

The Epidemiology of Antiretroviral Drug Resistance among Drug-Naive HIV-1–Infected Persons in 10 US Cities

Hillard S. Weinstock,^{1,a} Irum Zaidi,¹ Walid Heneine,² Diane Bennett,¹ J. Gerardo Garcia-Lerma,² John M. Douglas, Jr.,^{3,a} Marlene LaLota,⁴ Gordon Dickinson,⁵ Sandra Schwarcz,⁶ Lucia Torian,⁸ Deborah Wendell,⁹ Sindy Paul,¹⁰ Garald A. Goza,¹¹ Juan Ruiz,⁷ Brian Boyett,^{12,b} and Jonathan E. Kaplan^{1,2}

¹Division of HIV/AIDS Prevention, Surveillance and Epidemiology, and ²Division of AIDS, STD, and TB Laboratory Research, Centers for Disease Control and Prevention, Atlanta, Georgia; ³Denver Department of Public Health, Denver, Colorado; ⁴Florida Department of Health, Tallahassee, and ⁵University of Miami School of Medicine and the Miami Veterans Administration Medical Center, Miami, Florida; ⁶San Francisco Department of Public Health, San Francisco, and ⁷California Department of Health Services, Sacramento, California; ⁸New York City Department of Health, New York, New York; ⁹Louisiana Office of Public Health, New Orleans; ¹⁰State of New Jersey Department of Health and Senior Services, Trenton; ¹¹Michigan Department of Community Health, Lansing; ¹²Houston Department of Health and Human Services, Houston, Texas

Background. The prevalence and characteristics of persons with newly diagnosed human immunodeficiency virus (HIV) infections with or without evidence of mutations associated with drug resistance have not been well described.

Methods. Drug-naive persons in whom HIV had been diagnosed during the previous 12 months and who did not have acquired immune deficiency syndrome were sequentially enrolled from 39 clinics and testing sites in 10 US cities during 1997–2001. Genotyping was conducted from HIV-amplification products, by automated sequencing. For specimens identified as having mutations previously associated with reduced antiretroviral-drug susceptibility, phenotypic testing was performed.

Results. Of 1311 eligible participants, 1082 (83%) were enrolled and successfully tested; 8.3% had reverse transcriptase or major protease mutations associated with reduced antiretroviral-drug susceptibility. The prevalence of these mutations was 11.6% among men who had sex with men but was only 6.1% and 4.7% among women and heterosexual men, respectively. The prevalence was 5.4% and 7.9% among African American and Hispanic participants, respectively, and was 13.0% among whites. Among persons whose sexual partners reportedly took antiretroviral medications, the prevalence was 15.2%.

Conclusions. Depending on the characteristics of the patients tested, HIV-genotype testing prior to the initiation of therapy would identify a substantial number of infected persons with mutations associated with reduced antiretroviral-drug susceptibility.

Antiretroviral-drug resistance is an important cause of treatment failure in persons infected with HIV-1 and has been associated with increased mortality [1–4]. Although the transmission of drug-resistant strains of HIV has been well documented [5], the prevalence and characteristics

of persons with or without mutations associated with drug resistance are less clear. A number of studies have examined the prevalence of mutations associated with resistance in small samples of recently or acutely infected persons, mostly white men who have sex with men (MSM) [6–13]. Fewer studies have assessed the prevalence of mutations in drug-naive persons with newly diagnosed infections whose infections are, for the most part, of unknown duration; these persons are more typical of HIV-infected patients presenting for initial evaluation and treatment. In addition, few studies have been sufficiently large and representative of newly diagnosed HIV to describe the characteristics of persons infected with drug-resistant strains of HIV.

A concern with testing chronically infected patients is that, even if drug-resistant mutations are initially present, in the absence of drug selection pressures,

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^a Present affiliation: Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta.

^b Present affiliation: Division of HIV/AIDS Prevention, Surveillance and Epidemiology, Centers for Disease Control and Prevention, Atlanta.

Reprints or correspondence: Dr. Hillard S. Weinstock, Centers for Disease Control and Prevention 1600 Clifton Rd., MS E02, Atlanta, GA 30333 (hsw2@cdc.gov).

