Pretreatment HIV-drug resistance in Mexico and its impact on the effectiveness of first-line antiretroviral therapy: a nationally representative 2015 WHO survey

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Summary

Background WHO has developed a global HIV-drug resistance surveillance strategy, including assessment of pretreatment HIV-drug resistance. We aimed to do a nationally representative survey of pretreatment HIV-drug resistance in Mexico using WHO-recommended methods.

Methods Among 161 Ministry of Health antiretroviral therapy (ART) clinics in Mexico, the largest, including 90% of ART initiators within the Ministry of Health (66 in total), were eligible for the survey. We used a probability-proportional-to-size design method to sample 25 clinics throughout the country. Consecutive ART-naive patients with HIV about to initiate treatment were invited to participate in the survey; individuals with previous exposure to ART were excluded. We assessed pretreatment HIV-drug resistance by Sanger sequencing and next-generation sequencing of viruses from plasma specimens from eligible participants with Stanford University HIV Drug Resistance Database methods. We obtained follow-up data for a median of 9·4 months (range 6–12) after enrolment. We investigated possible relations between demographic variables and pretreatment drug resistance with univariate and multivariate logistic regression.

Findings Between Feb 3 and July 30, 2015, we screened 288 patients in 25 clinics, from whom 264 provided successfully sequenced viruses with no evidence of current exposure to antiretroviral drugs. With the Sanger method, of these 264 participants, 41 (15·5%, 95% CI 11·4–20·5) had pretreatment resistance to any antiretroviral drug and 28 (10·6%, 7·2–15·0) had pretreatment resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). At least low-level pretreatment resistance (Stanford penalty score ≥15) was noted in 13 (4 · 9%) of participants to efavirenz and in 23 (8·7%) to the combination tenofovir plus emtricitabine plus efavirenz. With next-generation sequencing, of 264 participants, 38 (14·4%, 95% CI 10·4–19·2) had pretreatment resistance to any antiretroviral drug and 26 (9·8%, 6·5–14·1) had pretreatment resistance to NNRTIs. After median follow-up of 8 months (IQR 6·5–9·4, range 5–11) after ART initiation, 97 (72%) of 135 NNRTI initiators achieved viral suppression (<50 copies per mL) compared with ten (40%) of 25 individuals who started with protease inhibitor-based regimens (p=0·0045). After multivariate regression considering pretreatment resistance and initial ART regimen as composite variables, people starting NNRTIs with pretreatment drug resistance achieved significantly lower viral suppression (odds ratio 0·24, 95% CI 0·07–0·74; p=0·014) than patients without NNRTI resistance.

Interpretation High levels of pretreatment drug resistance were noted in Mexico, and NNRTI pretreatment drug resistance significantly reduced the effectiveness of first-line ART regimens based on these drugs. Baseline HIV-drug resistance testing for initial ART follow-up and decision making should be considered.

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Introduction

Latin America is the region with the highest antiretroviral therapy (ART) coverage among low-income and middle-income countries.[1] Increasing ART exposure has resulted in the emergence of HIV-drug resistance and transmission of drug-resistant HIV strains in the region.[2] Prevention of emergence and transmission of HIV-drug resistance is a major challenge directly influencing future effectiveness and sustainability of ART programmes, and immediate programmatic action at a national level is needed.

To improve national representativeness of surveys of HIV-drug resistance, WHO has developed an improved comprehensive strategy for HIV-drug resistance surveillance and monitoring.[3] This strategy includes a protocol for pretreatment HIV-drug resistance surveillance, which has been identified as priority for Latin American countries, including Mexico.[4] Findings from surveillance inform selection of first-line regimens, introduction of HIV-drug resistance testing for patients initiating ART, and intensification of viral load monitoring in settings where high levels of resistance are detected, the findings also provide guidance for cost-effectiveness analyses.[5]

In Mexico, nationally representative studies that can inform public health decisions at the country level are warranted. The Mexican HIV Programme[6] has achieved

Research in context

Evidence before this study

Prevention of emergence and transmission of HIV-drug resistance is a major challenge that directly influences future effectiveness and sustainability of antiretroviral therapy (ART) programmes. In an effort to improve national representativeness of HIV-drug resistance surveys, WHO has developed an improved comprehensive strategy for HIV-drug resistance surveillance and monitoring, including a standardised protocol on pretreatment HIV-drug resistance surveillance. We searched PubMed with the terms ("HIV" AND ["pretreatment drug resistance" OR "transmitted drug resistance" OR "primary drug resistance"] AND "Mexico") for articles published in English and Spanish up to June, 2016. We identified seven results describing studies of HIV-drug resistance surveillance in ART-naive adults in Mexico, including three meta-analyses and one WHO survey in Mexico City. Other studies were regional or designed with convenience sampling (or both). So far, no nationally representative data were available in Mexico to inform policy making in this area.

Added value of this study

Ours is the first survey of pretreatment HIV-drug resistance with national representativeness done in Mexico in accordance with WHO guidelines. Our survey showed high levels of pretreatment HIV-drug resistance in Mexico, with dominance of resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI). Additionally, we showed a significant impact of NNRTI resistance on the effectiveness of first-line NNRTI-based ART regimens, which are widely used in Mexico and the rest of Latin America. We were also able to compare next-generation sequencing with standard Sanger sequencing and to show a clinical role of low-abundance drug resistance variants (>5%) in the effectiveness of first-line NNRTI-based ART regimens.

Implications of all the available evidence

Although a recommendation for a national change in first-line ART drugs from NNRTI to a different class is debatable, given the high efficacy of NNRTI-based regimens in the Mexican setting in individuals without NNRTI resistance, we recommend the integration of baseline testing for pretreatment HIV-drug resistance for initial ART follow-up and for its feasibility to be examined. Our results also emphasise the need to do standardised surveys of HIV-drug resistance surveillance.

good results in ART coverage: 118 000 individuals in Mexico were receiving ART at the end of 2015, of whom 73 782 (63%) received treatment at Ministry of Health clinics. The proportion of individuals staying on treatment and with virological suppression (<1000 RNA copies per mL) at 12 months were 84% and 92%, respectively, in 2014.[6] Nevertheless, nearly half of people with HIV in Mexico (an estimated 210 000) are unaware of their serological status. Baseline testing for HIV-drug resistance is not routine, and factors that can increase the prevalence of pretreatment drug resistance such as drug stock-outs (5·3% in 2014) and prescription of non-recommended ART regimens exist in the country.[6,7] We aimed to estimate pretreatment drug resistance levels among ART-naive individuals initiating ART in Mexico; to compare population-based Sanger sequencing and next-generation sequencing; to assess the effect of pretreatment HIV-drug resistance on the virological response to first-line regimens based on non-nucleoside reverse transcriptase inhibitors; and to understand whether low-abundance drug resistance variants predict virological failure.

