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Transmitted antiretroviral drug resistance in a low HIV prevalence setting

Thuy Thi Vu Nguyen
University of Iowa

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TRANSMITTED ANTIRETROVIRAL DRUG RESISTANCE IN A LOW HIV
PREVALENCE SETTING

by

Thuy Thi Vu Nguyen

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Epidemiology
in the Graduate College of
The University of Iowa

July 2012

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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To my family and loved ones

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ABSTRACT

Background: Antiretroviral drug resistance is steadily growing in populations of HIV treatment-naive individuals due to person-to-person transmission. However, Iowa-specific data for transmitted antiretroviral drug resistance-associated mutations prevalence has not been previously reported. We postulate that the prevalence of drug resistance in Iowa does not differ significantly between HIV risk groups.

Methods: Data were collected from electronic medical records and an HIV Program database between 2006 and 2011. Information included age, gender, risk exposure group, viral load, CD4 count, CD4%, and other HIV risk factors and behaviors.

Results: Transmitted drug resistance mutations (TDRM) were not associated with many risk factors, but rapid plasma reagin (RPR) screening for syphilis was significant ($p=0.02$) and used as a proxy for highest level of sexual risk behavior. RPR was used with minor NRTI and NNRTI along with intravenous drug use in logistic regression to model the likelihood of acquiring TDRM.

Conclusion: Some question the practicality of implementing genotypic ARV resistance testing guidelines because of uncertainty about the prevalence of ARV drug resistance among treatment-naïve patients but harboring resistance mutations puts patients at high risk of failing effective, first-line therapies. Hence, genotypic resistance testing at HIV diagnoses can not only improve disease management but also assist in surveillance.

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CHAPTER 1

INTRODUCTION

Background and History

The human immunodeficiency virus (HIV), a member of the retrovirus family, shares common characteristics with other ribonucleic acid (RNA) viruses such as the high mutation rate, number of offspring and small genome. Its mutation rate is 0.1-0.3 mutations per genome per replication cycle making it one of the fastest evolving organisms. Its poor replication fidelity is due to the error-prone reverse transcriptase, and template switching between the RNA genomes, which is estimated to occur 2-3 times per replication cycle. These high production and replication rates promote recombination and mutation leading to HIV genetic diversity in both the individual and the population [1]. HIV exists as a swarm of many genetically distinct strains, a quasi-species [2].

HIV-2 groups A and B are predominantly found in West Africa and global spread has been relatively limited. HIV-1 group M viruses, however, have been far more epidemiologically successful in persisting worldwide. Group M is divided into nine subtypes (A, B, C, D, F, G, H, J, K) with over 40 circulating recombinant forms (CRFs) [1,3].

The HIV-1 epidemic is a combination of old and new lineages with four predominant subtypes and two CRFs in circulation: Subtypes A-D and CRF01_AE and CRF02_AG, respectively. Other strains may have emerged from Africa but their dispersal was limited due to chance and fitness costs [1]. The subtype of HIV most commonly found in North America is clade B. Only approximately 3-5% of HIV in North America is non-B [3].

Evolution of a virus depends upon the rate of infection and epidemic spread in the population. Furthermore, its in-host evolution depends on the environment, which varies throughout the infection. New viral variants may arise and be neutralized by the humoral

immune response; however, it lags and fails to attack the even more contemporary strains [1].

Selective pressure by the host can propagate at the population level, for instance, transmitted drug resistance (TDR) [1]. Highly-active antiretroviral therapy (HAART) was introduced in 1996 and has dramatically increased longevity and decreased opportunistic infections making HIV a manageable chronic infection [1,4,5]. Antiretroviral therapy (ART) increases immune function and the recovery of immune system impairment [6]. Additionally, it has been shown to decrease the risk of HIV transmission [4]. Therapeutic options, however, are limited to drugs within five classes [1]: Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors (PI), Integrase Inhibitors, Fusion Inhibitors and CCR5 Antagonists. Resistance will likely continue to spread as antiretroviral (ARV) drugs reach more developing countries [7].

Transmissibility depends on infectivity and duration. Infectivity is greatest during the early and late stages of the disease where viral loads are highest. It is estimated that 5-50% of infections stem from individuals newly infected themselves [1]. Furthermore, infection risk depends upon viral quantity, STI coinfection, route of transmission and behavioral characteristics [1].

Stages of infection rely on the distinct patterns of evolution of HIV within the host. Positive or Darwinian selection is the evolution of HIV with the host immune response. It continuously selects for specific variants over the course of infection. Large variability in strains indicates that many mutations are tolerated [1].

Additional resistance may be compensatory and restore replication of formerly drug resistant variants to wild-type levels. This depends on host genetics, genetic barriers, treatment adherence, and replicative function [1]. Since drug resistant mutations come at a fitness cost, reversion to wild-type may confer survival advantage. For the example of a single drug resistant variant, back mutation is required instead of the re-

emergence of the wild-type strain [8]; however, some revert to wild-type over a relatively short time while other strains require many years. This may be due to differences in replicative fitness and compensatory mutations [1]. TDR in treatment-naïve patients usually differs from the original strain transmitted. The transmitted strain likely had more drug resistance mutations but either experienced partial or full reversion [1]. The rate and frequency of this backward evolution is important since most HIV-infected people present with chronic rather than early or acute infection; thus, resistant variants have had an opportunity to revert and become minority variants undetectable through genotypic assays [1,8].

In the HIV TDR variant, the virus becomes established in reservoirs after inoculating the majority of target cells [9]. Memory CD4 T-cells that are infected may harbor long-lived variants and act as a viral archive. These archived variants may re-emerge with sufficient drug pressure, both in wild-type and resistant strains. The re-appearance of archived resistance has been associated with virologic failure [1]. However, not all patients with resistance mutations experience virologic failure [10].

Resistance testing is recommended for all HIV patients at diagnosis regardless of whether ARV therapy will be initiated immediately [5,7]. The persistence of some mutations varies; thus, having a baseline will aid in therapy selection later to avoid suboptimal treatment as a result of archived mutations [7].

The most popularly prescribed first-line ARV regimens are NNRTI-based [10]. They boast high success rates, but genotyping would enable therapy customization to avoid treatment failure and the further accumulation of resistance mutations [10]. The current body of knowledge shows the effectiveness of using 2 NRTIs and 1 drug from another ARV class [4,5]. Single-dose fixed-formulations are the preferred form of initial therapy due to their convenience [4].

While TDR reduces the efficacy of traditional ART therapies, it is further complicated by cross-resistance within each ART class in the clinical management of

second-line therapy [1]. Estimated resistance in the US is between 1-27% and 2-52% in Europe [1,3,4,5,6,9,10]. Additionally, developing countries are seeing increasing numbers of TDR cases as ART becomes more readily available [1].

The clinical prevalence of TDR is important in the use and management of multi-drug resistant HIV; poor virologic responses to initial therapy may be a detriment to reduced disease progression and is likely to enhance secondary transmission. Empirical recommendations for treatment are therefore insufficient without guidance of clinical TDR prevalence [6]. This is related to the proportion of TDR, which indicates a treatment-experienced population and that transmission of resistance is common [9]. As ART becomes available globally, understanding the proportion, prevalence and transmission of drug resistance will become important in ensuring the sensitive and accurate detection of resistant strains for diagnoses and treatment [2,9].