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drug-resistant mutations may become undetectable if the infecting strains revert to wild type or become overgrown by fitter wild-type viruses; persisting as archived viruses or as minority species, they may not be detectable by current assays [14], even though they may become problematic with the introduction of therapy and selective pressures on the predominant wild-type strains. Consequently, resistance testing prior to initiation of antiretroviral therapy in persons with established HIV infection, although recommended by some when duration of infection or regional prevalence can be established [15], has not been widely recommended in the United States [16].

To describe the prevalence and characteristics of persons with mutations associated with reduced drug susceptibility, we systematically enrolled, in multiple venues across the United States, drug-naive persons in whom HIV infection had been recently diagnosed. As a better understanding of patterns of antiretroviral-drug resistance in persons presenting for care emerges, this kind of information may help to guide recommendations for baseline resistance testing and, possibly, the selection of initial antiretroviral regimens.

PATIENTS AND METHODS

Patients. HIV-1-infected persons in whom the condition had been diagnosed during the previous 12 months were enrolled consecutively from 39 selected HIV care clinics, HIV counseling and testing sites, and other clinical settings in 10 US cities during 1997–2001. Eligibility criteria included age ≥ 18 years, antiretroviral-drug-naive status according to medical chart review (if a chart was available) and personal interview, and no history of AIDS-defining conditions (including a CD4⁺ T cell count < 200 cells/mm³). After informed consent was obtained, demographic, risk-behavior, and clinical information was obtained from medical charts, if available, and from standardized interviews. Blood specimens were obtained from each consenting participant. The study was approved by the institutional review board at the Centers for Disease Control and Prevention and by the local human-subject review boards affiliated with the clinics or other sites where participants were enrolled.

Resistance testing. Reverse transcriptase (RT) and protease genotyping was conducted on the basis of HIV-amplification products, by automated sequencing (Applied Biosystems) at the Centers for Disease Control and Prevention (165 specimens [GenBank accession numbers AY471869–AY472017]), ViroLogic (249 specimens), and Virco (668 specimens) laboratories. The mutations included in the analysis that were identified by sequencing at the ViroLogic and Virco laboratories were based on the reports of mutations provided by those laboratories. The analysis considered (1) RT-gene mutations that have been associated with reduced susceptibility to RT inhibitors and (2) major protease-gene mutations that have been associated with

reduced susceptibility to protease inhibitors, as reported by the International AIDS Society–USA in June 2002 [17]: RT—M41L, A62V, K65R, D67N, T69D, 69 insert, K70R, L74V, V75I, V75T, V75M, V75S, V75A, F77L, L100I, K103N, V106A, V108I, Y115F, F116Y, Q151M, Y181C, Y181I, M184V, M184I, Y188C, Y188L, Y188H, Y188C, G190A, G190S, L210W, T215Y, T215F, K219Q, K219E, P225H, M230L, and P236L; protease—D30N, M46I, M46L, G48V, I50V, V82A, V82S, V82E, V82T, I84V, and L90M. In addition, all RT mutations at codon 215 that were different from wild-type T215 were included [17, 18], as were the T69A/N/S mutations.

Plasma specimens having an RT mutation or a major protease mutation associated with reduced drug susceptibility were tested phenotypically at the ViroLogic (29 specimens) or Virco (50 specimens) laboratories, by recombinant-virus assays [19, 20]. The following antiretroviral drugs approved by the Food and Drug Administration were tested: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, and nelfinavir. Fold changes in the IC₅₀ of the patient's virus, relative to that of the reference wild-type viruses, were used to categorize phenotypic results as either sensitive or resistant according to cutoff values established by the manufacturers [21, 22].

Additional HIV antibody testing for recency of infection.

All specimens for which informed consent was provided were also tested by a modified version of the HIV-1 enzyme immunoassay (Vironostika HIV-1 EIA; bioMérieux) [23, 24]. This assay is less sensitive than the standard HIV-1 enzyme immunoassay—it becomes reactive ~ 170 days after the standard EIA becomes positive; thus, HIV-infected persons in whom the virus does not react by the modified EIA are considered to have been infected during the previous 4–6 months (“recent infection”). All testing by this assay was conducted at 1 laboratory (San Francisco Department of Public Health), where assay performance was monitored [25].

