Public Health Surveillance of Transmitted Antiretroviral Drug Resistance, Oregon 2007–2011.

By

Sean Schafer, MD

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Sean D. Schafer

has been approved

Mentor/Advisor

Member

Member

Member

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Acknowledgments

Thesis committee:
Clyde Dent, PhD, Katrina Hedberg, MD, MPH, Bill Lambert, PhD (Advisor)

Oregon Public Health Division:
Jeff Capizzi, Lea Bush, JA Magnuson, Veda Latin, Ruth Helsley

Providence Hospital Microbiology Laboratory:
Mary Campbell, Microbiology Laboratory Director,

Quest Laboratories:
Rebecca Moore

HIV Providers
Diana Antoniskis MD, Eric Chang, MD, Todd Korthuis MD, MPH, Michael McVeigh MD, Melissa Murphy, MD

Seattle King County Public Health:
Susan Buskin, PHD

OSHU Public Health and Preventive Medicine Staff:
Theresa Triano,, Natalie Chin

Abstract

Surveillance of transmitted antiretroviral mutations detects emerging drug resistance, informs pre- and post-exposure prophylaxis, highlights HIV transmission from source cases already in care, and guides efforts to control resistance. We established a statewide surveillance system for transmitted antiretroviral resistance in Oregon that relies on laboratory reporting of nucleic acid sequences ascertained for clinical purposes. We evaluated reporting completeness and representativeness, compared Oregon to U.S. resistance estimates, and compared cases with transmitted resistance to newly-diagnosed cases without resistance. During 2007–2011, laboratories reported 49 percent (1,067/2,175) of expected tests, 73 percent (316/435) for 2011. Among 1,226 patients newly diagnosed with HIV during 2007–2011, resistance tests collected within 3 months of diagnosis were reported for 24.5 percent (300), and 39.6 percent during 2011 alone.. We observed no significant differences among newly-diagnosed patients with and without reported resistance tests. Overall, 17.3 percent of newly-diagnosed cases had at least one resistance mutation for any of three classes; this was not significantly different from a 2006 U.S. survey. We also found that 12.3 percent had at least one nucleoside reverse transcriptase inhibitor mutation (NRTI); 5.7 percent had at least one non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation, and 2.7 percent had at least one protease inhibitor (PI) mutation. The proportion of cases with NRTI resistance was twice the proportion reported by the earlier study ($p<0.001$); NNRTI and PI mutations were less frequent than expected, though not significantly so. We observed no significant differences among newly-diagnosed cases with and without evidence of resistance. This approach appears to accurately represent the proportion of newly infected cases with transmitted resistance and to have the potential to be replicable by other statewide HIV surveillance systems without substantial new costs.

Introduction

Public health surveillance of transmitted antiretroviral drug resistance can detect emerging antiretroviral drug resistance, contribute to public health efforts to prevent new infections by informing pre- and post-exposure drug prophylaxis and highlighting transmission from source cases that are in care, and measure the success of efforts to limit the spread of antiretroviral drug resistance. In the U.S., antiretroviral drug resistance surveillance has been conducted using samples of newly-diagnosed people from cohort studies or supplemental sampling from cases reported to public health authorities. These approaches can be costly and might not be representative of regions not included in the samples. This paper describes initial findings from a newly-developed, statewide, population-based system for surveillance of transmitted anti-retroviral drug resistance and distribution of viral subtypes among newly-diagnosed people. This approach has the potential to be replicable by other statewide HIV surveillance systems without substantial new costs.

Background

The public health value of transmitted antiretroviral drug resistance surveillance

Antiretroviral drug resistance can be transmitted from an infected person who already has antiretroviral drug resistance to a susceptible person through sexual, parenteral, and vertical routes.[1-3] This is called secondary, or transmitted, drug resistance. Among newly-diagnosed, treatment-naïve people, those with transmitted drug resistance experience longer delays from onset of therapy to viral suppression and greater frequency of treatment failure than their counterparts without transmitted drug resistance.[4]

Because antiretroviral drug resistance originates under selective pressure of antiretroviral drugs in people being treated for HIV infection, antiretroviral drug resistance in someone with

recent infection who has not yet been treated indicates that the infection was acquired from a source who was being, or had previously been, treated with antiretroviral drugs.[5] Being in treatment, that source would have been aware of his or her infection. Consequently, the proportion of cases with drug resistance in newly-diagnosed and still-untreated people is proportional to the number of new cases acquired from sources aware that they are infected—as opposed to acquisition from infected sources who do not yet know that they are infected. Surveillance of the proportion of newly-diagnosed cases with transmitted drug resistance over time enables public health officials to make inferences about the relative proportions of cases that are transmitted by sources who know they are infected and by sources who do not know they are infected as approaches to prevention differ depending upon whether source cases are aware of being infected. In addition surveillance of antiretroviral resistance informs public health officials about the success or failure of efforts to control or limit the extent of antiretroviral resistance.

Substantial transmission by sources already aware of their infection dictates different strategies for prevention of new infections than transmission occurring from sources that don't yet know they are infected.[6] When sources know they are infected, appropriate strategies to prevent additional new infections include increasing overall medication adherence and optimal selection of antiretroviral therapy, thereby reducing viral load and infectiousness among the population of people with known HIV infection. Other strategies for reducing transmission by people who already know they are infected include individual and system-level approaches to increase condom use, and pre- and post-exposure prophylaxis. In contrast, widespread and frequent HIV testing is the best available strategy for prevention if substantial transmission is occurring from sources who are not yet aware of their infection. Specific characterization of populations with transmitted antiretroviral resistance further enables public health agencies to target and tailor interventions specifically for groups among which higher levels of transmitted resistance are seen.

Detection of new, resistant viral strains in the population is another motivation for ongoing surveillance of transmitted drug resistance. When HIV drug resistance was first recognized, there was concern about potential emergence of a virulent strain of drug resistant HIV. However, drug-resistant HIV strains are now generally believed to be less fit then wild types, probably because the mutations that confer resistance generally reduce virulence and convey a survival disadvantage in the absence of treatment. Nevertheless, surveillance of transmitted drug resistance can be valuable for early recognition and public health response to the emergence of a virulent, resistant strain.