Objective

Drug resistance to first-line HAART or combination ARV therapies (cART) is steadily growing in treatment-naïve individuals due to person-to-person transmission of HIV strains with resistance-associated mutations [2,11]. These transmitted drug-resistance mutations (TDRM) have been encountered in the U.S. previously, but recently, its prevalence has increased [12]. In New York, NY, Shet et al discovered a TDRM rate of 24.1% between 2003 and 2004; however, other studies report rates varying from 8-23% [13]. This is clinically significant since TDRM among recently infected and treatment-naïve HIV patients delays virologic suppression [7].

There is, conversely, a major gap in knowledge about the proportion of TDRM in low HIV prevalence states. Data for the prevalence and type of TDRM are not reported. The proportion of ARV-naïve patients in Iowa carrying TDRM variants for the major ARV drug classes is unknown; hence, the health risks for utilizing these agents without resistance guidance require determining the TDRM frequency in low-HIV prevalence

regions. The 2010 guidelines set forth by the International AIDS Society-USA (IAS-USA) recognize that fixed-dose, once daily cART formulations are frequently prescribed as first-line therapies [4]. The cART regimen of emtricitabine, tenofovir, and efavirenz (trade name: Atripla®) that has become popular among Iowan providers and patients in recent years consists of two NRTIs with one NNRTI [J.L. Meier, personal communication, 2011].

Resistance to NNRTI agents are of particular concern since studies have reported stable rates of multi-drug resistance transmission rates for all drug classes except for NNRTI-resistance which has increased since 2005 [7].

We studied the clinical prevalence of drug resistance mutations in ARV-naïve, HIV-infected persons in Iowa and anticipated that, it is similar to regions with moderate-to-high HIV prevalence. We further postulated that the majority of resistance is to the NNRTI class of ARV therapies and that ARV drug resistance does not differ significantly between HIV risk groups and among the various risk factors.

CHAPTER 2

METHODS

Study Design

This retrospective cohort research study assessed a population of treatment-naïve patients. Subjects with known HIV infection were followed in time from the point of first clinical contact to whether their HIV strain was assayed for genotypic resistant to currently prescribed ART. Additionally, those that experienced TDRM were further analyzed.

The cross-sectional nature of this particular study design derives from the single data collection point. Information was abstracted on if there was evidence of a lab order for genotyping and whether results were available. Thereafter, no further data was gathered.

Population

A list of adult patients (≥ 18 years of age) new to the University of Iowa Hospitals and Clinics (UIHC) AIDS/HIV Clinic during January 1, 2006 to December 31, 2011 was screened for eligibility (N=372). Eligibility included a confirmed diagnosis of HIV-1 positive status (with or without a concurrent diagnosis of AIDS, defined as a CD4 count ≤ 200 cells/mm³ or >200 cells/mm³ with an AIDS-defining illness within 30 days of HIV-diagnosis) and deemed ARV treatment-naïve at their initial clinical visit. This was determined by standardized patient intake procedures including testing for viral load, CD4 count, and CD4%. Exclusion criteria comprised patients whose ARV status could not be determined or who were returning patients (n=210). Additionally, we excluded those patients whose medical records were incomplete because of loss to follow-up. Figure 1 shows the flowchart for study participant selection. The Institutional Review Board at the University of Iowa approved the protocol and the waiver of informed consent.

The demographic characteristics are shown in Table 1. These, in addition to baseline, laboratory and genotypic resistance results were abstracted from electronic medical records and the UIHC AIDS/HIV internal clinic database. Data for other risk factors collected appear in Tables 2-6 and included a history of illegal substance abuse (not including intravenous drug use), alcohol use and sexual transmitted infections (STI). Patients were classified by risk group exposure as men who have sex with men (MSM), injection drug users (IDU), heterosexual, or a combination risk group as depicted in Table 1.

Genotypic resistance testing is requested at the baseline clinical visit or prior to ARV initiation. Laboratory testing is performed to establish baseline values and recorded in patient electronic medical records. The major resistance types evaluated were the PI, NRTI and NNRTI classes of ARV drugs as dictated by the IAS-USA Panel which are displayed in Tables 7 and 8 [4,7].

To examine the corollary between transmitted drug-resistant mutations between low and high HIV prevalence regions, rural and urban classifications were based on zip codes and the corresponding definitions by the University of Washington Rural-Urban Commuting Area (RUCA) Codes. RUCA codes utilize U.S. Census tract Urbanized Area and Urbanized Cluster data in conjunction with work commuting data. It grades the transition from rural to urban and increases the sensitivity relative to county-level U.S. Census taxonomies [14].

Statistical Analysis

Univariate analyses were performed on the continuous variables of age, CD4 count, CD4% and viral load. Ages were grouped based on clusters of patients, whereas clinically significant cut points were used to categorize CD4 count, CD4% and viral load. Each variable of interest was compared among patients with and without resistance through univariate analyses after adjusting for covariates. These are shown in Table 1.

Patients were classified according to RUCA code definitions for the rural or urban classification strata and an association was surveyed based on TDRM. Cochran-Mantel-Hansel chi-square tests were performed on categorical data whenever possible; however, when expected frequencies were <5 , Fisher's Exact tests were used as an alternative. The Cochran-Armitage trend test was used to test for trends in non-major ARV, or minor, drug mutations. We also tested for heterogeneity among the major ARV resistance classes as well as for rural/urban classification, risk groups and behaviors. We determined statistical significance by using $\alpha=0.05$ to reject the null hypothesis and two-tailed tests for significance.

Logistic regression was used to test the association of compromised host immune status characterized by CD4 count and CD4% with viral load, risk groups and behaviors, and rural/urban classifications to model the likelihood of TDRM while controlling for the covariates of age, gender, race and ethnicity. Significant interactions for proxy risk behaviors such as the rapid plasma reagin (RPR) screening tests for syphilis with mutations to specific drug classes were considered. However, due to the small number of patients who tested positive for the RPR test, this interaction was omitted.

Propensity scores were used to account for the bias due to observational studies and to derive associations by controlling for the covariates of sex, age, exposure group, CD4 count, viral load, race, ethnicity and RUCA codes. The resulting model was contrasted with the logistic regression model. Propensity scores predicted the likelihood that a patient would be categorized as healthy or high-risk based on CD4% using the cut points of $\geq 29\%$ for normal and $< 29\%$ for at risk [18]. Analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

