

High Level of Pretreatment and Acquired Human Immunodeficiency Virus Drug Resistance in El Salvador: A Nationally Representative Survey, 2018–2019

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Background. Human immunodeficiency virus drug resistance (HIVDR) can negatively impact the effectiveness of antiretroviral therapy (ART). We aimed to estimate the prevalence of pretreatment HIVDR (PDR) among ART initiators and the prevalence of viral load (VL) suppression and acquired HIVDR among individuals receiving ART for 12 ± 3 months (ADR12) and ≥48 months (ADR48) in El Salvador.

Methods. Nationally representative cross-sectional PDR, ADR12 and ADR48 surveys were conducted among adults with HIV from October 2018 to August 2019, following World Health Organization-recommended methods. Demographic and clinic data and blood specimens were collected.

Results. Two hundred sixty participants were enrolled in the PDR survey, 230 in ADR12 and 425 in ADR48. Twenty-seven percent (95% confidence interval [CI], 17.1%–39.9%) of ART initiators had PDR to efavirenz or nevirapine. The prevalence of VL suppression was 88.8% (95% CI, 83.1%–92.8%) in ADR12 and 80.5% (95% CI, 76.6%–84.0%) in ADR48 surveys. Among people with HIV receiving a first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimens and with unsuppressed VL, the prevalence of ADR to efavirenz or nevirapine was 72.0% (95% CI, 32.3%–93.3%) and 95.0% (68.5%–99.4%) in the ADR12 and ADR28 surveys, respectively. ADR12 to boosted protease inhibitors (PI/r) or integrase strand transfer inhibitors (INSTIs) was not observed. ADR48 was 1.3% (95% CI, 0.2%–9.6%) and 2.1% (0.3%–13.7%), respectively.

Conclusions. Programmatic improvements in ART delivery are urgently needed in El Salvador to address the high levels of resistance to efavirenz or nevirapine among ART initiators and the low VL suppression prevalence among individuals on treatment.

Keywords. Central America; drug resistance surveillance; El Salvador; HIV; WHO.

Human immunodeficiency virus drug resistance (HIVDR) can increase the number of AIDS-associated deaths, new HIV infections, and the costs of the antiretroviral therapy (ART) program [1, 2]. Therefore, World Health Organization (WHO) recommends the surveillance of pretreatment drug resistance (PDR) among ART initiators and acquired HIV drug resistance

(ADR) among people with HIV (PHIV) receiving ART for 12 ± 3 months (ADR12) and ≥48 months (ADR48) [3–5].

Several Latin American and Caribbean countries have reported nationally representative estimates of PDR to nonnucleoside reverse transcriptase inhibitors (NNRTIs) >10% [6–11], the WHO-recommended threshold to urge transition to a non-NNRTI-containing first-line ART regimen [2]. Significantly higher levels of HIV resistance to NNRTI (odds ratio [OR], 3.4 [95% confidence interval {CI}, 1.8%–6.9%]; $P < .0001$) have been reported among ART initiators with previous exposure to antiretroviral (ARV) drugs (ie, ART discontinuation or HIV prophylaxis) compared with those without previous exposure to ARV drugs [12].

In 2008, the prevalence of PDR to any ARV drug in El Salvador was 10.3% (95% CI, 2.8%–24.2%), as reported in a study including 47 female sex workers and 9.0% (95% CI, 3.6%–17.6%) among 98 men who have sex with men [13]. In 2011, a sentinel study reported a prevalence of PDR to any ARV drug of 5.7% (95% CI, 2.4%–12.6%) among 88 ART

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initiators without previous exposure to ARV drugs, 2.3% (95% CI, .6%–7.9%) resistance to both nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs, and 1.4% (95% CI, .2%–7.6%) to boosted protease inhibitors (PI/r) [14].

To better understand the current HIVDR context in El Salvador, a nationally representative study was conducted among adults initiating and receiving ART from October 2018 to August 2019.

METHODS

The national HIV program has provided universal ART in El Salvador since 2018. At the time of the study, there were 24 000 PHIV in El Salvador, and 48% had started ART in 19 clinics [15]. According to the national ART guidelines, the regimen of tenofovir (TDF), emtricitabine or lamivudine (FTC/3TC), and efavirenz (EFV) was the preferred first-line ART, and a PI/r-based regimen was the preferred second-line ART [16].

Study Design

A nationally representative cross-sectional study was conducted following WHO-recommended methods [17, 18]. Clinics with a small number of adults receiving ART (representing <10% of the national cohort of adults receiving ART) were excluded from the sampling frame following the WHO-recommended methods [17, 18]. Twelve clinics were selected to participate in the study.

The sample size was calculated using the WHO standardized Microsoft Excel-based calculator [19]. The sample size per survey was calculated based on (i) ADR48: the total number of PHIV receiving ART 4 years before survey initiation (5842); (ii) ADR12: the number of ART initiators 2 years before survey initiation (1400); and (iii) PDR: the number of ART initiators 1 year before survey initiation (744). The default model assumptions recommended by WHO were used for sample size calculation: (i) 10% PDR; (ii) 85% viral load (VL) suppression; (iii) 25% previous ARV drug exposure prevalence among ART initiators; (iv) 100% individuals initiating NNRTI-based regimens; (v) 95% PHIV receiving a first-line ART regimen (ie, ARV drugs prescribed to initiate ART); (vi) 100% PHIV on first-line ART receiving NNRTI-based regimens; and (vii) a target CI half-width of $\pm 5\%$. The sample size was 249 for the PDR survey, 284 for the ADR12 survey, and 400 for the ADR48 survey. The sample distribution into the 12 clinics included in the sampling frame was proportional to the cohort of PHIV receiving ART in each clinic.

