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# Transmitted HIV Drug Resistance Is High and Longstanding in Metropolitan Washington, DC

**Seble G. Kassaye,[1] Zehava Grossman,[2,3] Maya Balamane,[1] Betsy Johnston-White,[4] Chenglong Liu,[1] Princy Kumar,[1] Mary Young,[1] Michael C. Sneller,[5] Irini Sereti,[5] Robin Dewar,[6] Catherine Rehm,[5] William Meyer III,[7] Robert Shafer,[4] David Katzenstein,[4] and Frank Maldarelli[3]**

[1]Department of Medicine, Georgetown University, Washington D.C.; [2]Department of Epidemiology, Tel Aviv University, Israel; [3]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, Maryland; [4]Department of Medicine, Stanford University, California; [5]Laboratory of Immunoregulation, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, [6]Leidos Biomedical Research, Frederick, and [7]Quest Diagnostics, Baltimore, Maryland

***Background.*** Washington, DC, has 2.5% human immunodeficiency virus (HIV) prevalence, 3.9% among African Americans. Antiretrovirals (ARTs) are the cornerstone for treatment and prevention. Monitoring changes in transmitted drug resistance (TDR) is critical for effective HIV care.

***Methods.*** HIV genotype data for individuals enrolled in research studies in metropolitan Washington, D.C., were used to identify TDR using the World Health Organization mutation list [Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PloS One 2009; 4:e4724]. HIV phylogenies were reconstructed using maximum likelihood and Bayesian methods. HIV transmission clusters were supported by 1000 bootstrap values >0.70 and posterior probability >0.95 of having a common ancestor.

***Results.*** Among 710 individuals enrolled in 1994–2013, the median age was 38.6 years, 46.2% were female, and 53.3% were African-American. TDR was 22.5% among 566 treatment-naive individuals; 15.8% had nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) resistance, 9.8% had nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance, and 4.2% had protease inhibitor (PI) resistance. Single class TDR was 10.0%, 5.1%, and 1.6% to NRTIs, NNRTIs, and PIs. Dual TDR to PI and NRTI was seen in 1.6%, NRTI and NNRTI in 3.4%, and triple class TDR in 0.9%. TDR frequency decreased from 1994–2006 (27.1%) to 2007–2013 (19.4%; $P = .02$). Only 6/79 (7.6%) individuals within transmission clusters had evidence of TDR.

***Discussions.*** We identified high prevalence of TDR among HIV-infected individuals in metropolitan Washington, DC, regardless of gender. Active surveillance for TDR is needed to guide ART usage and analyses of risk group contributions to HIV transmission and resistance.

**Keywords.** HIV; transmitted drug resistance; transmission dynamics; HIV clusters; women.

Washington, DC, has the highest prevalence of human immunodeficiency virus type 1 (HIV-1) in the United States: 2.5% among the general population and even higher (3.9%) among African Americans [1]. These stark statistics have led to a concerted effort to mobilize treatment and prevention efforts within the District of Columbia. Provision of effective combination antiretroviral therapy (cART), which decreases onward transmission of HIV [2], and preexposure prophylaxis (PrEP) [3, 4] are key components of the public health response [5].

Knowledge of transmitted drug-resistance (TDR) mutations is critical to decrease HIV replication and transmission. Primary infection with drug-resistant HIV may limit treatment options and could deter use of "one-pill once-daily" options that improve adherence and treatment success [6]. Nonsuppressive therapy fosters emergence of drug resistance and potential transmission of drug-resistant virus. Use of antiretrovirals (ARTs) for PrEP is increasing [7] and, although medication adherence correlates with efficacy [4], infection with drug-resistant HIV while on PrEP has rarely been identified in clinical studies [4, 8, 9]. Increasing PrEP use under nonstudy conditions may select for TDR, affecting treatment options for those who acquire HIV despite use of PrEP [10].

TDR is more frequent with increasing use of cART. The prevalence of TDR is lower in sub-Saharan Africa than in North America, Europe, and Australia, where cART was introduced earlier and is broadly accessible [11–14]. In the United States, reported estimates of TDR prevalence are 9.1%–13.7% [15, 16] and range from 12% to 18.9% among men who have sex with men (MSM) [17, 18]. These estimates, generated using population-based sequencing irrespective of timing of HIV acquisition, likely underestimate the true prevalence of TDR, as detection declines over time using this approach and often requires specialized methods that capture minority variants of HIV [19–21].

Temporal trends of TDR in Washington, DC, are not well documented. In 2005, the estimated prevalence of TDR was 7%, with no TDR reported among women [22]. Substantial TDR (17%) was detected between 2007 and 2010 [23].

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Correspondence: S. G. Kassaye, Georgetown University, Department of Medicine, 2115 Wisconsin Ave, NW, Ste 130, Washington D.C. 20007-2265 (sgk23@georgetown.edu).

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These studies primarily enrolled men (78%–81%), and the frequency of TDR among women remains uncertain. Almost 27% of HIV-infected individuals in Washington, DC, are women, 92.4% of whom are African American [1]. Additional data regarding the history of TDR in Washington, DC, especially among women, will be very useful.

Several studies have followed a large group of HIV-infected individuals in the Washington, DC, area. Here, we characterize the prevalence and transmission dynamics of drug-resistant HIV, including among a substantial number of women enrolled in observational and clinical research studies in the greater metropolitan Washington, DC, area over a 20-year period.

