HIV Drug Resistance Surveillance in Honduras after Ten Years of Widespread ART

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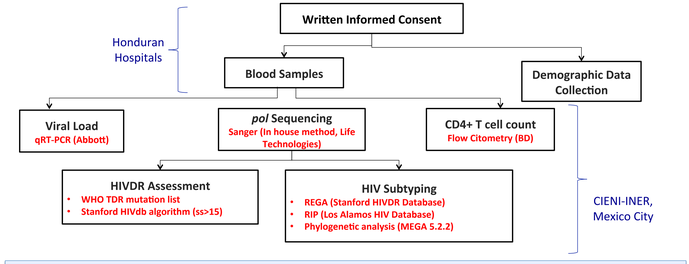
Background

- The recent expansion of antiretroviral treatment (ART) coverage in middle/low-income countries has been associated with increasing prevalence of HIV carrying antiretroviral (ARV) drug resistance (DR) mutations in individuals who have never been exposed to ARV drugs, usually referred to as "transmitted drug resistance" (TDR).
- HIVDR surveillance is key to maximizing the long-term effectiveness of ART regimens and to ensure the sustainability of ART programs.
- Honduras adopted national guidelines on ART for adults and children in 2003.
- We present results from a national study to assess the prevalence and trends of HIVDR in individuals failing ART (acquired DR, ADR) and in ART-naïve individuals (pre-ART DR, PDR) in Honduras, after 10 years of widespread availability of ART.

Methods

- 337 HIV-infected, ART-naïve, and 287 ART-experienced Honduran individuals were enrolled in 5 reference centers in Tegucigalpa, San Pedro Sula, Choluteca and La Ceiba, between April 2013 and December 2014.
- Plasma HIV protease-RT sequences were obtained using an in-house-developed protocol with a 3730xl Genetic Analyzer instrument (Life Technologies).
- HIVDR was assessed using the WHO HIV TDR surveillance mutation list and the Stanford algorithm (v7.0) (threshold score 15 for any ARV drug).
- Recently infected (RI) individuals were identified using a multiassay algorithm including incidence tests. Briefly, the BED HIV-1 Incidence EIA (Sedia) was applied to all individuals with CD4+ T cell counts >200 cells/µL and <1 year of HIV diagnosis and positive tests were confirmed with the HIV-1 Lag-Avidity EIA (Sedia).



Results

- PDR to any ARV drug was 11.6%, for the study period. NNRTI PDR prevalence (8.0%) was higher than NRTI (2.1%) and PI (2.1%) (p<0.0001).
- No demographic/clinical variables were associated with higher or lower PDR. Individuals with ADR were older, had lower VL and CD4 T cell counts than ART-naïve individuals.
- No significant trends in time were observed when comparing 2013 and 2014, nor using a moving average approach. Comparison of patients with >500 CD4 T cells/µL vs. <350 CD4 T cells/µL, did not show significant differences in PDR prevalence either.
- No significant differences were observed between TDR levels in RI individuals and individuals with chronic infection.
- The most prevalent PDR mutations were M46IL (1.5%) to PI, T215X (0.6%) to NRTI, and K103NS (5.6%) to NNRTI. E138X mutations, conferring DR to rilpivirine were also highly prevalent (5.0%), causing higher PDR estimations when using the Stanford DR definition (16.9% to any ARV drug).
- A cluster of viruses with NNRTI PDR from MSM from the same center was observed.
- The overall ADR prevalence for individuals with <48 months on ART was 89.3% and for the ≥48 months on ART group 79.3%. In both cases, PI ADR was lower, compared with NRTI and NNRTI (p<0.0001).
- ADR to two drug families was 70.6% and 56.2% (<48 and ≥48 months respectively) and ADR to three drug families 2.7 and 8.0%, with an increase in PI ADR in individuals with longer time on ART.
- M184V (74.7%, <48 months; 66.5%, ≥48 months), and K103N (49.3%, <48 months; 41.0%, ≥48 months) were the most frequent ADR mutations.
- PDR mutation frequency correlated with ADR mutation frequency both in individuals with <48 and ≥48 months on ART for PI and NNRTI (p<0.01 in all cases), but not for NRTI.