Patient Consent Statement

The institutional review boards of Universidad del Valle de Guatemala and the National Institute of Respiratory Diseases in Mexico (protocol number E02-17) approved the study. The staff involved in recruiting participants were trained on

appropriate study procedures. All participants provided written informed consent before enrollment.

Participant Enrollment

ART initiators (≥ 18 years of age), with or without previous exposure to ARV drugs, were eligible for the PDR survey. ART initiators were defined as individuals starting or restarting first-line ART. Previous exposure to ARV drugs was defined as ART discontinuation or HIV prophylaxis. PHIV (≥ 18 years of age) receiving ART for 12 ± 3 months were eligible for the ADR12 survey, and those receiving ART for ≥ 48 months were eligible for the ADR48 survey. Eligible individuals who consented were consecutively enrolled from October 2018 to August 2019. Deidentified demographic and clinical data and blood specimens were collected.

Laboratory Procedures

Blood specimens were collected from both adults initiating ART or receiving ART who were enrolled in the study. Plasma for VL testing was separated from whole blood within 6 hours of collection. VL (Abbott m2000 system, Abbott Molecular, Des Plaines, Illinois) and CD4 (Cytomics FC 500 system, Beckman Coulter, Brea, California) were measured in 2 reference laboratories in El Salvador.

Dried blood spots were prepared for HIVDR testing using either venous blood or finger pricks (depending on the participating centers' operational capabilities and staff availability). Specimens for HIVDR testing were handled and stored following the WHO/HIV ResNet Laboratory Operational Framework [20]. HIVDR testing was performed on specimens with VL ≥ 1000 copies/mL at the National Institute of Respiratory Diseases in Mexico, a WHO-designated regional laboratory for HIVDR surveillance. HIV RNA was extracted from 1 dried blood spot using silica columns (QIAamp Viral RNA Kit; Qiagen, Valencia, California). HIV protease and reverse transcriptase were amplified and sequenced using in-house validated protocols (HXB2: 2268-3302) [21]. Integrase was amplified and sequenced separately, using an in-house-validated protocol (HXB2: 4013-5265) [7, 22]. Sanger sequencing was performed on a 3730xl Genetic Analyzer (ThermoFisher, Waltham, Massachusetts). The sequences were assembled using ReCall (British Columbia Centre for Excellence in HIV/AIDS [BCCfE], Vancouver, Canada) [23]. The WHO/BCCfE HIVDR quality control tool was used for posttesting quality assurance [20]. Sequence clusters with genetic distance $< 0.5\%$ within the same sequencing batch were repeated and confirmed.

Data Analysis

As per WHO-recommended methods, VL suppression was defined as < 1000 viral copies/mL and HIVDR was defined as a penalty score ≥ 15 using the Stanford HIVdb tool (version

8.9) [17, 18, 24, 25]. HIV subtype was assigned using the Stanford HIVdb subtyping tool (version 8.9) and confirmed with the REGA HIV-1 subtyping tool (version 3) and the Recombinant Identification Program (version 3.0) [24, 26–28].

Weighted statistical analysis was performed using Stata version 15.1 (StataCorp, College Station, Texas), following the WHO recommendations [17, 18]. The weights were defined per participating clinic, considering the estimated eligible population sizes and the number of enrolled individuals. PDR analysis was weighted considering the number of specimens successfully sequenced. VL suppression estimates were weighted based on the VL results available. ADR analysis was weighted by the number of sequences available among the individuals who were not virally suppressed. VL suppression among PHIV receiving ART for 12 (\pm 3) months was adjusted by retention using the country estimate of adults receiving ART 12 months after ART initiation (706/800 [88.3%]) [29]. Retention-adjusted VL suppression was calculated as the product of the retention and VL suppression rates, and a Wald 95% CI was computed using the associated variance. The weighted analysis accounted for underenrollment, actual patient accrual rates, retention in care, and viral amplification failure.

Phylogenetic Analysis

An HIV transmission network analysis was performed to identify and characterize transmission clusters. After removing codons associated with HIVDR according to the Stanford HIVdb tool version 8.9 [24, 25], sequences from participants of the ADR and PDR surveys were aligned by codons using Mega v6 [30]. Phylogenetic trees were constructed using the maximum likelihood method based on the Tamura-Nei model with 1000 bootstrap repetitions. Reference sequences for HIV subtypes obtained from the Los Alamos HIV Sequences Database (<http://www.hiv.lanl.gov>) were included in the trees. The trees were visualized and colored in Mega v6 [30]. Using HIV-TRACE [31], putative transmission links were defined and resolved between individuals whose HIV sequences had a genetic distance <1.5%. A multivariable logistic regression model including gender, age, VL, state of residence, HIVDR, and previous ARV exposure was applied to find variables associated with clustering. Analyses were performed using Stata version 15.1 software (StataCorp, College Station, Texas).

RESULTS

Clinical and Demographic Characteristics

A total of 260 ART initiators were enrolled in the PDR survey (104% of the target sample size), 72.5% (95% CI, 64.0%–79.7%) were male, and 77.7% (95% CI, 69.7%–84.1%) were >25 years old (Table 1). Among ART initiators, 32.6% (95% CI, 25.6%–40.4%) had CD4 count <200 cells/ μ L, 44.6% (95% CI, 35.6%–

54.0%) had CD4 count 200–500 cells/ μ L, and 22.8% (95% CI, 15.7%–31.9%) had CD4 count >500 cells/ μ L. The majority (91.7% [95% CI, 87.6%–94.5%]) initiated ART with TDF, FTC/3TC, and EFV (Table 1). HIV-1 subtype B was observed in 99.5% (95% CI, 96.5%–99.9%) of ART initiators and the CRF12_BF in 0.5% (95% CI, .1%–3.5%).

