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Characterization of HIV drug resistance profiles in patients at a healthcare center in Quito: "A pilot study"

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Caracterización de los perfiles de resistencia a los medicamentos contra el VIH en pacientes de un centro de atención en Quito: "Un estudio piloto"

Abstract

HIV/AIDS is one of the most important chronic infectious diseases. Although ART therapies have decreased morbidity and mortality considerably, new cases continue to appear. HIV-1 drug resistance is one of the most important problems that delay 95-95-95 goals. The presence of drug resistance mutations in naïve and ART-experienced patients is considered a risk factor for treatment failure and the transmission of HIV-1 resistance strains. A cross-sectional study included naïve and ART-experienced patients from one health care center in Quito, Ecuador in 2019 and 2021. Demographic data was collected with blood samples for sequencing, genotyping, and resistance tests. In 42 patients recruited, the overall prevalence of HIV-1 DRM was 9.5 %, most related to NNRTI. A total of 42 mutations were found, 38.6 % related to PIs, 34.09 % to NNRTI/NRTIs, and 22.7 % to INSTIs, most of them considered as minor or accessories, producing PLLR, LLR, and, in one patient, HLR to NNRTIs. Although few drug resistance mutations that reduce ART susceptibility were identified, further studies are required to characterize HIV-1 drug resistance in Ecuador and its implications for clinical response.

Keywords: HIV, HIV-1, ART, drug resistance, HIV/AIDS, NNRTI, INSTI, DRM.

Resumen

El VIH/SIDA es una de las enfermedades infecciosas crónicas más importantes a nivel mundial en los últimos años. Aunque las terapias antirretrovirales (TAR) han reducido considerablemente la morbilidad y la mortalidad por VIH, existe un reporte continuo de nuevos casos. La resistencia a TAR contra el VIH-1 es uno de los problemas más importantes que retrasan los objetivos 95-95-95 establecidos por la Organización Mundial de la Salud, que como objetivo busca conseguir que los pacientes que viven con VIH (PVV) conozcan su diagnóstico, reciban tratamiento y eviten la transmisión del virus. La resistencia al TAR se da por la presencia de mutaciones en el genoma del VIH-1, tanto en pacientes que han recibido previamente tratamiento como aquellos que lo inician; constituyendo un factor de riesgo asociado al fracaso terapéutico y la transmisión de cepas resistentes al VIH-1. Se realizó un estudio observacional tipo transversal para determinar el perfil

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de resistencia a TAR que incluyó pacientes que recibieron o no previamente TAR en la unidad de VIH de un hospital en Quito, Ecuador, durante 2019 y 2021. Se recopilaron datos demográficos junto con muestras de sangre para secuenciación, genotipificación y determinación de mutaciones asociadas con la resistencia a TAR. En los 42 pacientes reclutados, la prevalencia mutaciones de resistencia a TAR (DRMs) para VIH-1 fue del 9.5%; siendo relacionadas con los fármacos inhibidores de la transcriptasa reversa no nucleosídicos (NNRTI). Se identificaron un total de 42 mutaciones: 38.6% relacionadas con los fármacos inhibidores de la proteasa (PIs), 34.09% con NNRTI/ inhibidores de la transcriptasa reversa nucleosídicos (NRTIs) y 22.7% con los fármacos inhibidores de la translocación de la integrasa (INSTIs), la mayoría de ellas consideradas menores o accesorias sin repercusión en la sensibilidad a TAR; ocasionando una potencial resistencia de bajo nivel (PLLR), resistencia de bajo nivel (LLR) y, en un paciente, alto nivel de resistencia (HLR) exclusivamente para los NNRTIs. Aunque se identificaron escasas mutaciones relacionadas con la resistencia a las diferentes combinaciones de TAR en el grupo de pacientes, se requieren estudios adicionales para caracterizar la resistencia a medicamentos del VIH-1 en Ecuador, su y sus implicaciones en la respuesta clínica.

Palabras clave: VIH, VIH-1, ART, resistencia a medicamentos, VIH/SIDA, NNRTI, INSTI, DRM.

INTRODUCTION

HIV/AIDS remains one of the most significant chronic transmissible diseases globally, affecting approximately 39 million people. In 2022, around 63,000 people died from HIV-1 infection, and at least 1.3 million new cases were reported annually, with the majority occurring in individuals over the age of 15 [1]. The introduction of antiretroviral therapy (ART) has greatly improved the management of HIV/AIDS cases by treating all diagnosed individuals, regardless of viral load or HIV/AIDS stage, improving access to care, and significantly reducing mortality and comorbidities in at least 51% [2]. New HIV cases have risen significantly worldwide in recent years (1.3 million), and 14% of people living with HIV (PLHIV) remain unaware of their status. Only 76% of diagnosed individuals have access to ART. This contributes to treatment delays, as late intervention often coincides with advanced HIV/AIDS, making treatment less effective and increasing the risk of opportunistic infections, complications, and viral transmission [3].

The World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) proposed the 95-95-95 strategy, which aims to ensure that by 2025, 95% of people living with HIV know their status, 95% of those diagnosed receive antiretroviral therapy (ART), and 95% of those on ART achieve undetectable viral loads [4]. While significant progress has been made, this goal has challenges that still need attention. Critical factors have been identified as challenges, including achieving undetectable viral loads and managing poor clinical outcomes and complications such as co-infections. These setbacks are often driven by factors such as patient adherence issues, high rates of treatment discontinuation due to social challenges, poor retention in care, and ART resistance [5,6]. ART resistance is particularly linked to the HIV-1 viral ability to replicate and, in the presence of selective drug pressure, generate drug

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resistance mutations (DRMs), which can compromise the efficacy of ART, leading to poor therapeutic responses and treatment failure [7].