Table 1. Demographic characteristics of HIV-1-infected Honduran individuals with PDR and ADR

	Individuals with PDR (n=39)	ART-naïve individuals without PDR (n=298)	Individuals with ADR (n=287)
Gender [n (%)]			
Male	24 (61.5)	171 (57.4)	148 (51.6)
Female	15 (38.5)	127 (42.6)	139 (48.4)
Age [years; median (IQR)]*	31 (24, 38)	34 (27, 43)	39 (34, 47)
CD4 T Cell Count [cells/µL; median (IQR)]*	309 (107, 521)	271 (93, 483)	162 (74, 301)
Plasma Viral Load [log copies/mL; median (IQR)]*	4.8 (4.0, 5.3)	4.6 (4.0, 5.2)	4.2 (3.5, 4.8)
Marital Status [n(%)]			
Free Union	12 (30.8)	87 (29.2)	64 (22.3)
Married	3 (7.7)	29 (9.7)	41 (14.3)
Single	23 (59.0)	165 (55.4)	163 (56.8)
Other	0 (0)	11 (3.7)	15 (5.2)
Unknown	1 (2.6)	6 (2.0)	4 (1.4)
Literacy [n (%)]			
Primary	19 (48.7)	144 (48.3)	168 (58.5)
High School	11 (28.2)	87 (29.2)	70 (24.4)
Degree/Technical	5 (12.8)	32 (10.7)	15 (5.2)
Cannot Read-Write/None	4 (10.3)	32 (10.7)	29 (10.1)
Unknown	0 (0)	3 (1.0)	5 (1.7)
Employment [n (%)]			
Employed	11 (28.2)	132 (44.3)	123 (42.9)
Unemployed	24 (61.5)	144 (48.3)	153 (53.3)
Student	2 (5.1)	15 (5.0)	7 (2.4)
Unknown	2 (5.1)	7 (2.3)	4 (1.4)
HIV Risk Factor [n (%)]			
MSM	8 (20.5)	37 (12.4)	17 (5.9)
Heterosexual	28 (71.8)	238 (79.9)	253 (88.2)
Blood Transfusion	2 (5.1)	1 (0.3)	3 (1.0)
Heterosexual/MSM	1 (2.6)	10 (3.4)	5 (1.7)
IVU	0 (0)	5 (1.7)	1 (0.3)
Other	0 (0)	4 (1.3)	6 (2.1)
Unknown	0 (0)	3 (1.0)	2 (0.7)
Collection Center [n (%)]			
INCP	8 (20.5)	61 (20.5)	81 (28.2)
HEU	4 (10.3)	31 (10.4)	47 (16.4)
HMCR	12 (30.8)	101 (33.9)	94 (32.8)
USM**	12 (30.8)	68 (22.8)	39 (13.6)
HS	3 (7.7)	37 (12.4)	23 (8.0)
Unknown	0 (0)	0 (0)	3 (1.0)

*Difference between PDR/ADR, and ART-naïve/ADR, (p<0.01), Mann-Whitney test; **Difference between PDR/ADR, and ART-naïve/ADR, (p<0.01), Fisher test; PDR, Pre-ART Drug Resistance; ADR, Acquired Drug Resistance; IQR, Inter Quartile Range.

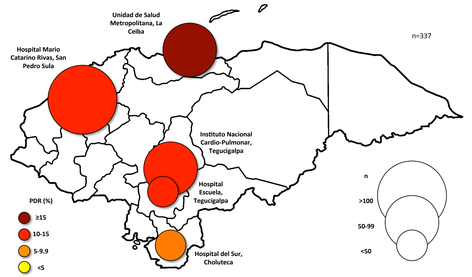


Figure 1. Geographic distribution of HIV PDR cases observed in the study. PDR was estimated for 337 PR-RT HIV sequences from antiretroviral treatment (ART)-naive Honduran individuals enrolled at five hospitals in Honduras. Using the WHO list of mutations for HIV drug resistance surveillance. The percentage of PDR cases by hospital is shown. Circle size represents the number of patients enrolled at each hospital; color reflects PDR level.

Table 2. PDR in a Honduran HIV-1-infected cohort in recently infected individuals and according to CD4+ T cell counts (2013-2014).