Statistical analysis. We used the Mantel Haenszel χ^2 test and, where appropriate, Fisher's exact test to compare proportions of different HIV-infected populations with mutations associated with reduced drug susceptibility. Because of the small numbers of persons enrolled in 1997 and 2001, trends were analyzed for 1998–2000 only. Only clinics submitting data for all 3 of these years were included in analyses of trends. All statistical analyses were performed by use of SAS version 6.12 (SAS Institute).

RESULTS

Of 1311 persons eligible for enrollment in this study, 1104 (84%) agreed to participate, and viral sequences from 1082 (83%) were successfully amplified; the analysis focused on these 1082 HIV-1-infected individuals. Table 1 summarizes demo-

graphic and clinical characteristics of the study population. Most were male, and 46% were African American, whereas 22% were of Hispanic origin. Most participants were enrolled from HIV care clinics. A substantial proportion of male participants, 60%, reported being MSM. A modified version of the HIV-1 enzyme immunoassay found 19% (182 of 949 persons who provided informed consent for additional testing) to have been recently infected with HIV.

Although the proportion of HIV-infected persons with mutations associated with reduced antiretroviral drug susceptibility did not vary significantly by age group, city, site of enrollment, CD4⁺ T cell count, or recency of infection, persons with these mutations were more likely to be white, to be MSM, and/or to have a partner taking antiretroviral medications (table 1).

The overall prevalence of mutations associated with reduced antiretroviral drug susceptibility was 8.3%; however, it was 6.1% and 4.7% in women and heterosexual men, respectively, and was 12% in MSM. The prevalence was 5.4% and 7.9% in African American and Hispanic persons, respectively, and was 13% in whites. Figure 1 shows that, for both sexual orientations and for both sexes, whites had the highest prevalence of these mutations; white MSM had the highest overall prevalence (14%), and Hispanic and African American heterosexual men and African American women had the lowest prevalences (4.3%, 4.6%, and 4.9%, respectively).

The prevalence of these mutations also varied according to the drug class with which they are associated (table 2). Of the 90 persons with RT mutations or major protease mutations, 69 (77%) had RT mutations associated with reduced susceptibility to the nucleoside reverse-transcriptase inhibitors (NRTIs); 40 of these 69 persons had 1, 24 had 2, 3 had 3, and 2 persons had 4. The most commonly observed NRTI resistance-associated mutations were M41L (19 persons), K70R (9 persons), M184V (9 persons), and D67N (7 persons). There were 4 persons with T215Y or T215F mutations; 27 other persons had T215D/S/C/E/I mutations, and 15 persons had T69D/N/S/A mutations. When persons with T215D/S/C/E/I or T69N/S/A mutations were excluded, 69 (6.4%) of 1082 persons had resistance-associated mutations, and 48 (4.4%) of 1082 had NRTI-associated mutations.

Nonnucleoside-associated reverse-transcriptase mutations were found in 18 (1.7%) of the 1082 study participants, and major protease mutations were found in 21 (1.9%). The most commonly observed nonnucleoside-associated mutation was K103N (10 persons), and the most commonly observed major protease mutations were L90M (10 persons) and M46I (8 persons). Only 14 persons (1.3%) had mutations associated with reduced susceptibility to antiretroviral medications in ≥2 drug classes; these 14 persons, 10 of whom were MSM, were enrolled from 7 of the 10 study cities.

The prevalence of mutations associated with reduced anti-

Table 1. Characteristics of total study population and of persons with mutations associated with reduced susceptibility to reverse-transcriptase (RT) or protease inhibitors, in 10 US cities during 1997–2001.