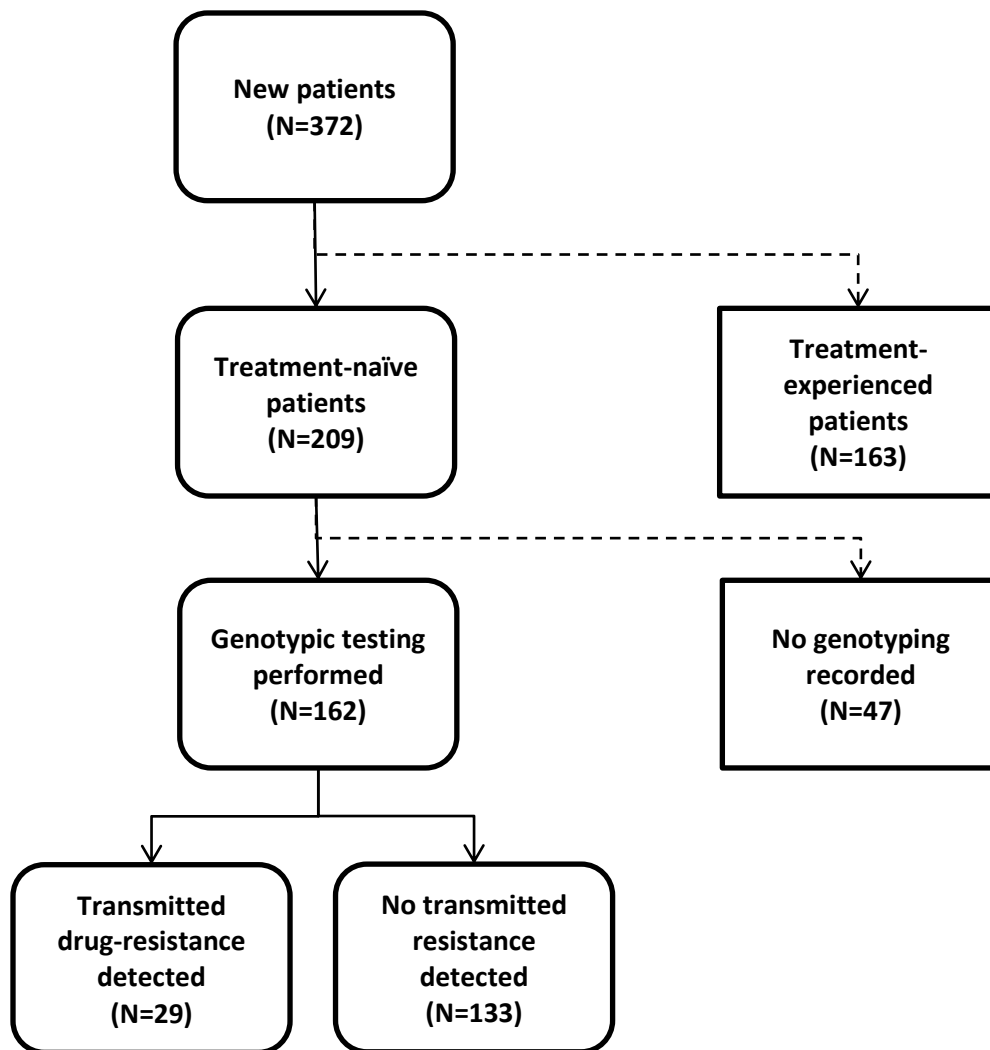


Figure 1 Flowchart of subject selection

CHAPTER 3

RESULTS

Study subjects were predominantly non-Hispanic White. There were a total of 131 males (81%) with an average age of 33.7 (± 9.8) years. The remainder included 31 females (19%) with a mean age of 34.5 (± 11.7) years. The mean CD4 T-lymphocyte count, CD4% and viral load for all subjects were 313 (± 209), 20% ($\pm 10\%$) and 220,000 ($\pm 490,000$), respectively.

The most common exposure was through MSM. Heterosexual exposure was the second most common and selected as the reference for being the lowest risk exposure group out of the MSM, IDU and combination exposure groups. There was no association between TDRM and exposure group.

The association of TDRM to the demographic characteristics for subjects is shown in Table 1. Every variable for each table shows an overall ordered p-value. The second set of p-values represents the association The age group with the highest frequency of cases was in the 25-34 group; however, the proportion with the highest TDRM was the ≥ 55 age group. Regardless, TDRM was not significantly different amongst age groups (overall $p=0.20$).

As shown in Table 2, most subjects with TDRM had CD4 T-lymphocyte counts ≤ 200 cells/mm³ which indicates severe disease stage and it is likely that these patients had viral loads $\geq 100,000$ copies/mL. However, neither of these characteristics—in addition to CD4% ($p=0.92$)—was significant to the association with TDRM ($p=0.74$, $p=0.34$, respectively).

While there were not significant differences between TDRM groups based on urbanicity or rurality (overall $p=0.44$) based on the RUCA coding shown in Table 3, subjects lived primarily in or near urbanized areas or clusters. Urban areas generally encompass an urban nucleus of $\geq 50,000$ people or a population density of 1,000 persons

per square mile. Urban clusters are defined similarly with the exception that they comprise at least 2,500 but less than 50,000 people [14].

Patients who had combination risk group exposure were dichotomized as either having had any combination of exposure (MSM, heterosexual and/or IDU) or none. There was not significant relationship ($p=0.63$); nor was there a relationship ($p=1.00$) when the combination risk group was categorized into mutually exclusive secondary exposures of either IDU or heterosexual. These were the only secondary options patients exhibited. Results are detailed in Table 4.

Risk factors for HIV infection and TDRM were taken from patients' medical records at their baseline clinical visit. These risk factors were based on behavioral and quantifiable aspects, which were measured through laboratory assays such as genotypic resistance and RPR testing.

Table 4 shows of those who were genotyped and whether patients exhibited possible or confirmed transmitted resistance mutations. Additionally, Figure 2 compares the frequency of TDRM in those that have been genotyped over the five year study period.

There was one instance of a patient that was not genotyped at baseline but had TDRM. This case was unique since the patient acquired HIV infection but by his second clinic visit, he had indicated he was stable on ARV therapy without the clinician having initiated specific ARV agents. He was formally prescribed an initial drug regimen when a genotypic resistance lab test was ordered after which it was discovered he potentially harbored resistance. Whether the patient was truly on therapy or the resistance was acquired or transmitted is unknown, but the time lapse between the first and second visit would have been insufficient for resistance to have become acquired.

Screening positive on the RPR test for syphilis was significantly associated with TDRM ($p=0.01$). Table 4 indicates that the RPR test overall was statistically significant ($p=0.02$) even though only a small number of cases tested positive for the screening.

Specific risk behaviors and characteristics that were tested are displayed in Table 5. There was no significant association among TDRM with tobacco, illicit drug, intravenous drug or alcohol use. There was also no relationship with the quantity of cigarettes smoked per day or with history of incarceration. There was borderline significance ($p=0.07$) among those with a history of STI. These included patients who were identified as having tested positive for an STI while under patient care or having self-reported prior infection.

Patients who reported exposure status on current or historic use of illicit drugs were categorized by specific illicit drug classes displayed in Table 6. It is interesting to note that 50% of those who took opioids and 50% of those who took prescription drugs had TDRM, while 100% of those who participated in taking club drugs also had TDRM. Despite these proportions, the actual frequencies were small (<5).

The estimated proportion, or clinical prevalence, of TDRM in the study population was 14% (95% Confidence Interval: 13.2,15.0). The proportion of TDRM in each major ARV class is presented in Table 7. The largest proportion of resistance was to the NNRTI class at 10.5%. Figure 3 presents the major mutations within each drug class that confers resistance to at least one FDA-approved ARV drug.

Minor mutations are recognized to occur, but they do not confer resistance to any ARV drugs. The relationship of these minor mutation to TDRM was statistically significant ($p<0.0001$) particularly for the NNRTI ($p<0.0001$) and NRTI ($p=0.01$) minor mutations (Table 8). An overwhelming majority of patients without transmitted major drug class resistance had acquired minor mutations to reverse transcriptase (92.9%) and/or PI (79.6%) genes.

The variables selected for the final logistic regression model is shown in Table 9. The overall fit of the model was excellent with a ROC c-statistic of 0.938 ($p=0.001$). The Hosmer-Lemeshow test was able to be rejected ($p=0.8817$) which indicated the goodness of fit of the proposed final model.

The large 95% confidence intervals for the odds ratios can be attributed to the small sample size due to the rarity of TDRM and a positive RPR screening test. RPR was kept in the model though it was not statistically significant due to its clinical significance as a proxy for the highest sexual risk behavior.