Methods

Study population

To achieve a nationally representative survey of pretreatment HIV-drug resistance in Mexico, we selected participating clinics according to the WHO pretreatment drug resistance surveillance concept note,[5] with the probability-proportional-to-size (PPS) sampling method. Briefly, we obtained a list of all Ministry of Health clinics that initiate ART in Mexico (161 in total), with their respective number of ART initiators during 2013 (the most recent available information at the time of survey planning) from the Mexican National Centre for HIV/AIDS Prevention and Control (CENSIDA). The largest clinics (no threshold for size) including 90% of ART initiators within the Ministry of Health (66 in total) were eligible for the survey. We calculated sample size with the WHO pretreatment drug resistance sample size calculator,[8] using a 32 clinic model and assuming 10% prevalence of HIV-drug resistance among all ART initiators, a genotyping failure rate of 20%, precision of the HIV-drug resistance prevalence estimate of 5%, and an estimate of 75% of individuals initiating ART with NNRTI-based regimens. The number of clinics to be included was selected by the user, according to the country's possibilities and internal logistics. In our case, we selected 32. Setting the number of clinics as 32, the number of participants was 288, including nine individuals per clinic. When clinics were selected twice, 18 individuals were included (appendix p 1). Clinics initiating patients on ART were ordered by size with their population size of eligible patients initiating ART over 6 months. The cumulative population size for each clinic listed was calculated. A sampling interval was determined dividing the total number of patients initiating ART over 6 months into the total number of clinics to be included. A random starting point was selected. Clinics were then selected based on the random starting point, sampling interval, and cumulative population size. The protocol was revised and approved by the Ethics Committee of the

***Figure 1*: Geographical distribution of participating clinics**
Probability-proportional-to-size (PPS) sampling with a 32 clinic model was applied to design the pretreatment HIV-drug resistance 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. CAPASITS=Centro Ambulatorio para la Prevención y Atención en SIDA e Infecciones de Transmisión Sexual.

National Institute of Respiratory Diseases (INER, Mexico City, Mexico; E10-10), the institution coordinating the survey. Consecutive adult patients (aged ≥18 years) about to initiate first-line ART at the sampled clinics were screened through a questionnaire and those reporting no previous exposure to antiretroviral drugs were invited to participate in the survey. We excluded individuals reporting previous exposure. All participants gave written informed consent before blood-sample donation.

Procedures

Data for demographic characteristics, including education level, and marital and employment status, risk factors for HIV infection, sex, and age were obtained through a questionnaire at the moment of blood-sample donation. Baseline CD4-positive T-cell counts and determinations of HIV plasma viral load were done for each participant with the same blood specimen donated for the survey. We extracted viral RNA from 1 mL of plasma and amplified and sequenced HIV protease reverse transcriptase with an in-house developed protocol as previously published,[9] using a 3730xl Genetic Analyzer instrument (ThermoFisher, Waltham, MA, USA). Sequences were assembled with the web-based automated sequence analysis tool RECall (University of British Columbia, Canada).[10] We did the sequencing at the WHO-accredited Centre for Research in Infectious Diseases laboratory of the INER, fulfilling procedural and infrastructure requirements for good laboratory practices and quality assurance in HIV genotyping.

HIV *pol* amplicons obtained for Sanger sequencing were also deep sequenced with a MiSeq instrument (Illumina, San Diego, CA, USA). DNA libraries were generated for the *pol* PCR products with Nextera XT DNA Sample Preparation Kit and Nextera XT Index Kit (Illumina), according to manufacturers' instructions. Multiplexed runs, each with 96 viral libraries, were done with 500-cycle MiSeq Reagent Kits v.2 (Illumina). We assessed the frequency of drug-resistant mutations within each patient's viral population from next-generation sequencing runs (fastq files) with HyDRA, an automated HIV-drug resistance analysis pipeline (National Microbiology Laboratory; Public Health Agency of Canada, Winnipeg, MB, Canada).[11] Aminoacid mutations were queried against a merged drug resistance mutation database including the WHO list of surveillance drug resistance mutations[12] and

	Complete cohort (n=264)	Individuals without pretreatment HIV-drug resistance (n=223)*	Individuals with pretreatment HIV-drug resistance (n=41)*
Sex			
Male	225 (85%)	194 (87%)	31 (76%)
Female	39 (15%)	29 (13%)	10 (24%)
Age (years)	31 (24–39)	31 (25–37)	33 (24–42)
CD4 count (cells per μL)	258 (86–428)	264 (101–407)	176 (50–522)
CD4 cell count (%)	13% (7–21)	14% (7–21)	11% (6–19)
Plasma viral load (log copies per mL)	4·8 (4·2–5·3)	4·8 (4·2–5·3)	5·1 (4·0–5·5)
Recent infection	64 (24%)	56 (25%)	8 (20%)
Marital status			
Single	182 (69%)	156 (70%)	26 (63%)
Married	27 (10%)	23 (10%)	4 (10%)
Domestic partnership	46 (17%)	38 (17%)	8 (20%)
NA	9 (3%)	6 (3%)	3 (7%)
Education			
Illiterate	5 (2%)	5 (2%)	0
Elementary	34 (13%)	27 (12%)	7 (17%)
High school	148 (56%)	130 (58%)	18 (44%)
Degree or technical qualification	66 (25%)	51 (23%)	15 (37%)
Postgraduate	6 (2%)	6 (3%)	0
NA	5 (2%)	4 (2%)	1 (2%)
Employment			
Employed	131 (50%)	111 (50%)	20 (49%)
Unemployed	98 (37%)	84 (38%)	14 (34%)
Student	23 (9%)	18 (8%)	5 (12%)
NA	12 (5%)	10 (4%)	2 (5%)
HIV risk factors			
Men who have sex with men†	136 (52%)	122 (55%)	14 (34%)
Heterosexual†	96 (36%)	75 (34%)	21 (51%)
Bisexual	11 (4%)	9 (4%)	2 (5%)
People who inject drugs	4 (2%)	4 (2%)	0
Other	1 (<1%)	1 (<1%)	0
NA	16 (6%)	12 (5%)	4 (10%)

Data are n (%) or median (IQR). NA=not available. *Pretreatment drug resistance defined with the Stanford University HIV-drug resistance database tool as individuals with a penalty score of at least 15 for any antiretroviral drug. All variables were compared between individuals with and without pretreatment drug resistance. †p<0·05 Fisher's exact test for discrete variables, Mann-Whitney test for continuous variables comparing individuals with and without pretreatment HIV-drug resistance. For men who have sex with men p=0·0175; for heterosexual p=0·0353.

***Table 1*: Demographic and clinical characteristics of survey participants**

the Stanford HIV Drug Resistance Database (HIVdb).[13] A conservative threshold of 2% was used to define the presence of drug resistance mutations. With our sequencing protocol, a sequencing depth of 1500–2000 times and a viral load of more than 2500 copies per mL (>2500 total input copies) would be needed in order to accurately identify variants at 1% frequency.

We assessed HIV-drug resistance using the Stanford Algorithm (version 7.0), with the HIVdb program.[13,14] Individuals with drug resistance were defined as those with at least low-level resistance (ie, a Stanford penalty score ≥15) to any antiretroviral drug. Additionally, we estimated the prevalence of pretreatment drug resistance with the Stanford Calibrated Population Resistance (CPR) method,[15] based on the WHO list of surveillance drug resistance mutations.[12] We did CPR and HIVdb analyses both with Sanger and next-generation 20% consensus sequences.

