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Primary Antiretroviral Drug Resistance in Newly Human Immunodeficiency Virus-Diagnosed Individuals Testing Anonymously and Confidentially

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The purpose of this study was to determine if anonymous and confidential testers differ in recency of human immunodeficiency virus (HIV) infection at time of testing and prevalence of antiretroviral drug (ARV) resistance. We examined data from the Centers for Disease Control and Prevention–sponsored Antiretroviral Drug Resistance Testing project, which performed genotypic testing on leftover HIV diagnostic serum specimens of confidentially and anonymously tested ARV-naïve persons newly diagnosed with HIV in Colorado ($n = 365$ at 11 sites) and King County, Washington ($n = 492$ at 44 sites). The serologic testing algorithm for recent HIV seroconversion was used to classify people as likely to have been recently infected or not. Type of testing, anonymous or confidential, was not significantly associated with either timing of HIV testing by serologic testing algorithm for recent HIV seroconversion or resistance rates. Mutations conferring any level of ARV resistance were present in 17% of testers, and high-level resistance mutations were present in 10%. Anonymous testers were significantly more likely to have CD4+ counts >500 cells per mm^3 (45% vs. 28%; $p = 0.018$), indicative of an early infection. This study indicates that anonymous testers have demographic differences relative to confidential HIV testers but were not more likely to exhibit drug resistance. Findings related to when in the course of disease anonymous testers are tested are inconsistent, but anonymous testers had higher CD4 counts, which indicates early testing and is consistent with other studies.

Introduction

Since the introduction of antibody testing for human immunodeficiency virus (HIV) in 1985, many public testing sites have offered both anonymous and confidential testing. Unlike confidential testing, where testers' names are recorded with their test results, there is no link between anonymous testers' names and their HIV status. Health departments introduced anonymous testing because of the unique stigma attached to HIV infection and out of concern that individuals would fear breaches in confidentiality and subsequently avoid getting tested. Although anonymous testing has been controversial, some studies suggest that people who seek anonymous testing would not otherwise get tested.[6,10–12,23] HIV is the only infection for which anonymous testing is publicly funded and as of 2008 40 states offered both confidential and anonymous publicly funded testing.

Previous studies have demonstrated that those who get tested anonymously differ from those who opt for confidential testing. The most common risk group to seek anonymous testing is men who have sex with men (MSM), but anonymous testers also tend to be younger, white, and more educated than confidential testers.[2,3,15,18] Both the Multistate Evaluation of Surveillance of HIV (MESH) study[2] and the Centers for Disease Control and Prevention (CDC)'s Supplement to HIV/AIDS Surveillance (SHAS) project[4] showed that anonymous testers seek testing and treatment earlier in the course of the disease.

Whether antiretroviral drug (ARV) resistance rates differ between anonymous and confidential testers has not been previously evaluated. Currently, the U.S. Department of Health and Human Services and the International AIDS Society—USA recommend that genetic testing for drug resistance be conducted before starting highly active antiretroviral therapy to test for primary, or transmitted, drug

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resistance.[19,24] They also recommend that testing be conducted for all patients upon entering into care for HIV. Since reversion of drug-resistant mutations to wild type occurs in the absence of drug selection pressure,[7,27] testing for transmitted drug resistance is most reliable when conducted early in the course of infection. It has been found that those who are diagnosed over a year after they were infected are less likely to test positive for resistance than those who were recently infected.[25] If anonymous testers tend to be diagnosed earlier, as the MESH and SHAS studies suggest, they may be more likely to show resistance. This could indicate the importance of offering anonymous ARV resistance testing as an option as well as provide evidence that previous estimates of primary drug resistance may be too low. To test the hypothesis that those who are tested anonymously show greater levels of resistance than those who test confidentially, we used HIV antiretroviral resistance surveillance data collected in Colorado and King County, Washington, on 857 treatment-naïve individuals.