The proportion of ART initiators without previous ARV drug exposure was 79.2% (95% CI, 73.1%–84.2%). The proportion of ART initiators who reported unknown previous ARV drug exposure was low (0.6% [95% CI, .6%–.64%]). Previous exposure to ARV drugs was reported by 20.3% (95% CI, 15.3%–26.4%) of ART initiators, 16.7% (95% CI, 11.6%–23.5%) among males and 30.7% (95% CI, 18.2%–46.8%) among females. All participants with previous ARV drug exposure reported ART discontinuation.

A total of 230 PHIV receiving ART were enrolled in the ADR12 survey (81% of the target sample size) and 425 in the ADR48 survey (106% of the target sample size). The percentage of females increased with follow-up time receiving ART (ADR12 = 22.6% [95% CI, 17.8%–28.2%] vs ADR48 = 47.1% [95% CI, 42.2%–52.0%]). The proportion of PHIV on a first-line NNRTI-based regimen was 99.7% for ADR12 and 83.4% for ADR48. The most frequently prescribed regimen among PHIV receiving ART for 12 \pm 3 was TDF, FTC/3TC, and EFV. Zidovudine (ZDV), FTC/3TC, and EFV was the most frequently prescribed regimen among PHIV receiving ART for \geq 48 months (Table 1). The proportion of PHIV receiving ART for \geq 48 months using second-line ART was 17.7% (95% CI, 13.8%–22.4%) among females and 14.8% (95% CI, 11.1%–19.4%) among males.

Pretreatment HIV Drug Resistance

The prevalence of PDR to EFV or nevirapine (NVP) was 27.0% (95% CI, 17.1%–39.9%), to any NRTI was 10.3% (95% CI, 4.1%–23.6%), to integrase strand transfer inhibitors (INSTIs) was 3.5% (95% CI, 2.9%–19.5%), and to PI/r was 1.2% (95% CI, .6%–2.4%) (Table 2). The prevalence of PDR to EFV or NVP was higher than 10% in both ART initiators with previous exposure to ARV drugs (37.4% [95% CI, 22.3%–55.4%]) and those without previous ARV exposure (24.4% [95% CI, 13.2%–40.7%]). The prevalence of PDR to EFV or NVP was 29.1% (95% CI, 17.7%–44.0%) among males and 14.9% (95% CI, 7.3%–28.0%) among females. K103NS was the mutation most frequently observed among ART initiators (20.1% [95% CI, 11.3%–33.2%]) (Supplementary Table 1).

The prevalence of PDR to TDF was 3.0% (95% CI, 1.0%–11.0%) and to FTC/3TC was 8.0% (95% CI, 3.0%–23.0%) (Table 2). Resistance to elvitegravir was 3.0% (95% CI, .3%–21.5%) and 0.5% (95% CI, .1%–2.6%) to raltegravir. The mutations associated with reduced susceptibility to elvitegravir and raltegravir were S147G (3.0% [95% CI, .3%–21.5%]), E138AKT (1.2% [95% CI, .5%–3.1%]), and Y143CHR (0.5% [95% CI,

Table 1. Demographic and Clinical Characteristics of Individuals Initiating and Receiving Antiretroviral Therapy in El Salvador, 2018–2019^a

Characteristic	ART Initiators (n = 260)		PHIV Receiving ART for 12 ± 3 mo (n = 230)		PHIV Receiving ART for ≥48 mo (n = 425)	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Gender						
Female	54	23.9 (17.3–32.2)	62	22.6 (17.8–28.2)	211	47.1 (42.2–52.0)
Male	200	72.5 (64.0–79.7)	164	75.6 (69.7–80.6)	213	52.6 (47.6–57.4)
Transgender	6	3.5 (1.4–8.7)	4	1.8 (.6–5.5)	1	0.4 (.1–2.3)
Age, y						
≤25	57	22.3 (15.9–30.3)	42	19.0 (11.3–30.2)	13	4.2 (2.4–7.3)
>25	203	77.7 (69.7–84.1)	188	81.0 (69.8–88.7)	412	95.8 (92.7–97.6)
Current ART line						
First-line	260	100.0 (98.5–100.0)	229	99.7 (98.6–99.9)	337	83.4 (80.4–86.0)
Second-line	NA	...	1	0.3 (.1–1.4)	87	16.5 (13.8–19.5)
Third-line	NA	...	0	0.0 (0–1.6)	1	0.2 (.0–.6)
Current ART regimen						
TDF + 3TC/FTC + EFV	235	91.7 (87.6–94.5)	216	93.5 (89.1–96.2)	113	23.8 (20.1–27.9)
TDF + 3TC/FTC + NVP	2	1.6 (.3–8.8)	1	0.2 (.2–.2)	10	2.3 (1.3–3.9)
ZDV + 3TC/FTC + EFV	9	2.9 (1.7–4.8)	7	4.5 (2.1–9.1)	167	44.9 (40.2–49.7)
ZDV + 3TC/FTC + NVP	2	0.6 (.2–1.5)	0	0.0 (.0–1.6)	30	8.5 (6.0–11.8)
PI-based	3	0.7 (.4–1.2)	1	0.3 (.1–1.4)	93	17.7 (15.0–20.7)
Other	9	2.5 (1.6–3.9)	5	1.5 (.8–3.0)	12	2.9 (1.6–5.2)

Abbreviations: 3TC/FTC, lamivudine/emtricitabine; ART, antiretroviral therapy; CI, confidence interval; EFV, efavirenz; NA, not applicable; NVP, nevirapine; PI, protease inhibitor; PHIV, people with human immunodeficiency virus; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

^aStudy design–weighted proportions and 95% CIs.