We identified recent infections with a multiassay algorithm designed to minimise false-recency results.[16] Individuals with recent infection were defined as those with less than 1 year of diagnosis, CD4 cell counts of more than 200 cells per mL, a plasma viral load of more than 400 RNA copies per mL, a BED HIV-1 Incidence EIA (Sedia, Portland, OR) ODn score of less than 1·0 and a confirmatory HIV-1-LAg-Avidity EIA (Sedia) ODn score of less than 1·0. This algorithm has a mean seroconversion period of 130 days (95% CI 118–142) and a low false-recency rate (0·4%).

For phylogenetic analyses, we aligned sequences in ClustalW (European Bioinformatics Institute, Cambridge, UK) and eliminated positions associated with drug resistance. We constructed a maximum likelihood tree with the General Time Reversible +I+γ model, with the MEGA 6·06 program, including reference sequences from the Los Alamos HIV Database.[17] The best substitution model was determined with the model selection method implemented in MEGA 6.06. We assessed confidence with 1000 bootstrap repetitions.

Statistical analysis

We obtained baseline and follow-up clinical data, including ART initiation date, first-line ART regimen, plasma viral load, and CD4 cell count determinations after ART initiation, changes in ART regimens, and mortality from the Mexican national HIV database (SALVAR) on Jan 29, 2016, 6 months after closing the survey. We investigated possible relations between demographic variables and pretreatment drug resistance with univariate and multivariate logistic regression. The model was adjusted by age, sex, and demographic variables that were significant in the univariate analysis ($p<0·05$). Additionally, we used univariate regression to assess the effect of pretreatment drug resistance and demographic variables on ART outcomes. We then did multivariate regression with two options: pretreatment drug resistance and initial ART regimen considered as individual variables, and pretreatment drug resistance and initial ART regimen combined as composite variables. The multivariate model was corrected by pretreatment CD4 cell count, plasma viral load, and significant variables from univariate analysis ($p<0·05$). Results were reported as odds ratios (ORs) with 95% CIs. Analyses were done in R, version 3.2.4.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 3 and July 30, 2015, we screened 288 patients in 25 clinics, from whom 270 (94%) viruses from plasma specimens were successfully sequenced with both Sanger and next-generation methods. Each sampled clinic contributed data for nine patients, except for seven clinics that were sampled twice and contributed data for 18 patients each (figure 1; appendix p 1). Six patients were excluded after revision of the SALVAR database because of evidence of current exposure to antiretroviral drugs, leaving 264 patients in the survey. Non-amplification was random, within the expected range, and not associated with plasma viral load. Most participants were young, male, single, with high-school education, and presented at advanced stages of HIV infection (table 1). Slightly more than half the participants self-identified as men who have sex with men. We obtained follow-up data for a median of 9·4 months (IQR 8·0–10·3, range 6–12) after enrolment.

On the basis of Sanger sequences, 31 viruses (11·7%; 95% CI 8·1–16·3) had surveillance drug resistance mutations; 17 (6·4%, 3·8–10·1) had resistance to NNRTIs (appendix p 2). Of 264 individuals, with the Stanford HIV-drug resistance database algorithm, 41 (15·5%, 95% CI 11·4–20·5) had pretreatment resistance to any antiretroviral drug, 28 (10·6%, 7·2–15·0) had resistance to NNRTI (table 2). The higher level of pretreatment resistance to NNRTI estimated with the HIVdb method than with the CPR approach was mainly associated with Glu138Ala (50% of cases) and Ala98Gly (20% of cases). Heterosexual transmission was the HIV risk factor for about a half of people with pretreatment drug resistance compared with a third of those without (OR 2·4, 95% CI 1·2–4·9; p=0·0165); men who have sex with men was a less common risk class in patients with pretreatment drug resistance than in those without (OR 0·4, 0·2–0·9; p=0·0268, table 1). These results were also significant for individuals with pretreatment resistance to NRTI (appendix p 3). Ten (25·6%, 95% CI 13·0–42·1) of 39 women and 31 (13·8%, 9·6–19·0) of 225 men had pretreatment drug resistance (p=0·0898).

Overall and class-specific pretreatment drug resistance levels based on Sanger sequencing were similar to those obtained with next-generation sequencing between the 20% and 10% sensitivity thresholds (appendix p 2). Pretreatment resistance to NRTI (p=0·0024) and protease inhibitors (p=0·0034), but not NNRTI (p=0·6140) increased further at the 2% threshold (figure 2, appendix p 4).

The most common pretreatment drug resistance mutation was Lys103Asn (4·2% frequency at 20% threshold; figure 3). Other frequent pretreatment drug resistance mutations for NNRTI included Lys101Glu (0·8%), Gly190Ala (0·8%), and Pro225His (0·8%). For NRTIs, thymidine analogue mutations (TAMs) were the most common, including Met41Leu (1·1%), Asp67Asn (0·4%), Thr215 revertants (1·5%), and Lys219GlnGlu (0·8%), in addition to the discriminatory mutations Met184Val (1·1%) and Met184Ile (0·4%; appendix p 5). Some non-TAMs occurred as low-abundance variants (<5%), including Lys65Arg, Thr69Asp, Lys70Glu, and Val75Ala (figure 3). For protease inhibitors, Leu90Met (1·9%) was the most frequent mutation, followed by Ile85Val (0·8%), Val82APhe (0·8%), Gly73Ser (0·4%), Ile54Val (0·4%), and Ile47Val (0·4%). As in the case with NRTI mutations, several protease-inhibitor mutations occurred as low-abundance variants only (<5%), including Asp30Asn, Met46LIle, Gly48Val, Phe53Tyr, and Asn88Asp (figure 3).

When analysing phylogenetic relations between circulating viruses, we noted geographically defined clusters of viruses with pretreatment drug resistance, suggesting both transmission among men who have sex with men and heterosexual transmission (appendix p 6).

	Sanger method*† (n=264)	Next-generation sequencing *‡ (n=264)
Any antiretroviral drug	41 (16%, 11·4–20·5)	38 (14%, 10·4–19·2)
NRTI	15 (6%, 3·2–9·2)	13 (5%, 2·6–8·3)
NNRTI	28 (11%, 7·2–15·0)	26 (10%, 6·5–14·1)
Protease inhibitors	7 (3%, 1·1–5·4)	8 (3%, 1·3–5·9)

Data are n (%, 95% CI) of individuals, divided by drug class. NRTI=nucleoside reverse transcriptase inhibitors. NNRTI=non-nucleoside reverse transcriptase inhibitors. *Pretreatment HIV-drug resistance defined with the Stanford HIVdb program as individuals with a penalty score of at least 15 for any antiretroviral drug of the corresponding class. †Drug resistance estimations based on sequences obtained by the Sanger method. ‡Drug resistance estimations based on next-generation sequencing consensus (20% threshold).

