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MAJOR ARTICLE

Statewide Longitudinal Trends in Transmitted HIV-1 Drug Resistance in Rhode Island, USA

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Background. HIV-1 transmitted drug resistance (TDR) remains a global challenge that can impact care, yet its comprehensive assessment is limited and heterogenous. We longitudinally characterized statewide TDR in Rhode Island.

Methods. Demographic and clinical data from treatment-naïve individuals were linked to protease, reverse transcriptase, and integrase sequences routinely obtained over 2004–2020. TDR extent, trends, impact on first-line regimens, and association with transmission networks were assessed using the Stanford Database, Mann-Kendall statistic, and phylogenetic tools.

Results. In 1123 individuals, TDR to any antiretroviral increased from 8% (2004) to 26% (2020), driven by non-nucleotide reverse transcriptase inhibitor (NNRTI; 5%–18%) and, to a lesser extent, nucleotide reverse transcriptase inhibitor (NRTI; 2%–8%) TDR. Dual- and triple-class TDR rates were low, and major integrase strand transfer inhibitor resistance was absent. Predicted intermediate to high resistance was in 77% of those with TDR, with differential suppression patterns. Among all individuals, 34% were in molecular clusters, some only with members with TDR who shared mutations. Among clustered individuals, people with TDR were more likely in small clusters.

Conclusions. In a unique (statewide) assessment over 2004–2020, TDR increased; this was primarily, but not solely, driven by NNRTIs, impacting antiretroviral regimens. Limited TDR to multiclass regimens and pre-exposure prophylaxis are encouraging; however, surveillance and its integration with molecular epidemiology should continue in order to potentially improve care and prevention interventions.

Keywords. HIV-1 drug resistance; longitudinal trends; surveillance; transmitted drug resistance; transmission networks.

Global progress in the fight against HIV/AIDS has been made by adopting early and universal life-saving antiretroviral therapy (ART) for prevention and treatment [1]. However, transmitted drug resistance (TDR) remains a roadblock toward ending the HIV epidemic, as it can impact prevention and clinical outcomes and compromise ART effectiveness. This may be true even with new medications with high barrier to resistance, and particularly considering more recent, guideline-recommended 2-drug regimens [2].

On an individual level, resistance testing at the time of ART initiation in resource-rich settings can help clinicians select efficacious regimens [2, 3]. On a population level, TDR surveillance can inform policy-makers and guidelines in resource-limited settings, where individual testing is limited [4]. Longitudinal TDR monitoring can inform time trends and impact clinical care and public health at the local, state, and country levels. However, longitudinal TDR data are limited in both resource-rich and resource-limited settings.

TDR global rates, patterns, and trends vary across diverse geographic areas and time periods [5–12]. In the United States, TDR patterns seem to be more consistent, with examples like an overall increase from 8% in 2000 to a peak in 2005–2007 (17%), and a decline since the late 2000s to 14% [7, 12]. However, heterogeneity remains; for example, overall decline in Washington DC (from 15% to 6% between 2004 and 2013) [10] and in North Carolina (from ~18% to ~8% between 2005 and 2014) [12]; an increase in Portland, Oregon (from 17% to 31% between 2005 and 2009) [13]; stability in San Diego (13%–14% between 1996 and 2013) [14]; and high levels among men who have sex with men (MSM) in Atlanta (21%), Baltimore (29%), Birmingham (53%), and Boston (26%) [15]. Dual- and triple-class TDR ranges from 1.5% to 5% and 0% to 0.9%, respectively; and TDR to integrase strand transfer inhibitors (InSTIs) has also been reported [16–18]. Longitudinal and particularly statewide TDR studies are limited, leaving recent trends unknown. Taken together, study diversity, limited geographic representation,

Received 7 September 2021; editorial decision 15 November 2021; accepted 6 December 2021; published online 7 December 2021.

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Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab587

timing, and changing ART regimens and guidelines form research gaps justifying a statewide longitudinal TDR assessment.

The same sequence data used to determine TDR can be used for molecular epidemiology and its incorporation into public health [12, 19–21]. Such analyses have allowed demonstration of spread of resistance within networks, as well as identification of sources of onward transmission [22, 23]. Integrating TDR surveillance with the emerging real-time molecular epidemiology analyses on a regional and/or state level could have a synergistic effect and further inform public health interventions [24, 25].

In this study, leveraging the small size of Rhode Island (RI) and the comprehensive availability of sequences from HIV drug resistance testing, we uniquely assessed the magnitude and longitudinal dynamics of TDR on a statewide level, estimated its impact on first-line ART, evaluated its potential associations, and assessed it in transmission networks. We hypothesized that current TDR in RI is still extensive and has increased over time, with potential impact on care and management, even in the era of broad use of ART with a high genetic barrier to resistance.

METHODS

Study Cohort

Study data included all available HIV-1 sequences (single earliest per person) obtained from ART-naïve people with HIV (PWH), who received clinical care in RI health care facilities from 2004 to 2020. Data collected from medical records included sociodemographic (gender, age at genotyping, ethnicity, race, MSM status, incarceration, psychiatric illness, illegal substance use, and country of birth) and clinical information (ART regimens, CD4 counts, and viral load). Records were reviewed to ensure ART-naïve status at genotyping. Annual numbers of newly HIV-diagnosed individuals in RI were provided by the Department of Health. The study was approved by the Lifespan Human Subjects Research Institutional Review Board.

HIV-1 Sequences and Sequence Analyses

HIV-1 protease and reverse transcriptase (PRRT; HXB2 nucleotide positions 2253–3268) and integrase (positions 4230–5093) sequences were obtained through provider-ordered resistance testing performed by commercial laboratories using Sanger sequencing as part of routine care. Additional sequence quality control was performed with SQUAT [26] and Stanford Database tools [27]. Multiple sequence alignment was performed with *mafft*, version 7.450 [28]. HIV-1 subtyping was performed with REGA [29] and COMET [30], with manually resolved discrepancies.