Timely knowledge of community levels of resistance to specific antiretroviral drugs can also be useful for prevention of new infections through pre-exposure and post-exposure prophylaxis, strategies that are likely to become more widespread. Much as they have used hospital-based surveillance of bacterial antimicrobial resistance to inform choices of empiric antibacterial therapy, clinicians can use accurate and timely estimates of the proportion of newly-diagnosed cases with transmitted antiretroviral drug resistance in the community to optimize choices of antiretroviral medications to use for pre- and post-exposure prophylaxis.[7]

Indirectly, surveillance of transmitted antiretroviral drug resistance that is done by collecting nucleic acid sequences reported by clinical laboratories enables identification of outbreaks or clusters of cases via molecular methods that can then be investigated epidemiologically. In addition to translating these sequences into a sequence of amino acids and comparing them to libraries of drug resistance mutations—a process provided for free via the internet by the Stanford HIV Database—public health authorities can use multiple sequence analysis software to examine the relatedness of newly-diagnosed cases, much like pulsed-field gel electrophoresis and polymerase chain reaction typing are used to identify clusters of foodborne and tuberculosis infections.[8] Epidemiologic investigation of cases with high degrees of phylogenetic similarity might lead to insights about community patterns of transmission, or suggest public health action to reduce transmission.[9]

Brief overview of antiretroviral drug resistance

Antiretroviral drug resistance is a reduction in the capacity of a drug to suppress HIV viral replication in a person with HIV infection. Antiretroviral drug resistance is an important public health problem because HIV drug resistance increases the rate of disease progression and shortens survival in people with HIV infection. For example, in one study, 19 month-survival was 91 percent among people with HIV without resistance to an entire class of HIV drugs and 73 percent among people whose virus exhibited resistance to 3 classes of HIV drugs.[10] Additionally, incidence of AIDS defining illness was observed to increase with the extent of HIV drug resistance. Drug resistance develops readily in patients who have incomplete viral suppression and are taking antiretroviral medications, and medication adherence doesn't prevent emergence of resistance if the drugs taken don't thoroughly suppress viral replication.[5] Bangsberg studied 57 patients with incomplete viral suppression (>50 copies/ml) on stable antiretroviral therapy and found that the rate of development of new mutations actually increased with increasing medication adherence.[11]

Primary HIV drug resistance develops *de novo* in an individual when mutations occur at the locations of the molecular targets of HIV drugs.[12] Mutations occur frequently because HIV replicates rapidly, its reverse transcriptase is unable to proofread sequences during replication, and because genetic recombination occurs when different viruses infect the same cell. Within each HIV-infected individual, over time, a 'quasispecies' comprised of many genetically distinct variations develops.[13] If a variant with a mutation at the location of the molecular target of a specific HIV drug is present, even in low numbers, and the individual is treated with that drug, a reproductive advantage can be conveyed to that variant and drug resistance emerges.

In addition, drug resistance can appear in newly-diagnosed people by direct transmission through sexual, parenteral, and vertical routes from an infected person who has HIV drug resistance.[1-3] This is called secondary, or transmitted drug resistance. Compared to treatment-

naive people without drug resistance, newly-diagnosed people with transmitted drug resistance experience longer delays from onset of therapy to viral suppression and greater frequency of treatment failure.[4] Because of its prognostic and clinical importance, the HIV Medical Association recommends that all patients have resistance testing performed at the outset of medical care, regardless of whether or not anti-retroviral drug therapy is planned.[14]

At the population level, prevalence of transmitted drug resistance appears to have increased with increasing antiretroviral use until the late 1990s. In 2002, Little and colleagues reported that transmitted HIV drug resistance in treatment naïve patients increased from 3.4 percent during 1995–1998 to 12.4 percent during 1999–2000 among 377 subjects with acute HIV infection recruited from 10 US cities.[4] Since then, use of multiple drug "cocktails" and emphasis on complete adherence has become standard, and prevalence of transmitted drug resistance may have stabilized.[14] In their analysis of data from ten U.S. cities during 1997–2001, Weinstock and colleagues fount that 8.3 percent of over 1,000 newly-diagnosed participants tested during 1997–2001 had mutations conferring reduced susceptibility to reverse transcriptase or protease inhibitors.[15] In 2010, Wheeler, et al. reported that 14.6 percent of newly-diagnosed individuals among over 2000 tested in 10 states during 2006 had acquired transmitted drug resistance mutations (TDRM).[16] In South Carolina, 14.4 percent of 1,277 people with recent infection tested from 2005 through 2009 had "major" antiretroviral drug mutations. However, direct comparison of resistance prevalence reported by various investigators might be misleading because reported prevalence depends upon the scheme used to determine whether or not a particular mutation is a polymorphism that occurs randomly or a mutation that occurs in response to treatment pressure. Moreover, different authorities categorize mutations differently as to the level of reduced drug susceptibility they confer.[12]

Descriptive epidemiology in Oregon

By area, Oregon is the 9th largest state, covering 97,813 square miles.[17]Oregon ranks 27th by population with an estimated 3.8 million residents in 2010.[18] Portland is Oregon's largest city with a population of 584,000 at the end of 2010.[19]Though it also includes Vancouver city in Washington State, 2.3 million people live in the Portland Metropolitan Statistical Area.[20] At the end of 2011, approximately 7,500 people with HIV lived in Oregon; 5,900 (155/100,000) had identified cases that had been reported to Oregon's Public Health Division.[21] In addition, an estimated 1,400 people, (37/100,000 people with HIV infection had yet to be identified and remain unaware of their infection. This estimate of unrecognized cases is based on a Centers for Disease Control and Prevention (CDC) estimate that 20 percent of people with HIV in the U.S. are unaware of their infection.

About 250 people are newly diagnosed with HIV annually in Oregon and 60 people with HIV die, an annual incidence and mortality that hasn't changed appreciably since 1996 when highly active antiretroviral therapy was introduced (Figure 1). Seventy-five percent of all cases occur in men. In 70 percent male cases, the men acknowledge that they have sex with other men. Six percent report injection drug use, and another nine percent sex with other men and injection drug use. Ten percent of cases have unknown risks and about five percent of cases (unlabeled) have presumed heterosexual acquisition from partners with known infections, or injection drug using partners (Figures 2 and 3).