***Table 2*: Pretreatment HIV-drug resistance prevalence in Mexico**

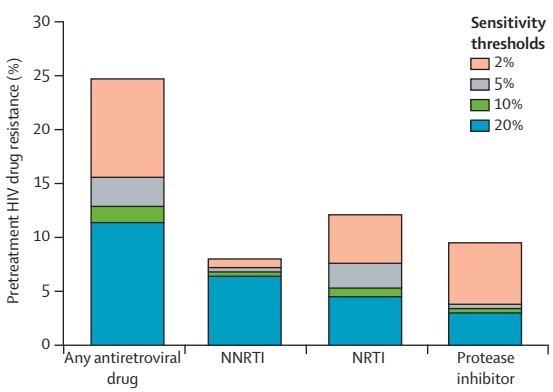

***Figure 2*: Pretreatment HIV-drug resistance at different sensitivity thresholds**
Levels were estimated with next-generation sequencing. Drug resistance was defined as the presence of any surveillance drug resistance mutation at the specified sensitivity threshold. NNRTI=non-nucleoside reverse transcriptase inhibitors. NRTI=nucleoside reverse transcriptase inhibitors.

See Online for appendix

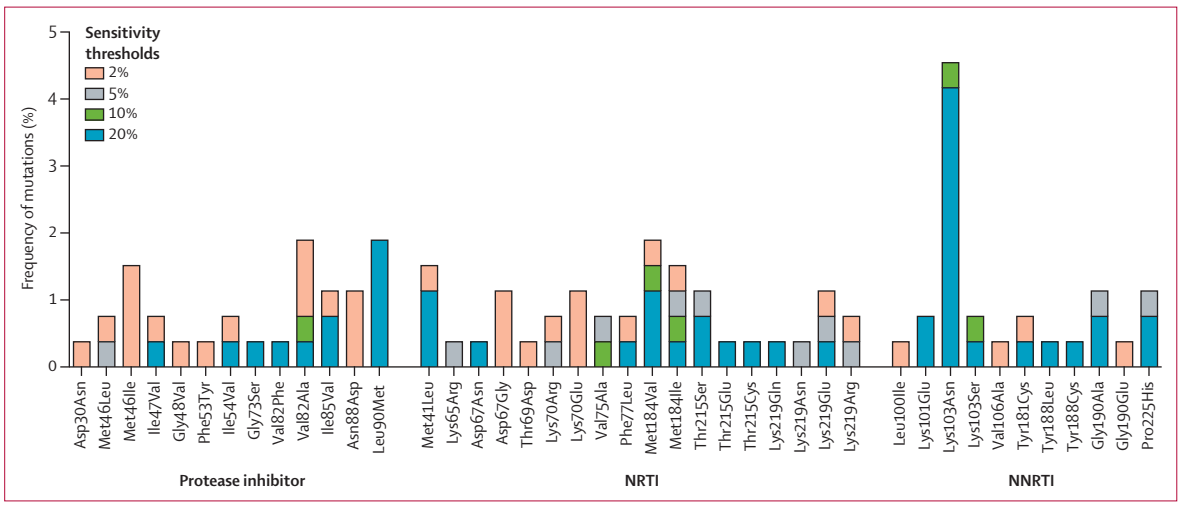

***Figure 3*: Pretreatment HIV-drug resistance mutation frequency at different detection sensitivity thresholds**
Frequency of mutations was determined with next-generation sequencing. Cumulative prevalence of pretreatment drug resistance mutations is shown for surveillance drugs only, classified by drug class. NRTI=nucleoside reverse transcriptase inhibitors. NNRTI=non-nucleoside reverse transcriptase inhibitors.

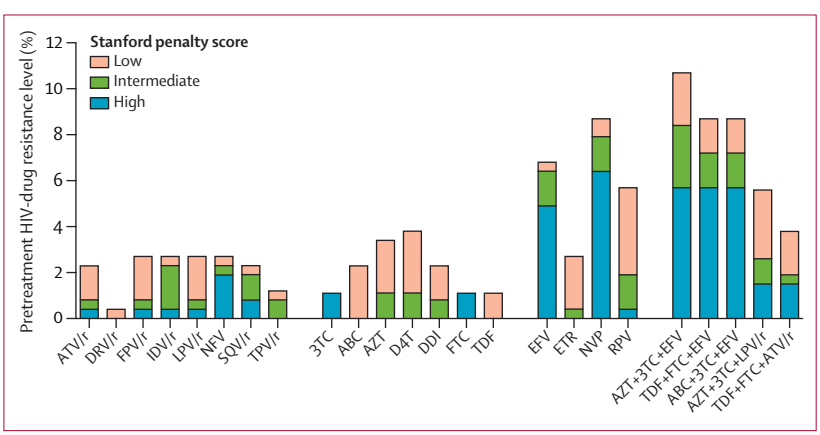

***Figure 4*: Pretreatment HIV-drug resistance divided by antiretroviral drugs and regimens**
Levels were calculated with the Stanford HIVdb program from Sanger sequences, and classified according to the Stanford Penalty Score as high (≥60), intermediate (30–59), or low (15–29). Only the most widely used antiretroviral drug regimens in Mexico are shown (appendix). Resistance to each regimen was defined as the presence of pretreatment drug resistance to any of the drugs in the combination, at the Stanford levels defined above. ATV/r=ritonavir-boosted atazanavir. DRV/r=ritonavir-boosted darunavir. FPV/r=ritonavir-boosted fosamprenavir. IDV/r=ritonavir-boosted indinavir. LPV/r=ritonavir-boosted lopinavir. NFV=nelfinavir. SQV/r=ritonavir-boosted saquinavir. TPV/r=ritonavir-boosted tipranavir. 3TC=lamivudine. ABC=abacavir. AZT=zidovudine. D4T=stavudine. DDI=didanosine. FTC=emtricitabine. TDF=tenofovir disoproxil fumarate. EFV=efavirenz. ETR=etravirine. NVP=nevirapine. RPV=rilpivirine.

High-level pretreatment drug resistance (ie, a Stanford penalty score ≥60) was more frequent with efavirenz (13/264, 4·9%) and nevirapine (17/264, 6·4%), and at least low-level resistance (ie, a penalty score ≥15) was noted in 6·8% (18/264) and 8·7% (23/264) of participants for each drug, respectively (figure 4). At least low-level resistance was noted in 5·7% (15/264) and 2·7% (7/264) to rilpivirine and etravirine, respectively. Regarding NRTIs, 1·1% (3/264) of participants showed high-level pretreatment drug resistance to lamivudine and emtricitabine. At least low-level resistance was noted in 3·4% (9/264) with zidovudine, 2·3% (6/264) with abacavir, and 1·1% (3/264) with tenofovir (figure 4). With protease inhibitors, at least low-level resistance was noted in 2·7% (7/264) and 2·3% (6/264) for lopinavir and atazanavir, respectively. Darunavir was the least affected drug with only low-level resistance in 0·4% (1/264) of cases (figure 4). Of note, pretreatment drug resistance to tenofovir plus emtricitabine plus efavirenz, the most frequently used ART regimen (appendix p 7), was 8·7% (23/264) (figure 4). 64 (24%) of 264 participants had recent infection. Of these, HIV-drug resistance was noted in eight (13%) of 64 participants, six (9%) associated with NNRTIs and two (3%) with NRTIs. This drug resistance level was not different from the overall pretreatment resistance level in individuals with long-standing infection (table 1).

