HIV Pre-Treatment Drug Resistance in Mexico: A Nationally Representative WHO Survey

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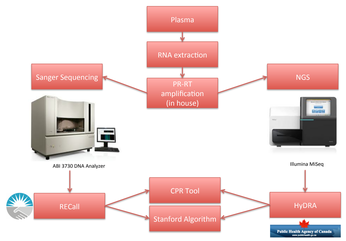
Background

- Prevention of emergence and transmission of HIV drug resistance (DR) is a major challenge directly influencing future effectiveness and sustainability of antiretroviral (ART) programmes, and requires immediate programmatic actions at country level. However, performing nationally representative HIVDR surveys that could inform policy making is challenging.
- In an effort to improve national representativeness of HIVDR surveys, the WHO and its partner organizations and experts of the HIVResNet group have developed an improved comprehensive strategy for HIVDR surveillance and monitoring, including a standardized protocol on pre-treatment HIVDR (PDR) surveillance.
- PDR surveillance informs selection of current first-line regimens, implementation of HIVDR testing, switch from NNRTI- to PI-based first line regimens, and intensification of viral load monitoring.
- Considering the national scenario with a relatively high ART coverage, predominance of first-line NNRTI-based ART regimens, as well as the existence of eventual drug stock-outs, bad prescribing practices and an estimated 33% prevalence of virally suppressed individuals from the total number of persons living with HIV, we present results of the first nationally representative PDR survey implemented in Mexico, following WHO recommendations.

Methods

- Participating clinics were selected according to the WHO HIV PDR surveillance concept notes, using the probability proportional to size (PPS) sampling method to achieve a nationally representative estimate of HIV PDR prevalence.
- A list of 161 Ministry of Health clinics representing 8,864 ART initiators was obtained and 95 clinics representing <10% of patients initiating ART were eliminated.
- A 32 clinic model was used to estimate sampling interval and sample size using the following parameters:
  - ✓ Proportion of ART initiators without prior ART exposure: 100%
  - ✓ Proportion of people initiating NNRTI-based regimens: 75%
  - ✓ Total number of clinics in the sampling table: 66
  - ✓ Total number of ART initiators in 2013 divided by two: 3,997
  - ✓ Desired CI for PDR outcomes: 5%
- PDR was assessed by Sanger and next generation sequencing (NGS) using the Stanford CPR and HIVdb tools.



Results

- A total of 264 viruses from 288 participants screened were successfully sequenced using both Sanger and NGS methods.

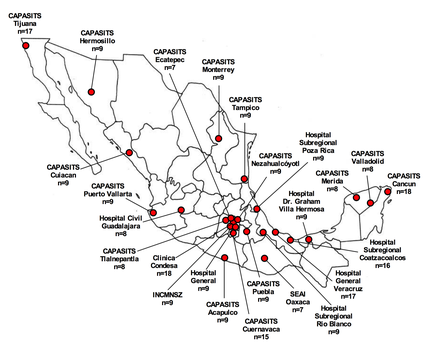
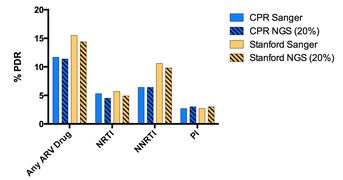


Fig 1. Geographic distribution of participating clinics.
Probability-proportional-to-size (PPS) sampling, using a 32-clinic model was applied to design the PDR survey according to WHO guidelines. Participants were recruited in 25 clinics selected by the PPS method, among all Ministry of Health institutions initiating ART in Mexico, from February to July 2015. Selected clinics and number of participants included per clinic are shown.

Results

- Based on Sanger sequences, PDR level to any ARV drug estimated using the CPR tool was 11.7%.
- When using the HIVdb algorithm, PDR to any ARV drug was 15.5%, reflecting an important, but not significant increase in NNRTI PDR (6.4% vs. 10.6%; p=0.12), mainly associated with E138AK (50% of cases) and A98G (20% of cases).
- Using the NGS consensus sequence, PDR estimations both using the CPR tool and the HIVdb algorithm were similar to the corresponding estimations using Sanger sequences.



