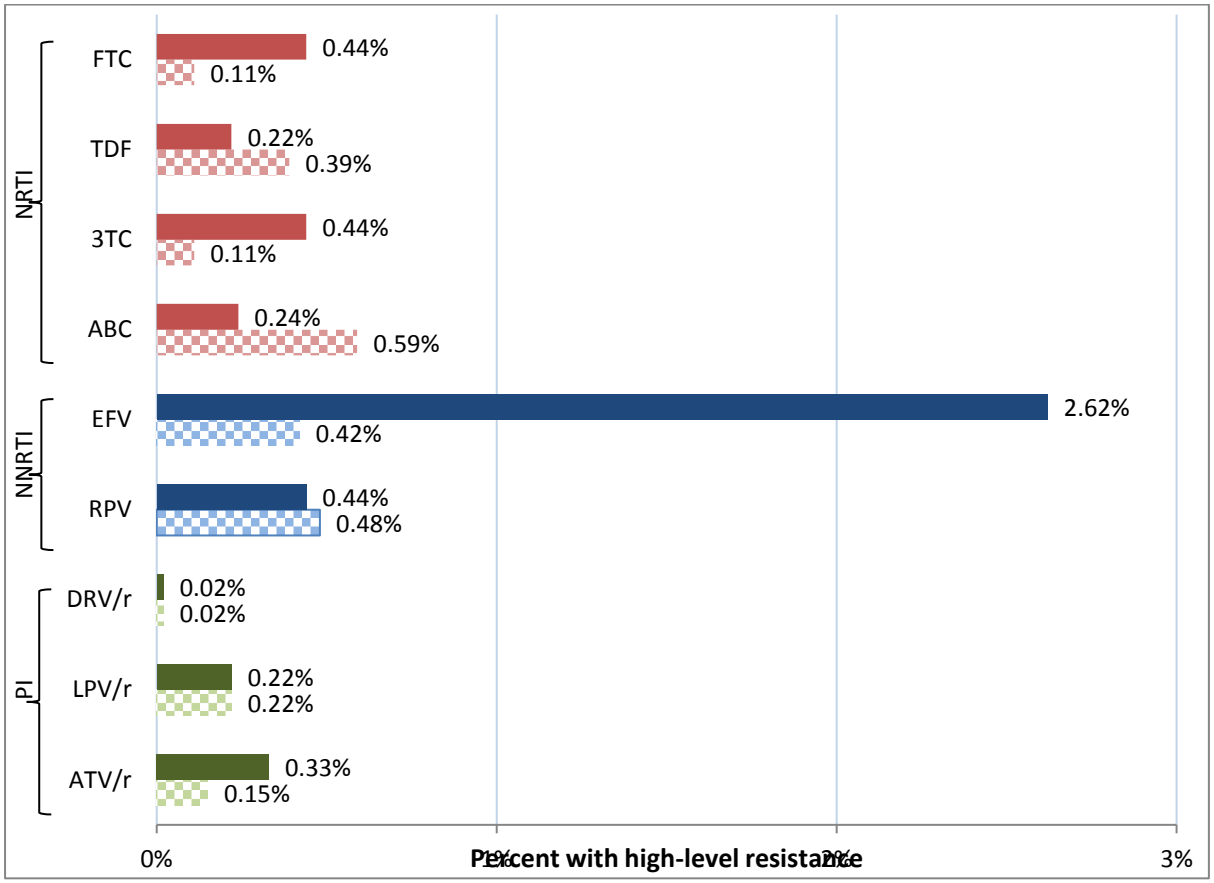
∓ p-value for heterogeneity

Figure 1: Temporal trends in transmitted drug resistance over time for individuals with at least one ART naive nucleotide sequence within one year of testing positive for HIV: CASCADE data of HIV seroconverters.



NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; TDR, transmitted drug resistance. Statistically significant decline ($P < 0.01$ for TDR, NRTI, and PI) in the prevalence of transmitted drug resistance over time using linear mixed models

Figure 2: High level resistance (Stanford scores >3; solid bars indicate a score of 5, checked bars indicate a score of 4) associated with first-line antiretroviral drugs recommended by the European AIDS clinical Society for individuals with at least one ART-naive nucleotide sequence within 1 year of testing positive for HIV: CASCADE data of HIV seroconverters.



ABC, abacavir; ATV/r, atazanavir; DRV/r, darunavir; EVF, efavirenz; FTC, emtricitabine; LPV/r, lopinavir; NRTIs, Nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; RPV, rilpivirine; TDF, tenofovir; 3TC, lamivudine. Adapted with permission [29]

Supplementary Table 1: Number of specific mutations for individuals with at least one ART naïve nucleotide sequence within one year of testing positive for HIV in the ART era using CASCADE data of HIV seroconverters

Mutation	Frequency	Mutation	Frequency	Mutation	Frequency
NRTIs	280	**NNRTIs**	185	**PIs**	144
41L	91	103N	119	90M	39
215S	61	190A	16	46I	31
215D	41	181C	13	46L	26
184V	34	188L	11	82A	16
67N	30	225H	10	85V	15
210W	28	103S	8	54V	13
219Q	27	190S	7	30N	13
77L	18	101E	7	88D	9
215E	16	100I	6	82L	6
70R	16	101P	6	53L	6
215C	15	106A	5	73S	6
215Y	15	230L	3	47V	5
65R	8	190E	2	83D	4
67G	8	188C	1	24I	4
184I	7	188H	1	32I	4
215F	7	106M	1	84V	3
69D	7	179F	1	54L	3
219N	5	181I	1	88S	2
115F	5	181V	1	54M	2
219E	4			73C	2
219R	4			50V	2
74V	4			76V	1
74I	3			82T	1
215I	2			82F	1
215V	2			50L	1
116Y	2			53Y	1
69	1			54A	1
75A	1			73T	1
151M	1			23I	1
67E	1			47A	1

Abbreviations: NRTIs = Nucleoside Reverse Transcriptase Inhibitors; NNRTIs = Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs); PIs = Protease Inhibitors

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Appendix

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