Data on ART initiation were available for 237 (90%) of 264 individuals, 178 (85%) of 210 initiating with NNRTI-based regimens and 30 (14%) of 210 with regimens based on protease inhibitors (table 3, appendix p 7). The median follow-up of patients on ART was 8 months (IQR 6·5–9·4, range 5–11). 27 (11%) of 237 participants had not started ART within 6 months of closing the survey, suggesting eventual delays in ART initiation. The median time for ART initiation after enrolment was 29 days (IQR 15–54). Of 179 individuals with follow-up for plasma viral load, 109 (61%) had less than 50 copies per mL 6 months after closing the survey. After multivariate correction, older age, high pretreatment plasma viral load, and pretreatment resistance to any drug were associated with lower viral suppression (table 4). More individuals with a plasma viral load less than 50 copies per mL starting with NNRTI-based regimens achieved viral suppression compared with starting with regimens based on protease inhibitors (p=0·0045; table 3, appendix p 8). This difference was still significant

	Complete cohort	All NNRTI initiators	NNRTI initiators without pretreatment drug resistance in reverse transcriptase	NNRTI initiators with pretreatment drug resistance in reverse transcriptase	NNRTI initiators with NNRTI pretreatment drug resistance	NNRTI initiators with NRTI pretreatment drug resistance
First ART regimen*	n=210	..	..	..	..	..
NNRTI based	178 (84·8)	..	..	..	..	..
Protease-inhibitor based	30 (14·3)	..	..	..	..	..
Integrase-inhibitor based	2 (1·0)	..	..	..	..	..
Plasma viral load within 6 months after ART initiation†	n=179	n=135	n=112	n=23	n=15	n=10
<1000 copies per mL	148 (82·7)	124 (91·9)	105 (93·8)	19 (82·6)	12 (80·0)	9 (90·0)
<200 copies per mL	141 (78·8)	121 (89·6)	104 (92·9)	17 (73·9)	10 (66·7)	8 (80·0)
<100 copies per mL	126 (70·4)	109 (80·7)	97 (86·6)	12 (52·2)‡	7 (46·7)‡	5 (50·0)§
<50 copies per mL	109 (60·9)	97 (71·9)	86 (76·8)	11 (47·8)‡	6 (40·0)‡	5 (50·0)
Change of ART scheme within 6 months of ART initiation*	n=210	n=178	n=152	n=26	n=16	n=12
All individuals	19 (9·0)	18 (10·1)	11 (7.2)	..	..	..
Individuals with pretreatment drug resistance	7 (3·3)	7 (3·9)	..	7 (26·9)‡	5 (31·3)‡	3 (25·0)

Data are n (%). ..=not applicable. ART=antiretroviral therapy. NNRTI=non-nucleoside reverse transcriptase inhibitors. *Data for first-line ART regimen available for 210 of 264 individuals; 27 individuals did not start ART within 6 months and information is unknown for a further 27 individuals. †Plasma viral load data within 6 months after closing the survey (median follow-up on ART 8 months (IQR 6·5–9·4, range 5–11) available for 179 of 264 individuals; when more than one determination was available, the last result was used. Pretreatment drug resistance defined with the Stanford HIVdb program as individuals with a penalty score of at least 15 for any drug in the corresponding category. ‡p<0·01 by Fisher's exact test, NNRTI initiators with *vs* without pretreatment drug resistance in reverse transcriptase (plasma viral load <50, p=0·0094; plasma viral load <100, p=0·0005) or NNRTI initiators with *vs* without NNRTI pretreatment drug resistance (plasma viral load <50, p=0·0052; plasma viral load <100, p=0·0010). §p<0·05 by Fisher's exact test, NNRTI initiators with *vs* without NRTI pretreatment drug resistance (plasma viral load <100, p=0·0107).

***Table 3*: Effect of pretreatment HIV-drug resistance on patient treatment outcomes**

for patients with viral load suppression at less than 1000 copies per mL (p=0·0097). NNRTI initiators with pretreatment resistance to NNRTI achieved significantly lower levels of suppression with multivariate option 2 (plasma viral load <50 copies per mL) as well as people starting protease inhibitors with pretreatment resistance to any drug (table 4).

Of 19 individuals with registered ART regimen change, seven (37%) had pretreatment resistance to any antiretroviral drug. Individuals without pretreatment drug resistance were less likely to change antiretroviral drug regimens than individuals with resistance in reverse transcriptase (OR 0·2, 95% CI 0·1–0·6; p=0·0067; table 3). Most individuals switched to a second-line ART regimen based on protease inhibitors including atazanavir (11), lopinavir (two), or darunavir (two).

From 22 participants with pretreatment resistance to NNRTI and information on first ART regimen, six started with regimens based on protease inhibitors (in all cases containing atazanavir). For the remaining 16 participants (all of whom started with efavirenz plus tenofovir plus emtricitabine), genotyping results were not taken into account initially for starting ART, but a switch to regimens based on protease inhibitors was recorded for five of these individuals within a median of 78 days.

Nine (4%) of 242 participants had died by 6 months after the survey had closed. Mortality was associated with older age, and higher pretreatment plasma viral loads (table 4). Associations between pretreatment resistance to NRTI, AIDS-defining illness at ART initiation, and lower pretreatment CD4 T-cell counts were noted, but significance was lost after multivariate correction (table 4).

Compared with NNRTI initiators without NNRTI pretreatment resistance, the proportion of NNRTI initiators with undetectable plasma viral load at 6 months after closing the survey was significantly lower in individuals with resistance mutations at sensitivity thresholds of 20% (p=0·019), 10% (p=0·0064), and 5% (p=0·015), but not 2% (p=0·074), suggesting a clinical role of low-abundance variants of at least 5% (figure 5). Low-abundance NRTI mutations had no significant effects. Because only two of 25 patients stating protease inhibitors had resistance mutations to these drugs, we could not assess of the possible effect of low-abundance resistance mutations on ART effectiveness.

Discussion

From our survey, we noted high levels of pretreatment HIV-drug resistance in Mexico, with dominance of NNRTI resistance that significantly reduced the effectiveness of first-line NNRTI-based ART regimens.

Our study was the first nationally representative survey of pretreatment HIV-drug resistance in Mexico. Its most important strength was its national representativeness; clinics selected by the PPS method reflected the