.1%–2.6%]). Mutations associated with resistance to dolutegravir (DTG), cabotegravir, and bicitegravir were not detected (Supplementary Table 1).

VL Suppression

The prevalence of VL suppression was 88.8% (95% CI, 83.1%–92.8%) among PHIV receiving ART for 12 (±3) months, decreasing to 78.4% (95% CI, 73.9%–83.0%) when adjusted by retention on ART after 12 (±3) months of ART initiation. The prevalence of VL suppression among PHIV receiving ART for ≥48 months was 80.5% (95% CI, 76.6%–84.0%), 82.6% (95% CI, 78.3%–86.3%) among those on first-line regimen and 69.8% (95% CI, 59.2%–78.7%) among those on second-line ART.

Acquired HIV Drug Resistance

Among PHIV on ART for 12 (±3) months receiving a first-line NNRTI-based regimen and with unsuppressed VL, the prevalence of ADR to EFV or NVP was 72.0% (95% CI, 39.1%–91.2%), and 61.2% (95% CI, 32.9%–83.5%) to any NRTI (Table 3). Resistance to PI/r or INSTI was not observed.

The PHIV on ART for ≥48 months receiving a first-line NNRTI-based regimen and with VL ≥1000 copies/mL had a prevalence of ADR to EFV or NVP of 95.0% (95% CI, 72.0%–99.3%), 1.3% (95% CI, .4%–4.9%) to PI/r, and 2.1% (95% CI, .8%–5.6%) to INSTI (Table 3). The prevalence of resistance to ZDV and FTC/3TC among PHIV on first-line

NNRTI and TDF-based regimens with VL ≥1000 copies/mL was 14.4% (95% CI, 2.7%–50.8%). On the other hand, resistance to both TDF and FTC/3TC was 47.0% (95% CI, 26.4%–68.7%) among the PHIV on first-line NNRTI and ZDV-based regimens with VL ≥1000 copies/mL.

The most frequent mutations among PHIV receiving ART for ≥48 months with unsuppressed VL were M184IV (77.2% [95% CI, 60.9%–88.1%]), K103NS (59.5% [95% CI, 42.7%–74.3%]), and T215FY (35.6% [95% CI, 22.9%–50.6%]) (Supplementary Table 2). The only INSTI-associated resistance mutation observed was R263K.

Among PHIV receiving ART for ≥48 months with VL ≥1000 copies/mL and receiving a PI/r-based second-line regimen, 29.0% (95% CI, 11.0%–57.4%) were resistant to ritonavir-boosted lopinavir or atazanavir, 6.1% (95% CI, 2.2%–15.5%) were resistant to ritonavir-boosted darunavir, and 14.9% (95% CI, 2.2%–57.1%) were resistant to any INSTI (Table 3).

Phylogenetic Analysis

Although all individuals with available HIV sequences participating in the ADR12, ADR48, and PDR surveys were included in the phylogenetic analysis, only clusters including individuals participating in the PDR survey were observed. Eighteen clusters of 2–3 individuals, including 39 individuals, were observed (Figure 1). Frequent links between persons with large age differences were observed, suggesting HIV transmission between younger and older persons. As expected, persons forming

Table 2. Prevalence of Pretreatment Human Immunodeficiency Virus Drug Resistance in El Salvador, 2018–2019^a

