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HIV drug resistance in a cohort of HIV-infected men who have sex with men in the United States

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Abstract

Objective: To analyze HIV drug resistance among men who have sex with men (MSM) recruited for participation in the HPTN 078 study, which evaluated methods for achieving and maintaining viral suppression in HIV-infected MSM.

Methods: Individuals were recruited at four study sites in the United States (Atlanta, Georgia; Baltimore, Maryland; Birmingham, Alabama; and Boston, Massachusetts; 2016–2017). HIV genotyping was performed using samples collected at study screening or enrollment. HIV drug resistance was evaluated using the Stanford v8.7 algorithm. A multi-assay algorithm was used to identify individuals with recent HIV infection. Clustering of HIV sequences was evaluated using phylogenetic methods.

Results: High-level HIV drug resistance was detected in 44 (31%) of 142 individuals (Atlanta: 21%, Baltimore: 29%, Birmingham: 53%, Boston: 26%); 12% had multi-class resistance, 16% had resistance to tenofovir or emtricitabine, and 8% had resistance to integrase strand transfer inhibitors (INSTIs); 3% had intermediate-level resistance to second-generation INSTIs. In a multivariate model, self-report of ever having been on antiretroviral therapy (ART) was associated with resistance ($p=0.005$). One of six recently-infected individuals had drug resistance. Phylogenetic analysis identified five clusters of study sequences; two clusters had shared resistance mutations.

Conclusions: High prevalence of drug resistance was observed among MSM. Some had multi-class resistance, resistance to drugs used for pre-exposure prophylaxis (PrEP), and INSTI resistance. These findings highlight the need for improved HIV care in this high-risk population, identification of alternative regimens for PrEP, and inclusion of integrase resistance testing when selecting ART regimens for MSM in the US.

Keywords

HIV drug resistance; phylogenetic analysis; men who have sex with men; United States; HPTN 078

INTRODUCTION

In the United States (US), the current administration's plan, "Ending the HIV Epidemic: A Plan for America", includes interventions such as early antiretroviral therapy (ART) to achieve viral suppression and pre-exposure prophylaxis (PrEP) [1]. Goals of this program and other programs for HIV treatment and prevention may be compromised by HIV drug resistance [2, 3]. Acquired drug resistance can emerge in HIV-infected individuals with sub-optimal adherence to ART, in individuals who become HIV-infected while taking PrEP [4, 5], and in those taking antiretroviral (ARV) drugs for other reasons. Individuals can also be infected with drug-resistant HIV (transmitted drug resistance [TDR]). Some studies have reported decreases in acquired drug resistance in high-income countries [6, 7], which may reflect increased use of drugs with high genetic barriers for resistance and increased use of HIV genotyping and viral load monitoring to guide treatment. However, the frequency of acquired drug resistance remains high in many settings, and TDR has also increased in some settings [8–10].

Approximately two thirds of new HIV infections in the US occur in MSM [11]. In 2015, only 62% of MSM diagnosed with HIV were receiving care, and only 52% were virally suppressed [12]. Surveillance data have shown that most HIV infections among MSM in the US are linked to infections in other MSM [13], and phylogenetic studies have found that active transmission clusters are more concentrated among MSM than other risk groups [14]. Data on drug resistance among MSM in the US are limited. In a study of Black MSM in six cities in the US (HIV Prevention Trials Network [HPTN] 061; enrollment 2009–2010), 28% of 169 HIV-infected study participants had drug resistance; 36% of those with resistance had ARV drugs detected, including many who did not report being in care [15]. Resistance to integrase strand transfer inhibitors (INSTIs) was not observed in that cohort [16]. Some studies have also reported higher rates of TDR among MSM compared to other risk groups [8, 17, 18].

HPTN 078 evaluated an HIV prevention strategy focused on achieving and maintaining viral suppression in HIV-infected MSM in the US (screening/enrollment: 2016–2017). The study enrolled HIV-infected individuals who were not virally suppressed at study sites in four US cities, including two study sites that participated in HPTN 061. Many individuals were recruited using respondent driven sampling (RDS), which has been shown to help identify most-at-risk populations, including Black and Hispanic MSM and MSM with lower socio-economic status [19–21]. In this study, we analyzed HIV drug resistance and the phylogenetic relationships of HIV among individuals recruited for participation in HPTN 078.