The probability of exposure is the propensity score for CD4 percentage. It enabled adjustment for factors that may contribute to differences between risk groups such as sex, age, exposure group, ethnicity, race, CD4 count, viral load and RUCA code. The mean of the propensity in the high risk group was greater than the normal group, 0.89 and 0.49, respectively. The maximum values for each category were close in magnitude, 1.00 and 0.97, respectively.

Several variations of the original model were modified by using the propensity score and other covariates. The propensity scores and the likelihood of a poor outcome were designated by the CD4%. The CD4% and propensity scores were tested with the variables that represented the propensity score to ensure it accounted for sex, age, exposure group, CD4 count, viral load, ethnicity, race and RUCA codes. Each of these variables was not significant; however, age was borderline significant with a $p=0.06$ (data not shown). Table 10 shows the parameter estimates and the associated p-values for the model with only the propensity score and CD4%. This model is not meaningful except for in comparing with the other models.

The CD4% and the propensity score were added to the model in Table 9. The most statistically non-significant variables were removed and the model was re-analyzed. The results of which are shown in Table 11 (Model A) for comparison with the model in Table 10. The omitted variables were for minor NNRTI and minor NRTI mutations, and these were removed after the first and second iterations, respectively. This was because the p-value for the minor

NNRTI was 0.99 and for the minor NRTI was 0.84 when minor NNRTI was first included and when it was not in Model A.

An analysis was performed after removing the RPR variable, but was chosen to be retained. RPR was kept in the model since the magnitudes of the parameter estimates for CD4% and the propensity score did not change by more than 4-6%. The magnitude of intravenous (IV) drug use decreased by approximately 26% while its p-value and the propensity score increased. The p-value for the CD4% did not change in magnitude by more than approximately 10%.

Lastly, another propensity score model (Model B) was analyzed using more variables for comparison. Visual inspection of the data allowed the inclusion of parameters with the least missing patient values. This included the covariates for the propensity score in Model A with the addition of whether a patient had been genotyped, currently uses alcohol, had ever been a smoker and had a history of any illicit drug use (excluding IV drug use). As before, the analyses included the variables RPR and IV Drug Use. Model B is shown in Table 12. RPR was not significant and was initially excluded, but Model B reflected the same trends as Model A, that is, dramatic increases or decreases in the magnitude and p-values were not observed for the CD4%, propensity score and IV drug use. The p-value for Model B was unchanged from the overall p-value given in Table 12. As a result the RPR variable was retained for full comparability to the parameter estimates and p-values in Table 11.

The c-statistic for each of the models containing the propensity score and covariates were comparable. The c-statistics for Model A and Model B were 0.692 and 0.690, respectively. The Hosmer-Lemeshow Goodness-of-fit Tests for each were 0.81 and 0.43, respectively, and there is sufficient agreement between

the observed data and the fitted models for each. There appears to be greater agreement with Model A, but at the 0.05 level, both models are adequate.

Table 1 Demographic and exposure characteristics

	N (%)	No TDRM (%)	TDRM (%)	p
Total	162	133 (82.1)	29 (17.9)	
Sex				0.18
Male	131 (80.9)	105 (80.2)	26 (19.9)	0.19
Female	31 (19.1)	28 (90.3)	3 (9.7)	Reference
Age				0.20
<25	40 (24.7)	35 (87.5)	5 (12.5)	Reference
25-34	54 (33.3)	47 (87.0)	7 (13.0)	0.13
35-44	38 (23.5)	29 (76.3)	9 (23.7)	0.70
45-54	23 (14.2)	18 (78.3)	5 (21.7)	0.94
≥55	7 (4.3)	4 (57.1)	3 (42.9)	0.11
Risk Group				0.68
MSM ¹	99 (61.1)	82 (82.8)	17 (17.2)	0.47
IDU ²	10 (6.2)	8 (80.0)	2 (20.0)	0.90
Heterosexual	47 (29.0)	39 (83.0)	8 (17.0)	Reference
Other/Unknown	6 (3.7)	4 (66.7)	2 (33.3)	0.37
Race				0.47
White	123 (75.9)	99 (80.5)	24 (19.5)	0.35
Other	39 (24.1)	34 (87.2)	5 (12.8)	Reference

¹Men who have sex with men

²Injection Drug Users

Table 2 Stage of HIV-1 infection

	N (%)	No TDRM (%)	TDRM (%)	p
CD4 (cells/mm ³)				0.74
≤200	53 (33.1)	42 (79.3)	11 (20.8)	0.53
201-350	42 (26.3)	33 (78.6)	9 (21.4)	0.40
351-500	34 (21.3)	29 (85.3)	5 (14.7)	0.68
>500	31 (19.4)	27 (87.1)	4 (12.9)	Reference
CD4%				0.92
Below 0.20	74 (45.7)	61 (82.4)	13 (17.6)	0.92
At or above 0.20	88 (54.3)	72 (81.2)	16 (18.2)	Reference
Viral Load (copies/mL)				0.34
<10,000	25 (18.2)	26 (92.9)	2 (7.1)	Reference
10,000-49,000	44 (28.6)	36 (81.8)	8 (18.2)	0.95
50,000-99,999	23 (14.9)	17 (73.9)	6 (26.1)	0.23
≥100,000	59 (38.3)	48 (81.4)	11 (18.6)	0.87

Table 3 Rural/Urban classification scheme based on University of Washington Rural-Urban Commuting Area (RUCA) Codes

	N (%)	No TDRM (%)	TDRM (%)	p
RUCA Definition				0.44
Metro area core: primary flow within an UA ³	84 (51.9)	69 (82.1)	15 (17.9)	Reference
Metro area high commuting: primary flow 30% or more to a UA ³	6 (3.7)	6 (100.0)	0 (0.0)	0.95
Micro area core: primary flow within an UC ⁴ of 10,000 through 49,999	31 (19.1)	26 (83.9)	5 (16.1)	0.95
Micro high commuting: primary flow 30% or more to a large UC ⁴	3 (1.9)	3 (100.0)	0 (0.0)	0.97
Small town core: primary flow within an UC ⁴ of 2,500 through 9,999	19 (11.7)	13 (68.4)	6 (31.6)	0.95
Small town high commuting: primary flow 30% or more to a small UC ⁴	2 (1.2)	1 (50.0)	1 (50.0)	0.93
Rural areas: primary flow to a tract outside a UA ³ or UC ⁴	17 (10.5)	15 (88.2)	2 (11.8)	0.91

³Urban Area

⁴Urban Cluster

Table 4 Risk groups

	N (%)	No TDRM (%)	TDRM (%)	p
Combination Risk Groups				0.63
Yes	8 (4.9)	6 (75.0)	2 (25.0)	0.59
No	154 (95.1)	127 (82.5)	7 (17.5)	Reference
Secondary Risk Group				1.00
IDU	3 (37.5)	2 (66.7)	1 (33.3)	0.68
Heterosexual	5 (62.5)	4 (80.0)	1 (20.0)	Reference
Genotyped				0.45
Yes	159 (98.2)	131 (82.4)	28 (17.6)	Reference
No	3 (1.9)	2 (66.7)	1 (33.3)	0.49
Concurrent AIDS Diagnosis				0.49
Yes	56 (35.0)	45 (80.4)	11 (19.6)	0.49
No	104 (65.0)	88 (84.6)	16 (15.4)	Reference
RPR Result				0.02
Positive	7 (4.6)	3 (42.9)	4 (57.1)	0.01
Negative	145 (95.4)	122 (84.4)	23 (15.9)	Reference