Materials and Methods

The CDC-funded Antiretroviral Drug Resistance Testing (ARVDRT) project was conducted between 2003 and 2007, using leftover HIV diagnostic serum specimens to perform genotypic testing. This study included data from Colorado Department of Public Health and Environment and Public Health Seattle-King County, where both confidential and anonymous testing are offered at publically funded sites. Individuals who tested positive for and were newly diagnosed with HIV-1 at participating sites were eligible for inclusion in ARVDRT if (1) a remnant sample was available for genotypic testing from a diagnostic specimen drawn within 3 months of the diagnosis, and (2) the patient was not known to have used an ARV either at the time of or preceding the collection of the genotype specimen. Aliquots of samples from eligible individuals were sent to either Stanford University (75%) or University of Washington (25%) for genotypic testing. The following analysis includes all individuals 14 years of age or older who were tested at public sites and whose samples were sent for genotyping, regardless of whether Colorado or King County received sequence results back.

We compared characteristics of ARVDRT eligible individuals who tested anonymously and confidentially and examined whether the type of HIV test was associated with the stage of disease at the time of HIV testing and whether the person's virus displayed characteristics of ARV resistance. Type of HIV testing was classified as anonymous or confidential depending on whether a name (confidential) or unique code (anonymous) was recorded with the diagnostic serum specimen. Demographic and clinical information for confidential testers were collected by linking patient records with the HIV/AIDS Reporting System (HARS). Through HARS, information was collected on the patient's sex, age at diagnosis, HIV transmission mode, and baseline $CD4^+$ cell count and viral load. Anonymous testers were not included in HARS and could not be linked to any confidential tests, but medical records, including demographic data and HIV risk data from laboratory requisition forms, were maintained for some anonymous testers under a nonidentifiable code that was linked to the specimen but not to the patient. Baseline $CD4^+$ cell counts and viral loads for anonymous testers were collected only by King County using this method.

To estimate when in the course of the disease individuals sought HIV testing, data were used from the HIV incidence study (HIVIS), conducted from 1998 to 2005, and the HIV Incidence Surveillance (HIS) project, which started in 2004 and is ongoing. Both of these projects utilize the serologic testing algorithm for recent HIV seroconversion (STARHS) to describe the proportion of those with newly diagnosed HIV infections who have recent or long-standing HIV infection. This testing was conducted on aliquots of the same remnant diagnostic serum specimens used for ARVDRT and thus could be linked to ARVDRT for both anonymous and confidential testers based on the identification number of the specimen. During the period of HIVIS, samples from confidential and anonymous testers were analyzed with the less-sensitive enzyme-linked immunosorbent assay (LS-EIA), which indicated that the person was recently infected if antibody concentrations were below a specified threshold. In April 2004, confidential testers began being tested as part of HIS and in 2005 HIS began using a new assay called the BED HIV-1 Capture EIA. This assay utilizes the ratio of HIV-specific IgG to the total IgG to determine an optical density, which distinguished recently infected from long-term infected individuals based on a predetermined threshold level. Testing under HIVIS for anonymous testers was conducted until 2005, when STARHS testing was discontinued for anonymous testers. The threshold levels for both assays were set to approximate whether the person was infected within 5 months of when the sample was taken.

Samples sent to Stanford University or the University of Washington were analyzed to identify differences from a consensus sequence of the HIV *pol* region, which was used to determine HIV subtype and whether major resistance mutations were present. ARVDRT sites either received sequence information from the labs or were notified if samples could not be amplified. Resistance was determined using the Stanford drug-resistance algorithm (versions 3.0–6.0, available at http://hivdb.stanford.edu). This algorithm is designed to infer an individual's level of resistance to each of the major drugs within the three most common classes; protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), and nonnucleoside reverse transcriptase inhibitors (NNRTI). First, the algorithm assigns a "penalty score" for each mutation that confers resistance to the drug. Then, it adds up the penalty scores and translates them into five levels of resistance; susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance. In this analysis, an individual was considered to have any resistance to a class of drugs is there was at least one drug within that class that the person was not classified as susceptible to. They were considered to have high level resistance to a class of drugs if they had high level resistance to at least one drug within the class. Samples with high level resistance to two or more classes of ARVs were noted as having multiclass drug resistance. The drugs considered in this analysis included ritonivir-boosted lopinavir, indinavir, saquinavir, atazanavir, nelfinavir, emtricitabine, lamivudine, zidovudine, didanosine, tenofovir, stavudine, abacavir, delavirdine, efavirenz, and nevirapine.