	Associated outcome	Univariate				Multivariate option 1*				Multivariate option 2*			
		OR	95% CI	p value	q value	OR	95% CI	p value	q value	OR	95% CI	p value	q value
Age (years)													
>45	Death	9·53	2·39–40·44	0·0013	0·0272	6·99	1·13–49·47	0·0384	0·4841	9·20	1·32–84·74	0·0295	0·3248
35–44	Plasma viral load <1000 copies per mL	0·42	0·19–0·99	0·0430	0·2733	..	..	..	..	..	..	..	..
35–44	Plasma viral load <200 copies per mL	0·39	0·18–0·85	0·0171	0·1472	0·29	0·10–0·80	0·0164	0·4841	0·29	0·11–0·80	0·0158	0·2444
35–44	Plasma viral load <100 copies per mL	0·44	0·22–0·91	0·0257	0·1272	..	..	..	..	0·38	0·16–0·92	0·0316	0·3248
HIV/AIDS status													
Asymptomatic	Death	0·21	0·04–0·80	0·0285	0·1360	..	..	..	..	..	..	..	..
Symptomatic	Death	5·45	1·29–21·65	0·0150	0·1222	..	..	..	..	..	..	..	..
Recent infection	Plasma viral load <1000 copies per mL	3·58	1·18–15·52	0·0446	0·2733	..	..	..	..	..	..	..	..
Recent infection	Plasma viral load <200 copies per mL	3·37	1·24–11·81	0·0304	0·1965	..	..	..	..	..	..	..	..
Recent infection	Plasma viral load <100 copies per mL	3·38	1·42–9·42	0·0103	0·0738	..	..	..	..	..	..	..	..
Recent infection	Plasma viral load <50 copies per mL	2·72	1·28–6·22	0·0122	0·1090	..	..	..	..	..	..	..	..
Recent infection	Gain in CD4 cell count at 6 months	1·00	0·99–1·00	0·0448	0·7115	..	..	..	..	..	..	..	..
Pretreatment status													
CD4 count ≥200 cells per mL	Death	0·09	0·00–0·49	0·0225	0·1222	..	..	..	..	..	..	..	..
CD4 count <50 cells per mL	Death	21·30	4·92–146·95	0·0002	0·0086	..	..	..	..	..	..	..	..
Log plasma viral load <3·5 copies per mL	Gain in CD4 cell count at 6 months	0·98	0·97–0·99	0·0136	0·5860	..	..	..	..	..	..	..	..
Log plasma viral load <3·5 copies per mL	Gain in CD4 cell count at 3 months	1·00	0·99–1·00	0·0144	0·1092	..	..	..	..	..	..	..	..
Log plasma viral load 4·5–4·9 copies per mL	Gain in CD4 cell count at 3 months	1·00	1·00–1·01	0·0361	0·1524	1·00	1·00–1·01	0·0187	0·4841	..	..	..	..
Log plasma viral load 5·5–5·9 copies per mL	Plasma viral load <50 copies per mL	0·34	0·14–0·79	0·0138	0·1090	0·28	0·11–0·71	0·0078	0·3245	0·24	0·09–0·58	0·0020	0·2213
Log plasma viral load 5·5–5·9 copies per mL	Death	10·09	2·37–50·95	0·0021	0·0303	6·76	1·20–45·74	0·0345	0·4841	..	..	..	..
Treatment													
NNRTI initiator	Plasma viral load <1000 copies per mL	3·91	1·31–11·19	0·0116	0·1112	..	..	..	..	..	..	..	..
NNRTI initiator	Plasma viral load <200 copies per mL	4·65	1·71–12·45	0·0022	0·0331	..	..	..	..	..	..	..	..
NNRTI initiator	Plasma viral load <100 copies per mL	2·86	1·17–6·86	0·0193	0·1185	..	..	..	..	..	..	..	..
NNRTI initiator	Plasma viral load <50 copies per mL	3·04	1·31–7·22	0·0099	0·1090	..	..	..	..	..	..	..	..
NNRTI initiator	CD4 gain at 3 months	1·00	0·99–1·00	0·0224	0·1217	..	..	..	..	..	..	..	..
Start with TDF+FTC+ATV/r	Plasma viral load <1000 copies per mL	0·18	0·06–0·60	0·0037	0·0786	..	..	..	..	..	..	..	..
Start with TDF+FTC+ATV/r	Plasma viral load <200 copies per mL	0·18	0·06–0·56	0·0023	0·0331	..	..	..	..	..	..	..	..
Start with TDF+FTC+ATV/r	Plasma viral load <100 copies per mL	0·32	0·11–0·90	0·0270	0·1272	..	..	..	..	..	..	..	..

(Table 4 continues on next page)

	Associated outcome	Univariate				Multivariate option 1*				Multivariate option 2*			
		OR	95% CI	p value	q value	OR	95% CI	p value	q value	OR	95% CI	p value	q value
(Continued from previous page)													
Start with TDF+FTC+ATV/r	Plasma viral load <50 copies per mL	0·21	0·07–0·57	0·0032	0·0741	..	..	..	..	..	..	..	..
Start with TDF+FTC+ATV/r	CD4 gain at 3 months	1·00	1·00–1·01	0·0303	0·1438	..	..	..	..	..	..	..	..
Protease-inhibitor initiator	Plasma viral load <1000 copies per mL	0·23	0·08–0·68	0·0064	0·0921	..	..	..	..	..	..	..	..
Protease-inhibitor initiator	Plasma viral load <200 copies per mL	0·19	0·07–0·52	0·0010	0·0331	..	..	..	..	..	..	..	..
Protease-inhibitor initiator	Plasma viral load <100 copies per mL	0·30	0·12–0·75	0·0087	0·0738	..	..	..	..	..	..	..	..
Protease-inhibitor initiator	Plasma viral load <50 copies per mL	0·27	0·11–0·64	0·0034	0·0741	..	..	..	..	..	..	..	..
Protease-inhibitor initiator	CD4 gain at 3 months	1·00	1·00–1·01	0·0055	0·0712	..	..	..	..	..	..	..	..
Pretreatment HIV-drug resistance status													
Any drug	Plasma viral load <200 copies per mL	–	–	–	–	0·21	0·05–0·98	0·0400	0·4841	..	..	..	..
Any drug	Plasma viral load <100 copies per mL	0·34	0·15–0·74	0·0066	0·0738	..	..	..	..	..	..	..	..
Any drug	Plasma viral load <50 copies per mL	0·43	0·19–0·92	0·0310	0·1667	..	..	..	..	..	..	..	..
NNRTI	Plasma viral load <100 copies per mL	0·29	0·12–0·73	0·0086	0·0738	..	..	..	..	..	..	..	..
NNRTI	Plasma viral load <50 copies per mL	0·32	0·12–0·79	0·0152	0·1090	..	..	..	..	..	..	..	..
NNRTI	CD4 gain at 3 months	1·00	0·99–1·00	0·0475	0·1642	..	..	..	..	..	..	..	..
NRTI	Death	5·32	0·74–24·79	0·0495	0·2129	..	..	..	..	..	..	..	..
Protease inhibitors	CD4 gain at 3 months	0·99	0·99–1·00	0·0075	0·0712	..	..	..	..	..	..	..	..
Any drug and NNRTI initiators	Plasma viral load <100 copies per mL	0·28	0·11–0·70	0·0056	0·0738	..	..	..	..	..	..	..	..
Any drug and NNRTI initiators	CD4 gain at 3 months	1·00	0·99–1·00	0·0211	0·1217	..	..	..	..	..	..	..	..
Any drug and start with TDF+FTC+EFV	Plasma viral load <100 copies per mL	0·37	0·15–0·91	0·0296	0·1272	..	..	..	..	..	..	..	..
Any drug and start with TDF+FTC+EFV	CD4 gain at 3 months	1·00	0·99–1·00	0·0429	0·1631	..	..	..	..	..	..	..	..
Any drug and protease inhibitor initiators	Plasma viral load <1000 copies per mL	0·08	0·01–0·37	0·0015	0·0625	..	..	..	..	0·08	0·01–0·42	0·0032	0·2213
Any drug and protease inhibitor	Plasma viral load <200 copies per mL	0·10	0·02–0·48	0·0041	0·0436	..	..	..	..	0·11	0·02–0·57	0·0088	0·2444
Any drug and protease inhibitor	Plasma viral load <100 copies per mL	0·20	0·04–0·97	0·0442	0·1575	..	..	..	..		..	..	..
Any drug and protease inhibitor	Plasma viral load <50 copies per mL	0·19	0·03–0·90	0·0495	0·2298	..	..	..	..	0·15	0·02–0·76	0·0301	0·3248
Any drug and start with TDF+FTC+ATV/r	Plasma viral load <1000 copies per mL	0·14	0·03–0·67	0·0129	0·1112	..	..	..	..	..	..	..	..
Any drug and start with TDF+FTC+ATV/r	Plasma viral load <200 copies per mL	0·18	0·04–0·88	0·0320	0·1965	..	..	..	..	..	..	..	..
NNRTI and NNRTI initiator	Plasma viral load <100 copies per mL	0·22	0·07–0·65	0·0061	0·0738	..	..	..	..	..	..	..	..