METHODS

Study cohort

The HPTN 078 (NCT02663219) clinical trial was conducted in Atlanta, Georgia; Baltimore, Maryland; Birmingham, Alabama; and Boston, Massachusetts. Individuals age 16 or older were recruited using deep-chain RDS (DC-RDS) and direct recruitment. DC-RDS recruitment was conducted by identifying "seeds" who referred members in their social and

sexual networks; this process was then repeated using referrals from sequential waves of RDS. Direct recruitment was conducted from clinical/hospital referrals, support group referrals, alliances with testing programs, study advertisement, and venue-based recruitment. HPTN 078 screened 1,305 MSM for study participation; 902 were HIV infected, and 864 had a viral load result from the screening visit (median age of 46; 79% Black; 82% were virally suppressed [22]). The study enrolled 144 MSM who were not virally suppressed (median age 39; 84% Black [23]). Transgender women were eligible for participation in HPTN 078 but were not specifically recruited for the study.

In this report, we analyzed samples from all HIV-infected individuals who were screened for HPTN 078 and had viral loads $\geq 1,000$ copies at study entry. Enrolled individuals included those who were newly diagnosed, aware of their status but not in care, or in care but not virally suppressed. Demographic and behavioral data were collected at the screening visit.

Laboratory testing

HIV diagnostic testing, HIV viral load testing, and CD4 cell count testing were performed at study sites. Other testing described below was performed at the HPTN Laboratory Center, Baltimore, MD. HIV genotyping was performed using the ViroSeq HIV-1 Genotyping System, v.2.0 and the ViroSeq HIV-1 Integrase Genotyping Kit, RUO (Abbott Molecular, Des Plaines, IL) using samples collected at study entry (screening or enrollment). These methods are based on population sequencing and generate consensus sequences for HIV protease, HIV reverse transcriptase (RT, amino acids 1–335), and HIV integrase. HIV drug resistance was assessed using the Resistance Calculator Program (Frontier Science Foundation, Stanford v8.7 algorithm); cases were classified as being susceptible or having low-, intermediate-, or high-level resistance to drugs in the four drug-classes analyzed (protease inhibitors [PIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs], nucleotide/nucleoside reverse transcriptase inhibitors [NRTIs], and INSTIs). This algorithm includes analysis of resistance to doravirine and bictegravir, which were recently approved for HIV treatment by the US Food and Drug Administration (FDA). Quality assurance testing for HIV viral load was performed using the RealTime HIV-1 Viral Load Assay (Abbott Molecular, Abbott Park, IL; lower limit of quantification of 40 copies/mL). A multi-assay algorithm was used to identify individuals who were likely to be recently infected at the time of sample collection [24]; the algorithm includes the HIV-1 LAg-Avidity EIA (Lag-Avidity assay; SEDIA Biosciences Corporation Portland, OR and Maxim Biomedical, Bethesda, MD), the Johns Hopkins modified version of the Genetic Systems 1/2+O ELISA (Bio-Rad Avidity assay; Bio-Rad Laboratories, Redmond, WA), CD4 cell count, and viral load. Samples were classified as recently infected if they had a LAg-Avidity result <2.9 normalized optical density units [OD-n], a BioRad-Avidity index $<85\%$, a viral load >400 copies/mL, and a CD4 cell count >50 cells/mm³; this multi-assay algorithm has a mean duration of recent infection (MDRI) of 146 days in populations with subtype B HIV [24].

Phylogenetic analysis

HIV subtyping was performed using three automated subtyping tools (REGA HIV-1 Subtyping tool, v3.0 [25]; COMET HIV-1, v2.3 [26]; Recombinant Identification Program [RIP] [27]) and phylogenetic analysis. Phylogenetic analysis included study sequences and

HIV subtype reference sequences obtained from the Los Alamos National Laboratory (LANL) HIV sequence database. Maximum likelihood trees were constructed using RAxML, v8.2.10 [28].