WHO Mutation List [a]	Complete cohort (2013-2014) (n=337)			Recently Infected individuals [c] (n=52)			>500 CD4+ T cells/µL (n=81)			350-500 CD4+ T cells/µL (n=63)			<350 CD4+ T cells/µL (n=193)		
	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]
Any ARV Drug	39	(11.6)	[8.4, 15.5]	7	(13.5)	[5.6, 25.8]	12	(14.8)	[7.9, 24.4]	5	(7.9)	[2.6, 17.6]	22	(11.4)	[7.3, 16.7]
NNRTI	27	(8.0)	[5.3, 11.4]	4	(7.7)	[2.1, 18.5]	8	(9.9)	[4.4, 18.5]	3	(4.8)	[1.0, 13.3]	16	(8.3)	[4.8, 13.1]
NRTI	7	(2.1)	[0.8, 4.2]	1	(1.9)	[0.0, 10.3]	2	(2.5)	[0.3, 8.6]	1	(1.6)	[0, 8.5]	4	(2.1)	[0.6, 5.2]
PI	7	(2.1)	[0.8, 4.2]	2	(3.8)	[0.5, 13.2]	3	(3.7)	[0.8, 10.4]	1	(1.6)	[0, 8.5]	3	(1.6)	[0.3, 4.5]

Stanford Score>15 [b]	Complete cohort (2013-2014) (n=337)			Recently Infected individuals (n=52)			>500 CD4+ T cells/µL (n=81)			350-500 CD4+ T cells/µL (n=63)			<350 CD4+ T cells/µL (n=193)		
	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]	n	(%)	[95% CI]
Any ARV Drug	57	(16.9)	[13.1, 21.4]	10	(19.2)	[9.6, 32.5]	15	(18.5)	[10.8, 28.7]	13	(20.6)	[11.5, 32.7]	29	(15.0)	[10.3, 20.9]
NNRTI	44	(13.1)	[9.6, 17.1]	7	(13.5)	[5.6, 25.8]	13	(16.0)	[8.8, 25.9]	10	(15.9)	[7.9, 27.3]	21	(10.9)	[6.9, 16.2]
NRTI	6	(1.8)	[0.7, 3.8]	1	(1.9)	[0.0, 10.3]	1	(1.2)	[0, 6.7]	1	(1.6)	[0, 8.5]	4	(2.1)	[0.6, 5.2]
PI	10	(3.0)	[1.4, 5.4]	2	(3.8)	[0.5, 13.2]	2	(2.5)	[0.3, 8.6]	2	(3.2)	[0.4, 11.0]	6	(3.1)	[1.1, 6.6]

[a] Pre-Antiretroviral Treatment Drug Resistance (PDR) estimated using the WHO HIV transmitted drug resistance surveillance mutation list. [b] PDR estimated with the Stanford algorithm (v7.0), with a threshold of ≥15 for at least one antiretroviral drug of the specified class. [c] Recently infected individuals were defined using a previously described multiassay algorithm including HIV incidence tests. AVR, Antiretroviral; NNRTI, Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI, Nucleoside Reverse Transcriptase Inhibitors; PI, Protease Inhibitors.

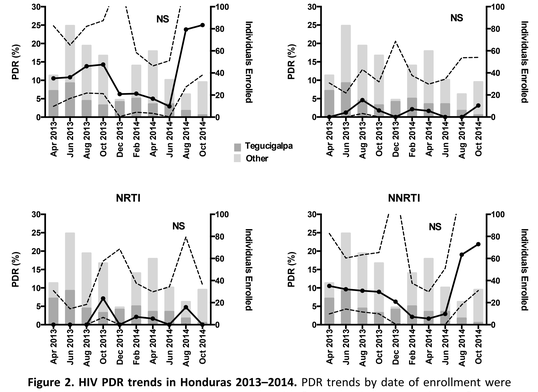


Figure 2. HIV PDR trends in Honduras 2013–2014. PDR trends by date of enrollment were estimated using a moving average approach, with 3-month windows, moving by 2-month intervals. PDR prevalence and 95% confidence intervals (CI) are shown. PDR, Pre-antiretroviral Treatment Drug Resistance; NRTI, Nucleoside RT Inhibitors; NNRTI, Non-nucleoside RT Inhibitors; PI, protease inhibitors.