Characteristic	No. (%) of persons		P ^a
	Total	With RT or major protease mutation	
Total	1082	90 (8.3)	
Age group			.07
<25	135	14 (10)	
25–34	453	41 (9.1)	
35–44	323	27 (8.4)	
>44	171	8 (4.7)	
Sex			.11
Male	802	73 (9.1)	
Female	280	17 (6.1)	
Race/ethnicity ^b			.002
African American	498	27 (5.4)	
Hispanic	240	19 (7.9)	
White	292	38 (13)	
Other	21	3 (9.5)	
Site type			.32
HIV care clinics	668	52 (8.6)	
HIV testing site	372	32 (7.8)	
Other clinical facility	42	6 (14)	
City			.22
San Francisco	75	9 (12)	
San Diego	39	7 (18)	
Denver	179	17 (9.5)	
Detroit/Grand Rapids	46	4 (8.7)	
Houston	187	9 (4.8)	
New York	127	12 (9.5)	
Newark	170	11 (6.5)	
New Orleans	137	10 (7.3)	
Miami	122	11 (9.0)	
HIV risk behavior			.001
Male-to-male sex	482	56 (12)	
Injection drug use ^c	110	9 (8.2)	
Heterosexual exposure ^d	490	25 (5.1)	
Recently infected			.07
Yes	182	21 (12)	
No	767	57 (7.4)	
CD4 count			.56
<350 cells	151	13 (8.6)	
≥350 cells	389	36 (9.3)	
Partner taking HIV medications			<.001
Yes	171	26 (15)	
No	736	45 (6.1)	

^a Based on χ^2 test.

^b Information about race/ethnicity was not known for 31 persons.

^c Does not include individuals who also reported male-to-male sex.

^d Does not include individuals who also reported male-to-male sex or injection drug use.

retroviral-drug susceptibility was higher in the 182 persons found to have been recently infected with HIV (12%) than in the rest of the study population (7.4%); however, this difference was not statistically significant. The prevalence of such mutations in MSM who had been recently infected with HIV was

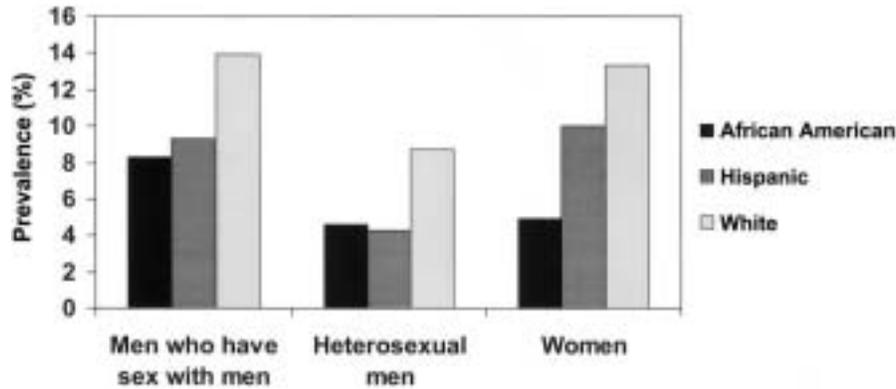


Figure 1. Prevalence of reverse-transcriptase and major protease mutations associated with reduced antiretroviral-drug susceptibility, by race/ethnicity and sexual orientation, in 1082 HIV-infected persons in 10 US cities during 1997–2001.

15%, whereas that in MSM whose infections were not recent was 11%; this difference too was not statistically significant. Similarly, recently infected heterosexual men had a higher, but not statistically different, prevalence of mutations than did heterosexual men whose infections were not recent (7.3%, compared to 4.4%); also, the prevalence of such mutations in women whose infections were recent was not statistically different from that in women whose infections were not recent (3.3%, compared to 5.8%), and within each racial or ethnic group, the prevalence in persons whose infections were recent was not different than that in persons whose infections were not recent.

Some mutations were more likely to be found in persons with recent infections. In persons in whom recency of infection was tested by the modified HIV-1 EIA, 6 of 8 with the M184V mutation and 5 of 8 with the K103N mutation were found to have been recently infected. In contrast, only 4 of 27 persons with HIV containing changes at codon 215, 2 of 13 persons

with changes at codon 69, 5 of 17 persons with the M41L mutation, and 3 of 10 persons with the L90M mutation were found to have been recently infected.