To assess association of TDR with molecular clusters, PRRT sequences were used to identify clusters by combining phylogeny (RAxML, version 8.2.10; bootstrap support ≥0.90) and pairwise distance (mean TN93 ≤0.015 substitutions/site), as described previously [31]. We estimated number and size of clusters, number and proportion of PWH in clusters, number and size of clusters that included PWH with TDR, and similarity of surveillance drug–resistant mutations (SDRMs) in clusters.

Drug Resistance Analysis

TDR was defined according to SDRM lists [32, 33]. Analyses included resistance to any class, dual-class, triple-class, class-specific, and individual SDRMs. Dynamics of individual SDRMs were plotted using 4-year bins spanning 2004–2007, 2008–2011, 2012–2015, and 2016–2020.

To assess the impact of TDR on ART, we first used detected SDRMs to compute prevalence of intermediate and higher predicted resistance levels to *all* relevant initial and subsequent treatment options, including the NNRTIs nevirapine, efavirenz, rilpivirine, etravirine, and doravirine; the NRTIs zidovudine, abacavir, emtricitabine, lamivudine, and tenofovir; the PIs lopinavir, atazanavir/ritonavir, and darunavir/ritonavir; and the InSTIs raltegravir, elvitegravir, dolutegravir, and bictegravir. We then assessed the impact of SDRMs on regimens individuals actually began taking. Resistance prediction was performed according to the Stanford Database algorithm, version 9 [27]. To assess changes over time, predicted resistance was plotted within the same 4-year bins (2004–2007, 2008–2011, 2012–2015, and 2016–2020).

Statistical Analysis

To quantify TDR time trends, we calculated the Mann-Kendall statistic based on TDR annual prevalence, which is the proportion of pairs of years where the later year has a higher TDR prevalence than the earlier year. A Mann-Kendall statistic of 1 indicates a strictly monotone TDR prevalence increase, and a Mann-Kendall statistic of –1 indicates a strictly monotone TDR prevalence decrease over the observed time period. Confidence intervals were calculated using the nonparametric bootstrap.

To estimate associations between TDR and sociodemographic and clinical variables, we used a main effects logistic regression. The model included age, MSM, illegal substance use, HIV-1 subtype, and clustering. These variables were selected based on previous studies [12, 15, 20, 25]. We also used main effects logistic regression to estimate associations between HIV-1 subtypes (B vs non-B) and sociodemographic and clinical variables. The model included age, MSM, illegal substance use, and molecular clustering. For both logistic regression models, inverse probability weighting was used to account for missing data. Tests of associations between TDR and clustering, and TDR and cluster size (treated as a categorical variable with 2, 3–9, and ≥10 members), were conducted using a chi-square test of proportions.

RESULTS

Study Population

A total of 2674 adults had HIV in RI by the end of 2019 [34]. Annual numbers of newly HIV-diagnosed individuals in RI

varied over time, with 31 in 1984, increasing sharply during the late 1980s, peaking to 323 in 1990, and steeply declining by 1995, with a steadier decline since to 62 in 2020 (~15% diagnosed outside of the state) (Figure 1, blue line). Over 2004–2020, in line with reduced annual numbers of diagnosed individuals (slope, –0.13), annual numbers of PWH with sequences also declined (slope, –0.16) (Figure 1, red line), while proportions of PWH with available sequences increased (slope, 0.39; median [interquartile range {IQR}], 86% [80%–93%]) (bars in Figure 1).

By the end of 2020, 1123/2674 (42%) PWH had available sequences before ART initiation (used for TDR assessment and susceptibility prediction). Initial regimens with starting dates were available for 1067 (95%; used for patient-specific prediction of susceptibility).

Sociodemographic and clinical characteristics of the cohort are presented in Table 1: 77% were male, 56% age 25–44 years, 60% White, 55% MSM, 59% US-born, mean CD4 count 400 cells/μL, 87% with HIV-1 subtype B and 13% non-B (CRF02_AG 4.7%, G 2.1%, C 1.3%, A1 1.1%, CRF01_AE 1%, and others <0.5% each). HIV-1 PRRT sequences were available for 1122, and integrase sequences were available for 49 people sampled over 2017–2020.

Longitudinal Dynamics of TDR

Among 1122 PRRT sequences, 162 (14%) had TDR, with a steady increase from ~8% in the mid-2000s to 26% in 2020 and annual fluctuations (Mann-Kendall test statistic, 0.47, 95% CI, 0.16 to 0.68) (Figure 2A). Dual-class TDR over 2004–2020 remained <4% with a slight increase over time (Mann-Kendall test statistic, 0.24; 95% CI, –0.022 to 0.46). No triple-class TDR was seen after 2010. Average class-specific TDR over 2004–2020, represented by SDRM occurrence, was 9% for NNRTIs, 4% for NRTIs, and 3% for PIs. NNRTI SDRMs contributed the most, with a steady increase from 5% in 2004 to 18% in 2020 (Mann-Kendall test statistic, 0.48; 95% CI, 0.16 to 0.69) (Figure 2B). NRTI SDRMs contributed less, with a smaller increase from 2% in 2004 to ~8% in 2020 (Mann-Kendall test statistic, 0.074; 95% CI, –0.21 to 0.32). PI TDR was <2% in the mid-2000s, fluctuated to 7% in 2008 and 5% in 2016, and dropped to ~2%–3% in 2019–2020 (Mann-Kendall test statistic, 0.21; 95% CI, –0.15 to 0.49) (Figure 2B).