Table 3. PDR in a Honduran HIV-1-infected cohort, 2013-2014

WHO Mutation List [a]	2013 (n=189)			2014 (n=148)			p value
	n	(%)	[95% CI]	n	(%)	[95% CI]	
Any ARV Drug	24	(12.7)	[8.3, 18.3]	15	(10.1)	[5.8, 16.2]	NS
NNRTI	17	(9.0)	[5.3, 14.0]	10	(6.8)	[3.3, 12.1]	NS
NRTI	4	(2.1)	[0.6, 5.3]	3	(2.0)	[0.4, 5.8]	NS
PI	5	(2.6)	[0.9, 6.1]	2	(1.4)	[0.2, 4.8]	NS

Stanford Score>15 [b]	2013 (n=189)			2014 (n=148)			p value
	n	(%)	[95% CI]	n	(%)	[95% CI]	
Any ARV Drug	33	(17.5)	[12.3, 23.6]	24	(16.2)	[10.7, 19.3]	NS
NNRTI	25	(13.2)	[8.7, 18.9]	19	(12.8)	[7.9, 19.3]	NS
NRTI	4	(2.1)	[0.6, 5.3]	2	(1.4)	[0.2, 4.8]	NS
PI	6	(3.2)	[1.2, 6.8]	4	(2.7)	[0.7, 6.8]	NS

[a] Pre-Antiretroviral Treatment Drug Resistance (PDR) estimated using the WHO HIV transmitted drug resistance surveillance mutation list. [b] PDR estimated with the Stanford algorithm (v7.0), with a threshold of ≥15 for at least one antiretroviral drug of the specified class. AVR, Antiretroviral; NNRTI, Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI, Nucleoside Reverse Transcriptase Inhibitors; PI, Protease Inhibitors; NS, Not significant (p>0.05).

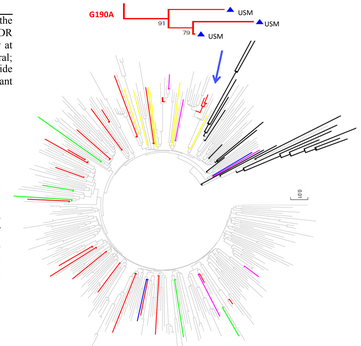


Figure 3. Phylogenetic relations between sequences with PDR in Honduras. Consensus Neighbor Joining tree with 1000 Bootstrap replications, built using the Tamura Nei model is shown. Viruses with PDR to Protease Inhibitors (PIs), Nucleoside RT Inhibitors (NRTIs), Non-Nucleoside RT Inhibitors (NNRTIs), and more than one ARV family are colored. B references and **no-B references** were obtained from the Los Alamos HIV Database. Clusters of viruses with TDR are shown and amplified. Blue triangle, MSM; USM, Unidad de Salud Metropolitana, La Ceiba.

Table 4. Frequency of pre-ART and acquired HIV drug resistance mutations in Honduras 2013-2014