At least 26% of HIV/AIDS patients who start antiretroviral therapy (ART) carry HIV-1 strains resistant to first-line treatments, especially to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) that are considered the backbone of ART. Several strategies have been implemented to address this issue, such as avoiding NNRTIs when the prevalence of drug resistance mutations (DRMs) exceeds 10%, strengthening adherence support, and improving patient retention in care [8]. The World Health Organization (WHO) has also established an effective HIV drug resistance surveillance program that monitors the most common DRMs worldwide and recommends resistance testing for all individuals who initiate or restart ART [9,10]. Additionally, international databases and collaborations also consolidate and analyze the described DRMs, assessing resistance levels and estimating the likelihood of treatment failure for ART-naïve and experienced patients [11].

The prevalence of pre-treatment drug resistance (PDR) mutations in ART-naïve patients to commonly used antiretroviral therapy drugs varies significantly depending on patient type, country, and other factors. It ranges from 5% to 30% in adults starting any ART, with 5.4% corresponding to Nucleoside Reverse Transcriptase Inhibitors (NRTIs), 12.9% to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), 0.4% to Protease Inhibitors (PIs), and 0.6% to Integrase Strand Transfer Inhibitors (INSTIs). These values increase in patients with prior ART experience. In Latin America, the prevalence of NNRTI resistance is notably higher, at 16.7% among treatment-naïve patients and 26.7% among those previously exposed to ART. This underscores the challenges of continuing NNRTI-based regimens in the region and highlights the need for regimens with drugs that have a high genetic barrier, such as dolutegravir (DTG). It also emphasizes the importance of ensuring that all people living with HIV (PLHIV) are included in national treatment programs [8,12].

In 2020, Ecuador reported 47,000 people living with HIV (PLHIV), including 2,823 new cases. From these, 85% knew their status, 74% received ART, and 65% achieved viral suppression. The HIV/AIDS mortality rate was 4.8 per 100,000 inhabitants, with 44 deaths. Most cases occurred in key and vulnerable populations, such as young adults (ages 19-49), sex workers, and men who have sex with men (MSM), primarily in the provinces of Guayas, Manabí, and El Oro [13]. National efforts have reduced mortality and morbidity and increased timely diagnosis through rapid testing. Care and follow-up are provided in national public hospitals offering ART regimens [14]. Since 2021, most healthcare centers have transitioned from NNRTI- to DTG-based regimens. ART resistance in naïve and experienced patients in Ecuador is not well characterized, potentially leading to poor clinical outcomes. This study aimed to determine the prevalence and profile of HIV-1 drug resistance mutations (DRMs) at an HIV healthcare center in Quito between 2019 and 2021.

MATERIALS AND METHODS

Study population

A cross-sectional study was conducted at the HIV healthcare center of Enrique Garces Hospital between April and May 2019 and from August to September 2021. The study

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included patients aged 18 and older with confirmed HIV/AIDS diagnoses, both treatment-naïve and ART-experienced, who regularly received care at the center. Participants were invited to join the study on different days, and those who voluntarily agreed provided written informed consent. They also completed a socio-demographic questionnaire. Each participant was assigned a unique code to ensure the anonymization of the data, which was used solely for research purposes.

Clinical data, including CD4+ cell counts, viral load, and ART history, were retrieved from the medical records provided by their physicians or through a study-specific questionnaire, and a blood sample was required by the patient for genotyping and identification of significant DRMs. The study was approved by the Ethics Committee of Universidad San Francisco de Quito USFQ (2021-085M) in compliance with the ethical principles outlined in the Declaration of Helsinki.

Collection Samples and RNA extraction

Blood specimens were obtained via venipuncture from the forearm (approximately 10 mL) and collected in ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes (BD, San Jose, CA) on the day of enrollment at the clinical care center, following WHO guidelines [15]. Each patient sample was assigned a unique identifier to ensure anonymity. Samples were processed immediately to separate plasma by centrifugation at 5,000 rpm for 10 minutes at 4°C, after which plasma was aliquoted and stored at -20°C until further use. For RNA extraction, a 1mL plasma aliquot per patient was centrifuged at 14,000 rpm for two hours at 4°C to concentrate HIV particles. The QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract RNA, following the manufacturer's protocol.

HIV-1 amplification and sequencing

Reverse transcription of a 1kb fragment of *pol* gene that includes protease PR (codons 6-99) reverse transcriptase RT (codons 1-251), nucleotides 2268-3303, and a second fragment of 0.8kb of integrase IN (codons 1-288) nucleotides 4231-5094 (WHO standard PR 10-93, RT 41-238, IN 51-263) [16] was performed using the Thermoscript Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Both gene fragments were amplified by nested polymerase chain reaction (PCR) using Platinum Taq polymerase (Invitrogen, Carlsbad, CA), 50uL with 8 mM MgCl2, 0.2 mM dNTP mix, 0.2 µM each primer [17]. The first PCR was made under the following conditions: 94°C, 2 min, 30 cycles at 94°C-20s, 50°C-20s, 72°C-90s, and the final extension of 72°C-6min. The second PCR was made under the following conditions: 94°C, 2 min, 40 cycles at 94°C-20s, 50°C-20s, 72°C-90s, and the final extension of 72°C-6min [17]. Direct cycle of Sanger sequencing was performed with seven overlapping segment primers using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing kit and an ABI PRISM 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA). Sequence fragments were assembled using Sequencher version 4.5 (GeneCodes, Ann Arbor, MI). Gene cutter tool in the Los Alamos database was used to align nucleotides and identified coding (https://www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html) to align nucleotides coding regions.