Drug Class	All		ART Initiators Without Previous ARV Drug Exposure		ART Initiators With Previous ARV Drug Exposure	
	no./No.	% (95% CI)	no./No.	% (95% CI)	no./No.	% (95% CI)
NRTI						
Any	14/209	10.3 (4.1–23.6)	7/159	9.6 (2.8–28.4)	7/47	13.3 (6.9–24.1)
ABC	9/209	8.0 (3.0–23.0)	2/159	7.0 (1.0–30.0)	7/47	13.0 (7.0–24.0)
3TC or FTC	9/209	8.0 (3.0–23.0)	2/159	7.0 (1.0–30.0)	7/47	13.0 (7.0–24.0)
TDF	3/209	3.0 (1.0–11.0)	1/159	2.0 (.0–17.0)	2/47	3.0 (1.0–9.0)
ZDV	6/209	2.0 (1.0–4.0)	5/159	2.0 (1.0–5.0)	1/47	2.0 (.0–9.0)
NNRTI						
EFV or NVP	39/209	27.0 (17.1–39.9)	21/159	24.4 (13.2–40.7)	18/47	37.4 (22.3–55.4)
DOR	23/209	12.0 (7.0–19.0)	10/159	9.0 (4.0–18.0)	13/47	23.0 (14.0–35.0)
EFV	38/209	26.8 (16.9–39.7)	21/159	24.4 (13.2–40.7)	17/47	36.3 (21.3–54.5)
ETR	14/209	10.0 (4.0–23.0)	6/159	9.0 (2.0–29.0)	8/47	14.0 (8.0–23.0)
NVP	39/209	27.0 (17.1–39.9)	21/159	24.4 (13.2–40.7)	18/47	37.4 (22.3–55.4)
RPV	21/209	12.0 (6.0–25.0)	13/159	12.0 (4.0–29.0)	8/47	14.0 (8.0–23.0)
PI/r						
ATV/r, DRV/r, or LPV/r	4/209	1.2 (.6–2.4)	4/159	1.5 (.7–3.1)	0/47	0.0 (.0–7.6)
ATV/r	3/209	1.0 (.0–2.0)	3/159	1.0 (.0–3.0)	0/47	0.0 (.0–7.6)
DRV/r	0/209	0.0 (.0–2.0)	0/159	0.0 (.0–2.0)	0/47	0.0 (.0–7.6)
LPV/r	2/209	1.0 (.0–2.0)	2/159	1.0 (.0–3.0)	0/47	0.0 (.0–7.6)
INSTI						
Any	2/197	3.5 (2.9–19.5)	1/149	3.9 (3.8–27.1)	1/45	2.1 (1.4–8.7)
BIC	0/197	0.0 (.0–1.9)	0/149	0.0 (.0–2.5)	0/45	0.0 (.0–7.9)
CAB	0/197	0.0 (.0–1.9)	0/149	0.0 (.0–2.5)	0/45	0.0 (.0–7.9)
DTG	0/197	0.0 (.0–1.9)	0/149	0.0 (.0–2.5)	0/45	0.0 (.0–7.9)
EVG	1/197	3.0 (.3–21.5)	1/149	3.9 (.4–27.1)	0/45	0.0 (.0–7.9)
RAL	1/197	0.5 (.1–2.6)	0/149	0.0 (.0–2.5)	1/45	2.1 (1.4–8.7)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ARV, antiretroviral; ATV/r, atazanavir/ritonavir; BIC, bictegravir; CAB, cabotegravir; CI, confidence interval; DOR, doravirine; DRV/r, darunavir/ritonavir; DTG, dolutegravir; EFV, efavirenz; ETR, etravirine; EVG, elvitegravir; FTC, emtricitabine; INSTI, integrase strand transfer inhibitor; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI/r, boosted protease inhibitor; RAL, raltegravir; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

^aStudy design—weighted proportions and 95% CIs. The “All” group includes 3 ART initiators with unknown previous ARV drug exposure. HIV drug resistance was defined as the presence of a penalty score ≥ 15 using the Stanford HIVdb algorithm.

clusters frequently resided in the same geographic area, which implies HIV is spreading locally. PDR prevalence in clustering individuals was 28% (11/39), with 3 clusters suggesting transmission of drug-resistant variants. Considering only participants of the PDR survey, after multivariable adjustment, persons aged 33–40 years were less likely to form clusters compared to persons aged <25 years (adjusted OR, 0.23 [95% CI, .07–.77]; $P = .017$) and persons with previous ARV exposure were less likely to be included in clusters (aOR, 0.14 [95% CI, .03–.65]; $P = .013$). Associations of clustering with gender, geographic state of residence, VL, or HIVDR were not observed after multivariable adjustment (Table 4).

DISCUSSION

As previously described in other low- and middle-income countries [6–11], this study suggests that the ART program in El Salvador faces important HIVDR challenges. The majority of PHIV initiated ART with an EFV- or NVP-based regimen.

The level of PDR to EFV and NVP was found to be high (24.4% in ART-naïve persons, 37.4% in persons with previous ARV drug exposure, and 27.0% overall), around 3-fold higher than the WHO-recommended threshold (10%) to urgently shift to a first-line non-NNRTI-containing regimen [2]. A systematic review informing the WHO guidelines on the public health response to PDR concluded that PHIV with PDR to NNRTIs who initiated with an NNRTI-based regimen were less likely to suppress VL, more likely to interrupt ART, and more likely to experience virologic failure or death when compared with PHIV with PDR to NNRTIs who initiated with a non-NNRTI-based regimen [2, 32]. Increases in AIDS-associated deaths, new HIV infections, and ART program costs have been predicted in settings with PDR levels >10% [1]. The results of this study were presented to El Salvador’s Ministry of Health and HIV Programme authorities in late 2019. Given the high rates of PDR to EFV and NVP, the Ministry of Health updated the national guidelines to prescribe the globally recommended DTG-based regimen as the preferred first-line option for ART initiators [2, 33, 34].

Table 3. Prevalence of Acquired Human Immunodeficiency Virus Drug Resistance Among Individuals Receiving Antiretroviral Therapy With Unsuppressed Viral Load in El Salvador, 2018–2019^a