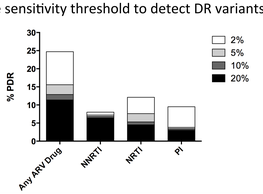
	CPR Sanger	CPR NGS (20%)	Stanford Sanger	Stanford NGS (20%)
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Any ARV Drug	11.7 (8.1, 16.3)	11.4 (7.8, 15.8)	15.5 (11.4, 20.5)	14.4 (10.4, 19.2)
NRTI	5.3 (2.9, 8.7)	4.5 (2.4, 7.8)	5.7 (3.2, 9.2)	4.9 (2.6, 8.3)
NNRTI	6.4 (3.8, 10.1)	6.4 (3.8, 10.1)	10.6 (7.2, 15.0)	9.8 (6.5, 14.1)
PI	2.7 (1.1, 5.4)	3.0 (1.3, 5.9)	2.7 (1.1, 5.4)	3.0 (1.3, 5.9)

Fig 2. Comparison of PDR levels estimated using the Stanford CPR and HIVdb tools on Sanger and next generation sequences.
A comparison of PDR prevalence estimations by drug family from Sanger vs. next generation consensus sequences is shown, using either the Stanford CPR or HIVdb tool. Estimations were based on 264 participants in all cases.

Table 1. Demographic and clinical characteristics of participants.

	Complete Cohort (n=264)	Individuals without PDR (n=233)	Individuals with PDR (n=31)
Gender [n (%)]*			
Male	225 (85.2)	203 (87.1)	22 (71.0)
Female	39 (14.8)	30 (12.9)	9 (29.0)
Age [years; median (IQR)]	31 (24, 39)	31 (25, 37)	33 (24, 41)
CD4 T Cell Count [cells/µl; median (IQR)]	258 (86, 428)	259 (100, 403)	192 (36, 544)
CD4 T Cell Count [%; median (IQR)]	13 (7, 21)	14 (7, 20)	12 (6, 22)
Plasma Viral Load [log copies/ml; median (IQR)]	4.8 (4.2, 5.3)	4.8 (4.2, 5.3)	5.2 (4.1, 5.5)
Recent Infection [n (%)]	48 (18.2)	44 (18.9)	4 (12.9)
Marital Status [n (%)]			
Single	182 (68.9)	163 (70.0)	19 (61.3)
Married	27 (10.2)	25 (10.7)	2 (6.5)
Domestic Partnership	46 (17.4)	38 (16.3)	8 (25.8)
NA	9 (3.4)	7 (3.0)	2 (6.5)
Literacy [n (%)]			
Cannot Read-Write/None	5 (1.9)	5 (2.1)	0 (0.0)
Primary	34 (12.9)	27 (11.6)	7 (22.6)
High School*	148 (56.1)	137 (58.8)	11 (35.5)
Degree/Technical	66 (25.0)	54 (23.2)	12 (38.7)
Graduate	6 (2.3)	6 (2.6)	0 (0.0)
NA	5 (1.9)	4 (1.7)	1 (3.2)
Employment [n (%)]			
Employed	131 (49.6)	119 (51.1)	12 (38.7)
Unemployed	98 (37.1)	84 (36.1)	14 (45.2)
Student	23 (8.7)	19 (8.2)	4 (12.9)
NA	12 (4.5)	11 (4.7)	1 (3.2)
HIV Risk Factor [n (%)]			
MSM	136 (51.5)	125 (53.6)	11 (35.5)
Heterosexual	96 (36.4)	81 (34.8)	15 (48.4)
Heterosexual/MSM	11 (4.2)	10 (4.3)	1 (3.2)
PWID	4 (1.6)	3 (1.3)	1 (3.2)
Other	1 (0.4)	1 (0.4)	0 (0.0)
NA	16 (6.1)	13 (5.6)	3 (9.7)

- The percentage of females was higher in individuals with PDR compared with individuals without PDR (p=0.03, OR 2.8 95% CI 1.2-6.6).
- NRTI (p=0.0024) and PI (p=0.0034) PDR levels, but not NNRTI PDR levels significantly increased when the sensitivity threshold to detect DR variants decreased.



	Sanger		20%		10%		5%		2%		p value [1]
	%	(95% CI)	%	(95% CI)	%	(95% CI)	%	(95% CI)	%	(95% CI)	(20% vs. 2%)
Any ARV Drug	11.7	(8.1, 16.3)	11.4	(7.8, 15.8)	12.9	(9.1, 17.5)	15.5	(11.4, 20.5)	24.6	(19.5, 30.3)	0.0001
NRTI	5.3	(2.9, 8.7)	4.5	(2.4, 7.8)	5.3	(2.9, 8.7)	7.6	(4.7, 11.5)	12.1	(8.4, 16.7)	0.0024
NNRTI	6.4	(3.8, 10.1)	6.4	(3.8, 10.1)	6.8	(4.1, 10.6)	7.2	(4.4, 11.0)	8.0	(5.0, 11.9)	NS
PI	2.7	(1.1, 5.4)	3.0	(1.3, 5.9)	3.4	(1.6, 6.4)	3.8	(1.8, 6.9)	9.5	(6.2, 13.7)	0.0034

Fig 3. PDR levels at different sensitivity thresholds.
PDR levels were estimated at different sensitivity thresholds, using next generation sequencing. Cumulative prevalence per drug family is shown.