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HIV-1 subtyping analysis and quality assurance

Base calling analysis and quality assurance was performed using RECall software/WHO HIVDR QC Tool (British Columbia Centre for Excellence in HIV/AIDS) [18], and a comparison of each was performed following WHO standards [16]; the presence of stop codons, APOBEC mutations, atypical mutations, and within-host similarity were checked. HIV-1 subtypes and circulating recombinant forms were identified using the Rega HIV subtyping tool V3 (https://rega.kuleuven.be) and the COMMENT online tool (https://comet.lih.lu). Recombination forms were confirmed using the RIP HIV Recombination Identification Program [19]. The mixture rate was also calculated according to the Stanford University algorithm, and HIVdb version 9.0 (https://hivdb.stanford.edu/hivdb/) was used [20].

Drug resistance mutations

HIV-1 mutations were identified and interpreted using the Stanford University HIVdb algorithm, version 9.0 (https://hivdb.stanford.edu/hivdb/), [20] which predicts resistance levels based on penalty scores assigned to each DRM: -level resistance (PLLR), 3) low-level resistance (LLR), 4) intermediate (I) and high-level resistance (HLR) to different ART. According to the penalty score, <10 is susceptibility, 10-14 potential low-level resistance, 15-29 low-level resistance, 30-59 intermediate resistance, and >60 high-level resistance. Those with "potential low-level resistance" were considered "susceptible," and "low-level, intermediate, and high-level" were considered "resistant" based on WHO recommendations [16,20]. Mutations without any drug susceptibility implications were considered polymorphisms, and those that may produce PLLR were not considered for the prevalence estimation.

Data Analysis

Demographic and clinical characteristics were summarized with 95% confidence intervals (CIs). A descriptive analysis was conducted with all variables considered normal data, using Shapiro Wilk (n<300). All the above data were collected using IBM SPSS Statistics for Mac, version 25, and graphics were created using Prism GraphPad (V9.5.1).

Nucleotide Sequence Accession Numbers

HIV-1 sequences were submitted to GenBank with the following accession numbers: OR543092- OR543131.

RESULTS

Study participant characteristics and descriptive analysis

Forty-two patients were enrolled in the study (22 from 2021 and 20 from 2019). The mean age was 34.8 years (31.7-37.8). 88.4% of participants were male, and 9.3% were female. 42.9% were identified as MSM (men having sex with men), 35.7% were heterosexual, 21.4% were self-defined as bisexual, and 23.23% of the subjects were foreign.

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Of the 42 subjects, 30.24% were categorized as ART drug-experienced and 67.4% as ART drug-naïve subjects. The average viral load and CD4+ count was $7.2x10^5$ copies/mL and 267.68 cells/mm^3, respectively. Also, 54,76% (23/42) were considered as only HIV-1 seropositive and 45.2% (19/42) as HIV/AIDS. Regarding coinfections, syphilis was found in 20% of MSM naïve patients and hepatitis B infection in 11.9%. All information was gathered from the clinical records and through a questionnaire. The remaining descriptive data are provided elsewhere (Table 1).

Regarding the selected ART regimens, among patients recruited in 2019, 38% (16/42) started with an Efavirenz (EFV)-based regimen as their first treatment, while in 2021, 21.4% (10/42) initiated a Dolutegravir (DTG)-based regimen. In both years, 14.2% (6/42) of patients received a protease inhibitor (PI)-based regimen as their first line of treatment. Among all ART-experienced patients, 38.4% were on an EFV-based regimen, and 23.8% were on a DTG-based regimen. Table 1 details the different regimens used in ART-naïve and experienced patients. In 2021, 60.5% of patients transitioned to a DTG-based regimen following national treatment guidelines.

TABLE 1. Antiretroviral regimens currently received by naïve and ART-experienced HIV patients attending a healthcare center in Quito.

ART regimens	Naive n (%)	Experience n (%)
TDF/FTC+RAL	6 (20.7)	1 (7.7)
TDF/3TC+DTG	6 (20.7)	2 (15.4)
TDF/FTC+DTG	1 (3.4)	1 (7.7)
ABC/3TC+ATV/r	0	1 (7.7)
TDF/FTC/EFV	14 (48.3)	5 (38.5)
ABC/3TC+LP/r	0	1 (7.7)
TDF/FTC+RAL+DRV/r	0	1 (7.7)
TDF/FTC+DRV/r	2 (6.9)	0
AZT/3TC+LP/r	0	1 (7.7)
TDF/FTC+RAL+DTG	0	1 (2.3)
TDF/FTC+LP/r	1 (2.3)	0
ABC/3TC+EFV	1 (2.3)	0
TDF/FTC+DRV/r+ATV/r	0	1 (2.3)