The estimated time of HIV-1 diagnosis was available for 1122/1123, with a median diagnosis-to-genotyping time (IQR) of 25 (13–138) days for those without SDRMs and 24 (12–71) days for those with SDRMs (P = .54).

Dynamics of individual SDRMs per antiretroviral class are presented in Figure 3. Among NNRTI SDRMs, K103N was the most prominent, with K103N/S prevalence increasing from 5% in 2004–2007 to 12% in 2016–2020, while other NNRTI SDRMs were lower (~1%), with minimal evolution over time. NRTI SDRM prevalence was low, with the most prominent being M41L, T215Y/I/S/D/E, and K219Q/N/R. The prevalence of K65R (0%) and M184V/I (0.5%), as well as PI SDRMs, was low.

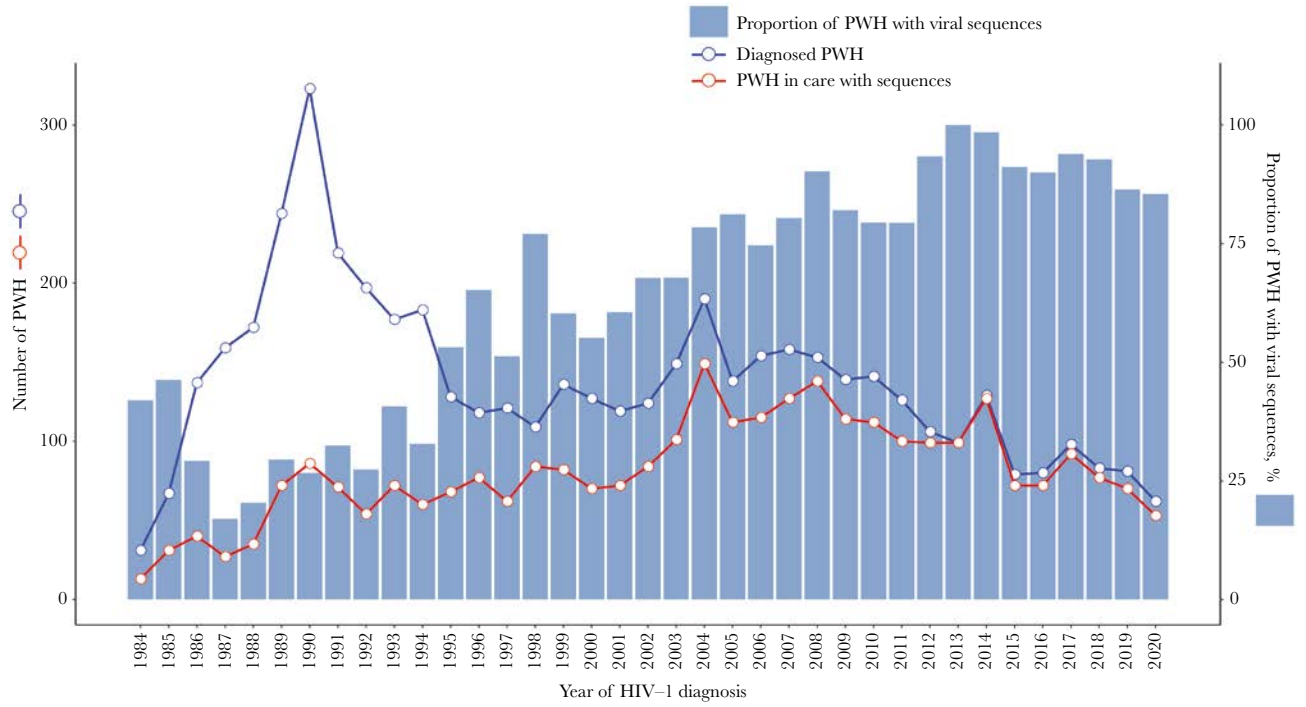

Figure 1. HIV-1 diagnoses and sequence availability in Rhode Island, USA. This graph demonstrates the number of newly diagnosed individuals (blue line) and those individuals in care with sequences (red line), living in RI (left y-axis), by year from 1984 to 2020 (x-axis). Bars depict proportions of PWH with sequences by year (right y-axis). Abbreviations: PWH, people with HIV; RI, Rhode Island.