The prevalence of mutations in recently infected persons varied by year (7.1% in 1998, 14% in 1999, and 8.9% in 2000); these differences were not statistically significant. This prevalence increased over time in persons not recently infected, from 3.2% in 1998 to 9.0% in 1999 to 12% in 2000 ($P = .004$, χ^2 for trend).

Of the 79 persons with an RT mutation or major protease mutation who were successfully tested phenotypically, only 31 (39%) showed phenotypic evidence of decreased susceptibility to the drugs with which their mutations have been associated (table 2). This finding varied by drug class. Only 13 (22%) of the 58 persons where viruses had NRTI mutations were found to have phenotypic evidence of resistance. In this group, the presence of a large number of viruses, with T215D/S/C/E/I mutations, that had wild-type susceptibility to zidovudine con-

Table 2. Prevalence of mutations associated with reduced drug susceptibility, by drug class and phenotypic confirmation of genotypic findings, in 1082 HIV-1-infected persons in 10 US cities during 1997–2001.

Drug class	No. of persons (% of study population [$N = 1082$]) with associated mutation ^a	No. (%) of persons with reduced susceptibility ^b
RT inhibitor or major protease inhibitor	90 (8.3)	31/79 (39)
Nucleoside RT inhibitor	69 (6.4)	13/58 (22)
Nonnucleoside RT inhibitor	18 (1.7)	14/18 (78)
Major protease inhibitor	21 (1.9)	10/20 (50)
≥2 drug classes	14 (1.3)	12/14 (86)

NOTE. RT, reverse transcriptase.

^a Mutation associated with reduced drug susceptibility [17].

^b The denominator reflects the number of persons with resistance associated mutations who were successfully phenotyped; 8 specimens with codon changes at 69 were not submitted for phenotypic testing; 3 specimens could not be successfully tested. Cutoff values for reduced sensitivity were defined by the manufacturers: Virologic, Inc.—4.5-fold for abacavir, 1.7-fold for didanosine and stavudine, and 2.5-fold for the others [21]; Virco—4.0-fold for zidovudine and neftinavir, 4.5-fold for lamivudine, 3.5-fold for didanosine, zalcitabine, and ritonavir, 3.0-fold for stavudine, abacavir, and indinavir, 8-fold for nevirapine, 10-fold for delaviridine, 6-fold for efavirenz, and 2.5-fold for saquinavir [22].

tributed to the low proportion with decreased susceptibility. Additionally, of the 19 persons with virus having the M41L mutation, only 3 showed evidence of decreased susceptibility to zidovudine, and 2 of those persons had virus with the T215Y mutation as well. On the other hand, 6 of 8 persons with virus carrying the M184V mutation had phenotypic evidence of resistance to lamivudine, and all 10 persons with virus with the K103N mutation had phenotypic evidence of resistance to non-NRTIs.

Persons with mutations associated with reduced antiretroviral drug susceptibility who also had phenotypic evidence of resistance were, like all persons with these mutations, statistically more likely to be white, to be MSM, and/or to report a partner taking antiretroviral medications than were persons with virus without these mutations. They were also more likely to have been recently infected (50%, compared to 18%; $P < .001$).

DISCUSSION

In patients infected with HIV, the first therapeutic regimen is the most important one for producing a maximal and durable virologic response [16]. Optimizing that initial regimen is therefore critical, and, for this reason, testing for the presence of drug-resistant strains of HIV prior to the initiation of therapy may be beneficial to the patient and cost effective [26]. The findings of the present study of drug-naive persons with newly diagnosed infections suggest that such a strategy of resistance testing would identify a substantial number of persons with HIV containing mutations associated with reduced antiretroviral-drug susceptibility.

These findings document that many mutations remain detectable 4–6 months after infection and support previous studies' observation, in a few patients, that transmitted drug-resistant virus may persist for months or years [27, 28]. The continued presence of these mutations and the ability to detect them have important implications both for resistance testing in drug-naive patients with established infection and for the conduction of surveillance of mutations associated with reduced antiretroviral-drug susceptibility in this population.