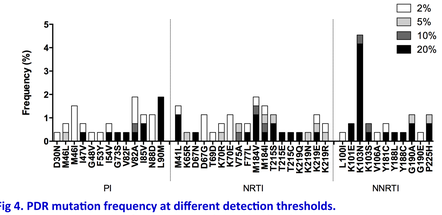


Fig 4. PDR mutation frequency at different detection thresholds.
Drug resistance mutation frequency was determined with next generation sequencing as explained in Methods. Cumulative prevalence of PDR mutations is shown. Only PDR mutations included in the WHO list are shown, and are classified by drug family.

- The most frequent PDR mutation was K103N (4.2% at 20% threshold). Other frequent NNRTI PDR mutations were K101E (0.8%), G190A (0.8%) and P225H (0.8%).
- For NRTI, TAMs were frequent, including M41L (1.1%), D67N (0.4%), T215 revertants (1.5%) and K219QE (0.8%) in addition to the discriminatory mutations M184V (1.1%) and M184I (0.4%). Interestingly, some non-TAMs were observed only as minority variants (under 5%), including K65R, T69D, K70E, and V75A.
- For PI, L90M (1.9%) was the most frequent mutation, followed by I85V (0.8%), V82AF (0.8%), G73S (0.4%), I54V (0.4%), and I47V (0.4%). Several PI mutations were found as minority variants only (<5%), including D30N, M46LI, G48V, F53Y, and N88D.

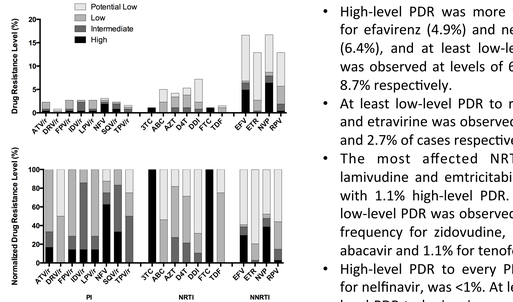


Fig 5. Drug resistance levels per antiretroviral drug.
PDR levels per drug were calculated using the Stanford HIVdb tool, from Sanger sequences and classified according to the Stanford Score (SS) as High: SS≥60, intermediate: SS 30-59, low: SS 15-29, potential low: SS 10-14. The top panel shows cumulative PDR levels per drug. The bottom panel shows normalized PDR levels per drug.

- High-level PDR was more frequent for efavirenz (4.9%) and nevirapine (6.4%), and at least low-level PDR was observed at levels of 6.8% and 8.7% respectively.
- At least low-level PDR to rilpivirine and etravirine was observed in 5.7% and 2.7% of cases respectively.
- The most affected NRTI were lamivudine and emtricitabine, both with 1.1% high-level PDR. At least low-level PDR was observed in 3.4% frequency for zidovudine, 2.3% for abacavir and 1.1% for tenofovir.
- High-level PDR to every PI, except for nelfinavir, was <1%. At least low-level PDR to lopinavir was observed in 2.7% of cases and to atazanavir in 2.3%. Darunavir was the least affected drug with only low-level PDR observed in 0.4% of cases.

- Geographically defined clusters of viruses with PDR were observed suggesting both MSM transmission and heterosexual transmission.

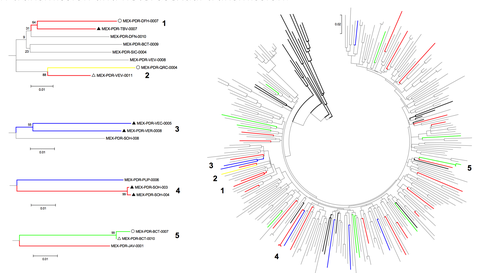


Figure 6. Phylogenetic relations between HIV sequences with PDR. A Maximum Likelihood tree built with the General Time Reversible + I + Γ model, without including DR positions is shown. 1000 Bootstrap repetitions were used to assess confidence. Viruses with PDR to PI (yellow), NRTI (green), NNRTI (red) and more than one ARV family (blue) are colored. Reference sequences were obtained from Los Alamos HIV Database (bold). Circle, female; triangle, male; full triangle, MSM.