Table 1. Cohort Demographic, Clinical, and Laboratory Characteristics

Variables	Total PWH (n = 1122), No. (%)	PWH Without SDRMs (n = 960), No. (%)	PWH With SDRMs (n = 162), No. (%)
Gender			
Males	860 (77)	727 (76)	133 (82)
Females	244 (22)	218 (23)	26 (16)
Transgender	11 (1)	11 (1)	0 (0)
Age, y			
<25	153 (14)	136 (14)	17 (10)
25–34	333 (30)	285 (30)	48 (30)
35–44	295 (26)	249 (26)	46 (28)
45+	341 (30)	290 (30)	51 (31)
Ethnicity			
Hispanics	284 (25)	246 (26)	38 (23)
Non-Hispanics	830 (74)	709 (74)	121 (75)
Unknown	1 (0)	1 (0)	0 (0)
Race			
White	675 (60)	571 (59)	104 (64)
Black or African American	319 (28)	279 (29)	40 (25)
Asians	26 (2)	24 (2)	2 (1)
Others	26 (2)	25 (3)	1 (1)
Unknown	69 (6)	57 (6)	12 (7)
Men who have sex with men			
Yes	618 (55)	522 (54)	96 (59)
No	487 (43)	426 (44)	61 (38)
Unknown	10 (1)	8 (1)	2 (1)
Ever incarcerated			
Yes	61 (5)	52 (5)	9 (6)
No	989 (88)	846 (88)	143 (88)
Unknown	62 (6)	55 (6)	7 (4)
Ever diagnosed with a mental health disorder			
Yes	404 (36)	338 (35)	66 (41)
No	650 (58)	564 (59)	86 (53)
Unknown	60 (5)	53 (6)	7 (4)
Ever substance use			
Yes	136 (12)	120 (12)	16 (10)
No	939 (84)	802 (84)	137 (85)
Unknown	40 (4)	34 (4)	6 (4)
Country of birth			
USA	663 (59)	555 (58)	108 (67)
Caribbean	145 (13)	130 (14)	15 (9)
Other	236 (21)	211 (22)	25 (15)
Unknown	71 (6)	60 (6)	11 (7)
HIV-1 subtype			
B	981 (87)	828 (86)	153 (94)
Non-B subtypes	141 (13)	132 (14)	9 (6)
G	24 (2.1)	22 (2.3)	2 (1.2)
C	15 (1.3)	13 (1.4)	2 (1.2)
A1	12 (1.1)	11 (1.1)	1 (0.6)
F1	5 (0.4)	5 (0.5)	-
D	3 (0.3)	2 (0.2)	1 (0.6)
Recombinants	82 (7.3)	79 (8.2)	3 (1.9)
CRF02_AG	53 (4.7)	52 (5.4)	1 (0.6)
CRF01_AE	11 (1)	11 (1.1)	-
Other recombinants	18 (1.6)	16 (1.7)	2 (1.2)
CD4 count, mean ± SD, cells/mm^3	400 ± 270	395 ± 266	426 ± 290

Abbreviations: PWH, people with HIV; SDRMs, surveillance drug resistance mutations.

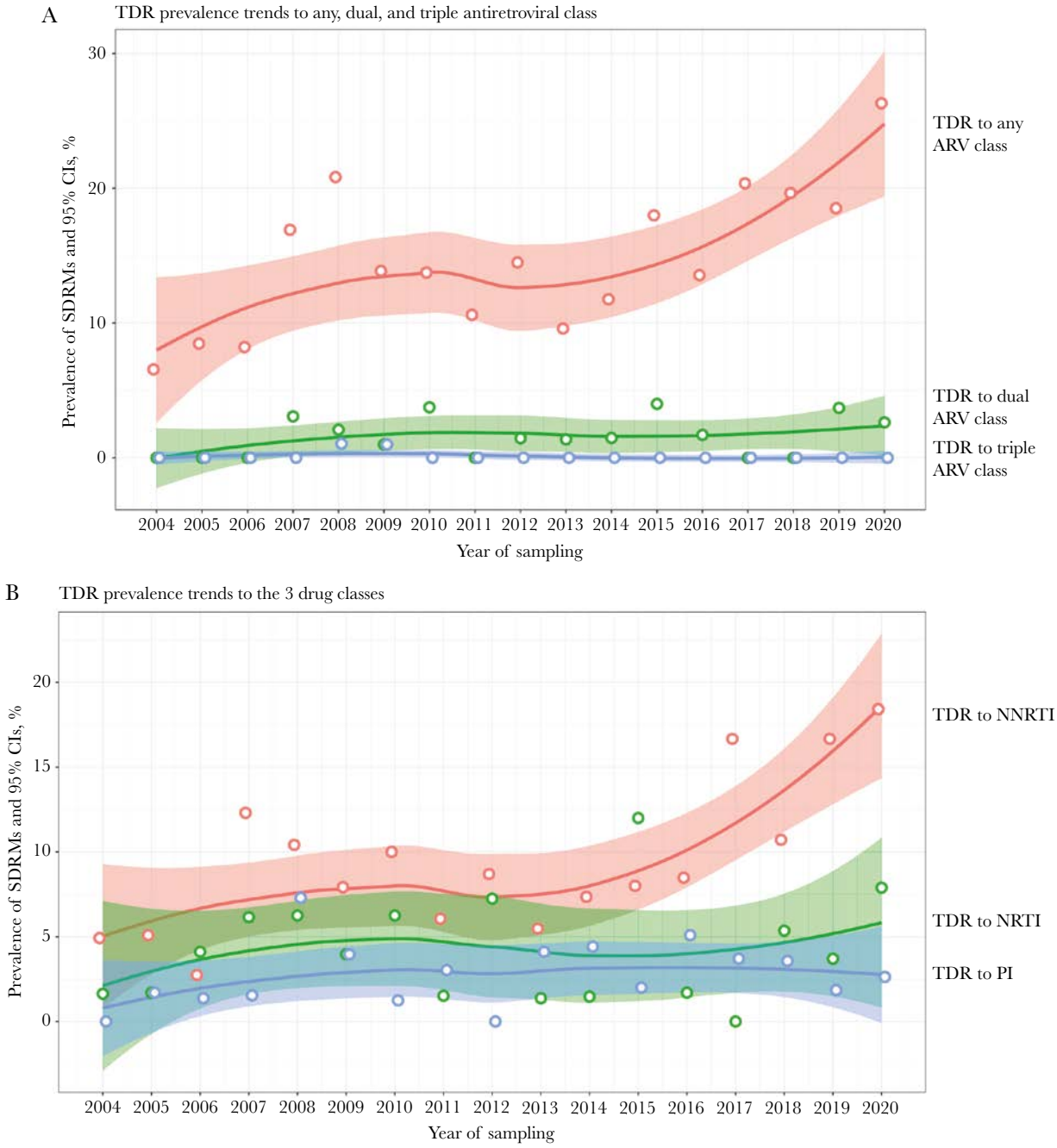

Figure 2. Dynamics of transmitted drug resistance in RI. These graphs demonstrate trends in TDR prevalence (y-axes) per year of sampling from 2004 to 2020 (x-axes). Regression curves were smoothed by the Loess method. Lines represent smoothed trends, each dot represents an annual value, and shades indicate 95% CIs. A, TDR prevalence trends to any, dual, and triple antiretroviral class. B, TDR prevalence trends to the 3 drug classes. Abbreviations: ARV, antiretroviral; NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; RI, Rhode Island; SDRM, surveillance drug–resistant mutation; TDR, transmitted drug resistance.