Table 5 Risk behaviors

	N (%)	No TDRM (%)	TDRM (%)	p
Prior STI				0.07
Yes	37 (56.1)	30 (81.1)	7 (18.9)	0.09
No	29(43.9)	28 (96.6)	1 (3.5)	Reference
Ever use of tobacco				0.87
Yes	94 (65.3)	76 (80.9)	18 (19.2)	0.87
No	50 (34.7)	41 (82.0)	9 (18.0)	Reference
Packs per day				0.92
<0.5	12 (18.8)	10 (83.3)	2 (16.7)	Reference
0.5 - <1	18 (28.1)	15 (83.3)	3 (16.7)	0.87
≥1	34 (53.1)	26 (76.5)	8 (23.5)	0.40
Current alcohol use				0.43
Yes	88 (59.5)	72 (81.8)	16 (18.2)	0.43
No	60 (40.5)	52 (86.7)	8 (13.3)	Reference
Prior illicit drug use				0.74
Yes	72 (51.1)	59 (81.9)	13 (18.1)	0.74
No	69 (48.9)	55 (79.7)	14 (20.3)	Reference
Prior intravenous drug use				0.14
Yes	15 (14.4)	10 (66.7)	5 (33.3)	0.11
No	89 (85.6)	75 (84.3)	14 (15.7)	Reference
Prior incarceration				0.48
Yes	14 (87.5)	7 (50.0)	7(50.0)	0.97
No	2 (12.5)	2 (100.0)	0 (0.0)	Reference

Table 6 Association of illicit drug exposure with transmitted drug resistance mutations

	N (%)	No TDRM (%)	TDRM (%)	p
Cannabinoids				0.95
Yes	55 (34.0)	45 (81.8)	10 (18.2)	0.95
No	107 (66.1)	88 (82.2)	19 (17.8)	Reference
Opioids				0.15
Yes	4 (2.5)	2 (50.0)	2 (50.0)	0.12
No	158 (97.5)	131 (82.9)	27 (17.1)	Reference
Stimulants				0.39
Yes	30 (18.5)	23 (76.7)	7 (23.3)	0.39
No	132 (81.5)	110 (83.3)	22 (16.7)	Reference
Club Drugs				1.00
Yes	3 (1.9)	0 (0.0)	3 (100.0)	0.98
No	159 (98.2)	130 (81.8)	29 (18.2)	Reference
Hallucinogens				0.55
Yes	4 (2.5)	3 (75.0)	1 (25.0)	0.71
No	158 (97.5)	130 (82.3)	28 (17.7)	Reference
Prescription Drugs				0.33
Yes	2 (1.2)	1 (50.0)	1 (50.0)	0.28
No	160 (98.8)	132 (82.5)	28 (17.5)	Reference

Table 7 Proportion of TDRM in each major ARV drug class

	TDRM (%)	p
Major Resistance Mutations		<0.0001
NRTI	6 (3.7)	
NNRTI	17 (10.5)	
PI	3 (1.9)	
Combo	3 (1.9)	

Table 8 Proportion of minor mutations

	N (%)	No TDRM (%)	TDRM (%)	p
Other Mutations				<0.0001
NRTI	19 (11.7)	11 (57.9)	8 (42.1)	0.01
NNRTI	21 (13.0)	3 (14.3)	18 (85.7)	<0.0001
PI	93 (57.4)	74 (79.6)	19 (20.4)	0.33
RT	28 (17.3)	26 (92.9)	2 (7.1)	0.12

Table 9 Logistic regression model

Variable	Parameter Estimate	p-value	OR	95% CI
Intercept	-4.46	<0.0001	-	-
RPR	2.51	0.14	12.4	(0.44, 348.10)
NRTI	3.36	0.01	28.9	(2.00, 415.26)
NNRTI	6.44	<0.0001	624.3	(32.6, >999.99)
IV Drug Use	3.10	0.01	22.2	(1.88, 262.38)

Table 10 Model with only the propensity score

Variable	Parameter Estimate	p-value
Intercept	-2.54	0.03
CD4%	0.44	0.54
Propensity Score	1.12	0.37

Table 11 Model A (p=0.09)

Variable	Parameter Estimate	p-value	OR	95% CI
Intercept	-5.06	0.01	-	-
CD4%	0.67	0.52	1.96	(0.25, 15.18)
Propensity Score	3.55	0.10	34.65	(0.48, >999.99)
RPR	1.57	0.17	4.81	(0.51, 45.65)
IV Drug Use	1.27	0.08	3.57	(0.85, 15.04)

Table 12 Model B (p=0.32)

Variable	Parameter Estimate	p-value	OR	95% CI
Intercept	-3.76	0.03	-	-
CD4%	0.66	0.58	1.94	(0.18, 20.80)
Propensity Score	2.17	0.23	8.73	(0.25, 299.76)
RPR	1.70	0.25	5.45	(0.30, 97.91)
IV Drug Use	1.22	0.15	3.38	(0.65, 17.51)