Phylogenetic analysis was also used to evaluate the relationships between HIV *pol* sequences. For this analysis, background sequences (ten sequences that were most closely related to each study sequence) were identified in the LANL database using a BLAST search. The Recombination Detection Program v4 (RDP4) [29] was used to identify recombinant sequences (including sequences with intra-subtype recombination). Potential recombinant sequences and duplicate background sequences were removed from further analysis. Codons that are the sites of drug resistance mutations were also removed from sequences before phylogenetic analysis [30]. Phylogenetic trees were constructed separately for protease/RT sequences and integrase sequences using RAxML, v8.2.10 (using GTR+G4 substitution model), accessed through the CIPRES Science Gateway [31]. Cluster Picker [32] was used to identify putative transmission clusters, using maximum genetic distance of 4.5% and 90% bootstrap support as thresholds; a maximum genetic distance threshold of 0.5% was used to identify recent transmission clusters [33]. Genetic distances among study sequences in clusters were calculated using “ape” package [34] in R. Phylogenetic trees were represented using iTOL (<https://itol.embl.de/>).

Statistical analysis

The association of HIV drug resistance with demographic, behavioral, and clinical variables was evaluated. Chi-square and Fisher’s exact tests were used for categorical variables; t-tests were used for continuous variables. Logistic regression was used for multivariate analysis.

Ethical considerations

Written informed consent was obtained from all individuals who were screened for participation in HPTN 078; this included consent for testing stored specimens. The study was approved by institutional review boards and ethics committees at each participating institution.

GenBank accession numbers

GenBank accession numbers of the sequences are [MK580177-MK580318](#) (HIV protease/RT) and [MK580319-MK580456](#) (HIV integrase).

RESULTS

Samples included in the resistance study

This study included analysis of samples from all HIV-infected individuals screened for participation in HPTN 078 who had a viral load $\geq 1,000$ copies/mL at study entry (144 who enrolled in HPTN 078 and 11 who did not enroll in the study). HIV genotyping was performed using samples obtained at study entry (screening or enrollment) from 145 individuals; ten were excluded from testing (seven did not have a sample available for testing, and three had discrepant viral load results from testing performed at the study site vs. the HPTN Laboratory Center). Protease/RT genotyping results were obtained for 142

(97.9%) of the 145 samples; three failed genotyping. Among the 142 individuals with results, 121 (85.2%) were Black/African American; 138 (97.2%) identified as male, one identified as female, two identified as transgender, and one identified as gender variant/non-conforming. Six of the 142 individuals were identified as recently infected using a multi-assay algorithm developed for cross-sectional HIV incidence estimation (two from Birmingham; four from Atlanta). Integrase genotyping results were obtained for 138 of the 142 cases (four failed analysis).

Analysis of HIV drug resistance

High-level resistance to at least one ARV drug was detected in 44 (31.0%) of the 142 cases; 30 (21.1%) had NNRTI resistance, 23 (16.2%) had NRTI resistance, and five (3.5%) had PI resistance; 11 (8.0%) of 138 cases had INSTI resistance (Figure 1, Table 1). Multi-class resistance was observed in 17 (12.0%) of the 142 cases (Figure 1). Ten individuals had resistance to two drug classes, six had resistance to three drug classes, and one had resistance to all four drug classes (Table 1).

The mutation detected most frequently was K103N (n=22) which causes high-level resistance to the NNRTIs, efavirenz and nevirapine. The Y188L mutation was detected in four cases; this mutation causes high-level NNRTI resistance, including resistance to the newly-approved NNRTI, doravirine. NRTI mutations associated with TDF and FTC resistance (M184I/V, K65R) were detected in 23 (16.2%) of the 142 cases (25% in Birmingham; 10–17% at the other sites). All 11 cases with high-level INSTI resistance had resistance to elvitegravir; four also had high-level resistance to raltegravir and intermediate-level resistance to the second-generation INSTIs, bictegravir and dolutegravir. All 11 individuals with INSTI resistance had NRTI resistance; five also had NNRTI resistance, and one also had PI resistance (Table 1).