(Table 4 continues on next page)

	Associated outcome	Univariate				Multivariate option 1*				Multivariate option 2*			
		OR	95% CI	p value	q value	OR	95% CI	p value	q value	OR	95% CI	p value	q value
(Continued from previous page)													
NNRTI and NNRTI initiator	Plasma viral load <50 copies per mL	0·30	0·09–0·87	0·0292	0·1667	..	..	..	..	0·24	0·07–0·74	0·0143	0·2444
NNRTI and NNRTI initiator	CD4 gain at 3 months	0·99	0·99–1·00	0·0054	0·0712	..	..	..	..	..	..	..	..
NNRTI and start with TDF+FTC+EFV	Plasma viral load <100 copies per mL	0·33	0·11–0·97	0·0429	0·1575	..	..	..	..	..	..	..	..
NNRTI and start with TDF+FTC+EFV	CD4 gain at 3 months	0·99	0·99–1·00	0·0069	0·0712	..	..	..	..	..	..	..	..
NRTI and NNRTI initiator	Plasma viral load <100 copies per mL	0·27	0·07–1·02	0·0476	0·1575	..	..	..	..	..	..	..	..
NRTI and NNRTI initiator	Death	7·72	1·02–41·16	0·0227	0·1222	..	..	..	..	..	..	..	..
NRTI and start with TDF+FTC+EFV	Death	8·82	1·19–44·27	0·0132	0·1222	..	..	..	..	..	..	..	..

Only variables with significant association to clinical outcome are shown. *Two options were used to do the multivariate analysis—option 1: pretreatment drug resistance and initial antiretroviral therapy (ART) regimen considered as individual variables and option 2: pretreatment drug resistance and initial ART regimen combined as composite variables. OR=odds ratio. NNRTI=non-nucleoside reverse transcriptase inhibitors. NRTI=nucleoside reverse transcriptase inhibitors. TDF=tenofovir disoproxil fumarate. FTC=emtricitabine. EFV=efavirenz. ATV/r=boosted atazanavir.

***Table 4*: Demographic and clinical variables associated with first-line ART outcome**

distribution of reported HIV-infected patients in the country.[18,19] The demographic characteristics of the cohort were also consistent with previous reports on the Mexican HIV epidemic, including mostly men[6,19] with a characteristic late presentation to clinical care.[20] This survey deviates from WHO guidelines in that it did not include patients with previous ART exposure (ie, restarters). This decision was made because of the observation that the proportion of restarters in the country is low (2 · 9%, 95% CI 2 · 8–3 · 0, from all individuals on ART up to 2015 according to the SALVAR database), to simplify inclusion criteria and avoid confusion. The most frequent reasons for ART discontinuation generally after revising the national database SALVAR were abandonment (57%), followed by migration (19%) and acquisition of social security health-care access (11%). These are important limitations that warrant improvement in future surveys. Another limitation was that only Ministry of Health clinics were sampled and no social security clinics were included. Nevertheless, 63% of individuals on ART in Mexico are affiliated to Ministry of Health clinics.

The worryingly high prevalence of pretreatment drug resistance, is in line with previous reports showing increasing pretreatment resistance trends in Mexico.[21–23] Pretreatment resistance transmission of HIV was higher in heterosexual individuals, and prevalence was 26% in women. We suggest that women and their male partners should be identified as target groups for programmatic actions. By contrast with previous studies and with comprehensive meta-analyses,[24,25] is our finding of the dominance of NNRTI pretreatment resistance over NRTI resistance. Indeed, the drugs most affected by pretreatment resistance were efavirenz and nevirapine, consistent with the common use of efavirenz-containing first-line ART regimens (appendix p 7). Given that 85% of individuals in our survey started with NNRTI-based regimens, and that 8·7% of individuals had resistance to the most widely used ART regimen tenofovir disoproxil fumarate plus emtricitabine plus efavirenz, this observation needs to be taken into account for policy making and selection of future first-line ART regimens, along with considering the high proportion of NNRTI initiators without NNRTI pretreatment drug resistance who achieved virological suppression. Although etravirine and rilpivirine are not used as part of first-line ART regimens in Mexico, we noted at least low-level pretreatment resistance to these drugs in 2·7% and 5·7% of individuals, respectively. Thus, the use of these drugs, particularly rilpivirine, in the Mexican setting should be examined with caution.

We noted high-level pretreatment resistance (Stanford penalty score ≥60) for the NRTI backbones that are most frequently used in ART regimens (ie, tenofovir disoproxil fumarate plus emtricitabine, abacavir plus lamivudine, and zidovudine plus lamivudine). For lamivudine and emtricitabine, resistance was largely a result of Met184Val mutations. Resistance to abacavir, tenofovir, and zidovudine (mostly intermediate-level or low-level, penalty score 15–59) was associated mainly with TAMs. Even with the high frequency of tenofovir disoproxil fumarate use, the absence of Lys65Arg was remarkable, and could be accounted for by viral fitness constraints and the bidirectional antagonism between the Lys65Arg and TAM pathways.[26,27] Of note, tenofovir and abacavir resistance, including Lys65Arg and Lys70Glu, mutations