## METHODS

### Study Population

We retrospectively studied individuals enrolled in HIV-related studies at the National Institutes of Health (NIH) or Georgetown University. Participants gave consent for the use of their data and/or samples in HIV-related research under protocols approved by institutional review boards at both institutions.

The following 4 groups were included: 207 women participating in the DC Women's Interagency HIV Study (DC-WIHS), a longstanding observational cohort of HIV-infected and high-risk HIV-uninfected women [24]; 154 individuals recruited from 2011 to 2013 into the Georgetown subunit of the DC Cohort, an observational clinic-based HIV study; 37 individuals enrolled in the AIDS Clinical Trials Group (ACTG) studies from 2001 to 2012 at Georgetown University; and 312 individuals living within 75 miles of Washington, DC, enrolled into several HIV-related studies at the NIH from 1998 to 2013. DC-WIHS women were enrolled in 3 phases during 1994–1995, 2001–2002, and 2011–2012 and were recruited from primary and tertiary care clinics, referrals from community outreach, women's support, drug rehabilitation, or HIV testing groups. NIH participants were referred from regional clinics and testing facilities and enrolled into a standard-of-care training clinic, a study of the immune reconstitution inflammatory syndrome among individuals with low CD4+ T-lymphocyte counts, and a study of early HIV infection. NIH participants had not undergone experimental procedures other than apheresis and were ART therapy naive.

### Data Collection

Demographics (age, gender, race/ethnicity), clinical information (CD4+ T-lymphocyte count and plasma HIV RNA level), and HIV acquisition risk factors (sexual preference and use of illicit drugs) were collected as part of the parent studies.

### HIV *pol* Sequence and Data Derivation

HIV *pol* sequences were derived from existing routine clinical genotypic resistance tests or generated from the earliest available stored samples. Available full HIV *pol* sequence data were digitized from commercial genotype reports using optical character recognition software to obtain a sequence in FASTA format (N = 85) and were manually reviewed and edited as necessary. Data on HIV drug-resistance mutations were extracted from commercial genotypic reports if sequence data were unavailable (N = 69).

Population-based sequencing was performed using plasma samples stored at −70°C for the remaining individuals. Validated HIV genotyping methods for population sequencing were applied at Stanford University (N = 207) or other ACTG-designated virology specialty laboratories (N = 37) [25]. Nucleotides were read as mixtures when the subpopulation exceeded 20% on chromatograms and were manually verified. HIV genotyping was performed at the NIH using the TRUGENE HIV-1 genotyping kit (N = 312) (Siemens Healthcare Diagnostics Inc., Berkeley, California).

### Identification of Transmitted Drug Resistance

TDR was identified using the Stanford HIV Drug Resistance Database (HIVDB.stanford.edu) and a calibrated population resistance tool [26] based on the World Health Organization TDR mutation list [27].

### Phylogenetic Analyses

HIV *pol* sequences were aligned using ClustalW and manually edited within BioEdit [28]. Forty-three TDR codons [27] were removed prior to phylogenetic analyses, resulting in a sequence length of 789 nucleotides. We used jModelTest version 2.1.4 [29], which revealed the general time reversible model (GTR) with gamma-distributed rate heterogeneity plus proportion of invariable sites (GTR + G + I) as the optimal nucleotide substitution model. We constructed maximum likelihood phylogenetic trees using MEGA6 version 6.06 [30]. HIV transmission clusters were identified if there was >70% bootstrap nodal support from 1000 replicates. We also conducted Bayesian analyses using Bayesian evolutionary analysis sampling trees (BEAST) [31]. A test run using a subset of the data compared the GTR + G + I and the HKY + G + I models in BEAST and generated similar results. The analyses using the full dataset presented here were conducted using the HKY + G + I model with uncorrelated log-normal relaxed molecular clock, tree coalescent GMRF Bayesian Skyride, and a run of 2 000 000 000 states to obtain an effective sample size of 203.6. Nodal support of ≥95% was used to verify transmission clusters. FigTree [32] was used to plot the summary phylogenetic tree. Subtype analysis was performed using the REGA subtyping tool [33], and analysis for recombination assessed using the Recombination Identification Program [34]. Chronicity of infection was estimated using the proportion of nucleotide positions with evidence for more than 1 nucleotide (an ambiguity); a sequence ambiguity score <0.44 was considered evidence for recent infection [35, 36].

### Statistical Analyses

Descriptive statistical tools were used to characterize the study population, and differences were assessed using the $\chi^2$ test for categorical variables and Kruskal–Wallis test for nonparametric

continuous variables. The following 3 distinct time periods were defined a priori based on availability of ART agents that influence treatment outcomes and drug-resistance mutations: 1994–1996, preceding the introduction of highly active ART therapy (pre-HAART); 1996–2006, cART era; and 2007–2013, cART era during which single-tablet regimens became available. We conducted 2-group comparisons to test for differences in prevalence of TDR between time periods.