Individuals who tested confidentially more than once were de-duplicated using their unique state surveillance identifier. If duplicate samples were found in ARVDRT, the earliest amplified sample was included in the analysis or, if no samples were able to be amplified, the earliest sample

was included. Those who tested anonymously more than one time in King County could only be de-duplicated if they went to the same place for testing each time and provided the same information for their anonymous code. Anonymous testing conducted at different sites, with different codes, or anonymous testers who also tested confidentially could not be linked together. Since testing site chart numbers were not recorded in Colorado, no anonymous testers there were able to be de-duplicated.

Data collected on HIV resistance and incidence through ARVDRT and HIVIS/HIS were initially approved by Institutional Review Boards (IRB), but were later switched to a surveillance designation and are now not considered research; thus, all HIVIS, HIS, and ARVDRT IRB reviews are now closed.

Differences between anonymous and confidential testers were analyzed either by the χ^2 test, for nominal variables, or the Cochran-Armitage test for trend, for ordinal variables. Significant associations were defined as those with a p-value <0.05. All analyses were conducted using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina).

Results

From all participating public testing sites in Colorado ($n=11$) and King County, Washington ($n=44$), between 2003 and 2007, 857 individuals who were eligible for ARVDRT had diagnostic serum specimens sent to labs for drug resistance testing. Of these, 195 (23%) tested anonymously and 662 (77%) tested confidentially (by name). More of the anonymous testers were reported from King County (86%) than from Colorado (14%), whereas similar amounts of confidential testers were reported from the two sites (Table 1).

The percentage of anonymous testers who were male (96%) was significantly higher than for confidential testers (91%). Anonymous testers were also significantly more likely to be younger ($p=0.0007$), white ($p=0.0001$), and MSM ($p=0.0001$). Country of birth was missing for 95% of anonymous testers, so this could not be effectively compared between the two groups.

Anonymous testers in King County were significantly more likely to have baseline CD4+ cell counts above 500 cells/mm^3 (45% vs. 28%; $p=0.018$), but had similar baseline viral loads as confidential testers (Table 2). HIV subtype distributions did not differ significantly with type of testing; however, anonymous testers were more likely to be missing viral subtype information. A slightly higher percentage of anonymous testers were designated as having a long-term infection based on the results of the STARHS testing (64% vs. 58%), however this result was not statistically significant. Among all testers, 41% were considered to have a recent infection and 59% to have long-term infection based on STARHS results.

On average, 90% of the samples sent for genotyping were able to be amplified and thus had resistance results sent back

Table 1. Characteristics of Persons Who Tested Positive for Human Immunodeficiency Virus Anonymously and Confidentially in the Antiretroviral Drug Resistance Testing Surveillance Project 2003–2007

	Anonymous	*Confidential*	*Total*	
	No. (%)	*No. (%)*	*No. (%)*	
Characteristic	(n = *195*)	(n = *662*)	(n = *857*)	p-*Value*
Sex				
Male	186 (96)	600 (91)	786 (92)	0.013
Female	7 (4)	60 (9)	67 (8)	
Age at diagnosis of HIV				
0–19 years	0 (0)	15 (2)	15 (2)	0.0007
20–29 years	38 (21)	222 (34)	260 (31)	
30–39 years	76 (42)	236 (36)	312 (37)	
40–49 years	50 (27)	135 (20)	185 (22)	
50–59 years	15 (8)	42 (6)	57 (7)	
60+ years	3 (2)	10 (2)	13 (2)	
Race/ethnicity				
White	111 (69)	377 (57)	488 (60)	0.0006
Black	26 (16)	119 (18)	145 (18)	
Hispanic	13 (8)	140 (21)	153 (19)	
Other	10 (6)	24 (4)	34 (4)	
HIV exposure category				
Men who have sex with men	130 (90)	458 (76)	588 (78)	0.0007
Injection drug user (IDU)	0 (0)	16 (3)	16 (2)	
Men who have sex with men–IDU	11 (8)	76 (13)	87 (12)	
Other	3 (2)	56 (9)	59 (8)	
Place of Birth				
Born in U.S.	1 (1)	539 (81)	540 (63)	<0.0001
Born outside U.S.	8 (4)	104 (16)	112 (13)	
Birthplace unknown	186 (95)	19 (3)	205 (24)	
HIV Testing Location				
Seattle, Washington	167 (86)	325 (49)	492 (57)	
Colorado	28 (14)	337 (51)	365 (43)	

HIV, human immunodeficiency virus.