We also analyzed cases of possible TDR. Of the 142 individuals, 117 reported that they had been tested for HIV and had received the test result. Eight of those 117 individuals reported that the test was negative, that they didn't recall the result, or that they preferred not to provide information about the test result. Resistance mutations were detected in three (37.5%) of those eight cases (one case from Baltimore [NRTI: M184V; INSTI: E92Q]; two cases from Atlanta [NNRTI: K103N in one case and NRTI: M184V; INSTI: T97A in the other case]). Resistance mutations were also detected in one of six individuals classified as recently infected using a multi-assay algorithm (NNRTI: K103N; NRTI: K219Q).

Factors associated with HIV drug resistance

We next evaluated the association of drug resistance with behavioral, demographic, and clinical factors (Table 2). Drug resistance was detected more frequently at the Birmingham site (53.1%) compared to the other study sites (20.8% to 28.6%, $p=0.018$, Table 2, Figure 1). Drug resistance was also significantly higher among those who reported ever taking ART ($p=0.011$, Table 2) and among those who were recruited using non-RDS methods ($p=0.044$, Table 2). In a multivariate model, self-report of ever taking ART was the only factor significantly associated with resistance ($p=0.005$, Table 2). The adjusted odds ratio for resistance was 13.0 (95% confidence interval [CI]: 2.45 to 69.2) for those who reported ever

taking ART vs. those who did not report ART, and was 3.06 (95% CI: 0.32 to 29.0) for those who reported “don’t know” or “prefer not to answer” vs. those who did not report ART.

Phylogenetic analysis of HIV-1 strains

Among the 142 individuals with protease/RT genotyping results, 138 (97.2%) had subtype B HIV (all subtyping tools in agreement); the other four sequences were potential inter-subtype recombinants (recombinant with REGA [n=3] and RIP [n=1]). All 138 integrase sequences were subtype B HIV.

Cluster analysis was performed using 132 of the 138 subtype B protease/RT sequences (six additional sequences were excluded from analysis; two had sequence ambiguity >5%; four had evidence of intra-subtype recombination with RDP4). The median pairwise genetic distance was 6.4% (interquartile range [IQR]: 5.6–7.2) among the 132 sequences and was similar across the four study sites. Study sequences from all four study sites were interspersed with background sequences, and no large clusters of study sequences were observed (Figure 2). Four small clusters of sequences were detected that included two study sequences with zero, one, or two background sequences (Supplemental Digital Content). Cluster analysis was also performed using all 138 integrase sequences. The median pairwise genetic distance among these sequences was 4.8% (IQR: 4.1–5.5). This analysis identified the same four clusters observed for protease/RT sequences and one additional cluster of two study sequences that was identified using integrase sequences only (Supplemental Digital Content). Three of the five clusters included sequences from individuals recruited at different study sites (Atlanta and Birmingham [n=2]; Atlanta and Boston [n=1]). None of the sequences from the six individuals identified as recently infected clustered with other study sequences.

We next evaluated whether any of the five clusters included drug-resistant HIV strains (note that codons associated with drug resistance were removed prior to phylogenetic analysis). In one cluster, K103N was present in both study and background sequences. The pairwise genetic distance between the study sequences in this cluster was <0.5% for protease/RT and integrase, indicating a recent transmission linkage (Supplemental Digital Content, Case 1). In another cluster, one study sequence had NRTI and INSTI resistance mutations, and the other study sequence had a shared NRTI mutation (T215C; Supplemental Digital Content, Case 2).

DISCUSSION

We evaluated HIV drug resistance among MSM in four US cities who were recruited for participation in the HPTN 078 study. Some individuals were identified for study screening using DC-RDS, in an attempt to reach those who were less likely to be engaged in care. HIV drug resistance was detected in 31% of the cases. Multi-class resistance was detected in 12% of the cases, including six individuals with 3-class resistance and one individual with 4-class resistance. Individuals with multi-class resistance have limited treatment options. Some individuals with multi-class drug resistance have achieved viral suppression when the post-attachment inhibitor, ibalizumab, was added to an optimized background regimen [35, 36].