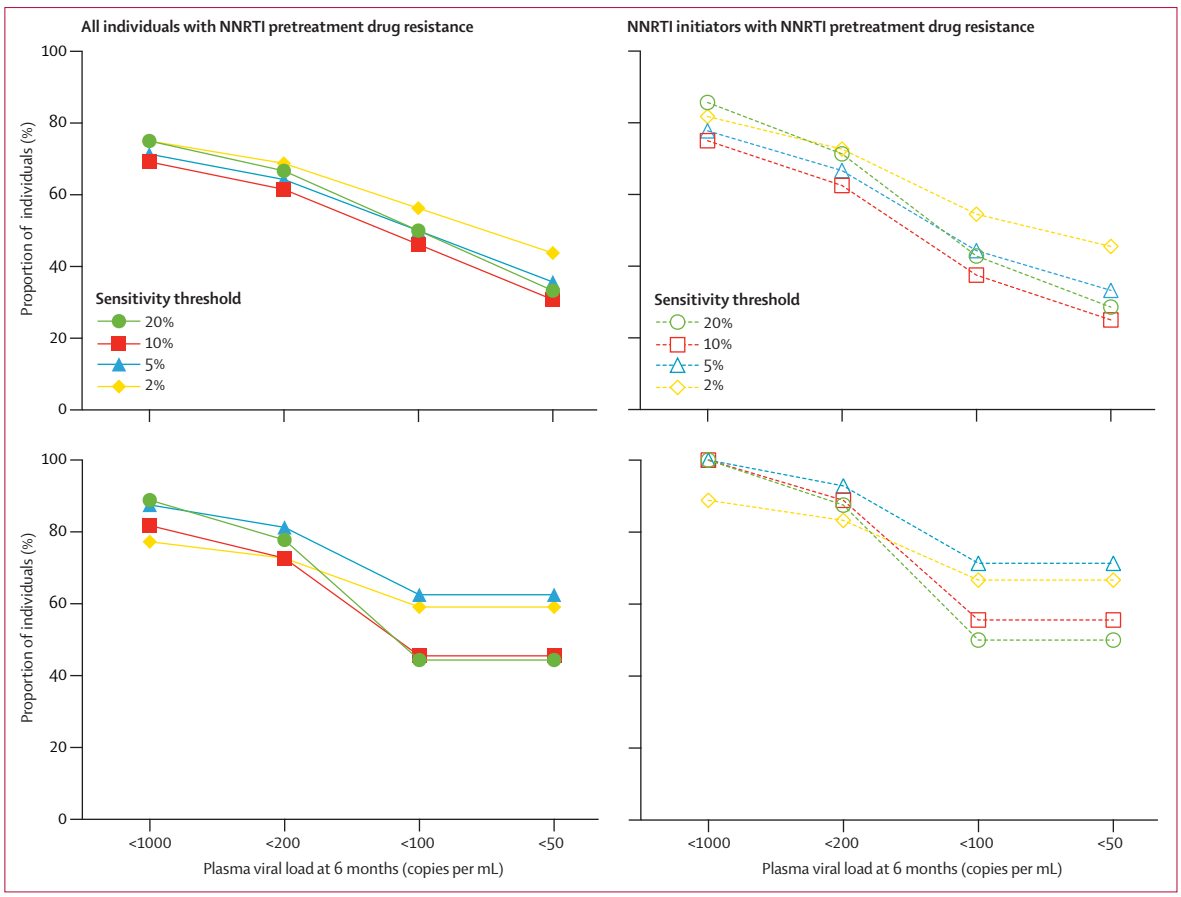

***Figure 5*: Effect of low-abundance drug resistance variants on first-line antiretroviral therapy**
The proportion of individuals achieving various levels of viral suppression was recorded for participants with available data for antiretroviral treatment initiation date and type of regimen, and follow-up viral load determinations up to 6 months after closing the survey. Effects of pretreatment drug resistance low-abundance variants of nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) at different sensitivity thresholds on viral suppression levels after initiation of first-line antiretroviral therapy are shown.

were exclusively low-abundance (<5%) variants. Nevertheless, all but one individual with Lys70Glu (who also had Lys103Asn and Pro225His) or Lys65Arg achieved virological suppression.

Although protease inhibitors used as first-line ART (atazanavir, lopinavir, and darunavir) showed low pretreatment resistance, the effect of resistance to these drugs on first-line ART effectiveness could not be assessed because of the low number of protease-inhibitor initiators with pretreatment resistance to protease inhibitors.

Similar to results of studies in other parts of the world, this survey showed a significant effect of pretreatment drug resistance on the virological response to first-line ART, especially pretreatment resistance to NNRTI in people starting NNRTIs.[28–31] Higher pretreatment resistance occurred in older individuals and heterosexual people, similar to findings in a large European cohort,[31] and we noted associations between lower pretreatment CD4 cell count and mortality and higher pretreatment plasma viral load and lower ART success, mortality, and immune reconstitution, similar to findings in southeast Asian and sub-Saharan cohorts.[29,30] Virological success rates were higher for NNRTI initiators than protease-inhibitor initiators. This observation was unexpected and might result from increased adherence to one-pill daily regimens (tenofovir disoproxil fumarate, emtricitabine and efavirenz). However, this finding could also be associated with a short follow-up. We noted no differences in baseline CD4 cell counts, plasma viral loads, or demographic variables between protease-inhibitor initiators and NNRTI initiators. Initiation of first-line ART with regimens based on protease inhibitors is a common practice in Mexico (14·3% of ART initiators) and the decision to use protease inhibitors over NNRTIs is mostly clinical, considering possible side-effects of efavirenz.

Although higher proportions of low-abundance drug resistance mutations for NRTI and protease inhibitors compared to NNRTI were noted in the cohort, NNRTI low-abundance variants at levels of 5% or more had a stronger role in virological suppression in individuals

starting with NNRTI-based regimens. Our observations are in agreement with previous studies[32,33] showing that NNRTI-based ART can result in virological success in individuals with very low-abundance drug resistance variants (<1%), and reporting increased risk of virological failure to first-line NNRTI-based regimens with NNRTI low-abundance variants at intermediate range (1–25%).[34] However, our observations contrast with those from other studies showing no clinical effect of low-abundance drug resistance variants (2–20%).[35]

Our analyses have limitations, including the use of a database without quality control and high rate of change, and that some important variables were not recorded, such as adherence, drug-associated side-effects, or psychosocial factors. Additionally, our survey allowed comparison of typical Sanger sequencing with next-generation sequencing for HIV-drug resistance testing, showing equivalent results for the 20% next-generation sequencing variant detection threshold. This fact is especially relevant for HIV-drug resistance surveillance, because processing a large number of samples per sequencing run can significantly reduce genotyping costs with increased sensitivity to detect drug resistance mutations.

Although recommendation for 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 pretreatment resistance to NNRTI, we recommend the integration of baseline HIV-drug resistance testing for initial ART follow-up and for its feasibility to be examined. If baseline HIV genotyping for all individuals starting ART is not feasible, targeting specific populations for HIV-drug resistance tests such as women and their male partners, or the possibility of intensifying the monitoring of plasma viral load should be considered and included in further cost-effectiveness analyses. Our work also emphasises the need to perform standardised HIV-drug resistance surveys.

Contributors

GR-T was the principal investigator and supervised the development of the survey. SÁ-R coordinated the survey, did the analyses, and wrote the manuscript. CG-M, DT-T, and VSQ-M coordinated logistics and did the Sanger sequencing. MM-F did the next-generation sequencing. KAR-M compiled data from national databases. MV-L provided epidemiological data from the national database and did the analyses. HR-G did the statistical analyses. HJ and PS provided training in assessment of HIV-drug resistance with next-generation sequencing and access to the HyDRA software. JC-R, JS-M, EAL-J, CM-R, and PU-Z supported logistics and survey implementation. All authors critically revised the manuscript.

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Declaration of interests

JS-M reports grants from Pfizer, Gilead, BMS, and ViiV outside the submitted work. He is also a speaker for Gilead and a consultant for Merck Sharp & Dohme. All other authors declare no competing interests.

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