TDRM Frequency and Percentage of Genotypic Testing

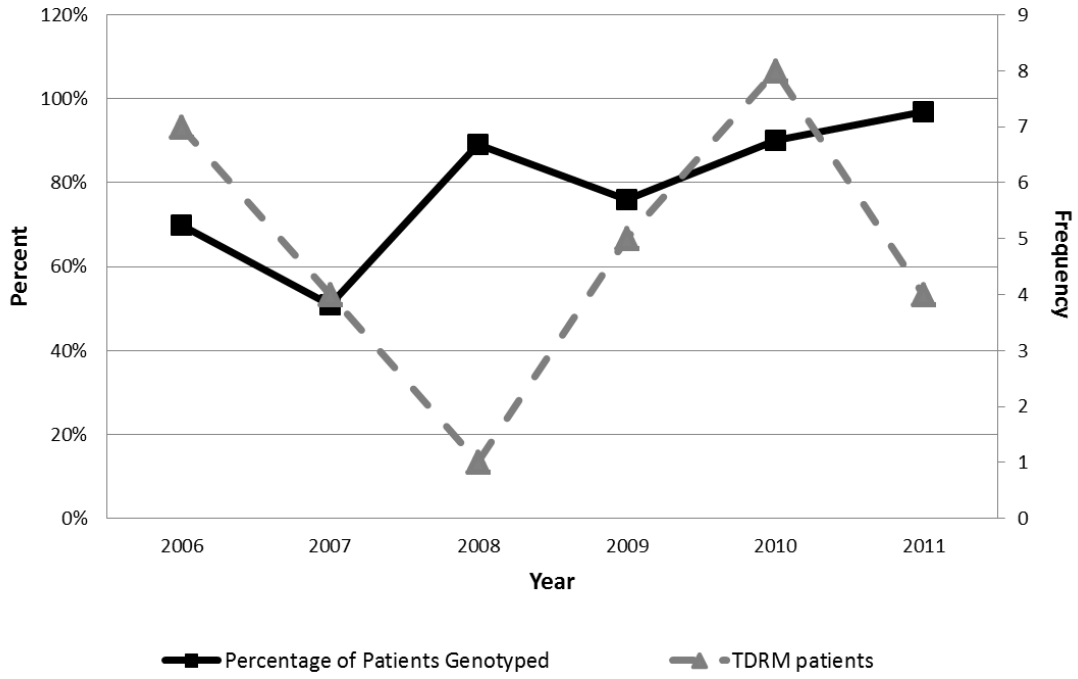


Figure 2 Frequency of TDRM and percentage of patients genotyped

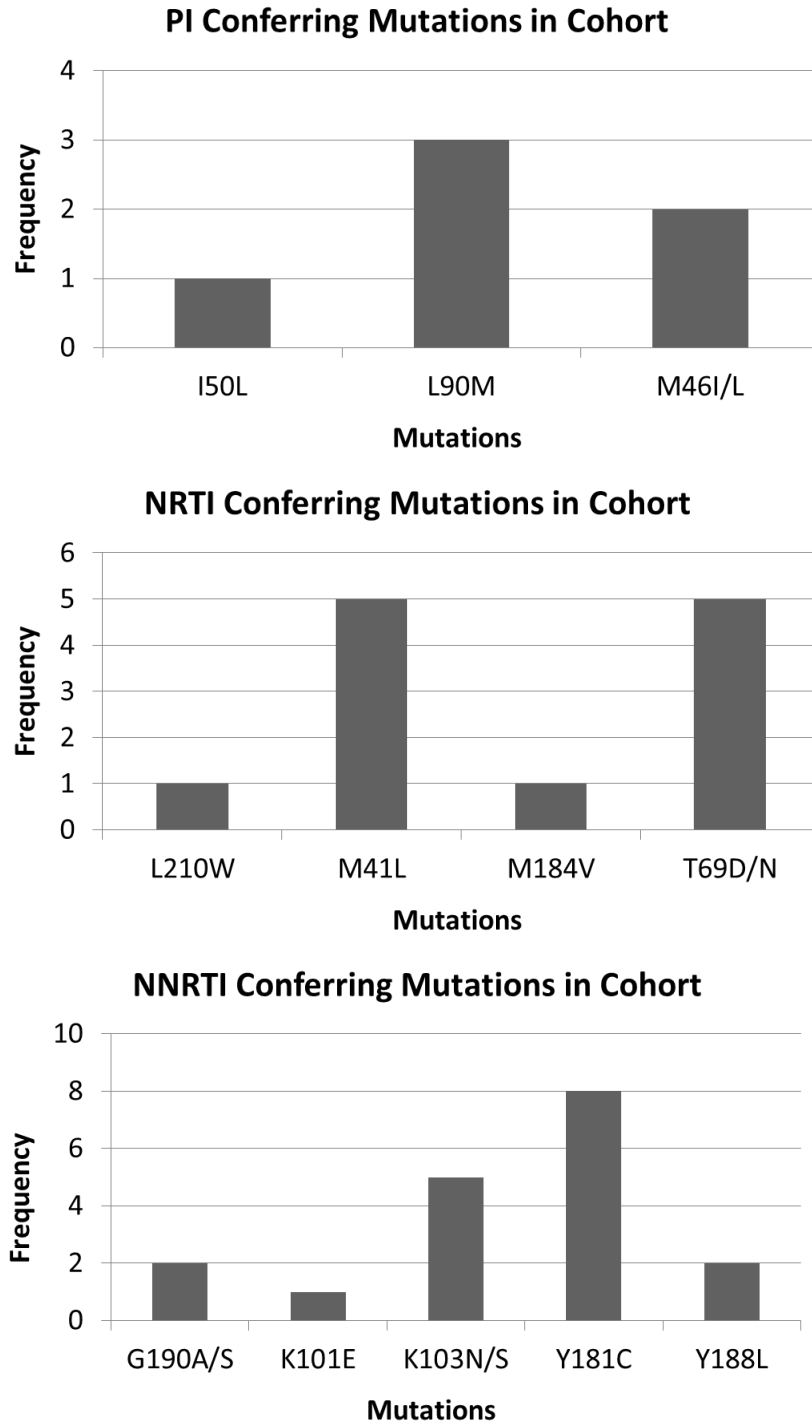


Figure 3 Known major resistance mutations conferring resistance to FDA-approved ARV drugs

CHAPTER 4

DISCUSSION

This research study is the first to investigate TDRM in a low HIV prevalence state. Furthermore, TDRM prevalence was calculated to be 14%, which aligns with a similar study conducted by Youmans et al in a large cohort in South Carolina [15], which is actually considered a high prevalence state for HIV infection. Jain et al, however, found a larger proportion; they found a 21% TDR [8]. We compared rural and urban populations based on RUCA codes and were able to demonstrate that TDRM is not different between these RUCA classification areas; hence, resistance trends are similar to high HIV prevalence metropolitan statistical areas. Such resistance mutations, especially those against NNRTI, confer 2-times greater risk of virologic failure on treatment-naïve patients [10].

As expected, the largest proportion of major HIV drug resistance was in the NNRTI class, which agrees with Huang et al where they found the proportion of drug resistance was 9.8% within the NNRTIs. However, TDRM among ARV-naïve HIV-positive patients is not associated with risk group exposure, alcohol use, or history of tobacco or drug use even after adjusting for age, gender, race, rurality, host immune status, and viral characteristics. This is in contrast to Youmans et al that found a statistical significant association of TDRM with age and risk exposure group [15]. However, our study is in accordance with Wheeler et al, which found no significant differences in TDRM based on sex, age and race/ethnicity categories [3].

Host immune status was determined by categorizing CD4 T-lymphocyte counts into clinically meaningful quartiles. At a count of >500 , the IAS-USA Panel recommends initiating therapy, however, it is not considered necessary until counts drop below 350 cells/mm^3 [4,7]. Moreover, a CD4 T-lymphocyte count of $\leq 200 \text{ cells/mm}^3$ is AIDS-defining. The guidelines for treatment for CD4 counts between 351 and 500

cells/mm³ are unclear and are often left to the discretion of the clinician [J Meier, personal communication].

For the analyses and logistic regression model, CD4% was dichotomized at or above and below the mean of 20%. While there is not a meaningful target value, a high CD4% is desirable. CD4% is often used because absolute CD4 T-lymphocyte counts tend to vary widely between measurements. CD4% gives clinicians a glimpse into the host immune system since it shows the relative number of CD4 cells present relative to other lymphocytes. The CD4% and absolute CD4 counts often correspond to the same clinical conclusions regarding strength of host immunity.

Viral load is important in monitoring the severity of the disease. While the target of therapy is to attain full HIV suppression by achieving an undetectable viral load (<50 copies/mL), it is usually taken as a proxy for the effectiveness of ARV therapy [4,7]. Decreases from the baseline measure are expected but may take up to several months to achieve. Little to no change in viral load measurements at the end of 3-4 months is indicative of virologic failure [4,7].

As of the 2010 Census Tract Data, 36% of the Iowa population is considered rural [16]. Using the small town and rural area RUCA definitions from Table 3 as the definition of 'rural,' 31% of patients affected by TDRM in the study accounted for a small but meaningful proportion of the 36% of rural Iowans.

This study is the first to examine the RPR syphilis test as a proxy for highest level of sexual risk exposure—beyond that for the typical high risk groups. Behaviors associated with a syphilis infection frequently overlap with behaviors that result in HIV infection and vice versa.