Figure 1. Reported HIV cases by year of diagnosis and deaths, Oregon, 1981–2010

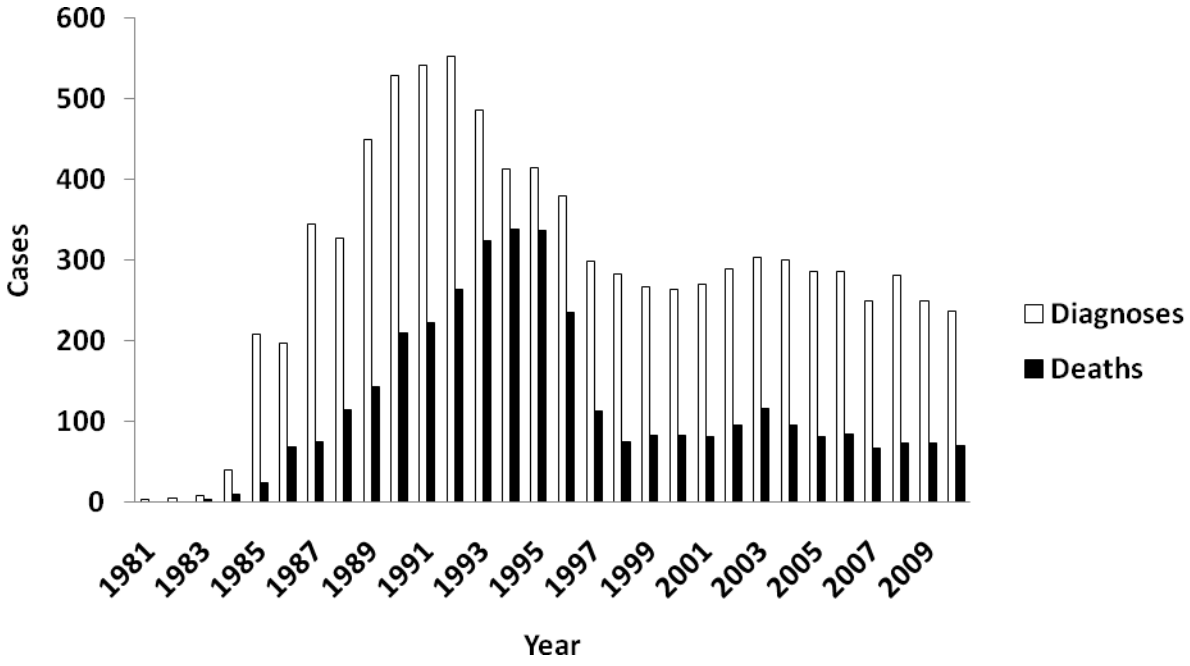
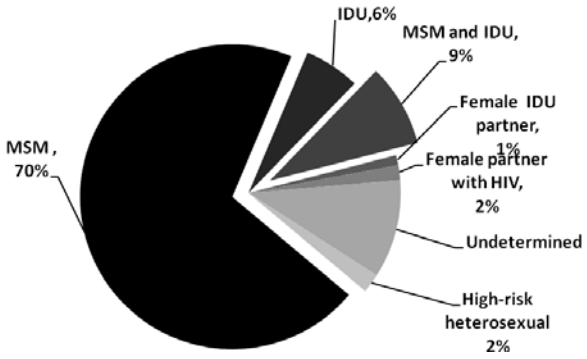
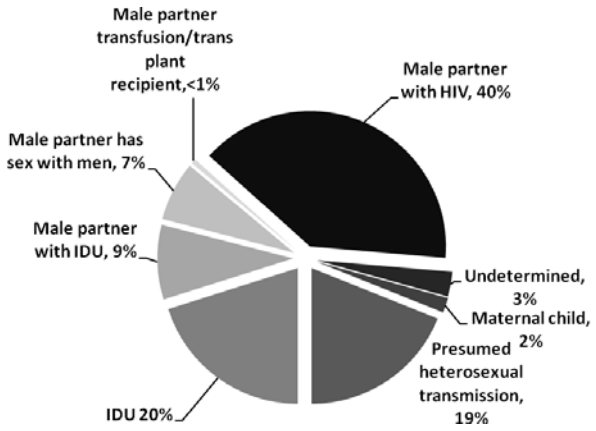


Figure 2. Presumed transmission categories among men (n=1,132) with reported cases of HIV diagnosed 2006–2010 , Oregon*



*Abbreviations: MSM=man who has sex with men; IDU=injection drug user

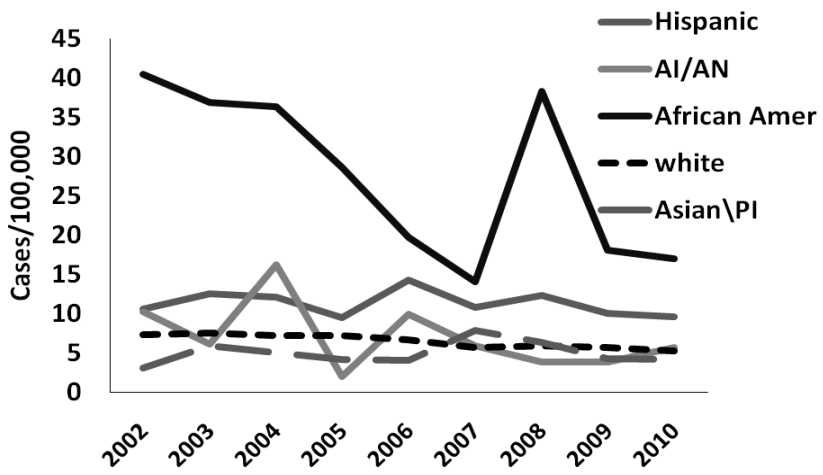
Figure 3. Presumed transmission categories among women (n=169) with reported cases of HIV diagnosed 2006–2010 , Oregon



*Abbreviations: IDU=injection drug user

Like other infectious diseases, risk of infection varies by race. During 2002–2010, African Americans experienced an average rate of about 25, Hispanics 10, and American Indians 10 per 100,000 compared to an average of 6 per 100,000 among whites) (Figure 4). Again, like other infectious diseases, HIV prevalence is disproportionately higher in metropolitan areas of Oregon. For example though it contains only about 19 percent of Oregon's total population, 56 percent of people with reported HIV resided in Multnomah County (location of Portland) at the time of their diagnosis.[22]

Figure 4. HIV diagnoses by year and race, Oregon, 2001–2010.



HIV Surveillance in Oregon

As in the other 49 U.S. states, HIV surveillance in Oregon is largely supported by funding from the U.S. government through the CDC. In Oregon, by law, physicians and others who treat illness must report cases of HIV to public health. In addition, laboratories must also report all results indicative of and specific for HIV. Such tests include CD4+ T-lymphocyte counts, quantitative and qualitative tests of viral particles in the blood commonly known as viral loads, diagnostic antibody tests, and tests for antiretroviral resistance. Any laboratory that conducts more than 30 tests a month must send these electronically to the Oregon Public Health Division. When the Health Division receives a result indicative of HIV, it compares it to existing case reports. If the case has previously been reported, the result is recorded with the person's existing record. Even after the case has been reported, the Public Health Division continues to accumulate reported results with the person's case information.

If a physician or other health care provider reports a new case of HIV or a test result indicative of HIV is received that does not appear to match a previous case, the information about

the “probable” case is forwarded to the local health department, which, in turn, contacts the provider and the patient to confirm the case, complete the case report and offer assistance with partner notification. Information collected for case reports includes information about date of earliest diagnosis, possible routes of HIV transmission (also known as ‘risk category’), HIV medication history, HIV care, residence, race and ethnicity, and age. If the person provides names and identities of recent sex partners, these are also recorded. All confirmed case reports are entered into a statewide integrated disease reporting system.

Study Aims

Low-cost, efficient, ongoing, state-level population-based surveillance for transmitted anti-retroviral drug resistance and distribution of viral subtypes among newly-diagnosed people has not been demonstrated in the U.S. Moreover, population-level estimates of transmitted antiretroviral drug resistance are not known for Oregon or for the Portland metropolitan area, and the characteristics of newly-diagnosed people with acquired transmitted drug resistance mutations (TDRM) are similarly unknown for the region. Accordingly, the aims of the current study were to:

1. Demonstrate public health capacity to estimate statewide levels of transmitted antiretroviral drug resistance via mandatory laboratory reporting of viral nucleic acid sequences from clinical drug resistance testing.
2. Estimate frequency of antiretroviral drug resistance among newly-HIV-infected people who have not yet been treated with antiretroviral drugs in Oregon during 2007–2011.
3. Describe the frequency of individual resistance mutations among people with TDRM.
4. Identify characteristics that distinguish newly-diagnosed cases of with transmitted antiretroviral drug resistance from newly-diagnosed cases without antiretroviral drug resistance. Specifically, is transmitted antiretroviral drug resistance among newly-diagnosed people in Oregon more common among:

a. men who have sex with men relative to women or men who have sex with women; people who have used injection drugs relative to those who have not used injection drugs;
b. people who are white, relative to people of other races;
c. Hispanics relative to non-Hispanics;
d. younger people relative to more aged;
e. people who have had previously reported cases of sexually transmissible infections relative to those who have not had previously reported cases of other sexually transmissible infections;
f. people who name a fewer number of traceable sexual partners at diagnosis relative to people who name more?