## RESULTS

The study population is described in Table 1. Among 710 individuals, the median age was 38.6 years, 328 (46.2%) were female, 380 (53.5%) were African American, 159 (22.4%) were Hispanic, and 64 (9.0%) were white. Among 69 ART-naive individuals for whom sequence data were not available, the median age was 41.0 years, 22 (31.9%) were female, 37 (53.6%) were African American, 11 (15.9%) were white, and 1 was Hispanic. Among 641 individuals with sequence data, the median age was 38.1 years, 306 (47.7%) were female, 380 (59.5%) were African American, 53 (8.3%) were white, 122 (19.0%) were Hispanic, and 86 (13.4%) were of other ethnicity; 568 (88.5%) had subtype B HIV-1, 73 (11.4%) had non-B HIV-1 subtypes, and 497 (77.5%) were drug naive. The median ambiguity score was 0.81, and 189/641 (29.5%) had ambiguity scores <0.44, suggestive of recent infection.

Among 566 ART-naive individuals, 69 had only drug-resistance mutations available for analysis, and 497 had the actual sequence data used to identify drug resistance. Included in this treatment-naive group were 235 (41.5%) females and 328 (57.9%) males; 3 were of unknown gender; 272 (48.1%) were African American, 157 (27.7%) were Hispanic, and 44 (7.8%) were white. HIV acquisition risks were heterosexual contact (44.3%), injection drug use (IDU; 6.5%), MSM (35.2%), blood transfusion (1.9%), and unknown (12.0%).

The overall TDR prevalence was 22.5%: 113/497 (22.7%) among those with subtype B HIV-1 and 13/69 (18.8%) among those with non-B HIV-1 subtypes ($P = .54$). Distribution of TDR by race/ethnicity was 11.4% non-Hispanic blacks, 3.4% Hispanic whites, 2.9% non-Hispanic whites, and 1.8% Hispanic blacks. High prevalence of TDR was a consistent finding across the study subgroups: 28.5% in the DC-WIHS, 22.2% in the DC cohort, 25% among ACTG participants, and 19.8% among NIH participants. The proportion of TDR among treatment-naive women and men was similar, 57/235 (24.3%) vs 68/328 (20.7%), respectively ($P = .32$). The proportions with TDR were also similar among individuals with heterosexual transmission (22.0%), IDU related (20.0%), MSM (21.9%), and unknown risk for HIV acquisition (15.1%).

Overall, 16.2% of individuals had resistance to 1 drug class: 9.8% had resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); 5.0% had resistance to nonnucleoside reverse-transcriptase inhibitor (NNRTIs); and 1.4% had resistance to protease inhibitors (PIs). The proportion with dual-class resistance was 5.4%: 3.4% to NRTIs and NNRTIs, 1.6% to NRTIs and PIs, and 0.4% to NNRTIs and PIs. The prevalence of TDR to all 3 classes was 0.9%. TDR varied across time (Figure 1), with prevalence of 28.9% (95% confidence interval [CI], .21–.38) during 1994–1996, 24.7% (95% CI, .16–.35) during 1997–2006, and 19.7% (95% CI, .16–.24) during 2007–2013.

We identified specific patterns in the prevalence of TDR that changed during the different eras of ART therapy. Observed TDR mutations occurring at frequencies above 1% are shown in Figure 2. Overall, TDR was most frequently associated with NRTIs (15.5%), followed by NNRTIs (9.6%) and PIs (4.1%). NRTI resistance was most common across all 3 periods but declined from 28.9% prevalence during 1994–1996 to 10.3% during 2007–2013. Frequency of TDR associated with NNRTIs peaked at 14.6% in the 1997–2006 period and stabilized at 11.1% during 2007–2013. PI resistance was not observed in 1994–1996, was 7.9% during 1997–2006, and then declined to 4.8% during 2007–2013. There was no significant difference in prevalence of TDR between the pre-ART or early HAART eras compared with the more recent cART era ($P = .09$). However, the prevalence of TDR declined significantly from the 1994–2006 period (27.4%) to the 2007–2013 period (19.4%; $P = .02$, Fisher exact test).

The pattern of individual TDR mutations varied across the study period, both between and within classes of ART drugs (Figure 2). Among NRTI-associated TDR, the thymidine-associated mutations predominated in the 1994–1996 period and declined over time. The TDR mutation M184V was present at 4.3%, and the prevalence of K65R was <0.5% in the most recent time period from 2007–2013. The most frequent NNRTI-associated TDR mutation was K103N/S, which present at 10.1% prevalence in 1997–2006 and then decreased to 8.6% between 2007 and 2013. Individual PI-associated TDR mutations remained low throughout the study period, all <3% except L90M, which was present at 4.5% prevalence during 1997–2006.

To identify patterns of HIV transmission, we investigated the presence of drug-resistance mutations within clusters of individuals with highly related viruses. Phylogenetic analyses were completed using 559 of the available 568 subtype B HIV-1 sequences; the remaining 9 sequences were not included in the analysis as they were significantly shorter. Our primary reason for including the maximal likelihood analysis as well as the Bayesian analysis (BEAST) was to ensure that inferences using the Bayesian approach did not overestimate transmission clusters. The maximum likelihood phylogenetic (data not shown) and BEAST trees of subtype B HIV-1–infected individuals (Figure 3) demonstrate a star-like phylogeny. Forty-three small clusters, comprising 92 individuals, were identified using the maximum likelihood approach. Thirty-seven HIV clusters, verified using BEAST, included 79 individuals in clusters of 2 or 3 individuals (Figure 3 and Supplementary Figure 1). The dyads