Selection of the variables to include in the logistic regression model involved incorporating the significant variables, which were RPR, minority NRTI mutations, minority NNRTI mutations and borderline variables which comprised prior history of STIs, prior IV drug use, history of opioid drug use and minor reverse transcriptase

mutations. In the logistic regression model, RPR was retained even though it was statistically not significant due to its clinical importance as a proxy for high risk behavior. NRTI and NNRTI were both significant; however, the high c-statistic may be attributed to the number of TDRM patients which also possessed minor NRTI and/or NNRTI mutations. Nevertheless, the overall number of these TDRM cases was small and non-TDRM cases were still likely to have acquired minor resistance mutations from transmission. The mechanism for intravenous drug use within the logistic regression model could potentially result from the risky habits of sharing needles and body fluids in addition to exchanging sexual encounters for drugs. It is also unlikely that these behaviors are conducive to adhering to ARV regimens; hence, any acquired drug resistance may be transmitted among sexual and needle-sharing partners.

Transmission of drug-resistant variants are an indicator that infected individuals are still engaging in high risk behaviors while aware of their HIV-positive status [3]. Efforts to control regional epidemics such as education, health promotion and interventions, in addition to advances in ART have not been sufficient to inhibit drug resistance from emerging in regions where treatment is available [17].

Newly infected patients with resistance have slower response to ART; plasma HIV-1 RNA levels are not readily suppressed and may give rise to additional resistance mutations after application of several different ARV drugs [6]. Clinical recommendations for resistance testing are to assist in initial therapy selection to achieve virologic suppression or plasma HIV-1 RNA levels of <50 copies/mL [7]. Therapy should maintain immune function, decrease transmission and delay disease progression [4]. The goal after first or multiple-regimen virologic failure is for plasma HIV-1 RNA to be below the level of detection for the most sensitive assays [4].

As expected, CD4 cell counts are lower in those with transmitted drug-resistance mutations than those without TDRM; however, one study showed that the rapid decline in the initial year of infection stabilizes and further declines slow [5]. The CASCADE

study found that treatment-naïve individuals with TDRM initially had higher CD4 cell counts than non-TDRM patients. However, the treatment-naïve TDRM patients showed dramatic decline in CD4 counts over the five-year study period. The loss in this initial difference indicates that the effects of TDRM on the natural history of untreated HIV are poor [7].

CD4 T-cell counts are calculated from the CD4%; the CD4% has been shown to be a more reliable measure of immune status since CD4 counts can fluctuate considerably within several hours, daily, seasonally or after certain medications [18]. CD4% by comparison is less likely to have this variation [18,19,20]. The normal range for CD4 spans between 500 to >1500. The corresponding CD4% for ≥ 500 cells/mm³ is $\geq 29\%$. The reference ranges for laboratories varies by site [18]. At the UIHC, the range is between 34-62% [J. Meier, email communication].

Choosing to use $>29\%$ to represent the healthy, reference population was based on the Johns Hopkins HIV Guide [18]. Table 13 displays the CD4 counts and their corresponding CD4% [18]. The range of 200-500 CD4 T-cell counts is still under variable interpretation depending on the care provider and the patient's overall health [J. Meier, personal communication]. However, earlier ART initiation has been shown to lead to faster virologic suppression and improved clinical outcomes [4,7].

Alternatively, in the prediction of TDRM using propensity scores and CD4% in Model A, age was borderline significant while testing the propensity score using the covariates it represented; nevertheless, the propensity score was sufficient at $\alpha=0.05$ in controlling for covariates. The parameter estimates and their p-values within Table 10 were used contrasted with Models A and B.

Model A does not include the variables for minor NNRTI and NRTI mutations the logistic regression model in Table 9 contains. These variables had large p-values which indicated that the propensity score controlled for the risk of a poor outcome due to HIV. The propensity score predicted the adverse outcome that is coupled with minor

mutations. Essentially, the propensity score and CD4% adjusted for the over-correction of patients who were at risk because they may have had exposure to IV drug use, screening positive for syphilis and/or harbored minor mutations.

The variables that went into consideration for the logistic regression model such as history of STI, concurrent AIDS diagnosis, and incarceration were also considered for Model B. However, the mean values between the risk and reference population when these variables were included created little overlap in the propensity scores of the two populations. The maximum values of the propensity score for each population showed a large difference in magnitude such that extrapolation would be outside the range of the data. Of the variables in consideration for the logistic regression model, those selected for Model B included genotyping, history of smoking or drug use and current alcohol use. In Model B, the RPR, IV drug use and propensity score values were not statistically significant. At least in Model A, there was borderline significance for IV drug use.

Overall, Model A is a better predictor of TDRM than Model B. The Wald χ^2 p-values for both models are not statistically significant but the p-value for Model A is closer to statistical significance compared to Model B. Furthermore, the propensity score for CD4% in Model A confers an odds ratio of 34.65 compared to the odds ratio in Model B of 8.73. Model A, therefore, predicts a greater likelihood of a poorer outcome for those in the at-risk group than the propensity score in Model B. In other words, there is a difference between patient populations by defining their risk through the CD4% based on sex, age, exposure group, viral load, CD4 count, race, ethnicity and urban or rural residence. Model A is more effective at characterizing this difference than Model B, which attempts to also control for baseline HIV strain genotyping, history of tobacco and/or illicit drug use, and current consumption of alcoholic beverages. These appear to be too stringent in predicting the propensity score.

The c-statistics for each model are <0.70 indicating that the predicted probability that TDRM occurs is poor. Model A is somewhat better than Model B since the

discriminatory power of Model A is borderline acceptable. Furthermore, while the odds ratio of Model A is extremely large and that of Model B is moderate to large, their 95% confidence intervals are considerably wide. The odds ratio for Model B, while nearly four times smaller in magnitude, is bounded unlike the confidence interval for Model A, which does not have a maximum bound.

The effect measures for RPR and IV Drug Use are less dramatic in Model A than the logistic regression model in Table 9. The purpose in using the propensity score was to take into account the likelihood that high risk characteristics could confound the factors that could be associated with TDRM. The discrepancy in the parameter estimates and the odds ratios for RPR and IV Drug Use show that there is residual confounding within the logistic regression model. The propensity scores from Model A demonstrate that patients with a poor CD4%, that is, poorly controlled HIV infection, have an association with TDRM that is independent of positive RPR test result or IV drug use.

We recognize several limitations of our study. There may be sampling bias due to the lack of recognition between HIV exposure and infection. Also, since symptoms are non-specific during acute infection, patients are unlikely to seek professional medical care and when they do present, do so with late-stage, chronic HIV disease. Results may, therefore, be an under-representation of the actual TDRM cases among ARV-naïve HIV infected patients.

The logistic regression model over-estimated the effect of positive RPR, minor NRTI mutations, minor NNRTI mutations and IV drug use since patients who tend to test positive for RPR and participate in IV drug use have higher than average risk behaviors. These behaviors could pre-dispose the risk population in acquiring TDR HIV infection.

Patients who often have drug resistant mutations to single or multiple ARVs typically harbor minor mutations. Unfortunately, the logistic regression model was unable to discriminate the minor mutations from TDRM patients with major mutations that also were affected by minor ones. All patients who were genotyped that had minor

mutations detected, whether or not they were identified as TDRM patients, were included in each of the minor mutation variables (minor NRTI, minor NNRTI, minor PI and reverse transcriptase mutations).

While some drug resistant mutations are resilient, others are not and revert to wild-type or replication-competent variants as soon as drug pressures are removed. Therefore, reversion may underestimate the true level of TDRM since they may fall below the limits of assay detection. Archived mutations may continue to persist and re-emerge with application of selective drug pressure [6].

Reversion of some mutant variants and late disease presentation may mean many minor strains were missed in genotyping. These low frequency variants are clinically relevant since they have been shown to significantly reduce the efficacy NNRTIs, which have a low genetic barrier (i.e. few mutations needed to acquire resistance) compared to PIs which have a high genetic barrier (several mutations required for resistance) [1].