Research hypotheses and rationale

Antiretroviral drug resistance develops spontaneously in people being treated for HIV infection, not in people with HIV who have not yet been treated.[5] The occurrence of antiretroviral drug resistance in a newly-diagnosed person is a marker for infection transmitted from a source who is being, or has been treated. The proportion of newly-diagnosed, untreated cases with antiretroviral drug resistance is thus a function of the relative use in the community of each of the available antiretroviral drugs to treat cases of HIV, the ratio of cases treated to cases not being treated, adherence to treatment by people being treated for HIV infection, replication capacity of wild-type vs. resistant HIV strains, and the relative number of transmission opportunities (behaviors that confer risk for transmission) among people who are aware of their infection and being treated and those who are infected but have not received treatment.

We anticipated that the prevalence of TDRM in Oregon would be similar to reports from other cities and states. In particular, Wheeler and coauthors from the Centers for Disease Control and prevention found the proportion of newly-diagnosed cases with any class of TDRM to be

14.6 percent, the proportion with any transmitted NRTI resistance mutation to be 5.6 percent, the proportion with any transmitted NNRTI resistance to be 7.8 percent, and the proportion with any transmitted PI resistance mutation to be 4.5 percent.[16] Accordingly, we hypothesized that:

1. the proportion of newly-diagnosed Oregon HIV cases during 2007–2011 with one or more major resistance mutation from any of three classes would be 14.6 percent;
2. the proportion of newly-diagnosed cases with a mutation conferring resistance to nucleoside reverse transcriptase inhibitors would be 5.6 percent;
3. the proportion with a mutation conferring resistance to non-nucleoside reverse transcriptase inhibitors would be 7.8 percent; and,
4. the proportion with a mutation conferring resistance to protease inhibitors would be 4.5 percent.

Demographic and behavioral factors may be related to the likelihood of TDRM in a newly-diagnosed person. In order to have TDRM, a newly-diagnosed person must have been infected by a source who is already aware of his or her infection and receiving treatment. The relative likelihood that one's infection was acquired from someone who was aware of his or her own infection and receiving treatment vs. having acquired infection from someone who was unaware and not in treatment is probably closely related to one's sexual behaviors with different categories of partners, one's sexual network and the proportion of people with HIV disease and in treatment in that network. For example, treated HIV infection is likely to be substantially more prevalent within the sexual network of someone who is himself a man who has sex with men, leading to a higher proportion of antiretroviral resistance among newly-diagnosed HIV infections in men who have sex with men. Conversely people with HIV infection who also use injection drugs are less likely to be receiving care and potentially, less likely than other sources to transmit resistant virus. Other factors might be associated with TDRM such as race and ethnicity, age, type or number of named sexual contacts and past history of other reportable sexually transmissible

diseases because they might plausibly be related to sexual network and likelihood that the source case was aware of their infection.

Existing studies of predictors of transmitted antiretroviral resistance have focused on demographic and behavioral characteristics of cases. Investigators in South Carolina found TDRM to be associated with younger age at diagnosis and mode of transmission (i.e., men who had sex with other men were more likely to have TDRM) when they studies people tested at public HIV testing sites, but not those diagnosed at private healthcare sites.[23] Weinstock and colleagues reported that prevalence of transmitted resistance mutations was 11.6 percent among men who had sex with men but was only 6.1 percent and 4.7 percent among women and heterosexual men, respectively. In addition, transmitted resistance prevalence was approximately twice as high among African American and Hispanic participants. And, consistent with the presumptive mechanism of secondary acquisition of HIV drug resistance, resistance prevalence was more than 15 percent among persons who knew that their sexual partners were taking antiretroviral medications.[15] In contrast, CDC reported no differences in the demographic characteristics of patients with transmitted antiretroviral drug resistance when compared with their counterparts without such resistance in 10 US cities.[16] To date, no one has studied relationship between previous cases of other reportable sexually transmitted disease or number of traceable partners and transmitted antiretroviral drug resistance. Thus, we hypothesized that"

1. TDRM would be significantly more common among men relative to women;
2. TDRM would be significantly more common among men with a history of having had sex with men relative to men who do not report sex with other men;
3. TDRM would be significantly more common among people who use injection drugs relative to those who don't use injection drugs;
4. TDRM would be significantly more common among whites relative to non-whites relative to non whites and among white non-Hispanics relative to Hispanics;

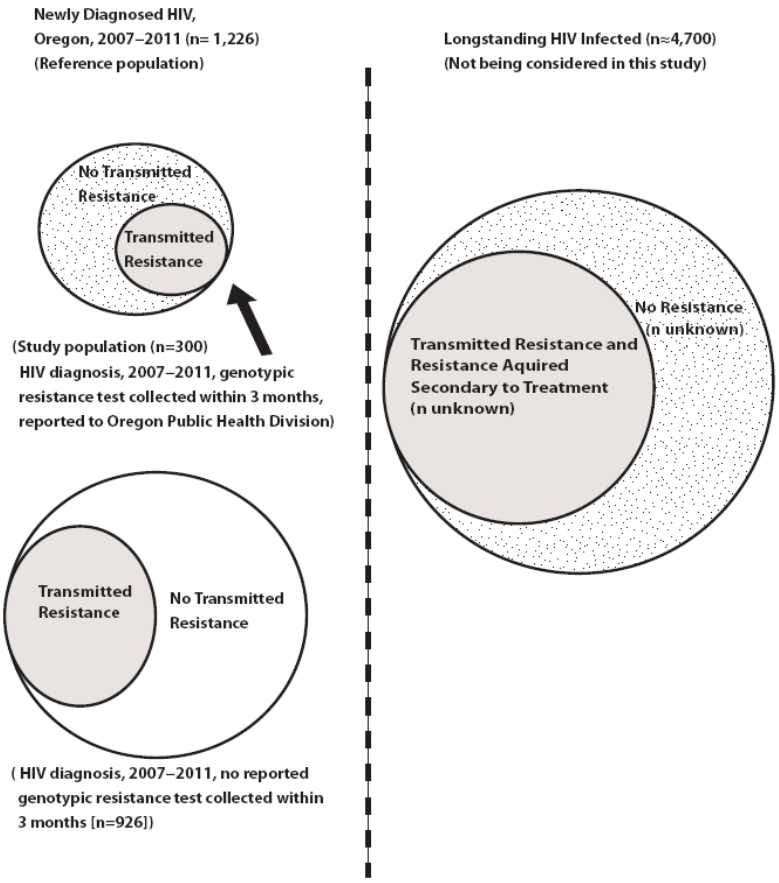
5. TDRM would be significantly more common among people aged less than 30 years relative to peopled aged at least 30 years; and
6. TDRM would be significantly more common among people with previously reported cases of sexually transmissible disease relative to those without previously reported cases of sexually transmissible disease.