Abbreviations: TDF/FTC+RAL: tenofovir/emtricitabine+raltegravir; **TDF/3TC+DTG:** tenofovir/lamivudine+dolutegravir; **TDF/FTC+DTG:** tenofovir/emtricitabine+dolutegravir; **ABC/3TC+ATV/r:** abacavir/lamivudine+ atazanavir boosted with ritonavir; **TDF/FTC/EFV:** tenofovir/emtricitabine/efavirenz; **ABC/3TC+LP/r:** abacavir/lamivudine+ lopinavir boosted with ritonavir; **TDF/FTC+RAL+DRV/r:** tenofovir/emtricitabine+raltegravir+ darunavir boosted with ritonavir; **TDF/FTC+DRV/r:** tenofovir/emtricitabine+ darunavir boosted with ritonavir; **AZT/3TC+LP/r:** zidovudine/lamivudine+ lopinavir boosted with ritonavir; **TDF/FTC+RAL+DTG:** tenofovir/emtricitabine+ raltegravir+ dolutegravir; **TDF/FTC+LP/r:** tenofovir/emtricitabine+ lopinavir boosted with ritonavir; **ABC/3TC+EFV:** abacavir/lamivudine+ efavirenz; **TDF/FTC+DRV/r+ATV/r:** tenofovir/emtricitabine+ darunavir and atazanavir boosted with ritonavir.

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Quality assurance, mixture rate, and HIV-1 subtyping analysis

Forty-two plasma samples were analyzed, with 97.6% (41/42) successfully sequenced for at least one region of the PR-INT-RT genes. However, two samples (4.76%) amplified only the RT-PR gene, while one (2.38%) amplified only the INT gene. No insertions or deletions were detected. Two stop codons were identified in two samples (RT_188: TAG and INT_Q168). Of the HIV-1 sequences, 78.57% were classified as subtype B and 16.66% as non-B subtypes, specifically circulating recombinant forms (CRF) from the A, B, and G subtypes (Table 2). A more than 1% mixture rate was observed in 26.1% (11/42) of the samples.

TABLE 2. HIV genotyping in ART-naïve and experienced patients from a healthcare center in Quito.

Genotypes(n=42)	n	%
B	33	78.57
CRF02_AG	5	11.9
CRF24_BG	1	2.38
CRF19_cxp	1	2.38

Abbreviations: CRF02_AG: circulating recombinant form 02_AG; **CRF24_BG:** circulating recombinant form 24_BG; **CRF19_cxp:** circulating recombinant form 19_cxp (subtypes D, A1 and G)

Surveillance, major, and accessory DRMs and polymorphisms in RT-PR and INT genes

All 42 samples contained at least one polymorphism, and 42 potential drug resistance mutations (DRMs) were identified. Of these, 38.6% were linked to protease inhibitors (PIs), 34.09% to NNRTIs/NRTIs, and 22.7% to INSTIs. However, only 30.9% (13/42) of these DRMs were classified as major, minor, or accessory mutations with a potential impact on ART susceptibility, while the role of 7.13% (3/42) remains unknown. The overall prevalence of DRMs was 9.52%, primarily associated with potential resistance to NNRTIs. All identified mutations and interpretations are detailed in Tables 2, 3, 4.

NRTI mutations

Neither surveillance drug resistance mutations (SDRMs) nor major drug resistance mutations were identified for NRTIs or thymidine analog mutations (TAMs). Most of the mutations were classified as minor or accessory. Notably, the polymorphic mutation S68G was observed in 14.28% (6/42) of the samples: four corresponded to the B subtype and two to the CRF02_AG.

NNRTI mutations

The major surveillance drug resistance mutation (SDRM) K103N and the non-polymorphic F227C mutation in one B subtype sample were identified. Additionally, several minor or accessory mutations were found: V179E/D (7.14%) in three subtype B samples, E138A (2.38%), V108I (2.38%), and V106I (2.38%) in three different B subtype samples. Another polymorphic RT mutation, V118I (7.14%), was observed in three B subtype samples. The rest of the mutations are described in Table 4.

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PI mutations

M46L was the only major surveillance drug resistance mutation (SDRM) identified in a subtype B sample. Additionally, the most prevalent PI accessory mutations were as follows: A71V (26.19%), K20R (4.76%), L10I (4.76%), K43T (2.38%), and A71T (2.38%); as well as the consensus mutations for the for CRF02_AG subtype V82I (16.66%), K20I (11.90%), T34S (2.38%), and V11VI (2.38%) (Table 3).

INSTI mutations

No major mutations were identified for INSTIs. The accessory mutations E157Q (7.14%) and L74M (4.76%) were identified in three subtype B samples and two subtype CRF02_AG samples, respectively. Additionally, other highly polymorphic mutations such as M50I (28.57%) in B subtype samples, L74I (14.28%) in five B subtype and one CRF02_AG samples, E138D (4.76%) in two B subtype samples, S119R (2.38%), and S230N (2.38%) were observed. Unusual mutations, including H183L (2.38%), L194LF (2.38%), C65CY (2.38%), L234Y(2.38%), and V75A (2.38%), were identified in three B subtype samples, and the APOBEC mutation R224RQ (2.38%) in one CRF02_AG sample was also detected, as detailed in Table 5.

TABLE 3. Mutations and frequency in PI gene in naïve and experienced HIV patients attending a clinic in Quito.