Results

- Information on first-line ART regimen was available for 237 (90%) individuals. From these, 210 had started ART up to January 2016, with 85% initiating with NNRTI-based regimens and 14% with PI-based regimens.
- Considering only individuals starting with NNRTI-based regimens, 72% (97/135) had VL<50 copies/ml, while among individuals starting with PI-based regimens, 40% (10/25) were undetectable (p=0.0045).
- Interestingly, among NNRTI-initiators with NNRTI PDR (defined using the WHO list of mutations), only 25% (2/8) had undetectable VL (p=0.0064). This reduced viral suppression rate was less pronounced, but still significant, in individuals starting NNRTI-based regimens with NRTI PDR (5/10, 50% individuals with VL<100 copies/ml; p=0.023).
- From 14 participants with NNRTI PDR, 5 (36%) started with PI-based regimens (in all cases containing atazanavir). For the remaining 9 (64%) participants (all of which started with Atripla), genotyping results were not taken into account initially for starting ART, but a switch to PI-based regimens was recorded for 4 (44%) of them in a median of 78 days.

Table2. NNRTI PDR effect on first-line ART regimen.

	Complete Cohort	All NNRTI initiators	NNRTI initiators without NNRTI PDR	NNRTI initiators with NNRTI PDR (Stanford)	NNRTI initiators with NNRTI PDR (WHO list)
First ART regimen [a]	**n=210**				
NNRTI-based	178 (84.8)				
PI-Based	30 (14.3)				
INI-Based	2 (1.0)				
VL within 6 months after ART initiation [b]	**n=179**	**n=135**	**n=120**	**n=15**	**n=8**
VL <1000 copies/mL after 6 months on ART	148 (82.7)	124 (91.9)	112 (93.3)	12 (80.0)	7 (87.5)
VL <200 copies/mL after 6 months on ART	141 (78.8)	121 (89.6)	111 (92.5)	10 (66.7)	6 (75.0)
VL <100 copies/mL after 6 months on ART	126 (70.4)	109 (80.7)	102 (85.0)	7 (46.7)**	3 (37.5)**
VL <50 copies/mL after 6 months on ART	109 (60.9)	97 (71.9)	91 (75.8)	6 (40.0)*	2 (25.0)**
Change of ART scheme within 6 months of ART initiation [c]	**n=210**	**n=178**	**n=162**	**n=16**	**n=9**
Total individuals changing ART regimen	19 (9.0)	18 (10.1)	13 (7.3)		
Individuals changing ART regimen with PDR	7 (3.3)	7 (3.9)		5 (31.3)*	4 (44.4)**
Deactivation from National Database [c]	**n=242**	**n=178**	**n=162**	**n=16**	**n=9**
Death	9 (3.7)	7 (3.9)	7 (4.3)	0	0
Other reasons	7 (2.9)	4 (2.2)	4 (2.5)	0	0

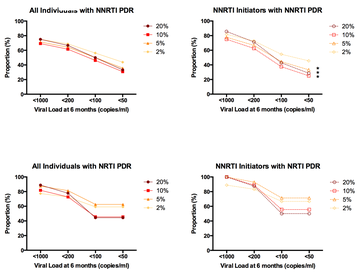


Fig 7. Effect of drug resistant minority variants on first-line ART
The proportion of individuals achieving various levels of viral suppression was recorded for participants with available data on ART initiation date and type of regimen, as well as follow-up viral load determinations up to 6 months after closing the survey. Effects of NRTI and NNRTI PDR minority variants at different sensitivity thresholds on viral suppression levels after initiation of first-line ART is shown.

- Compared to NNRTI initiators without NNRTI PDR, the proportion of individuals with undetectable VL was significantly lower in individuals with NNRTI mutations at 20% (p=0.019), 10% (p<0.01), and 5% (p=0.015), but not at 2% (p=0.074), suggesting an important clinical role of minority variants at least up to the 5% threshold.

Conclusions

- This is the first attempt to apply standardized and nationally representative WHO HIVDR surveys in Latin America.
- PDR trends in Mexico were similar to what is currently being observed in other low-/middle-income countries, with moderate overall PDR levels and NNRTI PDR dominance.
- This study also allowed to confirm the impact of NNRTI PDR in NNRTI-based first-line ART regimens, with a significant reduction in individuals achieving viral suppression.
- Our study also allowed the comparison of Sanger and next generation sequencing, suggesting an important clinical role for minority NNRTI PDR variants (>5%) in NNRTI-based ART regimens.
- Although recommending a national change in first line ART from NNRTI to a different class is debatable, given the high efficacy of NNRTI-based regimens in the Mexican setting in individuals without NNRTI PDR, the integration of basal HIVDR testing for initial ART follow-up and decision pipeline could improve ART efficacy.

Funding

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