Methods

Population

The study population included Oregon residents with reported cases of HIV infection diagnosed with HIV infection during 2007–2011 for whom a viral resistance test was collected within 3 months of diagnosis and subsequently reported to the Oregon Public Health Division (Figure 5).

Figure 5. Schematic of reference and study population for transmitted antiretroviral resistance surveillance, Oregon, 2007–2011.



Inclusion/exclusion criteria

To be included, cases must:

1) have been ≥18 years old at the time of HIV diagnosis;
2) have been a laboratory-confirmed case of HIV-infection occurring in an Oregon resident and reported to the Oregon Public Health Division;
3) have been reported during January 2007–December 2011; and
4) have an HIV RNA sequence reported to the Oregon Public Health Division collected within 3 months of diagnosis.

Data

The data for our analyses are comprised from two sources: 1) Public health case reports, and 2) reports of viral RNA sequencing done for purposes of genotypic resistance analysis and reported by clinical laboratories to the Oregon Public Health Division.

Case report data: As described earlier, public health case reports are completed by local public health staff after a probable case is identified, usually by a report of an HIV-related test by a clinical laboratory. Most information is obtained from the medical record held by the medical care provider. Case reports include information about date of earliest diagnosis, possible routes of HIV transmission (also known as 'risk category'), HIV medication history, HIV care, residence, race and ethnicity, and age. All case reports are entered into a statewide integrated disease reporting system.

Genotypic resistance reports: As described earlier, existing law in Oregon requires that laboratories report results of tests specific for and indicative of HIV to public health authorities. Laboratories that conduct more than 30 such tests must report testing data electronically through Oregon's electronic laboratory reporting system. During 2007, the Oregon Public Health Division surveyed HIV medical care providers and laboratories in Oregon and determined that three laboratories conducted meaningful numbers of genotypic antiretroviral resistance testing (GART) on behalf of HIV medical care providers in the state. One of these is the microbiology laboratory affiliated with a major integrated health system in Portland in Portland. Physicians affiliated with this system treat approximately 700 patients with HIV. The second laboratory is a national reference laboratory with regional labs around the U.S. and the third is also a national reference laboratory located in California that was acquired by the second laboratory during 2007, but operated under the original name until 2010. These two laboratories provide resistance testing for physicians treating approximately 4,000 Oregon patients

with HIV. The Public Health Division is not aware of any other laboratories conducting genotypic resistance testing of specimens from patients treated in Oregon. All 3 laboratories use "population DNA sequencing."[*] This process involves fragmenting and chemically labeling amplified DNA and determining the nucleotide sequence of the target *pol* gene region that encodes the reverse transcriptase and protease proteins. The lettered code is compared with consensus wild-type sequence, and mutations identified.[24] Each of the three reporting laboratories uses a different sequencing assay. These include "TRUGENE HIV-1," "HIV-1 GeneotypR PLUS," and "HIV-1 Genotype."[24] The three assays have high concordance.[24-26]

The Health Division worked with each laboratory to implement electronic reporting of viral sequences. All three laboratories reported these results for Oregon residents during 2007–2011. Viral sequences are stored with case report data in the electronic disease reporting system. Nucleic acid sequences are aligned and translated into amino acid sequences using Sierra, a program developed by the Stanford Online HIV Drug Resistance Database (HIVDB) team.[27] Mutations are in turn stored with the case report in the disease reporting system.

An analytic file was exported from the statewide disease reporting system. In addition to analytic variables listed below, the file contained a unique identifier for each individual whose test results were included among the data set. After the data were exported, the link from the disease reporting database to the analytic file identifier was destroyed. Other than county of residence, race-ethnicity, and age, the analytic file did not contain other personal identifiers such as name, street address or social security number. All analysis was completed on a password protected workstation located at the Oregon Public Health Division building in a restricted access area.

[*] Estimated numbers of patients in care by physicians affiliated with each laboratory based on estimates of patients in care during 2011 provided by Kari Greene, Project Coordinator for the Medical Monitoring Project, supplemental

Variables

Predictors. (obtained from case report): age at diagnosis, presumptive transmission category, sex, date of earliest diagnosis, HIV treatment history, number of recent partners reported, other reported infections that might have been sexually transmitted (viral hepatitis, shigellosis, syphilis, gonorrhea, giardiasis, chlamydia).

Outcomes. We considered four antiretroviral drug resistance outcomes: (1) presence of one or more nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or protease inhibitor (PI) mutations; (2) presence of one or more NRTI resistance mutations; (3) presence of one or more NNRTI resistance mutations, and (4) presence of one or more PI mutations. We considered a resistance mutation to be present if it was listed on a suggested global list of transmitted resistance surveillance mutations published in 2009.[28] In addition, we analyzed our data including mutations from a modified list used by Wheeler intended to optimize sensitivity to resistance mutations occurring among HIV subtype B.[16]

Analysis

Surveillance capacity and quality

To assess achievement of the first study aim—demonstrating public health capacity for transmitted antiretroviral drug resistance surveillance—we estimated reporting completeness and representativeness. We estimated reporting completeness by counting the total number of reports by laboratory and by year for all patients without regard to whether or not the test was a repeat test or an initial test collected more than three months after the date of diagnosis. We examined reporting trends by laboratory over time to make an empiric estimate of the expected number of tests conducted each year during the 5 years of observation then calculating the proportion of expected tests that had been reported. We estimated reporting representativeness by two

approaches. First, we counted the number of cases reported to the Oregon Public Health Division that had been diagnosed during 2007–2011. Then we calculated the proportion of cases diagnosed during 2007–2011 for which a genotypic resistance test collected within three months of the date of diagnosis had been reported to the Public Health Division. We also assesed representativeness of reporting by calculating and comparing frequencies of case characteristics among cases diagnosed during 2007–2011 for which a genotypic test conducted within three months of diagnosis and cases for which a reported genotypic test within three months of diagnosis was lacking.

Univariable

We calculated proportions of newly-diagnosed, treatment-naïve cases within several classes: 1) one or more transmitted resistance mutations associated with any of the three individual drug categories; (2) one or more transmitted NNRTI resistance mutations; (3) one or more NRTI resistance mutations; and (4) one or more PI resistance mutations. We calculated 95 percent confidence intervals for each and compared the proportion of cases with any resistance mutations to the null hypothesis (14.6 percent of cases with TDRM) using the exact method based on the binomial distribution.

Bivariable

Null hypotheses of no differences between cases with transmitted antiretroviral resistance and those without by behavior (men who have sex with men compared with men who don't have sex with men; people who use injection drugs compared with people who don't), race, ethnictity, age, past history of reported cases of sexually transmissible disease, number of traceable contacts reported were tested using the log-binomial model and the general linear model procedure in SAS® including only the single independent variable of interest and the Wald chi-square method to calculate p-values.