**Table 1. Characteristics of Individuals Enrolled Into Clinical Studies, 1994–2013**

| Characteristic | Overall (n = 710) | Treatment Naive | | | Treatment Experienced | | |
|---|---|---|---|---|---|---|---|
| | | Total (n = 566) | No TDR (n = 440) | With TDR (n = 126) | Total (n = 143) | No TDR (n = 80) | With TDR (n = 63) |
| Age | | | | | | | |
| median ± IQR | 38.6 ± 14 | 38.1 ± 15 | 38.0 ± 14 | 38.6 ± 15 | 40.2 ± 16 | 37.8 ± 15 | 43.0 ± 15 |
| Gender | | | | | | | |
| Number (%) | | | | | | | |
| Male | 379 (53.3%) | 328 | 260 | 68 | 50 | 26 | 24 |
| Female | 328 (46.2%) | 235 | 178 | 57 | 93 | 54 | 38 |
| Unknown | 3 | 3 | 2 | 1 | 0 | 0 | 0 |
| Risk | | | | | | | |
| Number (%) | | | | | | | |
| Hetero | 301 (42.4%) | 251 | 199 | 52 | 50 | 27 | 23 |
| Injection drug use | 62 (8.7%) | 37 | 30 | 7 | 25 | 15 | 10 |
| Men who have sex with men | 223 (31.4%) | 199 | 156 | 43 | 24 | 14 | 10 |
| Blood transfusion | 13 (1.8%) | 11 | 6 | 5 | 2 | 1 | 1 |
| Unknown | 111 (15.6%) | 68 | 49 | 19 | 42 | 23 | 19 |
| ethnicity | | | | | | | |
| Number (%) | | | | | | | |
| Black non- Hispanic | 380 (53.5%) | 272 | 208 | 64 | 108 | 61 | 47 |
| Black Hispanic | 40 (5.6%) | 39 | 29 | 10 | 1 | 1 | 0 |
| Other | 119 (16.7%) | 43 | 33 | 10 | 7 | 3 | 4 |
| White non- Hispanic | 64 (9.0%) | 44 | 28 | 16 | 20 | 10 | 10 |
| White Hispanic | 51 (7.2%) | 118 | 99 | 19 | 1 | 0 | 1 |
| Unknown | 56 (7.9%) | 50 | 43 | 7 | 6 | 5 | 1 |
| Human immunodeficiency virus RNA level ($Log_{10}$ copies/mL) | | | | | | | |
| median ± IQR | 4.17 ± 4.85 | 4.20 ± 4.88 | 4.11 ± 4.83 | 4.46 ± 4.99 | 3.95 ± 4.77 | 4.06 ± 4.77 | 3.88 ± 4.75 |
| CD4 counts (cells/μL) | | | | | | | |
| median ± IQR | 338 ± 379 | 341 ± 385 | 351 ± 358 | 304 ± 437 | 324 ± 348 | 346 ± 375 | 277 ± 334 |

Abbreviations: IQR, interquartile range; TDR, transmitted drug resistance.

[a] Treatment history was not reported for 1 patient.

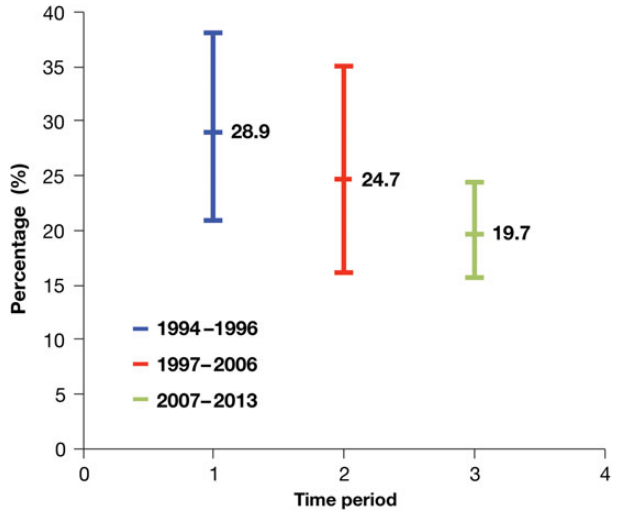


**Figure 1.** Time trends in the frequency of transmitted drug resistance–associated mutations, 1994–2013. Point estimates for each time period are shown with 95% confidence intervals.

were comprised of 2 males in 20 of the clusters; 2 women in 4 of the clusters; 1 male and 1 transgender female in 1 of the clusters; and 7 clusters with 1 male and 1 female. Three of the triads comprised only males; 1 triad was comprised of 2 males and 1 female; and 1 triad was comprised of 2 males and 1 transgender female. Transmission risk was concordant in 22 clusters and discordant in 6 clusters. The 6 discordant clusters included 3 dyads with an MSM and a heterosexual male; 1 dyad with an IDU female and heterosexual female; and 2 triads with 2 MSM and either a heterosexual male and/or heterosexual female. Demographic and HIV risk acquisition characteristics associated with clustering are shown in Table 2. Men were more likely to be in clusters than women, 20% vs 6%, $P < .0001$. Similarly, MSM were more likely to fall into a transmission cluster (22%) compared with heterosexuals (14%; $P = .046$. IDU was not associated with falling in a transmission cluster (2%). There was no significant difference in the proportion within a cluster by race. Six individuals within clusters had TDR (6.5%), 2 females and 4 males, including DC Cohort and NIH participants. Two dyads demonstrated concordant TDR, 1 pair with the L210W mutation and another pair with K103N. The remaining 2 individuals with TDR were in a discordant dyad, 1 infected with T69AD and a second individual with K103N and K219E TDR variants.