No InSTI SDRMs were found among the 49 available sequences (5 sampled in 2017, 2 in 2018, 17 in 2019, and 25 in 2020). Five individuals, all with HIV-1 subtype B, had accessory InSTI mutations (4 E157Q; 1 Q95K).

Dual- and Triple-Class Resistance

Seventeen individuals had TDR to 2 (n = 15; diagnosed 1999–2018) or 3 (n = 2; diagnosed 2008–2009) antiretroviral classes (Supplementary Table 1). Most individuals with dual/triple-class TDR had NRTI (n = 14; 82%) and NNRTI (n = 13; 76%) SDRMs, while PI SDRMs were found in 7 (41%) individuals.

Supplementary Figure 1 demonstrates the dynamics of viral load, CD4 counts, and antiretrovirals in selected individuals with dual-class TDR. One individual (Supplementary Figure 1A) with 2-class SDRMs (NNRTI K103N with high-level efavirenz resistance, plus NRTI D67N and K219Q with potential tenofovir

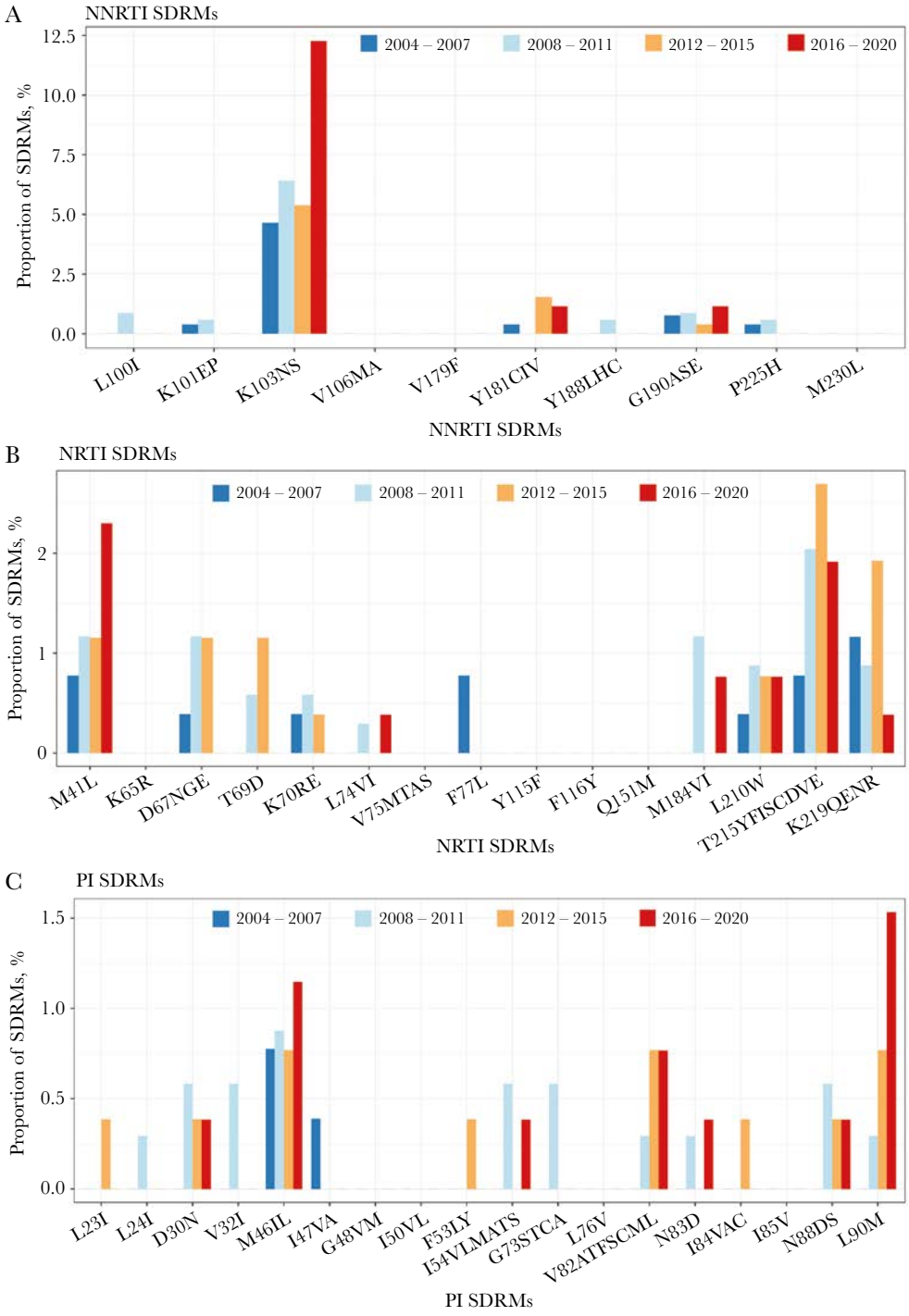

Figure 3. Transmitted drug resistance mutations in Rhode Island by antiretroviral class. These graphs demonstrate the proportions (y-axes) of specific transmitted drug resistance mutations (x-axes) by 4-year time intervals from 2004 to 2020 according to the color-coding bins shown in the legends at the top of each graph of the graphs. Each mutation is represented by the wild-type amino acid, followed by the amino acid position and the mutated amino acid/s. A, NNRTI-associated mutations. B, NRTI-associated mutations. C, PI-associated mutations (note different scales of y-axes). Abbreviations: NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; RI, Rhode Island; SDRM, surveillance drug–resistant mutation; TDR, transmitted drug resistance.

low-level resistance) responded rapidly to their first-line regimen, a pattern that was similar among 12/17 (71%) individuals with dual/triple-class TDR. After initiating TDF/emtricitabine/efavirenz, this person had an undetectable viral load for ~2 years. However, after adherence problems and a few viral blips (129–158 copies/mL), a repeat genotype showed a similar resistance profile, and the patient was switched to atazanavir/ritonavir/TDF/emtricitabine and has remained suppressed since.