Mutation	n	Frequency (%)
PI		
M16L	1	2.38
ACCESSORY		
K47T	1	2.38
OTHERS		
A71V	9	21.43
V82I	6	14.29
A71T	5	11.90
K20R	5	11.90
K20I	5	11.90
T74S	1	2.38
L10V	1	2.38
L10LI	1	2.38
L33I	1	2.38
V82VI	1	2.38
L10I	5	11.90
A71AITV	1	2.38
L33V	1	2.38
L10IV	1	2.38
V11V1	1	2.38

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TABLE 4. Mutations and frequency in RT gene in naïve and experienced HIV patients attending a clinic in Quito.

Mutation	n	Frequency (%)
RT		
NNRT		
V179E	2	4.76
E138A	1	2.38
K103KN	1	2.38
F227C	1	2.38
V108I	1	2.38
V179D	1	2.38
V106I	1	2.38
NRTI		
S68G	6	14.29
T39TA	1	2.38
OTHERS		
K101K	1	2.38
T69N	1	2.38
V118I	3	7.14
V179I	3	7.14
V90VI	1	2.38
Y188*	1	2.38

TABLE 5. Mutations and frequency in IN gene in naïve and experienced HIV patients attending a clinic in Quito.

IN	n	Frequency (%)
ACCESSORY		
E157Q	3	7.14
L74M	2	4.76
OTHERS		
S230N	4	9.52
M50I	11	26.19
L74I	8	19.05
E138D	1	2.38
H183HL	1	2.38
L104LF	1	2.38
C65CY	1	2.38
L234Y	1	2.38

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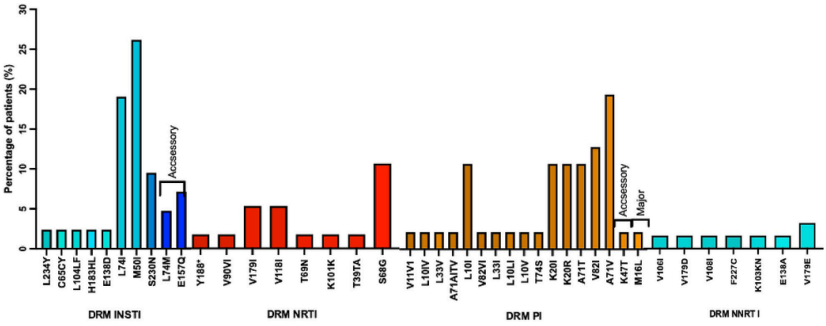

FIGURE 1. Mutation frequencies of HIV-1 for INSTI, PI, NNRTI, and NRTI based on Stanford database (HIVdb) identification in naïve and experienced HIV patients attending a healthcare center in Quito. On the X axis are the mutations for each HIV viral protein, and on the Y axis is the frequency of the identified HIV mutations. Light blue and blue represent accessory mutations for the INT gene, red is the NRTI mutations of the RT gene, brown and light brown are the mutations of the PI gene, and turquoise is the NRTI mutation of the RT gene.

Prediction of ART susceptibility

Of the 13 identified drug resistance mutations (DRMs), 30.76% (4/13) were either not predicted to impact ART susceptibility or were considered polymorphisms according to the Stanford University algorithm (score below 9). For NNRTI, S68G was identified in four ART-experienced patients on DTG, ATV/r, and EFV-based regimens, and T39TA in one ART-experienced patient on a DTG regimen. For INSTIs, L74M (4.76%) was found in two patients under DTG and RAL regimens, while K43T was in one treatment-naïve patient on an EFV-based regimen.

Potential low-level resistance (PLLR)

Of the drug resistance mutations (DRMs) analyzed, 53.8% (7/13) were associated with a prediction of potential low-level resistance (PLLR) to NNRTIs (score ranging from 10 to 14). The V179E/D mutation (7.14%) was identified in two ART-naïve patients receiving an FTC/EFV regimen and one ART-experienced patient on a TDF/FTC+RAL regimen. The E138A mutation (2.38%) was detected in one ART-experienced patient on TDF/FTC+RAL, while the V108I (2.38%) and V106I mutations were observed in two ART-naïve patients on DRV/r and TDF/FTC/EFV regimens, respectively. Furthermore, the presence of the E157Q mutation predicted PLLR for INSTIs in two ART-naïve patients on RAL and EFV regimens and in one ART-experienced patient on LP/r. For protease inhibitors (PIs), only the SDRM M46L mutation was identified in one ART-naïve patient on an EFV-based regimen.

Low-level, intermediate, and higher resistance (LLR, INTR, and HR)

Among NNRTIs (15.38%), the previously mentioned V108I mutation was associated with low-level resistance (LLR) (score 15-29) to Rilpivirine (RPV) and was observed in the same previous patient. The surveillance drug resistance mutation (SDRM) K103N was identified in an ART-experienced patient with low adherence to a TDF/FTC/EFV regimen, conferring high-level resistance (HLR) (score >60) to Nevirapine (NVP), Efavirenz (EFV), and Doravirine (DOR). Additionally, the F227C mutation was identified in this patient,

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and it is associated with intermediate resistance (INTR) (score 30-59) to RPV and Etravirine (ETR). A detailed susceptibility prediction of these drug resistance mutations (DRMs) is provided in Table 6.