Drug Class	PHIV on ART for 12 ± 3 mo		PHIV on ART for 12 ± 3 mo Receiving First-Line NNRTI-Based Regimen		PHIV on ART for ≥48 mo		PHIV on ART for ≥48 mo Receiving First-Line NNRTI-Based Regimen		PHIV on ART for ≥48 mo Receiving Second-Line PI/r-Based Regimen	
	no./No.	% (95% CI)	no./No.	% (95% CI)	no./No.	% (95% CI)	no./No.	% (95% CI)	no./No.	% (95% CI)
NRTI										
Any	9/15	61.2 (32.9–83.5)	9/15	61.2 (32.9–83.5)	50/61	83.5 (72.0–90.8)	39/42	91.2 (77.5–96.9)	11/19	65.7 (42.4–83.3)
ABC	9/15	61.2 (32.9–83.5)	9/15	61.2 (32.9–83.5)	50/61	83.5 (72.0–90.8)	39/42	91.2 (77.5–96.9)	11/19	65.7 (42.4–83.3)
3TC or FTC	9/15	61.2 (32.9–83.5)	9/15	61.2 (32.9–83.5)	47/61	77.2 (65.1–86.1)	39/42	91.2 (77.5–96.9)	8/19	45.3 (24.0–68.5)
TDF	8/15	49.1 (24.1–74.5)	8/15	49.1 (24.1–74.5)	28/61	42.4 (30.8–55.0)	21/42	47.4 (33.4–62.0)	7/19	31.0 (14.2–54.9)
ZDV	0/15	0.0 (0.0–20.4)	0/15	0.0 (0.0–20.4)	26/61	45.9 (33.5–58.7)	19/42	48.8 (34.3–63.6)	7/19	39.1 (19.0–63.7)
NNRTI										
EFV or NVP	12/15	72.0 (39.1–91.2)	12/15	72.0 (39.1–91.2)	53/61	84.8 (71.5–92.5)	41/42	95.0 (72.0–99.3)	12/19	61.5 (36.9–81.3)
DOR	9/15	62.8 (34.2–84.5)	9/15	62.8 (34.2–84.5)	39/61	63.4 (50.7–74.5)	35/42	80.8 (64.2–90.8)	4/19	23.7 (8.4–51.3)
EFV	12/15	72.0 (39.1–91.2)	12/15	72.0 (39.1–91.2)	52/61	83.8 (70.7–91.8)	41/42	95.0 (72.0–99.3)	11/19	58.4 (34.6–78.9)
ETR	6/15	48.1 (23.3–73.9)	6/15	48.1 (23.3–73.9)	18/61	29.3 (19.6–41.4)	16/42	37.7 (24.7–52.8)	2/19	10.2 (3.0–30.0)
NVP	12/15	72.0 (39.1–91.2)	12/15	72.0 (39.1–91.2)	53/61	84.8 (71.5–92.5)	41/42	95.0 (72.0–99.3)	12/19	61.5 (36.9–81.3)
RPV	7/15	53.3 (27.3–77.6)	7/15	53.3 (27.3–77.6)	28/61	43.5 (32.2–55.6)	22/42	52.9 (38.1–67.2)	6/19	22.2 (11.1–39.4)
PI/r										
ATV/r, DRV/r, or LPV/r	0/15	0.0 (0.0–20.4)	0/15	0.0 (0.0–20.4)	5/61	9.8 (3.7–23.3)	1/42	1.3 (0.4–4.9)	4/19	29.0 (11.0–57.4)
ATV/r	0/15	0.0 (0.0–20.4)	0/15	0.0 (0.0–20.4)	5/61	9.8 (3.7–23.3)	1/42	1.3 (0.4–4.9)	4/19	29.0 (11.0–57.4)
DRV/r	0/15	0.0 (0.0–20.4)	0/15	0.0 (0.0–20.4)	3/61	2.8 (1.3–5.9)	1/42	1.3 (0.4–4.9)	2/19	6.1 (2.2–15.5)
LPV/r	0/15	0.0 (0.0–20.4)	0/15	0.0 (0.0–20.4)	5/61	9.8 (3.7–23.3)	1/42	1.3 (0.4–4.9)	4/19	29.0 (11.0–57.4)
INSTI										
Any	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	1/61	5.1 (1.3–18.7)	1/44	2.1 (0.8–5.6)	1/16	14.9 (2.2–57.1)
BIC	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	1/61	3.6 (0.5–21.4)	0/44	0.0 (0.0–8.0)	1/16	14.9 (2.2–57.1)
CAB	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	1/61	3.6 (0.5–21.4)	0/44	0.0 (0.0–8.0)	1/16	14.9 (2.2–57.1)
DTG	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	2/61	3.6 (0.5–21.4)	0/44	0.0 (0.0–8.0)	1/16	14.9 (2.2–57.1)
EVG	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	2/61	5.1 (1.3–18.7)	1/44	2.1 (0.8–5.6)	1/16	14.9 (2.2–57.1)
RAL	0/17	0.0 (0.0–18.4)	0/17	0.0 (0.0–18.4)	2/61	5.1 (1.3–18.7)	1/44	2.1 (0.8–5.6)	1/16	14.9 (2.2–57.1)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ATV/r, atazanavir/ritonavir; BIC, bictegravir; CAB, cabotegravir; CI, confidence interval; DOR, doravirine; DRV/r, darunavir/ritonavir; DTG, dolutegravir; EFV, efavirenz; ETR, etravirine; EVG, elvitegravir; FTC, emtricitabine; INSTI, integrase strand transfer inhibitor; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI/r, boosted protease inhibitor; PHIV, people with human immunodeficiency virus; RAL, raltegravir; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

^aStudy design—weighted proportions and 95% CIs. HIV drug resistance was defined as the presence of a penalty score ≥15 using the Stanford HIVdb algorithm.

The HIV transmission network analysis showed clusters of drug-resistant variants that suggest PDR spread among ART-naive persons and locally. Of note, persons with ADR were not observed in the network, suggesting either a limited role of this group in HIVDR transmission in El Salvador or a limited sampling density in the network. A similar observation was made for persons 33–40 years of age, who were less likely to form clusters than persons under 25, which has also been observed in other Latin American countries [35]. These observations, although limited by sample size, can help support and design HIV prevention policies and activities such as preexposure prophylaxis for adolescents and young adults.