A second response pattern was seen in 5/17 (29%) individuals, who demonstrated slower responses to first-line regimens

and/or subsequent failure. One individual (Supplementary Figure 1B) with dual-class TDR (NRTI K70R and K219Q with zidovudine intermediate-level resistance and abacavir and tenofovir potential low-level resistance; and PI D30N, I85V, and N88D, with high-level nelfinavir resistance and atazanavir potential low-level resistance) responded initially to a tenofovir/emtricitabine/efavirenz regimen switch from zidovudine/lamivudine/lopinavir/ritonavir, started during pregnancy. The patient demonstrated gradual increase of viral load to 2181 copies/mL accompanied by new NNRTI K103N and V108I and NRTI M184V, requiring another regimen switch to tenofovir/emtricitabine/darunavir/ritonavir for ~3 years, with no adherence issues but some drug toxicity and a viral load blip (1343 copies/mL).

TDR Impact on First-line Regimens

Of 1122 individuals with PRRT sequences, 12% (n = 129) demonstrated intermediate–high predicted resistance to at least 1 analyzed drug, including 10% (n = 110) to NNRTIs, 2% (n = 25) to NRTIs, 0.5% (n = 6) to PIs, 1% (n = 11) to 2 classes, and 0.1% (n = 1) to 3 classes, demonstrating the impact of the detected SDRMs. While the prevalence of predicted intermediate–high resistance levels to any class remained <10% until 2015, it increased to 17% during 2016–2020, demonstrating the combined (rather than individual) impact of SDRMs (Figure 4A). Predicted resistance profiles and their dynamics over time paraleled individual mutation patterns, with particularly high and increasing predicted resistance to the NNRTIs efavirenz and nevirapine (Figure 4B), lower but still increasing rates to non-lamivudine/emtricitabine NRTIs (Figure 4C), and stable low rates to PIs (Figure 4D).

Of 162 PWH with SDRMs, initial regimens were known for 155 (96%). Of those, 120 (77%) had intermediate- to high-level predicted resistance to at least 1 drug, including 25 (16%) with intermediate–high resistance to a drug in their initial regimen (15 to efavirenz, 5 to emtricitabine, 3 to tenofovir, and single occurrences to abacavir, lamivudine, and zidovudine) (Supplementary Table 2). To address whether regimen switch was driven by TDR, we evaluated time to virologic suppression and time to regimen switch. After excluding 4 cases with missing viral loads, we identified 3/21 (14%) participants (#17, #18, and #20 in Supplementary Table 2) who could not reach viral suppression on regimens that contained predicted intermediate- to high-level resistance and required regimen switch; 2 of them