TABLE 6. Interpretation of DRM identified in samples from naïve and experienced HIV patients for NRTI, NNRTI, INSTI, and PI

Type of ART	Mutation	Interpretation*
NRTI	S68G	Susceptible to ABC, AZT, FTC, 3TC and TDF
	T39TA	
INSTI	L74M	Susceptible to BIC, CAB, RAL, DTG, and EVG
	E157Q	PLLR to RAL and EVG
PI	K43T	Susceptible to ATV/r, LPV/r and DRV/r
	M46L	PLLR to ATV/r and LPV/r
NNRTI	K103KN	HLR to NVP, EFV and DOR
	F227C	INTR to RPV and ETR
	V108I	PLLR: DOR, EFV/LLR to NVP
	E138A	PLLR to ETR and LLR to RVP
	V179D/E	PLLR to EFV, ETR, NVP and RPV
	V106I	PLLR to DOR, ETR, NVP and RPV

* Only LLR, INT, and HLR are considered for the prevalence calculation. PLLR are not considered.

Abbreviations: ATV/r: atazanavir boosted with ritonavir; **ABC:** abacavir; **AZT:** zidovudine; **BIC:** bictegravir; **CAB:** cabotegravir; **DOR:** doravirine; **DRV/r:** darunavir boosted with ritonavir; **DTG:** dolutegravir; **EFV:** efavirenz; **ETR:** etravirine; **EVG:** elvitegravir; **FTC:** emtricitabine; **HLR:** high-level resistance; **INSTI:** integrase strand transfer inhibitors; **INTR:** intermediate resistance; **LLR:**low-level resistance; **LPV/r:** lopinavir boosted with ritonavir; **NRTI:** nucleoside reverse transcriptase inhibitors; **NNRTI:** non-nucleoside reverse transcriptase inhibitors; **NVP:** nevirapine; **PI:** protease inhibitors; **PLLR:** potential low-level resistance; **RAL:** raltegravir; **RVP:** rilpivirine; **3TC:** lamivudine; **TDF:** tenofovir.

DISCUSSION

In this study, we described the presence of HIV-1 drug resistance mutations (DRMs) at a healthcare center in Quito, Ecuador, along with the circulating subtypes and their potential impact on various ART regimens over two different years. To our knowledge, this is the most recent study in Ecuador to report on the prevalence of HIV DRMs across different drug classes (NNRTIs, NRTIs, PIs, and INSTIs) before and during the transition to DTG-based regimens. We identified an overall DRM prevalence of 9.52% associated with low-level, intermediate, and high resistance prediction to the different available ART regimens. Most were primarily associated with NNRTIs, while none were detected for INSTI drugs. According to a recent meta-analysis, the worldwide prevalence of resistance to NNRTIs is 26.31% (95% CI, 20.76–32.25) [21].

Subtype B is the most prevalent HIV-1 subtype in Western and Central Europe, North America, Latin America, and the Caribbean, accounting for 60.5% of cases in these regions [8]. In the Andean region, the prevalence can reach up to 99% [22]. In our study,

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subtype B was detected in 78.57% of the samples, aligning with previous reports from Ecuador [23]. Circulating Recombinant Forms (CRFs) are considered HIV-1 strains that result from the recombination of different HIV subtypes and represent approximately 29% of HIV infections worldwide [24]. Among these, CRF02_AG was identified in 11.9% of our samples, confirmed by the presence of the K20I signature mutation in the PR gene [20]. This form has a global prevalence of 7%, while Latin America is less common, with a prevalence of 0.9% [8](World Health Organization, 2021). A previous Ecuadorian study reported a 4% prevalence of CRF02_AG in antiretroviral therapy (ART) treated patients during 2018 [23].

CRF19_cxp and CRF24_BG, recombinant forms of subtypes D-A and B-G, respectively, were previously reported in countries like Cuba and Spain but had not been documented in Ecuador [25,26]. These CRFs are particularly significant due to their association with rapid disease progression to AIDS. In our study, both CRFs were identified, with one case found in an ART-experienced patient from Cuba who exhibited persistent low-level viremia. Migration is known to play a critical role in the introduction and spread of HIV subtypes [24]; Ecuador has experienced significant migration from countries such as Cuba, Spain, and Venezuela in recent decades, likely explaining the detection of these CRFs in our population. However, the specific impact of these recombinant forms on HIV-1 transmission dynamics and ART resistance in Ecuador requires further investigation.

Despite this, our study has several limitations. First, the small sample size was due to resource constraints and did not include other national healthcare centers; this may lead to significant variations in our findings compared to national estimations. Geographic variability and selection bias are also concerns; although there is a significant proportion of HIV cases in Quito-Pichincha (19.56%), other provinces such as Guayas (75%), Manabí (8.46%), and Esmeraldas (4.86%) have reported higher prevalence rates in Ecuador [27]. These findings indicate that the prevalence of circulating mutations warrants further attention. In recent years, next-generation sequencing (NGS) technologies have significantly enhanced the accurate identification of drug resistance mutations (DRMs) compared to traditional Sanger sequencing, which only detects variants with DRMs at levels exceeding 20% of the viral population across various samples. This limitation of the Sanger method may help to explain the low prevalence of DRMs observed in our study. Despite this, Sanger sequencing is still recommended as the standard method for HIV DRM surveillance by the World Health Organization (WHO) [16]. Additionally, patient adherence to antiretroviral therapy (ART) and the transition to dolutegravir (DTG)-based regimens may not fully reflect DRM trends from previous years, potentially introducing further limitations to our analysis.

In 2023, the Pan-American Health Organization (PAHO) reported that 1.7 million people who live with HIV (PLHIV) were receiving ART with a treatment coverage of 73% for the Americas and the Caribbean [28]. While a definitive cure has not yet been found, ART has significantly improved the quality of life and life expectancy of HIV patients through long-term viral suppression and reducing clinical symptoms and complications. During the same year, in Ecuador, at least 85% of PLHIVs knew their status and were under an ART regimen [27], remarking the continuous efforts to improve and reach the established goals for 2030. However, at least 36% of patients under ART could not reach viral suppression (less than 1,000 copies/mL), increasing the risk of treatment failure and drug resistance.