Suboptimal therapy, health care and subtype differences may create conditions that will increase trends in drug resistance. Cross-resistance to other drugs in ARV classes is important, particularly, in resource-limited settings when PI's are the only choice after failure of first- or second-line therapies. Suboptimal ART may put patients at risk for the selection of major NNRTI-resistance [17].

Treatment-naïve patients are typically more resistant to the NNRTI class, which suggests that these have superior fitness and transmissibility compared to other resistance mutations [5]. When virologic failure includes an NNRTI, it is recommended to substitute with a new class of ARV or boosted-PI when not possible [4]. Etravirine may be effective for NNRTI-failure but only if combined with another potent drug and boosted with a PI [4]. The K103N mutation occurs in drug-binding pockets and confers resistance to all NNRTIs [Brenner]. However, the mutation that occurred most frequently within the study population was the Y181C, which historically, has been rare in other populations [9]. Y181C and K103N are common mutations seen with first-

generation NNRTI drugs; Y181C is usually selected for by efavirenz [22], which is a component of Atripla®, whereas, K103N is usually selected for by nevirapine when zidovudine is not coadministered [3,22]. Y188L is a low frequency mutation; however, this single mutation leads to resistance to most approved NNRTIs [22].

The rapid replacement of the NRTI mutation M184V coincides with the poor fitness of the mutational variant in the absence of drug pressure [8]. Lack of M184V mutation in newly infected, treatment-naïve HIV patients may indicate the rapid reversion of the mutation [8]. It is likely this is why our study cohort had a low frequency of this mutation. The M41L mutation is a thymidine analog mutation that affects the second-line drugs zidovudine and stavudine [3]. T69D/N confers resistance to all NRTIs when accompanied by 41, 210 and 215 [22], which is very relevant since M41L and L210W are two mutations that are seen in the study cohort.

The high frequency of the PI mutation L90M, which is selected by saquinavir/ritonavir and nelfinavir [3], is likely coupled with NNRTI mutation Y181C or NRTI mutation M41L as part of combination therapy. While a small proportion (2%), dual mutations to 2 drug classes were encountered in a study by Johnson et al [9]. The case-control study included primarily non-Hispanic White men on the impact of treatment response in newly infected TDRM patients [9]. Additionally, L90M confers resistance to all PIs except commonly used Darunavir & Tipranavir [22]. M46I/L is selected by Indinavir, which is no longer a preferred first-line ART [22]. Since PIs have a high genetic barrier, it is logical that the reversal of PI mutations would equally require multiple back mutations for reversion.

A pooled analysis by Li et al found that minority NNRTI mutations increase the risk of virologic failure by 2-3 times [10]. A large proportion of patients, whether affected by TDR or not, had acquired non-drug resistant mutations. Since NNRTIs are frequently prescribed commonly as fixed-formulations and NRTIs are recommended for therapy by the CDC and IAS-USA, it is not surprising that the majority of non-drug

resistant mutations belong to these ART classes. There is a significant interaction among minor variants, virologic failure and the higher levels of adherence. This may be due to the partial masking of minor variants in non-adherent patients who were at highest risk of virologic failure whether or not they harbored minor resistance mutations [21].

Prevalence estimates are innately confounded because the number of true treated patients is unknown. Additionally, the variance in the existing measurements of TDR prevalence is based on the population, types and availability of ART, sensitivity of resistance assays, criteria for defining resistance, and time points when resistance testing occurred [5]. Other factors that may attribute to variations in prevalence include differences in therapies at the population level, selection bias from non-representative samples, different risk behaviors and differential access to therapy among different exposure groups [7].

It is notable that drug-resistant HIV is most prevalent among MSM, a group which has historically higher access to health care relative to other risk groups. Reports show that increasing rates of STIs and HIV among the MSM exposure group suggests high risk behaviors may increase HIV incidence and transmission of TDRM [6]. Also, infection via intravenous transmission has been shown to reflect a multiplicity of variants whereas sexual transmissions frequently are caused by single virions; thus, rapid replacement of mutations may occur in cases involving IDU [8].

Rapid spread within a population indicates the virus that has not adapted to the host immune system since transmission occurs during the early infectious period. At the population-level, the evolution of the virus is slow and results in the spread of a similar strain. Alternatively, a rapidly evolving strain with heterogeneous variants is a slow-spreading virus where infection occurs in the chronic stage of disease and the virus has had time to adapt to host immune pressures [1]. This inherent limitation is not isolated to HIV alone but could affect any virus in any epidemic.

There are limitations inherent to HIV and in its detection. Technical limitations include commercial and home-brew assays unable to detect resistance when the viral load is <500 copies/mL [4,7]. Furthermore, HIV exists as a quasi-species and any TDR strain less than 20% of the population is undetectable by these assays [4,7].

Genotypic testing assays employ PCR techniques and are able to detect HIV variants that exist within the range of 15-25% of the viral population [10]. Standard resistance assays have a minimum limit of detection of $\geq 20\%$ prevalence within a clinical sample [2]. The technological bias of assays cannot distinguish undetectable minority variant populations; this contributes to a possible underestimation of minority variants on virologic failure [10].

While the detection limit of conventional assays can detect certain mutations at frequencies as low as 10%, this is not reliable for minor variant populations, which is a concern since most patients rarely present with acute infection; allowing time for any detectable transmitted drug resistant variants to decay to undetectable levels [9].

Furthermore, genotypic resistance interpretation is challenging due to complex interactions of mutations and the frequency with which they may occur [7]. For example, the NRTI mutation M184V partially reverses thymidine analog mutations while conferring hypersensitivity to tenofovir, zidovudine and stavudine [22]. Steps, however, are being taken to improve methods to remedy the issue. The CDC is taking steps to investigate and develop more sensitive methods to detect minor population HIV variants and the World Health Organization recommends that drug resistance surveillance should be incorporated into HIV ART rollout programs [2].

Future studies of TDR prevalence in other populations, particularly, in developing countries which are the center of communicable disease infections such as HIV will be essential to better understanding the natural history of acquired drug-resistance mutations. Research in the contact networks of sexual transmission will also enable population-specific interventions and preventative education. These can be somewhat

mapped through interviews and partner notification to identify recent infections but larger networks are more difficult to investigate [1]. Nevertheless, the greater our understanding of HIV transmitted drug-resistance, the more effective efforts will be at preventing its transmission and anticipating emerging virulent HIV strains. This would be important to incorporate into existing HIV surveillance programs to establish a baseline and to examine trends prior to and after interventions.

Table 13 CD4 counts and their corresponding CD4%
[18,19]

CD4 T-cell Count (cells/mm ³)	CD4%	Clinical Interpretation
>500	>29%	Normal
200-500	14-28%	
<200	<14%	Definition of AIDS; aggressive ART recommended

CHAPTER 5

CONCLUSION

The current HIV incidence is a combination of high transmission rates with effective ART [1], but despite effective HAART regimens to suppress HIV replication, TDRM is a growing clinically significant problem. Genotypic resistance testing guidelines have been revised by the IAS-USA Panel to include recommendations for all patients prior to receiving antiretroviral therapy. Using this as part of standardized clinical care could potentially lead to surveillance of emerging problematic TDRM strains and improvements in disease control. Studies in the contact networks of sexual transmission and TDR prevalence in populations, specifically, within developing countries will improve our understanding of the natural history of HIV transmitted drug resistance.

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