The prevalence of NNRTI, NRTI, and PI resistance in this cohort was similar to what we observed among MSM in an older study (HPTN 061: 28%; data from 2009–2010) [15]. In this study, more than half of those with drug resistance had resistance to at least one of the NRTI drugs approved for PrEP (TDF/FTC). This is concerning, since resistance to these drugs may reduce the efficacy of PrEP for high-risk HIV-uninfected MSM. INSTI resistance was detected in 8% of the individuals in this study. This is high compared to the rate of INSTI resistance in other reports. INSTI resistance was not observed in the older HPTN 061 study [16]. INSTI resistance was only detected in <2% of HIV-infected individuals living in Washington, DC (2013), while the overall prevalence of resistance was high [6]. A surveillance study among newly-diagnosed individuals in the US from 2010–2014 showed that integrase testing was performed more frequently among MSM than other risk-groups; in that study, the prevalence of INSTI resistance was low (0.4%) [37].

Three new INSTIs were recently approved by the US FDA for HIV treatment and INSTI-containing ART regimens are now used for first-line HIV treatment in the US [38]. The first-generation INSTIs, elvitegravir and raltegravir, have a lower barrier to drug resistance and have overlapping resistance profiles [39]. Second-generation INSTIs, such as dolutegravir or bictegravir [40], have different resistance profiles. In this study, we identified 11 individuals with high-level INSTI resistance, including four who had intermediate resistance to dolutegravir and bictegravir. All individuals with INSTI resistance also had high-level NRTI resistance; five also had high-level NNRTI resistance, and one also had high-level PI resistance, further limiting their treatment options. The mutation Q148R, which causes high-level resistance to several INSTIs, was detected in one case; this mutation has been observed in individuals who experience virologic failure after taking cabotegravir, which is being evaluated for HIV treatment and prevention [41, 42]. While dolutegravir, bictegravir, and cabotegravir have been shown to have activity against INSTI-resistant HIV [43], persons with both INSTI and NRTI resistance may not respond to co-formulated regimens that include a second-generation INSTI with emtricitabine and tenofovir or tenofovir alafenamide.

Some guidelines do not recommend routine testing for INSTI resistance in drug-naïve individuals [44]. Detection of resistance to first- and second-generation INSTIs in this cohort highlights the importance of including baseline integrase resistance testing when selecting ART regimens for MSM in the US. While regimens that include dolutegravir and bictegravir may be useful in this population, ~10% of those in this cohort with drug resistance also had intermediate resistance to these drugs, highlighting the urgent need to address issues related to drug resistance in this population.

Findings from this study suggest that some individuals may have been infected with drug-resistant HIV. We detected drug resistance in three of the eight individuals who reported that they did not have a prior HIV positive test and in one of six individuals who were classified as recently infected using a multi-assay algorithm. These cases may reflect TDR. However, it is also possible that one or more of these individuals was aware of their HIV status and had taken ART, but chose not to report this to study staff; they may also have used ARV drugs for another reason (e.g., PrEP, hepatitis treatment, recreational use). In a study of newly-diagnosed MSM attending a community clinic in Los Angeles, the rate of drug

resistance among recently-infected persons was similar to those with longer-standing infection [45].

In other studies, the rate of HIV drug resistance among MSM in the US, was higher among MSM who were older [15], Black, or had transgender partners [45]. In this study, age and race were not associated with resistance. The rate of resistance was higher in Birmingham (53%), compared to the other three study sites (21% to 29%). Resistance was also more frequent among those who were directly recruited than among those who were recruited through DC-RDS. However, neither of these factors (study site, recruitment method) was statistically associated with resistance in a multivariate model. In the multivariate model, self-report of ever taking ART was the only factor independently associated with resistance. In contrast, self-report of currently taking ART was not associated with drug resistance. This suggests that many of those with resistance may have received suboptimal care or had poor ART adherence at some point in the past. Future ARV drug testing is planned to investigate the reasons for lack of viral suppression in the study cohort (e.g., non-adherence vs. good adherence with drug resistance).

This study also included phylogenetic analysis of HIV strains. The analyses revealed no geographic clustering, suggesting a high degree of mobility. Four clusters that included at least two study sequences were identified using both the protease/RT and integrase sequences; a fifth cluster was identified using the integrase sequences only. The HIV integrase region is more conserved compared to protease/RT, which may explain the identification of one additional cluster using integrase sequences [46]. In this study, the median genetic diversity was lower in integrase compared to protease/RT (4.76% vs. 6.41%, respectively). Three of the five clusters included individuals recruited from different cities (two cases: Birmingham and Atlanta; one case Atlanta and Boston). Study investigators were aware that some participants traveled between sites/cities. In one cluster, the genetic distance between study sequences was below the 0.5% threshold; suggesting a recent transmission linkage [33]. Two clusters included individuals with drug resistance. None of the clusters included individuals identified as recently infected.