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The emergence of antiretroviral therapy (ART) resistance in recent decades has significantly compromised the benefits of treatment, particularly for the most widely used regimens. Determining and characterizing patterns of HIV-1 drug resistance mutations (DRMs) and polymorphisms is crucial for understanding viral transmission dynamics, adaptation, and resistance to current antiretroviral therapy (ART) regimens. This knowledge aids in developing accurate interventions. It is well-established that resistance to NNRTIs, such as nevirapine (NVP) or efavirenz (EFV), is the most common and can develop in at least 10% of ART-naive patients receiving these drugs as first-line therapy (ranging from 9-14% depending on the country), with this rate tripling in ART-experienced patients [12]. According to the WHO 2021 [8], the overall prevalence of pre-treatment drug resistance (PDR) for NNRTIs in countries such as Colombia, Argentina, Brazil, Mexico, and Cuba ranges from 6 to 22% [9,28,29] and in neighboring countries like Peru, the prevalence of pre-treatment drug resistance in 2021 was reported at 9.8% [30]. This is consistent with our study, which identified a prevalence of 9.52% for NNRTs, and half of them were considered as transmitted, reflecting the widespread use of EFV-based regimens as first-line regimens before 2021. Furthermore, a previous study conducted in Latin America reported a prevalence of drug resistance in a limited number of samples from Ecuador during 2012, which was 4.3%, suggesting the progressive increase of DRMs over the years [23].

In our study, half of the NNRTI resistance mutations (DRMs) were classified as transmitted, primarily consisting of minor, accessory, or polymorphic mutations with minimal impact on ART susceptibility. One such mutation is V108I. It was detected in a treatment-naive patient on a darunavir/ritonavir (DRV/r) regimen. This mutation, selected by most NNRTIs, contributes to reduced susceptibility only when combined with other DRMs, leading to low-level resistance (LLR) to nevirapine (NVP), potential low-level resistance (PLLR) to efavirenz (EFV), and etravirine (DOR) [31]. This mutation has been reported in 50% of drug resistance mutations (DRMs) in Argentina but shows a lower prevalence in Mexico and Colombia (<5%) [8,20,32]. In our study, it was observed in 2.38% of cases. Also, the E138A mutation was identified in one treatment-naive patient under an INSTI-based regimen. It could be found in 2-5% of treatment-naive patients under rilpivirine (RPV) and etravirine (ETR) regimens, with prevalence varying by subtype and region. This mutation confers potential low-level resistance (PLLR) to ETR and low-level resistance (LLR) to RPV but does not significantly impact ART efficacy [8,20,33,34].

In contrast, protease inhibitors (PIs) and integrase strand transfer inhibitors (INSTIs) exhibit lower resistance rates due to their higher genetic barrier. Nonetheless, their frequent use and viral characteristics still impact their effectiveness. Resistance rates for PIs ranged from 0.1-1.4%, and for INSTIs, available data showed rates of 7.6% for Argentina and 0.5% for Mexico [8]. For PIs, one important DRM was found in an ART-naïve patient under an NRTI-based regimen. The M46L mutation is classified as a surveillance DRM (SDRM). It can occur in various HIV-1 subtypes among both ART-experienced or naive patients, with a reported prevalence of 0.37% in subtype B samples. M46L impacts the structural integrity of the enzyme binding site, conferring resistance to indinavir, nelfinavir, and saquinavir [35,36,37]. It has been documented in multiple countries, including the United States, Argentina, Brazil, Peru, Spain, and Cuba [12,38]. For our study, the prevalence of PI drug resistance was 2.38%.

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No major DRMs were identified for INSTIs; however, minor accessory mutations like E157Q were found in two ART-naïve patients receiving RAL and efavirenz EFV regimens, as well as in one ART-experienced patient on an LP/r regimen. The E157Q mutation occurs in 1-5% of INSTI-naïve patients and has a minimal impact on susceptibility to RAL, EVG, or DTG when combined with other mutations [39]. It has been reported in African studies with a prevalence of 1.56% [40]. Additionally, L74M is a common polymorphic mutation found in 1-5% of naive patients, especially in the CRF02_AG subtype. It was found in two naive patients on RAL and DTG-based regimens, though it did not affect susceptibility.

The low prevalence of DRMs for PIs and INSTI regimens in the present study would be explained by their less frequent use as first-line regimens during 2019-2021 in Ecuador. It could also be attributed to several factors, including high viral suppression rates, good treatment adherence, limited ART exposure, and the geographical and epidemiological characteristics of ART-naive patients in Quito. Other potential explanations include the small sample size or the recent introduction of highly effective dolutegravir (DTG)- based regimens. Similar findings have been reported in previous studies from Sweden, Iceland, China, and the U.S., where low levels of transmitted DRMs have been associated with strong ART adherence and near-complete viral suppression, particularly concerning NRTIs, PIs, and INSTIs [41,42,43]. This suggests that the widespread use of potent regimens and comprehensive treatment strategies may continue to play a key role in reducing transmitted drug resistance across various populations [41,43,44,45].