## DISCUSSION

We identified a high prevalence of TDR (22.5%) in metropolitan Washington, DC, between 1994 and 2013. To our knowledge,

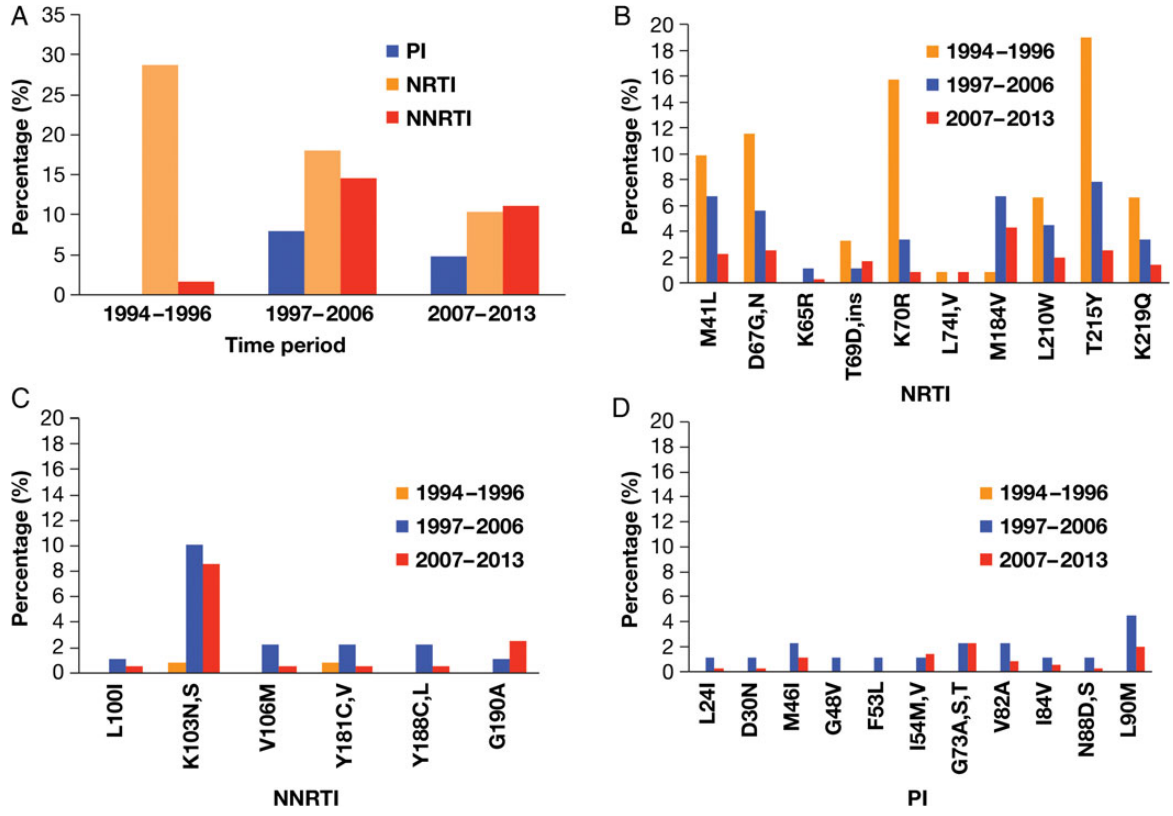


**Figure 2.** Frequency of transmitted drug resistance (TDR) across 3 time periods: (*A*) Overall frequency of TDR by drug class. (*B*) Frequency of nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)–associated TDR over time. (*C*) Frequency of nonnucleoside reverse transcriptase inhibitor (NNRTI)–associated TDR over time. (*D*) Frequency of protease inhibitor (PI)–associated TDR over time. *T215Y includes the following revertants: L215Y/F/D/S.