	All ART-naive, n(%) (n=337)	PDR, n(%) (n=39)[a]	ADR ≤48 months, n(%) (n=85)	ADR >48 months, n(%) (n=237)
		Protease Inhibitors (PI)		
L10F	1 (0.3)	0 (0)	1 (1.3)	12 (5.7)
V11IL	0 (0)	0 (0)	0 (0)	2 (0.9)
K20IT	2 (0.6)	1 (14.3)	1 (1.3)	2 (0.9)
L24I	0 (0)	0 (0)	1 (1.3)	4 (1.9)
D30N	0 (0)	0 (0)	0 (0)	1 (0.5)
V32I	0 (0)	0 (0)	0 (0)	2 (0.9)
L33F	3 (0.9)	0 (0)	1 (1.3)	8 (3.8)
E35G	1 (0.3)	0 (0)	0 (0)	0 (0.0)
K43T	0 (0)	0 (0)	0 (0)	4 (1.9)
M46IL	5 (1.5)	5 (71.4)	1 (1.3)	18 (8.5)
I47V	1 (0.3)	1 (14.3)	0 (0)	4 (1.9)
I50V	0 (0)	0 (0)	0 (0)	2 (0.9)
F53L	0 (0)	0 (0)	0 (0)	1 (0.5)
I54VA	0 (0)	0 (0)	1 (1.3)	13 (6.1)
I54M	0 (0)	0 (0)	0 (0)	1 (0.5)
Q58E	2 (0.6)	2 (28.6)	1 (1.3)	10 (4.7)
G73CSTA	0 (0)	0 (0)	0 (0)	4 (1.9)
T74S	3 (0.9)	0 (0)	0 (0)	1 (0.5)
L76V	0 (0)	0 (0)	0 (0)	4 (1.9)
V82A	0 (0)	0 (0)	1 (1.3)	6 (2.8)
V82F	0 (0)	0 (0)	0 (0)	4 (1.9)
V82T	0 (0)	0 (0)	0 (0)	1 (0.5)
N83D	0 (0)	0 (0)	0 (0)	1 (0.5)
I84VAC	0 (0)	0 (0)	0 (0)	3 (1.4)
N88D	0 (0)	0 (0)	0 (0)	2 (0.9)
L90M	1 (0.3)	1 (14.3)	0 (0)	7 (3.3)
		Nucleoside Reverse Transcriptase Inhibitors (NRTI)		
M41L	0 (0)	0 (0)	21 (28.0)	47 (22.2)
A62V	1 (0.3)	0 (0)	3 (4.0)	2 (0.9)
K65N	0 (0)	0 (0)	0 (0)	1 (0.5)
K65R	0 (0)	0 (0)	1 (1.3)	4 (1.9)
D67HT	1 (0.3)	1 (14.3)	0 (0)	0 (0)
D67NG	0 (0)	0 (0)	17 (22.7)	39 (18.4)
D67E	1 (0.3)	1 (14.3)	0 (0)	0 (0)
T69D	1 (0.3)	1 (14.3)	2 (2.7)	5 (2.4)
T69NG	0 (0)	0 (0)	3 (4.0)	14 (6.6)
K70NT	0 (0)	0 (0)	0 (0)	3 (1.4)
K70R	0 (0)	0 (0)	6 (8.0)	33 (15.6)
K70E	0 (0)	0 (0)	0 (0)	1 (0.5)
L74I	0 (0)	0 (0)	6 (8.0)	19 (9.0)
L74V	0 (0)	0 (0)	3 (4.0)	10 (4.7)
V75L	1 (0.3)	1 (14.3)	0 (0)	3 (1.4)
V75I	0 (0)	0 (0)	2 (2.7)	7 (3.3)
V75M	0 (0)	0 (0)	1 (1.3)	3 (1.4)
F77L	0 (0)	0 (0)	0 (0)	2 (0.9)
Y115F	0 (0)	0 (0)	1 (1.3)	6 (2.8)
M184VI	0 (0)	0 (0)	56 (74.7)	141 (66.5)
L210W	1 (0.3)	1 (14.3)	10 (13.3)	23 (10.8)
T215Y	0 (0)	0 (0)	24 (32.0)	43 (20.3)
T215A	1 (0.3)	0 (0)	0 (0)	0 (0)
T215F	0 (0)	0 (0)	11 (14.7)	24 (11.3)
T215CDESIV	2 (0.6)	2 (28.6)	1 (1.3)	10 (4.7)
K219QEN	1 (0.3)	1 (14.3)	9 (12.0)	40 (18.9)
K219R	1 (0.3)	1 (14.3)	2 (2.7)	1 (0.5)
		Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI)		
A98G	0 (0)	0 (0)	3 (4.0)	21 (9.9)
L100I	0 (0)	0 (0)	5 (6.7)	4 (1.9)
K101HV	0 (0)	0 (0)	6 (8.0)	2 (0.9)
K101E	4 (1.2)	4 (14.8)	8 (10.7)	15 (7.1)
K101P	0 (0)	0 (0)	1 (1.3)	10 (4.7)
K103NS	19 (5.6)	19 (70.4)	37 (49.3)	87 (41.0)
K103R	0 (0)	0 (0)	0 (0)	5 (2.4)
V106A	1 (0.3)	1 (3.7)	0 (0)	5 (2.4)
V106M	0 (0)	0 (0)	1 (1.3)	2 (0.9)
V108I	3 (0.9)	0 (0)	9 (12.0)	23 (10.8)
E138KQ	1 (0.3)	0 (0)	1 (1.3)	1 (0.5)
E138GAR	16 (4.7)	3 (11.1)	2 (2.7)	7 (3.3)
V179AT	5 (1.5)	1 (3.7)	2 (2.7)	3 (1.4)
V179D	11 (3.3)	3 (11.1)	3 (4.0)	27 (12.7)
V179E	0 (0)	0 (0)	4 (5.3)	13 (6.1)
Y181C	1 (0.3)	1 (3.7)	9 (12.0)	10 (4.7)
Y188L	0 (0)	0 (0)	3 (4.0)	15 (7.1)
Y188H	0 (0)	0 (0)	1 (1.3)	0 (0)
Y188F	0 (0)	0 (0)	0 (0)	1 (0.5)
Y188C	0 (0)	0 (0)	0 (0)	1 (0.5)
G190S	0 (0)	0 (0)	11 (14.7)	12 (5.7)
G190A	3 (0.9)	3 (11.1)	12 (16.0)	27 (12.7)
G190E	0 (0)	0 (0)	1 (1.3)	1 (0.5)
G190CQ	0 (0)	0 (0)	1 (1.3)	2 (0.9)
H221Y	1 (0.3)	0 (0)	8 (10.7)	9 (4.2)
P225H	3 (0.9)	3 (11.1)	8 (10.7)	39 (18.4)
F227L	0 (0)	0 (0)	1 (1.3)	2 (0.9)
M230L	0 (0)	0 (0)	5 (6.7)	5 (2.4)
K238T	0 (0)	0 (0)	3 (4.0)	10 (4.7)
Y318F	1 (0.3)	1 (3.7)	2 (2.7)	2 (0.9)