In 2018, the WHO recommended the inclusion of DTG in first-line regimens, highlighting the need for strategies to prevent HIV drug resistance and the transmission of viral populations carrying associated mutations [46]. It reported high levels of HIV viral load suppression (>90%) in populations receiving dolutegravir (DTG)-based antiretroviral therapy as a first-line regimen. According to PAHO data, Ecuador is one of the countries where more than 80% of PLWH currently receive a DTG-based regimen [46]. Still, from 2020, the DTG switch was performed according to international guidelines as a first-line treatment [47]. During our study, this transition was under implementation, finding that 48% of patients received an EFV and 24.1% DTG regimens. However, no national surveillance studies evaluate the direct impact of DRMs and their clinical response when EFV and DTG regimens are used [27].

On the other hand, acquired drug resistance mutations (ADRs) are typically observed in ART-experienced patients, where certain viral populations develop resistance under drug-selective pressure, often leading to a virological failure (VF) [47]. These mutations are frequently associated with NNRTI regimens, with a prevalence of approximately 27%, and in some African countries, this can rise to as high as 50% [48,49]. A 2018 study conducted in Ecuador involving 101 HIV-positive adults and children with VF found a prevalence of ADRs of 34.9% [23]. In contrast, our study observed a lower ADR prevalence of 4.74%, which may reflect a lower proportion of patients experiencing VF in the healthcare center. However, this could be different on a national scale.

The most common acquired drug resistance (ADR) mutation to NNRTIs is K103N. It was identified in an HIV-1 subtype B sample from a low-adherent, ART-experienced patient. K103N is considered a surveillance drug resistance mutation (SDRM), occurring in approximately 1.15% of treatment-naive patients and up to 37.11% of ART-experienced

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patients. This mutation alters the binding site of NNRTIs to the reverse transcriptase (RT) enzyme, significantly reducing the efficacy of NNRTIs like efavirenz (EFV) and nevirapine (NVP), with a 20- to 50-fold decrease in susceptibility. However, the presence of K103N does not impact other NNRTIs [20]. The importance of this mutation was noted in one meta-analysis performed in Latin America [28]. Another study suggested that this mutation may cause reduced viral suppression in patients under dolutegravir (DTG)-based regimens, particularly in South African populations, raising concerns about the potential for multi-class drug resistance [50]. This underscores the importance of continuous surveillance and tailored ART regimens to prevent treatment failure in the presence of specific mutations.

F277C is a non-polymorphic mutation selected in persons receiving DOR and rarely in persons receiving ETR and RPV. It usually occurs in combination with other DRMs and, in this setting, has been related to the high level of resistance for DOR. The mutation is traditionally associated with moderate to high-level reductions in susceptibility to NNRTIs such as nevirapine (NVP), efavirenz (EFV), etravirine (ETR), and rilpivirine (RPV). In the earlier study from Ecuador by [23], this ADR mutation was not reported. In our findings, the F277C mutation was identified in a single subtype B sample from an ART-experienced patient on an EFV regimen, but it did not result in any significant reduction in susceptibility to NNRTIs.

Although INSTI drugs have a high genetic barrier, a concerning increase in acquired drug resistance has been reported in some countries. One study performed in Mexico in 2020 identified a cross-resistance between different drugs of this group and VF to raltegravir and a lower proportion to dolutegravir [51]. In Sudan, DTG drug resistance mutations have been observed at a prevalence of less than 0.2% (95 % CI: 0.0-1.2 %); however, acquired drug resistance (ADR) surveys suggest that resistance rates to DTG may be below 3 % globally, though in low- and middle-income countries, prevalence can reach 4.8 %, and in certain African countries, it can rise as high as 19.6% [12]. Given these concerns, the WHO recommends routine drug resistance surveillance as the global use of DTG-based regimens expands [12].

In conclusion, our findings indicate that the prevalence of drug resistance mutations (DRMs) among the studied population is low, and most of these mutations do not significantly compromise susceptibility to key antiretroviral drug classes, including INSTIs, NRTIs, and PIs. However, some non-nucleoside reverse transcriptase inhibitor (NNRTIs) mutations could produce PLLR to LLR. The clinical impact of these results should be evaluated based on individualized responses to ART. Finally, it is important to highlight the potential role of viral mutations classified as polymorphisms. Although they are often not associated with resistance, it has been observed that they could contribute to resistance in the context of cumulative mutations or when they occur in certain HIV subtypes [52].

Our results also suggest that, given the low prevalence of DRMs, maintaining current ART regimens is a viable option. However, further studies are required to assess this approach nationally. Since HIV-1 DRM testing is not yet mandatory for treatment-naïve or experienced patients in many settings, implementing routine surveillance and follow-up of drug resistance is crucial. Countries like Brazil, Mexico, Argentina,

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Peru, and Honduras form part of the WHO HIVResNET for drug resistance, enhancing their responsiveness in the control and prevention of HIV transmission; although the economic resources required vary, Ecuador should consider its implementation as a public policy. Additionally, understanding the impact of switching to dolutegravir (DTG)-based regimens when necessary will ensure long-term treatment success.

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AUTHOR CONTRIBUTION STATEMENT

The authors confirm the contribution to the paper as follows: study conception and design: E. Teran, P. Quirola; data collection: P. Quirola, N. Cevallos; analysis and interpretation of results: P. Quirola, P. Espinosa; draft manuscript preparation: P. Quirola, E. Teran. All authors reviewed the results and approved the final version of the manuscript. Some sentences were improved using ChatGPT.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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