Results

Reporting completeness—genotypic resistance testing and reporting

During 2007–2011, the three major laboratories performing GARTs on specimens collected from Oregon residents reported 1,067 results for reported HIV cases in Oregon (Table 1) including tests of specimens collected from treatment naïve cases at any interval from diagnosis, repeat tests and cases already established in treatment. The total number of results reported increased from 175 during 2007 to 316 during 2011. Inspection of reporting by laboratories revealed that Laboratory A reported over 100 tests each year during 2007–2009, fewer during 2010 and none during 2011. Laboratory A underwent an equipment upgrade and internal security review during 2010–2011 leading to underreporting. They resumed reporting during 2012. Laboratories B and C reported not at all, and intermittently during 2007–2010 a time during which they were implementing regular sequence reporting using Health Level 7 (HL7) formatted messages and undergoing a merger. They each reported regularly during 2011. For estimation of expected reporting, we assumed that laboratory A was reporting completely during 2007–2009, and that laboratories B and C reported completely during 2011. We estimated that expected complete reporting would be the average of reporting by laboratory A during 2007–2009 (119 per year) and the total reported by laboratory B (175) and laboratory C (141) during 2011, yielding an expected average annual number of reports of 435 per year. Over 5 years, the expected number of reports was 2,175, and 1,067 were reported, an estimated overall completeness of reporting of 49 percent. However, during 2011, 316/435 (73 percent) of expected tests were reported.

Table 1. HIV Genotypic Resistance Test Reports by Laboratory and Year, Oregon 2007–2011.*

	Year					
	2007	**2008**	**2009**	**2010**	**2011**	**Total**
Laboratory A	128	108	121	86	0	443
Laboratory B	1	0	0	164	175	340
Laboratory C	46	79	0	18	141	284
Total	175	187	121	268	316	1067

***Includes tests performed on specimens collected from treatment-naïve cases and cases established in treatment.

Reporting representativeness—genotypic resistance testing and reporting

A total of 1,226 reported HIV cases among Oregon residents were initially diagnosed with HIV infection during 2007–2011, an average of 245 cases per year. Overall, 300 (24.5 percent) of these had GART results collected within 3 months of reported HIV diagnosis and reported to the Oregon Public Health Division. The number of reports of GART tests collected within 3 months of diagnosis increased from 17.1 percent of cases diagnosed during 2007 to 39.6 percent of cases diagnosed during 2011 (p for trend <0.0001) (Table 2). Among cases for which GART results within three months of diagnosis were available, proportions did not vary significantly by age, sex, sexual orientation, drug use history, or history of other reportable sexually transmitted infections. Proportions of cases for which GART results were available did range from eight percent among American Indians to 40 percent among Pacific Islanders, but numbers of cases among these groups were quite small and, overall, testing did not vary significantly by race (Table 3). The majority of reported cases diagnosed during 2007–2011 were white, male, non-Hispanic, and aged greater than 30 years. Overall, 70 percent of cases were men who reported having had sex with men at some point prior to HIV diagnosis. Fourteen percent of all diagnosed cases reported a history of injection drug use, and 22 percent had at least one other reported infection that might have been sexually transmitted.

Table 2. Reported genotypic resistance testing within three months of HIV diagnosis by year, Oregon 2007–2011.

Year	New diagnoses	GART* available (%)
2007	234	40 (17.1)
2008	274	51 (18.6)
2009	248	41 (16.5)
2010	240	77 (32.1)
2011	230	91 (39.6)
Total	**1226**	**300 (24.5)**

*Genotypic antiretroviral resistance testing collected within 90 days of date of HIV diagnosis and reported to Oregon Public Health Division

Table 3. Genotypic resistance testing collected within three months of HIV diagnosis and reported to Oregon Public Health Division, =2007–2011 (n=1,226).

	GART* within three months of HIV diagnosis		
Category	**No (%)**	**Yes (%)**	**Total**
All	926 (75.5)	300 (24.5)	1,226
Sex			
Female	114 (72.6)	43 (27.4)	157
Male	812 (76.0)	257 (24.0)	1,069
Race			
American Indian/Alaska Native	11 (91.7)	1 (8.3)	12
Black	62 (75.6)	20 (24.4)	82
Mixed	43 (79.6)	11 (20.1)	54
Pacific Islander	3 (60.0)	2 (40.0)	5
White	801 (75.3)	263 (24.7)	1,064
Ethnicity			
Hispanic	161 (76.7)	49 (23.3)	210
Non-Hispanic	732 (75.3)	240 (24.7)	972
Age Group			
<30 yr.	304 (78.4)	84 (21.7)	388
≥30 yr.	622 (74.2)	216 (25.8)	838
Any other co-morbid reportable infection**			
Yes	201 (74.4)	69 (25.6)	270
No	725 (75.9)	231 (24.2)	956
Man who reports sex with other men			
Yes	646 (75.5)	210 (24.5)	856
No	280 (75.7)	90 (24.4)	370
Injection drug use history			
Yes	130 (76.9)	39 (23.1)	169
No	796 (75.3)	261 (24.7)	1057

*Genotypic antiretroviral resistance testing.

** Including viral hepatitis, shigellosis, syphilis, gonorrhea, giardiasis, chlamydia

Proportion of cases with transmitted drug resistance mutations.

Overall, the proportion of newly-diagnosed cases with any TDRM was 17.3 percent (95% confidence interval: 13.2%–22.1%), not significantly different than the hypothesized value of 14.6 percent (Table 4). Among the 300 cases with reported results collected within 3 months of diagnosis, 248 (82.7 percent) had no TDRM, 44 (17.3 percent) had one or more mutations conferring resistance to a single drug class, 6 had mutations for resistance to two classes and 2 had at least one mutation for resistance within all three drug classes. Except for 2007, when 40 reports were received and a lower than average proportion of resistance (12.5 percent) observed, higher proportions of resistant cases were observed in years during which relatively fewer sequences were received. Temporally, more reports were received and lower proportions of resistance were observed during 2010 and 2011, compared with 2008 and 2009. Within drug classes, NRTI mutations were more common (12.3 percent of cases with any TDRM) during 2007–2011 than NNRTI mutations (5.7 percent) or PI mutations (2.3 percent). The proportion of NRTI mutations was significantly higher ($p<.0001$) than expected (5.6 percent), while the proportion of NNRTI and PI mutations were significantly lower ($p<.0001$ for both) than expected (7.8 percent for NNRTIs and 4.5 percent for PIs).

Table 4. Newly-diagnosed cases with transmitted drug resistance by year, Oregon, 2007–2011.[*]

Year	Any Class	NNRTI[**]	NRTI[†]	PI[‡]
2007	5/40 (12.5)	1/40 (2.5)	3/40 (7.5)	2/40 (5.0)
2008	12/51 (23.5)	4/51 (7.8)	10/51 (19.6)	1/51 (2.0)
2009	13/41 (31.7)	1/41 (2.4)	12/41 (29.3)	1/41 (2.4)
2010	12/77 (15.6)	6/77 (7.8)	6/77 (7.8)	2/77 (2.6)
2011	10/91 (11.0)	5/91 (5.5)	6/91 (6.6)	2/91 (2.2)
Total	52/300 (17.3)	17/300 (5.7)	37/300 (12.3)	8/300 (2.7)

*Numbers in table represent count of people with transmitted drug resistance mutations within class divided by the number of newly-diagnosed cases during with year with available early genotypic resistance testing results. Numbers in parentheses represent the calculated proportions.