In summary, we found a high prevalence of HIV drug resistance and multi-class drug resistance among viremic MSM from diverse regions in the US, with a resistance rate >50% in Birmingham, Alabama. Many MSM had INSTI resistance, and some had resistance to second-generation INSTIs. Resistance to TDF/FTC was also observed, which could impact use of PrEP in this population. These findings underscore the need for continued surveillance of drug resistance and improved HIV care in this high-risk population, for identification of alternatives to TDF/FTC for PrEP, and for including baseline integrase resistance testing when selecting ART regimens for MSM in the US.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. HIV drug resistance among HIV-infected men who have sex with men in four cities in the United States.

The figure shows the percentage of individuals with resistance to at least one antiretroviral (ARV) drug (any resistance); resistance to drugs in two or more ARV drug classes (multi-class resistance); and resistance to each drug class analyzed (non-nucleoside reverse transcriptase inhibitors [NNRTIs], nucleoside/nucleotide reverse transcriptase inhibitors [NRTIs], protease inhibitors [PIs], and integrase strand transfer inhibitors [INSTIs]). Protease/RT resistance was assessed in 142 individuals; INSTI resistance was assessed in 138 individuals.

Figure 2. Phylogenetic trees showing genetic relationships of protease/RT and integrase sequences.

Phylogenetic trees show the relationships between HIV sequences from individuals who were recruited to participate in the HPTN 078 study (colored lines) and background sequences from a public database (black lines). Colors indicate the geographic location of the study sites. Clusters that include at least two study sequences are shaded in grey; numbers correspond to cluster numbers shown in Supplemental Digital Content. Study sequences containing HIV drug resistance mutations are shown with black dots. Study sequences from the six individuals classified as recently infected are shown with red asterisks. Panel A includes 132 protease/reverse transcriptase (protease/RT) study sequences and 310 background protease/RT sequences. Panel B includes 138 integrase study sequences and 401 background integrase sequences.

Table 1.

Patterns of HIV drug resistance.

Case	Study site	Log ₁₀ VL ^a	Major drug resistance mutations				High-level resistance			
			NNRTI	NRTI	PI	INSTI	NNRTI	NRTI	PI	INSTI [intermediate-level resistance] ^b
1	MA	4.5	Y181L, G190A	Q151M, M184V	V32L, M46L, I54L, I84V	T66A, E138K, Y143C, S147G	EFV, ETR, NVP, RPV	ABC, AZT, ddI, d4T, FTC, 3TC, TDF	ATV, DRV, FVP, IDV, LPV, NFV, SQV	EVG, RAL [BIC, DTG]
2	MA	4.2	Y188L	D67N, K70R, M184V, T215F, K219E	L90M		DOR, EFV, NVP, RPV	ABC, AZT, ddI, d4T, FTC, 3TC	NFV	
3	MD	5.2	K101E, Y181C, G190A	K65R, Q151M, K219E	V32L, M46I/L, I47V, I54M, V82A, I84V, L90M		EFV, ETR, NVP, RPV	ABC, AZT, ddI, d4T, FTC, 3TC, TDF	ATV, DRV, FVP, IDV, LPV, NFV, SQV, TPV	
4	AL	5.7	K103S	K65R, M184V		E92Q, E138K, S147G, N155H	EFV, NVP	ABC, ddI, FTC, 3TC, TDF		EVG, RAL [BIC, DTG]
5	AL	3.4	G190A	K65R, M184V		E92Q	NVP	ABC, ddI, d4T, FTC, 3TC, TDF		EVG [RAL]
6	AL	4.5	K103N, Y188L	K65R, M184V		E92Q	DOR, EFV, NVP, RPV	ABC, ddI, FTC, 3TC, TDF		EVG [RAL]
7	AL	5.4	K103N	M184V		E92Q	EFV, NVP	FTC, 3TC		EVG [RAL]
8	MD	4.0		K65R, M184V		E92Q, S147G		ABC, ddI, FTC, 3TC, TDF		EVG [RAL]
9	MD	4.1		D67N, K70R, M184V, K219Q		E92Q		ABC, FTC, 3TC		EVG [RAL]
10	MD	4.8		M184V		E92Q		FTC, 3TC		EVG [RAL]
11	MD	5.4		M184V		E92Q		FTC, 3TC		EVG [RAL]
12	GA	4.3		M184V, T215Y		E138K, S147G, Q148R		FTC, 3TC		EVG, RAL [BIC, DTG]
13	GA	3.6		M184V		G140S, Q148H		FTC, 3TC		EVG, RAL [BIC, DTG]