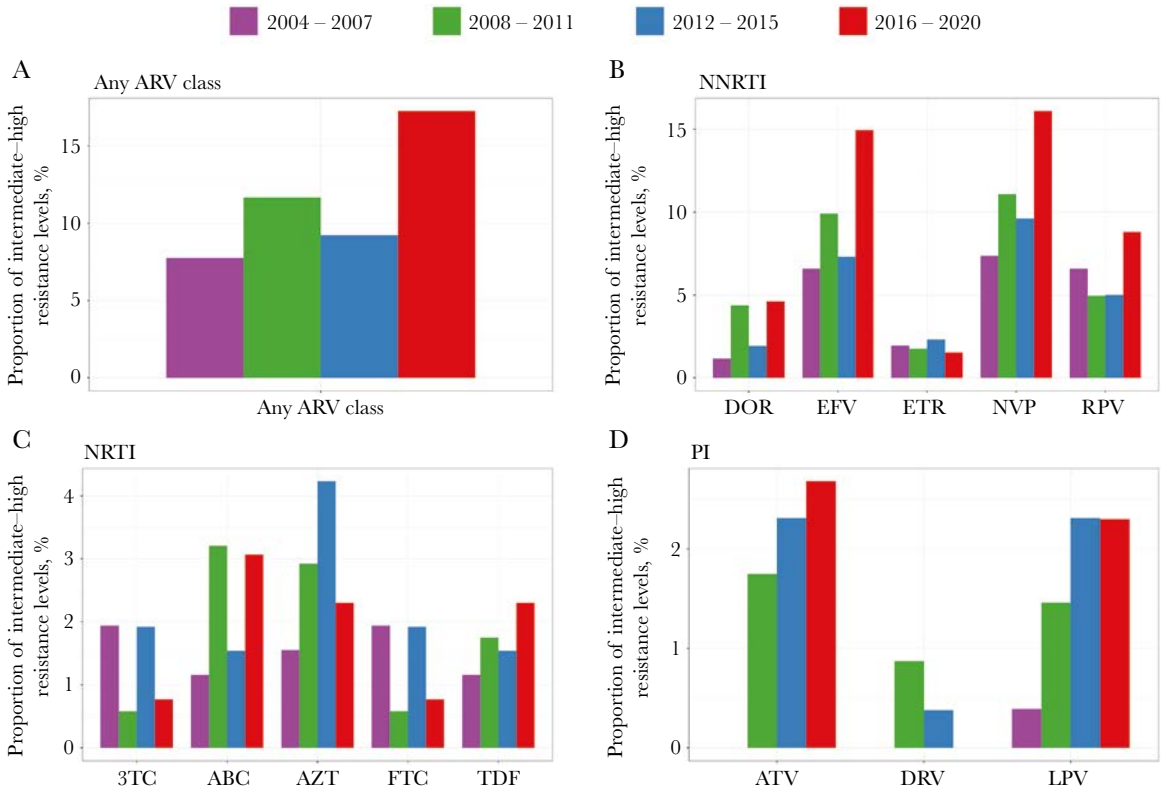

Figure 4. Intermediate to high predicted levels of transmitted resistance mutations in Rhode Island by antiretroviral class. These graphs demonstrate the proportions of predicted intermediate–high levels of resistance (y-axes) by antiretroviral class (x-axes) by 4-year time intervals from 2004 to 2020 according to the color-coding bins shown in the legend at the top. A, Resistance to any antiretroviral class. B, Resistance to the NNRTIs DOR, EFV, ETR, NVP, and RPV. C, Resistance to the NRTIs ABC, AZT, FTC, 3TC, and TDF. D, Resistance to the PIs ATV, DRV, and LPV (note different y-axis scales). Abbreviations: 3TC, lamivudine; ABC, abacavir; ARV, antiretroviral; ATV, atazanavir; AZT, zidovudine; DOR, doravirine; DRV, darunavir; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; LPV, lopinavir; NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleotide reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate.

reached viral suppression within 3 months of switch, while the third remained unsuppressed for >4 years. The remaining 18/21 (86%) participants became suppressed after a median (IQR) of 34 (24–75) days on the regimen that contained drug with intermediate- to high-level predicted resistance. Half of these 18 (including 2 with dual-class SDRMs M184V + K103N/P225H and G73CS + L100I) did not require regimen switch, while regimen switch in the other 9 participants was performed for reasons not directly related to viral suppression or TDR.

TDR Association With Clinical/Sociodemographic Variables

Supplementary Table 3 presents odds ratios (ORs) and associated 95% CIs from the multivariate logistic regression analysis relating TDR to selected clinical or sociodemographic variables. The only tested variable associated with TDR was HIV-1 subtype B (OR, 3.12; 95% CI, 1.43 to 6.81). The results from the multivariate logistic regression relating HIV-1 subtype (B vs non-B) to clinical or sociodemographic variables are presented in Supplementary Table 4, demonstrating that older age, MSM, and being in a molecular cluster are associated with HIV-1 subtype B.

Phylogenetic Clusters and TDR

We identified 123 clusters with 376/1122 (34%) individuals (2–12 per cluster) (Table 2). Most (n = 91; 74%) clusters included only individuals without TDR; 15 (12%) included both individuals with and without TDR; and 17 (14%) included only individuals with TDR, all of which shared SDRMs.

Proportions of individuals with SDRMs were similar among clustered (15%) and unclustered individuals (14%; $P = .56$). Among those who clustered, individuals with SDRMs were more likely in small clusters ($P = .025$). Among clustered individuals with SDRMs, 41% were in dyads, 59% were in clusters with 3–9 members, and none were in clusters with 10+ members. Among clustered individuals without SDRMs, 35% were in dyads, 54% were in clusters with 3–9 members, and 11% were in clusters with 10+ members.

DISCUSSION

Leveraging the near-centralized HIV care and increasing availability of sequences with linked data in RI, we assessed statewide longitudinal TDR trends, their impact, and their association with transmission networks. Confirming our hypothesis, we demonstrated a gradual increase in TDR prevalence from 8% to 26% over 2004–2020, with increasing potential impact on ART. This increase was driven mostly by NNRTIs, while SDRMs related to 2-class ART regimens, as well as dual- or triple-class TDR, were uncommon. Integrating TDR with transmission networks demonstrated that most of the 34% of individuals who clustered had no TDR; however, the 14% who had TDR shared similar SDRMs. These somewhat discordant results, of increasing TDR trends and occurrence in transmission

Table 2. Characteristics of Identified Molecular HIV Clusters

Cluster Size, Members per Cluster	No. of Clusters (No. of Members)	No. of Clusters Without TDR (No. of Members)	No. of Mixed Clusters (No. of Members)	No. of Clusters With TDR Only (No. of Members)	Shared DRM/S in Clusters With TDR Only (No. of Clusters)
12	3 (36)	3 (36)	0	0	
9	1 (9)	1 (9)	0	0	
8	1 (8)	1 (8)	0	0	
7	1 (7)	1 (7)	0	0	
6	2 (12)	0	2 (12)	0	
5	5 (25)	4 (20)	1 (5)	0	
4	16 (64)	12 (48)	3 (12)	1 (4)	K103NS
3	27 (81)	17 (51)	3 (9)	7 (21)	K103N (5); M41L/T25DE (1); G190A (1)
2	67 (134)	52 (104)	6 (12)	9 (18)	K103NS (6); M46L (1); G190A (1); T215D (1)

Abbreviations: DRM, drug resistance mutations; TDR, transmitted drug resistance.

networks but with potential decreasing significance considering better ART regimens, are reassuring; however, they justify the continued need for TDR surveillance.

The strengths of this study are its timeliness and comprehensiveness, as recent, longitudinal, and especially statewide TDR data from the United States are scarce, and existing reports are partial [7, 10, 12–15, 17, 35, 36]. Due to high diversity of study cohorts and methodologies, heterogenous results are expected. Indeed, our results, which are based on a high proportion of available RI sequences over time and demonstrate increasing TDR trends by 2020 that are mostly driven by NNRTI resistance, with some NRTI resistance and minimal to no PI and InSTI resistance, are supported by some reports, but not others. The comprehensiveness of our results should provide confidence in their ability to represent a more realistic and reliable snapshot of statewide TDR. Continued efforts to maximize comprehensive evaluations of TDR are essential.