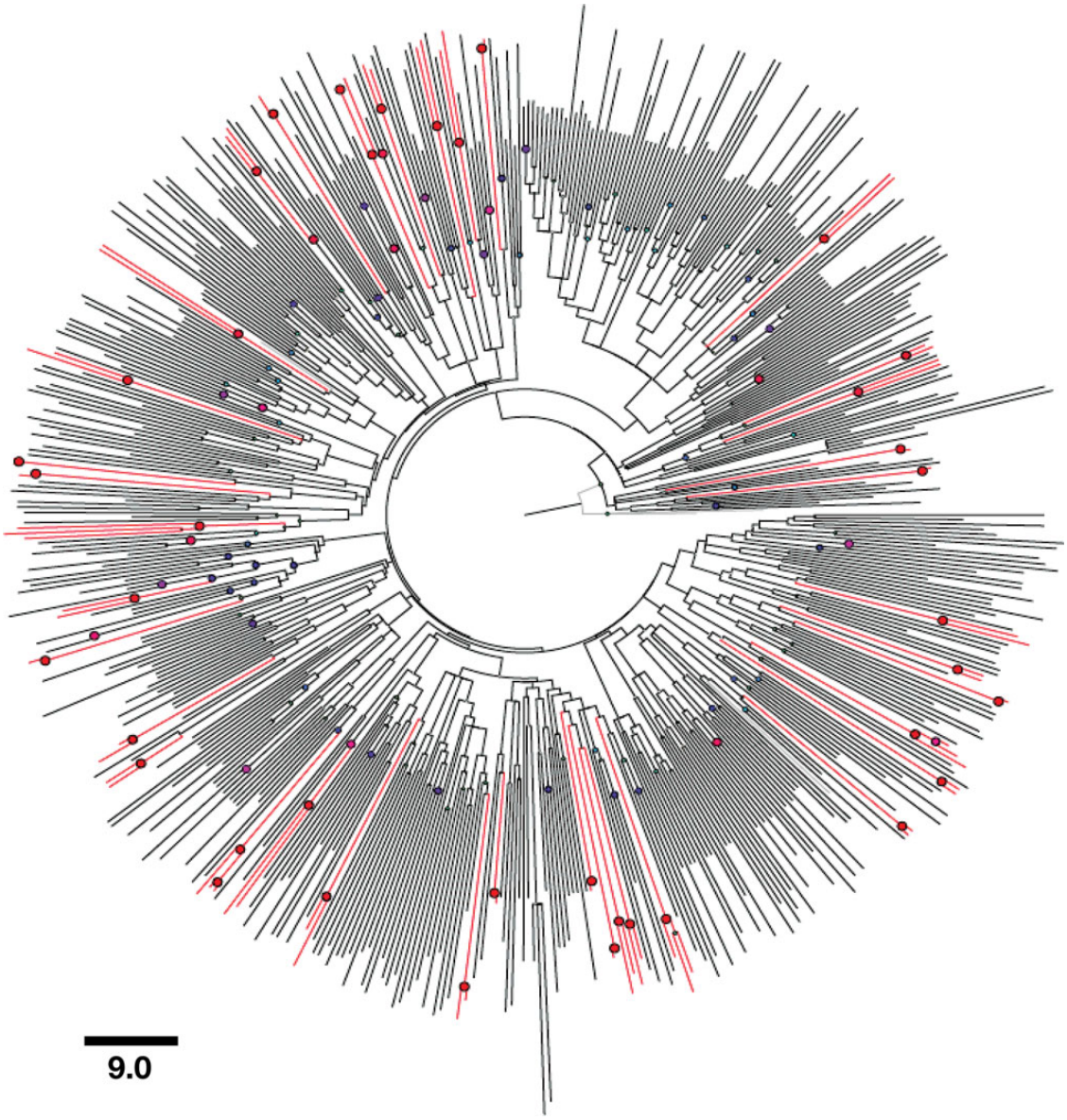


**Figure 3.** Bayesian phylogenetic tree of 559 subtype B human immunodeficiency virus type 1 (HIV-1) sequences. Bayesian evolutionary analysis sampling trees [31] analysis was performed with the following specifications: HKY + G + I model, uncorrelated log-normal relaxed molecular clock, tree coalescent GMRF Bayesian Skyride, and run of 2 000 000 000 states to obtain an effective sample size of 203.6. FigTree [32] was used to plot the summary Bayesian phylogenetic tree and demonstrates the star-like phylogeny of subtype B HIV-1 sequences. Nodal support of 95% or greater was required to verify transmission clusters, and these are indicated with red lines and circles. These clusters consist of 2 to 3 individuals.

this is the highest prevalence of TDR mutations reported within the United States or in any region within Europe or Australia, where ART therapy use has been long-standing. The relatively high frequency of non-B subtypes identified in our study likely reflects immigration patterns, especially from African countries, to the metropolitan Washington, DC, area. Most individuals in our study were chronically infected, evidenced by the high sequence ambiguity scores, with the implication that our results may underestimate TDR, as some but not all TDR mutations are known to wane over time following infection. The pattern of drug resistance that we observed, particularly the T215 revertants, is consistent with primary resistance mutations rather than related to nonsuppressive ART therapy [37]. Our phylogenetic analyses did not link TDR identified more recently (2007–2013) with TDR detected earlier (1994–1996 or 1997–2006) through a shared common ancestor. These data suggest that mutations such as T215Y that were commonly identified in 1994–1996 were not likely the source of current 215Y mutations. Our study provides a longitudinal perspective of TDR from a diverse group of treatment-naive participants enrolled

**Table 2. Sociodemographic and Human Immunodeficiency Virus Acquisition Risk Factors Associated With Highly Related Viral Sequences**

| Risk Factor | Total (N = 559) | Number Clustered[a] | % Clustered[a] | *P* Value |
|---|---|---|---|---|
| Gender | | | | |
| Male | 304 (54.4%) | 61 | 0.20 | |
| Female | 252 (45.1%) | 16 | 0.06 | <.0001 |
| Unknown/ Transgender | 3 (0.5%) | 2 | 0.66 | |
| Risk | | | | |
| Men who have sex with men [14] | 178 (31.8%) | 40 | 0.22 | |
| Heterosexual | 202 (36.1%) | 29 | 0.14 | .046 |
| Injection drug use | 57 (10.2%) | 1 | 0.02 | |
| Unknown risk | 113 (19.7%) | 9 | 0.08 | |
| Transfusion | 9 (1.6%) | 0 | 0 | |
| Ethnicity | | | | |
| White | 165 (29.5%) | 29 | 0.18 | NS |
| African American | 316 (56.5%) | 40 | 0.13 | *P* = .2 |
| Hispanic | 24 (4.3%) | 3 | 0.13 | |
| Unknown | 37 (6.6%) | 7 | 0.05 | |
| Other[b] | 17 (3.0%) | 0 | 0 | |

[a] Clusters are defined as groups of individuals with highly related human immunodeficiency virus sequence by maximal likelihood and verified using Bayesian methods with posterior probability >95% of having a shared common ancestor.