Concerning the PHIV receiving ART, the retention-adjusted VL suppression estimate among PHIV receiving ART for 12 (±3) and the VL suppression rate among PHIV receiving ART for ≥48 months were below the global 2020 target of 90% of all PHIV receiving ART with VL suppression [36]. In

addition, PHIV receiving ART with VL ≥1000 copies/mL in El Salvador had high rates of HIVDR to NNRTI-based regimens, the most frequently used in the country. Considering these observations, a comprehensive action framework is needed to strengthen the performance of the national ART program and improve the quality of HIV services in El Salvador to improve VL suppression rates. For example, as per WHO recommendations, PHIV receiving NNRTI-based first-line ART regimens should be transitioned to an optimal DTG-based regimen [33, 37]. In addition, the routine monitoring of early warning indicators of HIVDR should be used to identify clinics with suboptimal performance and to design focused interventions [38–40]. For example, the assessment of attrition from ART should trigger focused interventions to identify and overcome the barriers to retention in care and treatment [41, 42]. Also, the VL testing coverage in El Salvador was 66%, considerably below the WHO-recommended target of >95%

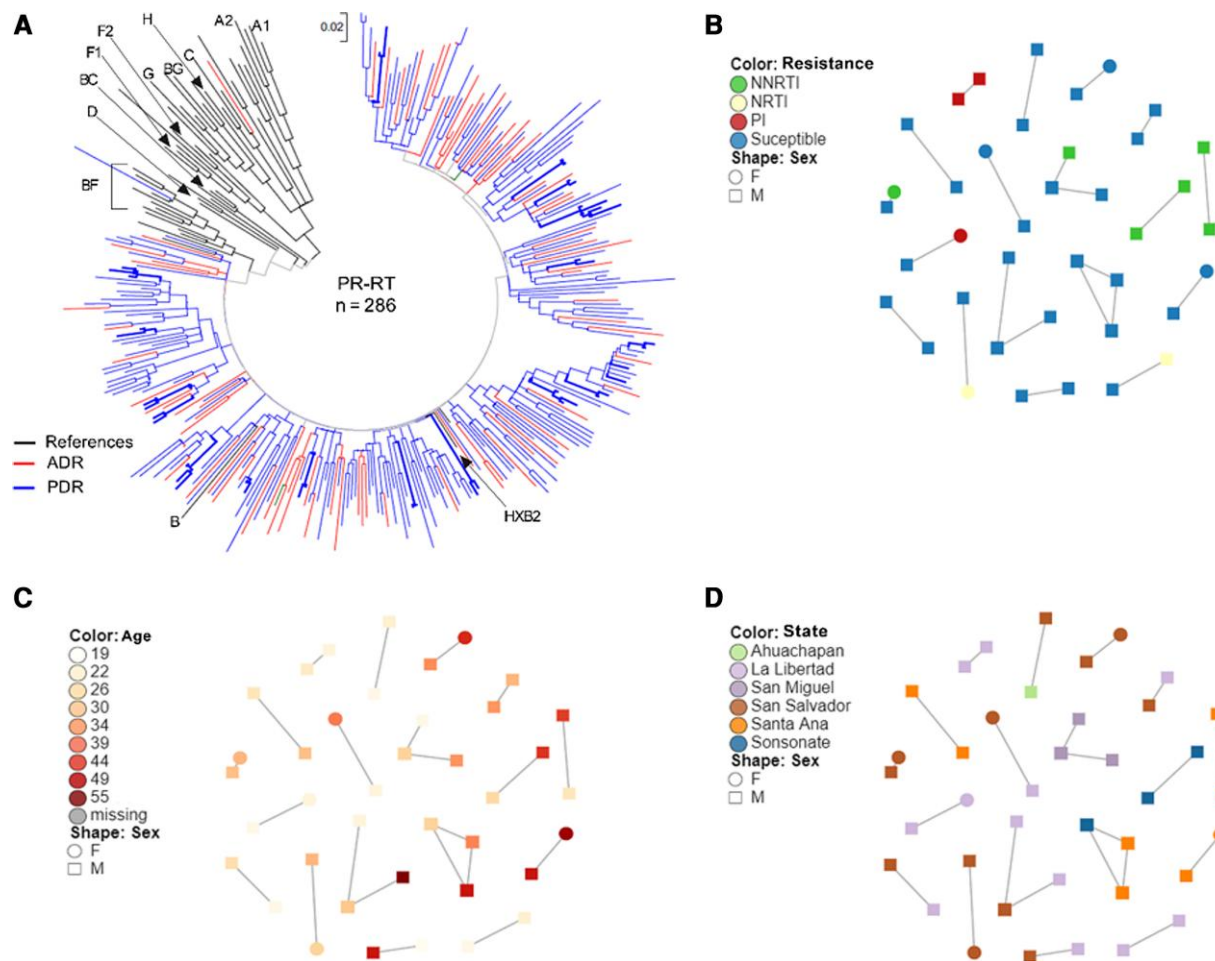


Figure 1. Phylogenetic tree and clustering. *A*, Sequences from 286 individuals included in the pretreatment drug resistance and acquired drug resistance surveys were included in the analysis. Reference human immunodeficiency virus (HIV) type 1 sequences of different subtypes obtained from the Los Alamos HIV Database were also included. Sequences were aligned by codon using ClustalW on Mega v6 after removing codons associated with HIV drug resistance according to the Stanford HIVdb tool (v8.9). A maximum likelihood tree was constructed based on the Tamura-Nei model with 1000 bootstrap repetitions. Using HIV-TRACE, putative transmission links were defined between individuals whose HIV sequence had a genetic distance <1.5%. Sequences forming clusters are shown in bold in the tree. Putative transmission links are detailed by drug resistance (*B*), age (*C*), and state of residence (*D*). Abbreviations: ADR, acquired drug resistance; F, female; M, male; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PDR, pretreatment drug resistance; PI, protease inhibitor; PR-RT, protease–reverse transcriptase.