Several observations can be made regarding the predominance of NNRTI TDR. First, its persistent increase despite the decline of NNRTI-containing regimens in RI since 2010 may be surprising but is not a new observation [12]. K103N, the most common NNRTI-associated transmitted mutation in our as well as other studies, likely predominates due to its low fitness cost and transmission capacity close to a wild-type virus, which may cause it to persist rather than decay [37]. Considerations of minority resistance variants or mutation linkage across viral genomes might shed further light on this phenomenon [38]. Second, although the predicted impact of TDR on first-line regimens was high (77% intermediate–high predicted resistance among individuals with SDRMs), most individuals with predicted resistance to drugs in their regimen became virologically suppressed. This suppression or resuppression phenomenon despite existing resistance has been previously observed and demands further consideration, to improve use of resistance testing in clinical care [39, 40]. Whether and how this phenomenon relates to the different virologic response patterns in the presence of TDR described here, likely also associated with parameters like adherence, which was not estimated here, remain to be determined. Importantly, resistance testing before ART initiation continues to be informative and assists in regimen selection. Third, whether NNRTI TDR could reduce viral suppression and be associated with long-term failure of InSTI-containing first-line regimens, as has been suggested, should be further investigated, in particular as InSTIs are increasingly recommended and used [41]. Lastly, our findings are in line with World Health Organization (WHO) recommendations to avoid using NNRTIs in settings with high TDR levels [42].

NRTI TDR in this study was low, but with a steady, albeit slow, increase (from 2% in 2004 to 8% in 2020). The low NRTI TDR levels support low risk in using dual-class regimens recommended by guidelines [2]. However, increasing NRTI TDR could negatively affect these 2-drug regimens, as well as alter the efficacy of expanding programs for pre-exposure prophylaxis (PrEP). It is encouraging that K65R and M184I/V were rarely transmitted in RI and that a dual regimen with a high resistance barrier like dolutegravir + lamivudine was reported effective in maintaining virologic control despite lamivudine resistance; however, the trend of increasing NRTI TDR and its impact should continue to be monitored [43, 44].

While we found no major InSTI SDRMs in RI, the increased use of this class since 2009, in the United States and globally, demands attention. First, 8% (4/49) of individuals with integrase genotypes harbored E157Q, a polymorphic mutation occurring in ~2%–5% of ART-naïve individuals depending on HIV-1 subtype [27] that could increase dolutegravir resistance [45]. Second, prevalence of InSTI TDR has increasingly been reported [46–48]. Third, InSTIs have been relatively recently introduced into care, and with their high barrier to resistance, resistance development may take time. Fourth, relevant mutations outside of integrase and novel resistance pathways may exist [49, 50]. Lastly, integrase resistance ordering and testing are commonly separate from PRRT and have not yet been incorporated into guidelines, making their monitoring challenging. Taken together, InSTI TDR surveillance is essential and should be considered a routine part of clinical care.

Using available sequence data for phylogeny allowed for exploration of TDR within transmission networks. Clustering in this ART-naïve cohort (34%) was similar to the entire RI HIV cohort (31%) [25]. While individuals with TDR were not members of transmission networks more than those without TDR, they were more likely to be found in small rather than large clusters, and some clusters exclusively included individuals with TDR who shared the same SDRMs. These data suggest at least some viral transmission with SDRMs within networks and support routine local and statewide TDR surveillance, which can inform interventions and promote early treatment. Integrating TDR surveillance with real-time molecular epidemiology approaches, ongoing in RI and other jurisdictions and recommended by the US Centers for Disease Control and Prevention [19], would allow statewide and regional analyses toward HIV and TDR prevention.

This study has several limitations. First, the extent of TDR in undiagnosed and unsampled individuals remains unknown, and sequences were available only since 2004, when routine resistance testing in RI started, both limiting extrapolation and generalization of findings, even in this statewide comprehensive data set. Second, while we could use records to derive ART-naïve status, cases of undocumented use of ART cannot be fully excluded. Third, full treatment histories were limited for earlier years, preventing analysis of the time between introduction of specific drugs and resistance development. Fourth, InSTI resistance was absent in the small number of available integrase sequences, justifying caution with trend estimation. Lastly, despite its associated strengths, RI is a small state with relatively

few PWH, challenging some TDR assessments (eg, limited integrase sequences) and associations (eg, with HIV-1 subtype).

In summary, analysis of longitudinal TDR trends in the densely sampled statewide HIV epidemic in RI over 2004–2020 revealed an ongoing increase in TDR prevalence with impact on ART. By the end of 2020, TDR reached a high of 26%, which was primarily, but not solely, driven by NNRTI resistance, which supports the WHO's efforts for routine global TDR surveillance. Limited TDR related to multiclass regimens and PrEP are encouraging, particularly considering current high–resistance barrier ART. However, clinical management challenges remain, and routine TDR surveillance of all antiretroviral classes, including InSTIs, and integration with molecular epidemiology approaches are important and can improve characterization on both the individual and population levels, toward development of public health interventions.

Acknowledgments

Financial support. This research was supported by National Institutes of Health grants R01AI136058, K24AI134359 and P30AI042853.

Potential conflicts of interest. M.H. reports fees from Competition Economics and The Miriam Hospital for consulting, outside the submitted work. R.K. reports receiving a research grant from Gilead Sciences for work that is not related to HIV or this paper. All other authors declare that they have no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Patient consent. The study does not include factors necessitating patient consent, and it was approved by the Lifespan Human Subjects Research Institutional Review Board in Providence, Rhode Island, USA.

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