			Major drug resistance mutations				High-level resistance			
Case	Study site	Log ₁₀ VL ^a	NNRTI	NRTI	PI	INSTI	NNRTI	NRTI	PI	INSTI [intermediate-level resistance] ^b
14	MA	3.3		M41L, M184V, L210W, T215Y	V32I, M46I, I47V, I54L, V82T, L90M			ABC, AZT, ddI, FTC, 3TC, TDF	ATV, DRV, FPV, IDV, LPV, NFV, SQV, TPV	
15	AL	3.8	K103N	M184V			EFV, NVP	FTC, 3TC		
16	MA	4.7	L100I	L74I, M184I			EFV, NVP, RPV	ABC, ddI, FTC, 3TC		
17	GA	5.2	K103N	L74I, M184V/I			EFV, NVP	ABC, ddI, FTC, 3TC		
18	MD	4.3			I84V				ATV, FPV, IDV, NFV, SQV	
19	AL	3.4	K103N, Y188L				DOR, EFV, NVP, RPV			
20	AL	3.9	Y188L				DOR, EFV, NVP, RPV			
21	AL	3.1	G190S				EFV, NVP			
22–38 ^c	AL (n=6)	4.3 (3.0–5.8)	K103N				EFV, NVP			
	MA (n=3)									
	GA (n=5)									
	MD (n=3)									
39–44	AL (n=3)	3.8 (3.2–4.8)		M184V/I				FTC, 3TC		
	MD (n=1)									
	GA (n=2)									

The table shows the study site, viral load, drug resistance mutations, and resistance patterns for 44 individuals who had high-level resistance to at least one antiretroviral drug.

^aViral load results (log₁₀ HIV RNA copies/mL) are shown for individual cases (Cases 1–21); the median and range for viral load are shown for Cases 22–38 and Cases 39–44.

^bIntermediate-level resistance to the second-line integrase strand transfer inhibitors (INSTIs), bictegravir and dolutegravir, was detected in four cases, based on the following mutation patterns: Case 1: T97A, E138K, Y143C, E157Q, S230R; Case 4: E92Q, E138K, N155H; Case 12: E138K, Q148R; Case 13: G140GS, Q148H. Note that the mutation, S230R (noted in Case 1), is not a major resistance mutation, but confers intermediate-level resistance when it is present with other mutations observed in this case.

^cOne individual who had HIV with the K103N mutation only did not have results for INSTI resistance.

Abbreviations: NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside/ nucleotide reverse transcriptase inhibitor; PI: protease inhibitor; INSTI: integrase strand transfer inhibitor; MA: Massachusetts (Boston); MD: Maryland (Baltimore); AL: Alabama (Birmingham); GA: Georgia (Atlanta). NNRTIs: EFV: efavirenz; ETR: etravirine; NVP: nevirapine; DOR: doravirine; RPV: rilpivirine.

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NRTIs: ABC: abacavir; AZT: zidovudine; d4T: stavudine; ddI: didanosine; FTC: emtricitabine; 3TC: lamivudine; TDF: tenofovir disoproxil fumarate. PIs: ATV: atazanavir; DRV: darunavir; FVP: fosamprenavir; IDV: indinavir; LPV: lopinavir; NFV: nelfinavir; SQV: saquinavir; TPV: tipranavir. INSTIs: EVG: elvitegravir; BIC: bictegravir; DTG: dolutegravir.

Table 2.

Factors associated with HIV drug resistance.