Table 2. Comparison of Clinical Characteristics of Human Immunodeficiency Virus-Infected Individuals Who Tested Anonymously and Confidentially in the Antiretroviral Drug Resistance Testing Surveillance Project 2003–2007

	Anonymous	*Confidential*	*Total*	
	No. (%)	*No. (%)*	*No. (%)*	p-*Value*
Baseline CD4 cell count (cells/mm^3)[a]				
<200	6 (14)	102 (21)	108 (20)	0.035
200–499	17 (40)	254 (51)	271 (51)	
≥500	19 (45)	138 (28)	157 (29)	
Baseline Viral Load (copies/ml)[a]				
<1,000	3 (7)	21 (5)	24 (5)	0.86
1,000–9,999	7 (17)	98 (21)	105 (21)	
10,000–99,999	20 (48)	207 (44)	227 (45)	
≥100,000	12 (29)	140 (30)	152 (30)	
HIV Subtype				
B	149 (76)	559 (84)	708 (83)	0.025
Non-B	10 (5)	28 (4)	38 (4)	
Unknown	36 (18)	75 (11)	111 (13)	
Serologic testing algorithm for recent HIV seroconversion result				
Recent infection	25 (36)	181 (42)	206 (41)	0.36
Long-standing infection	45 (64)	255 (58)	300 (59)	

[a]Data collected from King County only.

to King County or Colorado (Table 3). Samples from anonymous testers were significantly more likely to be amplified (95%) than confidential testers (89%; $p = 0.017$). Among the 774 samples that amplified, 17% had any resistance to at least one of the three major drug classes and 10% had high level resistance. These resistance rates did not differ significantly between anonymous and confidential testers. The prevalence of resistance-associated mutations was highest among NNRTIs (11%) and was lower for NRTIs (6%) and PIs (3%). The most common PI mutation was L90M, the most common NNRTI was K103N, and the most common NRTI was M41L (Table 4).

Discussion

In this comparison of individuals newly diagnosed with HIV confidentially versus anonymously at public sites in Colorado and King County, Washington, from 2003 to 2007, anonymous testers had substantially different demographic characteristics relative to confidential testers, but we found no consistent differences in markers for recency of infection nor any marked differences in the prevalence of HIV genetic mutations associated with drug resistance. Anonymous testers were more likely to have CD4+ cell counts above 500 cells per mm^3, indicative of more recent infections among these antiretroviral-naïve individuals. Resistance rates for both anonymous and confidential testers were similar to those seen in a larger number of urban U.S. populations during this period.[14,26]

Some of these results may be explained by study limitations. Most importantly, anonymous testers were not able to be de-duplicated in this study, except for the rare occasion where they tested anonymously more than once at the same location in King County and provided the same unique

Table 3. Antiretroviral Resistance Test Results for Human Immunodeficiency Virus-Infected Individuals Who Tested Anonymously and Confidentially in the Antiretroviral Drug Resistance Testing Surveillance Project 2003–2007

	Anonymous	*Confidential*	*Total*	p-*Value*
Samples could not be amplified, %	5	11	10	0.017
Resistance (%)				
Any	17	17	17	0.99
PI	3	4	3	0.51
NNRTI	10	11	11	0.67
NRTI	8	6	6	0.23
High level resistance (%)				
Any	11	10	10	0.71
PI	1	2	2	0.69
NNRTI	8	8	8	0.94
NRTI	4	2	2	0.21
Multiclass drug resistance (%)	2	2	2	0.75

PI, protease inhibitors; NRTI, nucleoside and nucleotide reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitors.

Table 4. Ten Most Common Antiretroviral Resistance Mutations for Human Immunodeficiency Virus-Infected Individuals in the Antiretroviral Drug Resistance Testing Surveillance Project 2003–2007

	Total No. (%)
PI	
L90M	7 (1)
NNRTI	
G190A[a]	8 (2)
K101E[a]	5 (1)
K103N	30 (4)
Y181C[a]	14 (3)
NRTI	
D67N	8 (1)
K219Q/E	5 (1)
L210W	5 (1)
M41L	14 (2)
T215Y/F	12 (2)

[a]Data collected from King County only.

identifier. For confidential testers, only the first positive test was included, but for anonymous testers, we may have incidentally included later tests for the person if they did not report that they had previously tested positive for HIV or had not received results from a previous positive test. This may explain why we did not consistently find that anonymous testers were more likely to test earlier in the course of infection.