[b] Other includes Native American (N = 1), Asian (N = 5), and self-designated "other" (N = 11).

in clinical and observational studies in which treatment status was reliably assessed using criteria broadly accepted by the research community. The majority of participants were enrolled prior to the widespread use of ARTs for PrEP. The low prevalence of K65R makes it unlikely that use of PrEP, with the currently used combination of tenofovir–disoproxil–fumarate and emtricitabine (Truvada), accounts for the drug-resistance mutations found in our study population.

Our finding of persistently high and longstanding TDR within a generalized epidemic reflects the challenge of access to and retention in care. Recent estimates from Washington, DC, suggest that 62% of HIV-infected individuals are retained within care and only 47% have achieved viral suppression [1]. Delays in treatment initiation, suboptimal treatment adherence, and interruptions in care provide opportunities for selection of drug-resistance mutations and HIV transmission with potential to alter patterns of TDR [38]. We observed a downward trend in the overall prevalence of TDR compared with the early period, but continued high levels of drug resistance are evidence of fragmented care. Variation in TDR mutation patterns across time mirrors evolving ART availability and usage, implicating ongoing inadequate engagement and retention in care as drivers of transmitted drug-resistant HIV. A continuum of care, both treatment and prevention, in conjunction with active surveillance for TDR as part of effective clinical program implementation is critical to ensure treatment success and mitigate HIV transmission and TDR.

Nearly one-half of our study participants were women, and elevated rates of TDR for many years in this group was unexpected. While previous studies demonstrated resistance among MSM, our data suggest women have been affected by transmission of drug resistance for years. It remains uncertain whether this observation is the effect of "bridging," that is, transmission of virus to women by men who had contacts with treated HIV-infected MSM. We did find some evidence of cross-risk transmissions, with pairs of individuals reporting different risk factors for acquisition of HIV. In surveys with high sampling density of affected populations, such bridging phenomena were prominent and represented a strong driving force for ongoing HIV transmission [39]. Comprehensive sampling of contemporaneous populations would better characterize underlying transmission networks and remains an essential and compelling imperative. Such studies may guide focused biomedical treatment and prevention approaches. For example, despite the sampling limitations of the data, our study highlights important gender differences in HIV transmission. Although there was no relationship between TDR and gender, we observed a striking higher likelihood of men falling within a cluster than women. While this gender difference has been observed within the United States and elsewhere [39–41], it is usually described in MSM populations. In the data here, men reporting heterosexual risk for HIV were present in clusters as well as MSM.

Most HIV-infected women acquire HIV through heterosexual transmission, and fewer related to IDU. A high proportion of women in our study did not report high-risk behaviors and were unaware of high-risk behaviors among their partners. While this could be underreporting by self-report, the observation of discordant HIV acquisition risk factors within some transmission clusters supports the concern that HIV acquisition risk is not solely that of the individual but that of the individual's partners, especially in the setting of a generalized epidemic. Current biomedical prevention strategies are broadly recommended for MSM, active IDUs, and women "at risk for HIV acquisition." However, our findings highlight the distinct challenge in identifying "at-risk" women who would benefit from targeted biomedical prevention interventions.

## Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

## Notes

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## References

1. District of Columbia DoHHA, hepatitis, STD, and TB administration (HASTA). Annual epidemiology and surveillance report: surveillance data through December 2013, **2015**.
2. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med **2011**; 365:493–505.
3. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med **2012**; 367:399–410.
4. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med **2010**; 363:2587–99.
5. Centers for Disease Control and Prevention. Update to interim guidance for preexposure prophylaxis (PrEP) for the prevention of HIV infection: PrEP for injecting drug users. MMWR Morb Mortal Wkly Rep **2013**; 62:463–5.
6. Raffi F, Yazdanpanah Y, Fagnani F, Laurendeau C, Lafuma A, Gourmelen J. Persistence and adherence to single-tablet regimens in HIV treatment: a cohort study from the French National Healthcare Insurance Database. J Antimicrob Chemother **2015**; 70:2121–8.
7. Flash C, Landovitz R, Giler RM, et al. Two years of Truvada for pre-exposure prophylaxis utilization in the US. J Int AIDS Soc **2014**; 17(4 suppl 3):19730.
8. Liegler T, Abdel-Mohsen M, Bentley LG, et al. HIV-1 drug resistance in the iPrEx preexposure prophylaxis trial. J Infect Dis **2014**; 210:1217–27.
9. Lehman DA, Baeten JM, McCoy CO, et al. Risk of drug resistance among persons acquiring HIV within a randomized clinical trial of single- or dual-agent preexposure prophylaxis. J Infect Dis **2015**; 211:1211–8.
10. Hurt CB, Eron JJ Jr, Cohen MS. Pre-exposure prophylaxis and antiretroviral resistance: HIV prevention at a cost? Clin Infect Dis **2011**; 53:1265–70.
11. Ambrosioni J, Sued O, Nicolas D, et al. Trends in transmission of drug resistance and prevalence of non-B subtypes in patients with acute or recent HIV-1 infection in Barcelona in the last 16 years (1997–2012). PloS One **2015**; 10:e0125837.
12. Rhee SY, Blanco JL, Jordan MR, et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. PLoS Med **2015**; 12:e1001810.
13. Frange P, Assoumou L, Descamps D, et al. HIV-1 subtype B-infected MSM may have driven the spread of transmitted resistant strains in France in 2007–12: impact on susceptibility to first-line strategies. J Antimicrob Chemother **2015**; 70:2084–9.
14. Baxter JD, Dunn D, White E, et al. Global HIV-1 transmitted drug resistance in the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. HIV Med **2015**; 16(suppl 1):77–87.
15. Pham QD, Wilson DP, Law MG, Kelleher AD, Zhang L. Global burden of transmitted HIV drug resistance and HIV-exposure categories: a systematic review and meta-analysis. AIDS **2014**; 28:2751–62.
16. Torian LV, Forgione LA. Transmitted antiretroviral drug resistance in New York City, 2006–2010: the first five years of routine genotype surveillance. J Acquir Immune Defic Syndr **2013**; 63:e119–22.
17. Ocfemia MCB, Hernandez AL, Saduvala N, Oster AM, Hall HI. Epidemiology of HIV-1 transmitted drug resistance among men who have sex with men in the United States. In: XXIV International HIV Drug Resistance Workshop. Seattle, Washington. Global Antiviral Journal, **2015**:81–2.
18. Chen I, Connor MB, Clarke W, et al. Antiretroviral drug use and HIV drug resistance among HIV-infected black men who have sex with men: HIV Prevention Trials Network 061. J Acquir Immune Defic Syndr **2015**; 69:446–52.
19. Buckton AJ, Harris RJ, Pillay D, Cane PA. HIV type-1 drug resistance in treatment-naive patients monitored using minority species assays: a systematic review and meta-analysis. Antivir Ther **2011**; 16:9–16.
20. Castro H, Pillay D, Cane P, et al. Persistence of HIV-1 transmitted drug resistance mutations. J Infect Dis **2013**; 208:1459–63.
21. Jain V, Sucupira MC, Bacchetti P, et al. Differential persistence of transmitted HIV-1 drug resistance mutation classes. J Infect Dis **2011**; 203:1174–81.
22. Boyd AC, Herzberg EM, Marshall MM, et al. Antiretroviral drug resistance among treatment-naive HIV-1-infected persons in Washington, D.C. AIDS Patient Care STDS **2008**; 22:445–8.
23. Swierzbinski MJ, Kan VL, Parenti DM. Human immunodeficiency virus (HIV) in Washington, D.C.: prevalence of antiretroviral resistance in treatment naïve patients from 2007 to 2010. J AIDS HIV Res **2015**; 7:49–54.
24. Bacon MC, von Wyl V, Alden C, et al. The Women's Interagency HIV study: an observational cohort brings clinical sciences to the bench. Clin Diagn Lab Immunol **2005**; 12:1013–9.
25. Winters MA, Coolley KL, Girard YA, et al. A 6-basepair insert in the reverse transcriptase gene of human immunodeficiency virus type 1 confers resistance to multiple nucleoside inhibitors. J Clin Invest **1998**; 102:1769–75.
26. Gifford RJ, Liu TF, Rhee SY, et al. The calibrated population resistance tool: standardized genotypic estimation of transmitted HIV-1 drug resistance. Bioinformatics **2009**; 25:1197–8.
27. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PloS One **2009**; 4:e4724.
28. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **1999**; 14:95–8.
29. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods **2012**; 9:772.
30. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol **2013**; 30:2725–9.
31. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol **2012**; 29:1969–73.
32. A. R. FigTree v1.4.2: Tree Figure Drawing Tool, **2015**. Available at: http://treebioedacuk/software/figtree/. Accessed 28 April 2015.
33. Pineda-Pena AC, Faria NR, Imbrechts S, et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. Infect Genet Evol **2013**; 19:337–48.
34. Siepel AC, Halpern AL, Macken C, Korber BT. A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. AIDS Res Hum Retroviruses **1995**; 11:1413–6.
35. Andersson E, Shao W, Bontell I, et al. Evaluation of sequence ambiguities of the HIV-1 pol gene as a method to identify recent HIV-1 infection in transmitted drug resistance surveys. Infect Genet Evol **2013**; 18:125–31.
36. Kouyos RD, von Wyl V, Yerly S, et al. Ambiguous nucleotide calls from population-based sequencing of HIV-1 are a marker for viral diversity and the age of infection. Clin Infect Dis **2011**; 52:532–9.
37. Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W. Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons. Proc Natl Acad Sci USA **2001**; 98:13907–12.
38. Marzel A, Shilaih M, Yang WL, et al. HIV-1 Transmission during recent infection and during treatment interruptions as major drivers of new infections in the Swiss HIV Cohort Study. Clin Infect Dis **2016**; 62:115–22.
39. Grossman Z, Avidor B, Mor Z, et al. A population-structured HIV epidemic in Israel: roles of risk and ethnicity. PloS One **2015**; 10:e0135061.
40. Levy I, Mor Z, Anis E, et al. Men who have sex with men, risk behavior, and HIV infection: integrative analysis of clinical, epidemiological, and laboratory databases. Clin Infect Dis **2011**; 52:1363–70.
41. Oster AM, Wertheim JO, Hernandez AL, Banez Ocfemia MC, Saduvala N, Irene Hall H. Using molecular HIV surveillance data to understand transmission between subpopulations in the United States. J Acquir Immune Defic Syndr **2015**; 70:444–51.