We did find that the prevalence of mutations associated with reduced antiretroviral-drug susceptibility in persons whose infections were recent is higher than that in persons whose infections were not. Although this difference is not statistically significant when all mutations are considered, some mutations, particularly those that could be confirmed phenotypically, are more likely to be found in recently infected persons. The shorter persistence of some mutations may be explained by decreased viral fitness and, thus, a faster rate of reversion to a more replication-competent variant [29, 30].

Of persons with detectable viral mutations identified in the present study, only 39% had phenotypic evidence of resistance.

The inclusion of mutations that indicate prior drug exposure but do not actually confer drug resistance may account, in part, for the lower prevalence of phenotypic resistance observed in this and other studies [6, 7]. Although the presence of genotypic or phenotypic markers of resistance in recently infected persons has generally been linked to reduced virologic responses to antiretroviral therapy [6, 7], the clinical implications of many resistance-associated mutations are not fully defined. We therefore caution against using these prevalence data to imply rates of virologic failures that would occur in patients initiating therapy; however, close monitoring of treatment responses in patients infected with viruses with these mutations may be warranted.

The largest proportion (41%) of persons with NRTI-associated mutations had mutations at codon 215, such as T215D/S/C/E/I, that differ from either wild type or the zidovudine/stavudine-selected T215Y/F. These mutations are known to be revertants of T215Y, and, although they are phenotypically sensitive to zidovudine, the mutant viruses can acquire T215Y *in vitro* more rapidly than can wild-type HIV-1, likely reflecting the fact that only 1 nucleotide change is necessary for evolution to T215Y [17]. Preliminary data suggest that patients with these mutations, after initiating therapy, do have an increased risk of virologic failure [31].

The prevalence of mutations associated with reduced antiretroviral-drug susceptibility varies depending on the particular population being tested. We found that the prevalence of mutations associated with reduced drug susceptibility was higher in whites and MSM than in other populations. We also found that the prevalence of these mutations was higher in persons reporting partners who took antiretroviral medications, suggesting that these viruses may have been transmitted directly from treated persons. The higher prevalence in whites and MSM may reflect better access to health care and treatment in these populations [32, 33]. Our results help to explain the prevalence of mutations found by others, whose study populations consisted mostly of recently or acutely infected white MSM [6, 7, 13].

Although we did not observe evidence of an increasing prevalence of mutations associated with reduced drug susceptibility, over time, in recently infected individuals, the number of recently infected individuals in our study is small. We did see an increasing prevalence in persons who were not recently infected; however, these trend data should be regarded with some caution, because they are limited to just 3 years.

We did not study a random sample of HIV-infected individuals; nevertheless, to date, this is the largest, most diverse population in the United States that has been studied for antiretroviral-drug resistance and, with the exception of the oversampling of Hispanics (only 11.3% of persons reported with HIV nationally are Hispanic, compared to 22% in the present study), our study well reflects the demographic characteristics

of persons reported with HIV as well as the proportion of MSM [34]. It should be noted that the persons in this study had CD4 T cell counts ≥ 200 cells/mm³; according to current guidelines, many may not have had indications for starting antiretroviral therapy.

Although the epidemiology of antiretroviral-drug resistance may reflect the HIV-infected populations that are seeking and receiving antiretroviral therapy, it also reflects, as others have noticed, the health care system's failures to prevent HIV transmission from these treated populations [6]. Recently reported increases in risky sexual behaviors, as evidenced by increases in sexually transmitted diseases in MSM with high rates of HIV coinfection [35–37], suggest that HIV infection rates may also be increasing in this group. Health care providers who are caring for HIV-infected patients can play a prominent role in helping to prevent the further transmission of HIV, including drug-resistant virus.

In summary, HIV genotypic testing prior to the initiation of therapy in patients with newly diagnosed HIV infections and without AIDS would identify a substantial number of persons with virus with mutations associated with reduced antiretroviral-drug susceptibility. The prevalence of these mutations varies depending on the characteristics of the patients tested and the duration of their infections. Continued surveillance for antiretroviral-drug resistance in sufficiently large, representative samples of persons with newly diagnosed HIV will be necessary to monitor changes, over time, in the prevalence and the populations affected.

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