**Non-nucleoside reverse transcriptase inhibitors.

†Nucleoside reverse transcriptase inhibitors.

‡Protease inhibitors.

Among NRTI mutations, TDRMs were observed at 8 positions (Table 5). Positions 41, 215 and 219 accounted for 86.5 percent of TDRM mutations. No other positions accounted for more than 4 TDRMs. Among NNRTI mutations, TDRMs were observed at 5 positions with position 103 accounting for 68.4 percent. No other position accounted for more than 2 TDRMs. Among PI mutations, TDRMS were observed at 9 positions; more than one TDRM was reported at only position 90.

Table 5. Transmitted Drug Resistance Surveillance Mutation Frequency, Oregon, 2007–2011[*]

Non-nucleoside reverse transcriptase inhibitor (NNRTI) (n=19)		Nucleoside reverse transcriptase inhibitor (n=59) (NRTI)		Protease inhibitor (n=12) (PI)	
Mutation	**Count (%)**	**Mutation**	**Count (%)**	**Mutation**	**Count (%)**
L100I	2 (10.5)	M41*KLM	1 (1.7)	L24I	1 (8.3)
K103KN	2 (10.5)	M41L	18 (30.5)	D30N	1 (8.3)
K103N	10 (52.6)	M41LM	1 (1.7)	M46I	1 (8.3)
K103S	1 (5.3)	D67E	1 (1.7)	I54V	1 (8.3)
V106AV	1 (5.3)	D67N	3 (5.1)	V82A	1 (8.3)
Y181I	1 (5/3)	T69ADNT	1 (1.7)	I84V	1 (8.3)
G190A	2 (10.5)	F77FL	1 (1.7)	I85IV	1 (8.3)
		M184AGV	1 (1.7)	N88D	1 (1.9)
		L210W	1 (1.7)	L90M	4 (33.3)
		T215C	3 (5.1)		
		T215D	1 (1.7)		
		T215DE	1 (1.7)		
		T215S	19 (32.2)		
		T215ST	1 (1.7)		
		K219E	2 (3.4)		
		K219KQ	1 (1.7)		
		K219Q	2 (3.4)		
		K219R	1 (1.7)		

Denominator is the number of sequences with any transmitted drug resistance mutation within the same class (NNRTI=19; NRTI=59; PI=12).

Subtype B was the most prevalent subtype, occurring in 95 percent of cases (Table 6). Among the 5 percent of cases with non-B subtypes only subtypes CRF01_AE(3) CRF02_AG(4) and G(3) were present in more than a single individual (Table 6). The Sierra database provided subtyping based on both the protease and the reverse transcriptase sequences. In 3 individuals, the subtype based on the protease sequence was D, while the reverse transcriptase subtype was B.

Table 6. HIV subtypes among newly-diagnosed cases, Oregon 2007–2011.

Subtype*	Count (%)
--/B	1 (0.3)
A/A	1 (0.3)
B/--	1 (0.3)
B/B	283 (94.4)
C/C	1 (0.3)
CRF01_AE/CRF01_AE	3 (1.0)
CRF02_AG/CRF02_AG	4 (1.3)
D/B	3 (1.0)
G/G	3 (1.0)

*[based on protease gene]/[based on reverse transcriptase gene]

Predictors of transmitted drug resistance mutations.

Among the 300 newly-diagnosed people for whom resistance testing within 3 months of diagnosis was reported no statistical differences among those with and those without resistance mutations were observed (Table 7). Point estimates of relative resistance prevalence were generally of low magnitude except for point estimate of 4.8-fold higher prevalence among men who had sex with men relative to men who did not report sex with other men, though only 24 men did not report sex with other men.

Table 7. Any transmitted drug resistance mutation by age, sex, race, ethnicity, other reported sexually transmitted disease, behavior, Oregon, 2007–2011.

Characteristic	Number with TDRM* (%)	RR**	p-value
Age group (yrs.)			
≤29	17/84 (20.2)		
≥30	35/216 (16.2)	0.95 (0.84–1.08)	0.43
Sex			
Female	5/43 (11.6)		
Male	47/257 (18.3)	1.57 (0.66–3.73)	0.30
Race			
Non-white	3/35 (8.6)		
White	48/263 (18.3)	2.13 (0.70–6.47)	0.18
Ethnicity			
Non-Hispanic	44/240 (18.3)		
Hispanic	5/49 (10.2)	0.56 (0.23–1.33)	0.19
Other reported STD			
No	43/231 (18.6)		
Yes	9/69 (13.0)	0.70 (.36–1.36)	0.30
Injection drug use			
No	36/215 (16.7)		
Yes	8/39 20.5)	1.34 (0.56–3.21)	0.51
Sex with men (males)			
No	1/24 (4.2)		
Yes	42/210 (20.0)	4.8 (0.69–33.32)	0.11

*Transmitted drug resistance mutations.

**Relative risk

Discussion

We aimed to demonstrate public health capacity to estimate statewide levels of transmitted antiretroviral drug resistance via mandatory laboratory reporting of viral nucleic acid sequences from clinical resistance testing. We were able to identify three laboratories that conducted these tests on behalf of Oregon patients and to induce all three laboratories to commence electronic reporting. We established the capacity to collect raw nucleic acid sequences and store these with other disease reporting data, and to exploit an internet–based resource to translate the sequences into mutations that could than be compared to a published list of suggested surveillance mutations. From 2007 through 2011, we collected reports for nearly half of the expected number of tests. Except for one year, the number of tests reported increased annually. Among newly-diagnosed cases, a resistance test collected within three months of the diagnosis was available for 24.5 percent during 2007–2011. However, the proportion of newly-diagnosed cases for which an early resistance test was available increased over time: among cases diagnosed during 2011, a resistance test collected within 3 months of diagnosis was available for 39.6 percent. Newly-diagnosed cases with a resistance test collected within three months of diagnosis were comparable to their counterparts without a reported early resistance test. These findings argue for the completeness and representativeness of the newly established surveillance system and ultimately our capacity to sustain the system.

Current national treatment guidelines recommend that medical treatment providers obtain GART at the initial visit for all newly-diagnosed patients.[29] As this recommendation is increasingly followed by providers and reporting improves, we expect that the proportion of cases with GART reported within 3 months of diagnosis will increase. However, the underlying proportion of cases with GART testing completed within 3 months of diagnosis is unknown.

Undoubtedly, some newly-diagnosed patients do not enter care within 3 months of diagnosis and others who do enter care rapidly do not receive resistance testing within such a brief interval.

In our population, transmitted resistance to commonly used antiretroviral drug classes occurred in 17.3 percent of cases tested within 3 months of HIV diagnosis in Oregon during 2007–2011. This is not significantly different than *a priori* estimates of 14.6 percent, based upon published data from 10 U.S. states by Wheeler, et al. that included data from the state of Washington, Oregon's neighbor to the north. Our findings are also comparable to recent estimates of transmitted antiretroviral resistance from South Carolina for a similar period of time.[16, 23] However proportions of mutations within classes varied substantially from expected based on the Wheeler data. In particular NRTI mutations were about 40 percent more prevalent than expected. Also, we observed wide variation in resistance prevalence by class and by year. For example, during 2009, 29.3 percent of newly-diagnosed cases had NRTI resistance compared to only 6.6 percent of newly-diagnosed cases with NRTI resistance mutations during 2011. Ultimately, the reason for our finding of proportionally greater overall NRTI resistance and the inter-year variation is not known. Random variation is certainly a possibility. Being a low-incidence state, we experienced relatively small numbers of newly diagnosed cases during the observation period.