Variables	Total N	n/N (%) with resistance	Univariate p value ^a	Multivariate p value ^a
Site			0.018	0.202
Atlanta, GA	48	10/48 (20.8%)		
Baltimore, MD	35	10/35 (28.6%)		
Birmingham, AL	32	17/32 (53.1%)		
Boston, MA	27	7/27 (25.9%)		
Recruitment method			0.044	0.430
RDS early wave (0–6)	84	20/84 (23.8%)		
RDS late wave (>6)	14	4/14 (28.6%)		
Direct ^b	44	20/44 (45.5%)		
Age at screening			0.362	
<25	13	3/13 (23.1%)		
25–34	51	13/51 (25.5%)		
35–44	26	7/26 (26.9%)		
>44	52	21/52 (40.4%)		
Race			0.683	
Black/African American	121	38/121 (31.4%)		
White	14	5/14 (35.7%)		
Other	7	1/7 (14.3%)		
Education			0.735	
High school or less	59	18/59 (30.5%)		
BA or BS degree or some college	79	24/79 (30.4%)		
Masters or other advanced degree	4	2/4 (50.0%)		
Employment			0.223	
Full time	24	5/24 (20.8%)		
Part time	20	9/20 (45.0%)		
Unemployed	98	30/98 (30.6%)		
Income (year)			0.706	
\$0–\$19,999	94	27/94 (28.7%)		
\$20,000–\$29,999	19	7/19 (36.8%)		
>\$30,000	29	10/29 (34.5%)		
Stable living situation			0.900	
Yes	123	39/123 (31.7%)		
No	16	4/16 (25.0%)		
Missing data	3	1/3 (33.3%)		
Number of sex partners in the past 6 months			0.961	
0–1	43	13/43 (30.2%)		

Variables	Total N	n/N (%) with resistance	Univariate p value ^a	Multivariate p value ^a
>1	87	28/87 (32.2%)		
Prefer not to answer	12	3/12 (25.0%)		
Sexual partner type (ever)			0.560	
Men only	59	21/59 (35.6%)		
Men and women	82	23/82 (28.0%)		
Prefer not to answer	1	0/1 (0%)		
Ever tested for HIV			0.388	
Yes	135	44/135 (32.6%)		
No	5	0/5 (0%)		
Don't know/Prefer not to answer	2	0/2 (0%)		
Result of last HIV test ^c			0.713	
Positive	109	35/109 (32.1%)		
Negative/Prefer not to answer/Don't know	8	3/8 (37.5%)		
Ever taken ART for HIV ^d			0.011	0.005
Yes	92	35/92 (38.0%)		
No	23	2/23 (8.7%)		
Prefer not to answer/Don't know	11	2/11 (18.2%)		
Currently taking ART for HIV ^e			0.135	
Yes	65	28/65 (43.1%)		
No	23	5/23 (21.7%)		
Prefer not to answer/Don't know	4	2/4 (50.0%)		
Baseline CD4 cell count (cells/mm ³)			0.354	
<350	75	24/75 (32.0%)		
350–500	28	11/28 (39.3%)		
>500	39	9/39 (23.1%)		
Baseline viral load, mean (SD) ^f			0.924	
All individuals	142	4.33 (0.72)		
Individuals with HIV drug resistance	44	4.33 (0.69)		

^aChi-square and Fisher's exact tests were used to evaluate associations between categorical variables; t-test was used to assess associations with continuous variables. Logistic regression was used for multivariate analysis. P-values <0.05 are shown in bold text.

^bMethods used for direct recruitment are described in the Methods section.

^cThis question was only asked if individuals reported that they had a prior HIV test and had received the result from their last test.

^dThis question was only asked if individuals reported that they had a prior positive HIV test or reported that they thought they were HIV positive.

^eThis question was only asked if individuals reported that they had ever been on ART.

^fThis variable shows the mean and standard deviation for baseline viral load (log₁₀ HIV RNA copies/mL) for all individuals and for individuals with resistance.

Abbreviations: N: total number; n: number with HIV drug resistance; GA: Georgia; MD: Maryland; AL: Alabama; MA: Massachusetts; RDS: respondent-driven sampling; BA: Bachelor of Arts degree; BS: Bachelor of Science degree; ART: antiretroviral treatment; SD: standard deviation.

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