There may have been other differences in the comparability of confidential and anonymous testers that influenced the results. Although the ARVDRT study officially changed to Variant Atypical and Resistant HIV Surveillance (VARHS), which was similar in protocol but excluded anonymous samples from resistance testing, at the beginning of 2008, each site had a different time point when they stopped including anonymous testers. For instance, surveillance of anonymous testers was concluded in King County in late 2006. Secular trends in testing behavior or drug resistance could cause confounding due to anonymous testers not being included in the final year of the study. However, it is unlikely that secular changes affected the results to a great extent, especially since antiretroviral resistance rates remained fairly constant during the study period.[26] King County residents were also overrepresented among anonymous testers; however, when the same analysis was conducted among only King County residents, similar results were found (results not shown). Other potential differences between anonymous and confidential testers were reduced by including only facilities at the two sites which offered anonymous testing, so that the individuals should have only differed on whether or not they chose to give their name to be linked with the test result.

The accuracy of results from the assays used to assess length of time since infection may also be questioned since this testing produces a substantial number of false-positives and false-negatives classifications at an individual level.[16] This may indicate that CD4+ cell counts are a more reliable way to assess recency of infection at an individual level. The criteria for being considered recent or long term may also have changed over time, since the assay was changed from the LS-EIA to the BED HIV-1 Capture EIA in 2005, which could have led to a differential misclassification since fewer anonymous testers were included in the later year of the study. Studies in the United States have found excellent agreement between the two assays, suggesting that this may not have greatly influenced our results.[21]

Our findings that anonymous testers were not diagnosed with HIV infection earlier than confidential testers differed from the MESH and SHAS studies when using STARHS to define recency of infection.[2,4] However, using a high CD4 count ($\geq$500 cells per mm^3) our findings were similar to those of MESH and SHAS. MESH and SHAS were able to deduplicate their anonymous testers, used a different method to determine whether an individual had a recent infection, and encompassed a broader range of study areas. We did find similar demographic differences between anonymous and confidential testers as have been seen in other studies.[2,3,15,18] One interesting finding that warrants more study is that anonymous testers had samples that were more likely to amplify. Since commercially available HIV genotyping assays were optimized for B subtypes,[1] they may not work as well for foreign born individuals, who are more likely to have a non-B subtype. If anonymous testers are less likely to be foreign born, this could be an explanation for better amplification rates among anonymous testers. Due to missing data and small numbers, our data were insufficient to determine if anonymous testers were less likely to be foreign born, have a different distribution of subtypes, or have higher viral loads—factors that may impact amplification success.

Results from this study may not be generalizeable to other regions of the country or other demographic or HIV risk behavior groups. The West Coast HIV epidemic at the time of the study was composed of a higher proportion of whites, males, and especially MSM relative to other geographic areas.[5] MSM may test for HIV more frequently than some other risk groups[13] and Western MSM may further test for HIV more frequently than other MSM,[9,22] which may explain the greater proportion of STARHS recent infections in our study relative to that found by other researchers.[8]

Despite the limitations, this study may indicate that anonymous testers are not more likely to have ARV resistance; however, further research is warranted. Preliminary research suggests that resistance mutations may persist for several years in the absence of drug selection pressure and this may be especially pronounced in populations where NNRTIs are the primary resistance mutation types, since these may confer fewer fitness disadvantages and thus last longer.[17,20] A lack of association between STARHS recent infection and ARV-drug resistance in our study (analysis not shown) supports the conclusion that timing between HIV infection and testing may not greatly impact drug resistance rates.

This research contributes to the growing body of evidence that anonymous testers differ greatly from confidential testers. Since anonymous testers are currently excluded from CDC-funded resistance surveillance as well as other HIV surveillance systems, more research into anonymous testing behavior may help to accurately understand the impact of the epidemic in this population. Future research should

examine whether anonymous testers who receive positive results follow-up with a confidential test or go straight into HIV care and, if they do receive confidential testing, how long a lag there is between this and their first positive anonymous test. As a significant proportion of the testing population, it is important to further elucidate the ways in which anonymous testers differ from confidential testers.

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Disclosure Statement

The authors declare no conflicts of interest.

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