Misclassification is possible explanation as well. We used the reported date of diagnosis from the case report. To the extent that cases were diagnosed earlier than the recorded diagnosis date they would have been misclassified as newly-diagnosed within 3-months. Such misclassification might have led us to count previously treated cases as treatment-naïve. If our findings of greater regional transmitted NRTI resistance are valid, they might reflect unrecognized regional U.S. or temporal differences in selection of antiretroviral treatment regimens for patients in treatment. Another possible explanation is a circumstance in which one or a small number of treatment-experienced cases with extensive NRTI resistance were responsible for a disproportionate number of transmissions—a cluster of related infections.

The distribution of individual TDRMs that we observed was very similar to that reported by Wheeler, et al.[16] We observed 42.4 percent of cases with any NRTI mutation to have a mutation at position 215 and 33.9 percent to have a mutation at position 41very comparable to the 42.8 percent at position 215 and the 31.5 percent at position 41 observed by Wheeler, et al. Mutations at positions 41, 210 and 215 comprise one common pattern of thymidine analogue mutations.[28] Effect of thymidine analogue mutations is cumulative. Generally, if a person has fewer than three, some susceptibility to NRTIs in general and to thymidine analogues in particular is retained. We observed no instances of 69 insertion complex, and 151 complex, NRTI resistance patterns that are known to be rare.[30]

We observed that 68.4 percent of cases with any NNRTI mutation had a mutation at position 103. Mutations at position 103 (K103N and K103S) confer resistance to 2 of the 4 drugs in the NNRTI class. All of the observed transmitted NNRTI mutations confer resistance to at least 2 of the 4 drugs in this class. Y 181 confers resistance to all 4.[30]

PI mutations were uncommon; L90M was observed in 4 patients. L90M confers broad cross class-protease inhibitor resistance, as do several of the other protease mutations observed, albeit only one instance of each.[30]

We found no significant differences among cases with and cases without transmitted antiretroviral resistance. The point estimate for prevalence of transmitted resistance among men who acknowledged sex with other men was nearly five times greater than the estimate for transmitted resistance among men who did not report sex with other men. This statistic approached statistical significance. However, only 24 of the 234 newly-diagnosed men for whom this information was available did not report sex with other men. Several earlier authors did conclude that transmitted antiretroviral drug resistance was more common among men who reported sex with other men than among those who denied sex with other men, probably because this group is more likely to have acquired HIV from a source who was aware of his infection and in treatment. Somewhat to our surprise, transmitted resistance mutations were not more common

among newly-diagnosed people who had had another reported infectious disease that might have been sexually transmitted than it among people without another reported infection. In fact, though not statistically significant, the group with another reported sexually transmissible disease was slightly less likely to have transmitted drug resistance.

Our study is subject to limitations. In particular, selection bias is a possibility. Only 300 tests were available among over 1,200 new cases. However cases with available tests were quite comparable to cases without such testing. In addition, the proportion of new cases with reported tests collected within the first 3 months after diagnosis increased over the course of the project to 39% during 2011. As the proportion of new cases increases, the potential for selection bias naturally declines..

Another possible source of bias is misclassification. In particular, if the date of diagnosis was incorrectly reported, then cases classified as newly diagnosed might have been infected for longer and possibly already treated with antiretrovirals. This would have biased our estimate of transmitted resistance upward. Future efforts will need to focus on validating the case report date of diagnosis by confirming it with the patient and treating physician in a subsample.

Case report data were frequently missing for behavioral attributes. For example, though we didn't report it here, we initially wished to analyze the relationship between an existing case report variable about whether a person had a sex partner who was known to be HIV positive and the presence of transmitted antiretroviral resistance. However this variable was quite often missing, limiting its utility for this purpose. Our data about history of sex with men and injection drug use were similarly limited but to a lesser extent. This is likely to be an inherent limitation to case report data. Nevertheless, resistance surveillance would benefit from optimally complete and accurate case report data

Use of different genotyping assays by each of the three reporting laboratories is another potential source of error in estimation of proportions of newly-diagnosed cases with resistance within and across antiretroviral drug classes. We believe such error to have been small. All three

labs use deoxynucleotide population sequencing approaches that have been demonstrated to have high concordance.[24-26]

In addition, we studied, as did all of our predecessors, NRTI, NNRTI, and PI mutations. However, integrase is now an option for a backbone therapy for treatment naïve patients. Some transmitted resistance has already been observed. Integrase is coded on the *pol* gene and reported by Stanford. We intend add integrase resistance surveillance in future reports. Fusion inhibitors are not used in patients without extensive resistance. Resistance to binding inhibitors is tested by another mechanism and will require us to ask our laboratories to separately report this test.

Conclusion

This is the first demonstration of the use of statewide electronic laboratory reporting of HIV genotypic testing results combined with standard case surveillance to conduct systematic surveillance of antiretroviral drug resistance. This was accomplished without special funding for this purpose, suggesting that is feasible to implement in other states under existing HIV surveillance funding. By collecting genetic sequences from reporting labs, translating these using an available web-resource built for this purpose, and comparing the mutations to a published list of mutations recommended for surveillance, we were able to compile estimates of the proportion of newly-diagnosed people in Oregon with important antiretroviral drug resistance. Retaining the sequences allows for reanalysis should existing polymorphisms be added in the future to the list of significant resistance mutations. In addition, genetic sequences offer the potential for examining strain relatedness in the context of epidemiologic investigation of outbreaks or clusters.

Table 7. Any transmitted drug resistance mutation by age, sex, race, ethnicity, other reported sexually transmitted disease, behavior, Oregon, 2007–2011.

Characteristic	Number with TDRM* (%)	RR**	P-value
Age group (yrs.)			
≤29	17/84 (20.2)	1.25 (0.74–2.10)	
≥30	35/216 (16.2)	Ref.	0.43
Sex			
Female	5/43 (11.6)	0.64 (0.27–1.51)	
Male	47/257 (18.3)	Ref.	0.30
Race			
Non-white	3/35 (8.6)	0.47 (0.15–1.43)	
White	48/263 (18.3)	Ref.	0.18
Ethnicity			
Non-Hispanic	44/240 (18.3)	0.56 (0.23–1.33)	
Hispanic	5/49 (10.2)	Ref.	0.19
Other reported STD			
No	43/231 (18.6)	1.07 (0.96–1.19)	
Yes	9/69 (13.0)	Ref.	0.30
Injection drug use			
No	36/215 (16.7)	Ref.	
Yes	8/39 20.5)	1.34 (0.56–3.21)	0.51
Sex with men (males)			
No	1/24 (4.2)	Ref.	
Yes	42/210 (20.0)	4.8 (0.69–33.32)	0.11

*Transmitted drug resistance mutations.

**Relative risk

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