[43, 44]. Therefore, enhancing the VL coverage and monitoring the VL testing cascade at the national and clinic levels could help identify programmatic gaps and underserved populations related to VL coverage, ART adherence counseling, and timely ART change for PHIV who are not suppressed [45–49].

In 2020, El Salvador updated the national ART guidelines adopting the WHO-recommended DTG-based second-line ART regimen in combination with an optimized NRTI backbone for those failing a first-line NNRTI-based regimen, prescribing ZDV (with FTC/3TC) if TDF was used in the failing first-line regimen and vice versa [33, 34]. DTG, administered with at least 1 fully active NRTI, has been reported to be superior to ritonavir-boosted lopinavir-based regimens in individuals failing an NNRTI-based first-line regimen [50]. In this study, we observed dual resistance to NRTIs used as the

backbone in second-line ART among PHIV receiving first-line NNRTI-based regimens with unsuppressed VL. HIVDR testing could be a valuable tool in El Salvador to avoid a functional DTG monotherapy among PHIV with dual resistance to the NRTI backbone, which could lead to DTG resistance [51–53]. To preserve the NRTI backbone among PHIV receiving ART in El Salvador, prolonged exposure to suboptimal ART should be avoided. Prompt clinical response to high VL and timely ART switch prevents drug resistance–associated mutation accumulation [54–57].

As previously reported by other countries in Central America [8, 10], a limitation of this study was the underenrollment for the ADR12 survey (81.0% [230/284] sample goal attainment) despite an extension of the enrollment period due to logistic constraints experienced by many clinics and slow

Table 4. Variables Associated With Clustering in Individuals Initiating Antiretroviral Therapy Within El Salvador's Human Immunodeficiency Virus Genetic Network

Variable	Clustering No. (%)	Crude OR (95% CI)	P Value	Adjusted OR (95% CI) ^a	P Value
Gender					
Male (n = 160)	33 (20.6)	1.00		1.00	
Female (n = 43)	6 (14.0)	0.62 (.24–1.61)	.325	0.93 (.32–2.74)	.901
Transgender (n = 6)	0 (0.0)	
Age category, y					
≤25 (n = 51)	16 (31.4)	1.00		1.00	
26–32 (n = 47)	10 (21.3)	0.59 (.23–1.49)	.261	0.59 (.21–1.66)	.320
33–40 (n = 55)	5 (9.1)	0.22 (.07–.69)	.004	0.23 (.07–.77)	.017
>40 (n = 55)	8 (14.6)	0.37 (.14–.99)	.040	0.55 (.19–1.64)	.285
Viral load category					
Quartile 1 (n = 51)	10 (19.6)	1.00		1.00	
Quartile 2 (n = 52)	11 (21.2)	1.10 (.42–2.89)	.846	1.25 (.43–3.61)	.675
Quartile 3 (n = 51)	4 (7.8)	0.35 (.10–1.23)	.086	0.38 (.10–1.44)	.156
Quartile 4 (n = 52)	13 (25.0)	1.27 (.53–3.50)	.513	2.03 (.69–5.97)	.197
State of residence					
San Salvador (n = 95)	12 (12.6)	1.00		1.00	
Other (n = 114)	27 (23.7)	2.15 (1.01–4.56)	.042	1.74 (.75–4.05)	.196
Any HIVDR					
No (n = 162)	28 (17.3)	1.00		1.00	
Yes (n = 47)	11 (23.4)	1.46 (.66–3.23)	.344	2.47 (.97–6.33)	.059
Previous ARV exposure					
No (n = 162)	37 (22.8)	1.00		1.00	
Yes (n = 47)	2 (4.3)	.15 (.03–.67)	.004	0.14 (.03–.65)	.013

Statistically significant associations ($P < .05$) are marked in bold.

Abbreviations: ARV, antiretroviral; CI, confidence interval; HIVDR, human immunodeficiency virus drug resistance; OR, odds ratio.

^aLogistic regression model including gender, age category, viral load, state of residence, presence of HIVDR, and previous ARV exposure. Number of observations in the model: 199. Missing data for age: 1, viral load: 3.

enrollment response. This limitation was accounted for by weighting the available data per clinic, considering the estimated eligible population sizes, the number of individuals enrolled, and the number of successful genotypes. Similarly, the study was designed to provide nationally representative PDR and ADR prevalence estimates, but not subnational or subpopulation-specific estimates that could help guide focused interventions. Finally, risk behavior data were not collected, limiting more in-depth analysis of the HIV transmission network in El Salvador.

In conclusion, important challenges for the ART program were observed in the first nationally representative HIVDR study in El Salvador, including high levels of resistance to EFV and NVP among ART initiators and low VL suppression prevalence among individuals on treatment. The prompt response of the Ministry of Health introducing DTG-based regimens as preferred first-line and alternative second-line ART [34] is expected to contribute to addressing HIVDR in the country. Other prioritizing actions are the transition of PHIV receiving ART to an optimal DTG-based regimen and monitoring the VL cascade to ensure that individuals with confirmed virologic failure are rapidly switched to an optimal regimen to avoid the accumulation of ARV-associated resistance

mutations. Additional studies to identify programmatic issues in specific geographical areas or populations are warranted to further adapt and strengthen responses to HIVDR in El Salvador.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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