[a] Frequency in individuals with pre-ART drug resistance (PDR, defined with the WHO list of mutations for HIV drug resistance surveillance) to the corresponding drug class (PI, n=7; NRTI, n=7; NNRTI, n=27). Mutations that contribute with drug resistance penalty scores in the Stanford algorithm are shown. Only mutations found in the cohort are shown. Mutations considered for the analysis are as follows:
NRTIs: M41L, A62V, K65R, D67T, D67H, D67N, D67G, D67E, T69A, T69D, T69ins, T69N, T69C, T69I, T69G, T69S, K70Q, K70N, K70R, K70E, L74I, L74V, V75I, V75L, V75A, V75T, V75S, V75M, F77L, Y115F, F116Y, V118I, Q151M, M184VI, L210W, T215Y, T215A, T215F, T215CDESIV, K219QEN, K219R.
NNRTIs: V90I, A98G, L100I, K101E, K101P, K103NS, V106A, V106M, V108I, E138KQ, E138GAR, V179AT, V179D, V179E, V179L, V179F, Y181IV, Y181C, Y188L, Y188H, Y188C, G190S, G190A, G190E, G190C, P225H, F227L, M230L, K238T, Y318F.
PIs: L10F, K20I, L23I, L24I, D30N, V32I, L33F, E35G, K43T, M46IL, I47A, I47V, G48VM, I50L, I50V, F53I, F53Y, I54VA, I54L, I54M, I54ST, Q58E, G73CSTA, T74S, L76V, V82A, V82F, V82T, V82S, V82M, V82C, V82L, N83D, I84VAC, I85V, N88D, N88S, L90M.

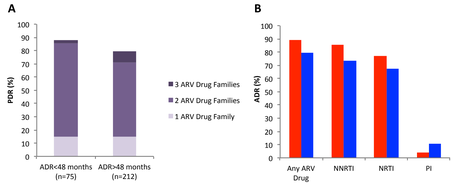


Figure 4. Acquired Drug Resistance (ADR) in Honduras by number of families affected (A) and by ARV drug family (B), 2013-2014. ADR was estimated using the Stanford algorithm with the HIVdb program. ADR frequency was calculated for individuals with <48 months and ≥48 months on ART.

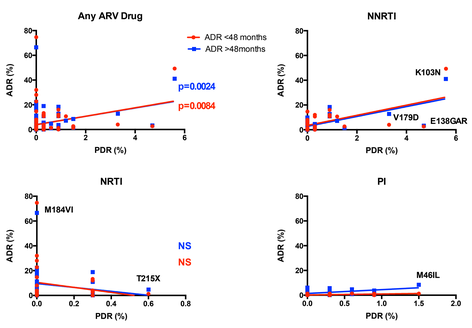


Figure 5. Correlations between PDR and ADR mutation frequency in Honduras. PDR mutation frequency was correlated with ADR mutation frequency at <48 and ≥48 months on ART, for the whole study period, for all DR mutations together and dividing them into ARV families (Pearson). Each point represents one mutation. Some of the most relevant DR mutations are shown. PDR, pre-antiretroviral treatment drug resistance; ADR, acquired drug resistance; NRTI, Nucleoside RT Inhibitors; NNRTI, Non-nucleoside RT Inhibitors; PI, protease inhibitors.

Conclusions

- The global PDR prevalence in Honduras remains at the intermediate level, according to WHO thresholds, after 10 years of widespread availability of ART, with NNRTI being the most affected ARV drug family.
- Using the Stanford HIVDR definition, PDR in Honduras would reach the high level WHO threshold, which would warrant the implementation of basal HIVDR tests.
- No increasing or decreasing PDR trends in time were observed.
- PDR mutation frequency was highly influenced by ADR mutation frequency.
- Further HIVDR surveillance studies with higher representativity are warranted.

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