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Genetic Diversity and Drug Resistance Mutations in HIV-1 pol Gene Sequences in the Philippines: A Retrospective Genomic Analysis

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Abstract

The Philippines has experienced a significant increase in HIV-1 infections in recent years, with a growing epidemic driven by the CRF01_AE strain. Understanding the genetic diversity of HIV-1 in the Philippines is crucial for the development of effective treatment strategies and the prevention of drug resistance. This study analyzed comprehensive data on common resistance mutation patterns from 2009 to 2017, revealing an increasing trend of mutations observed in NRTI and NNRTI resistance among the predominant CRF01_AE strains. The most common NRTI mutations observed were M184V, K65R, and S68G, whereas the most common NNRTI mutations were K103N, Y181C, and G190A. The study also found a high prevalence of M184V minority variants (0.5-20%) in treatment-naive patients, which could increase the risk of virological failure in 3TC-containing regimens. The findings of this study highlight the importance of comprehensive drug resistance surveillance and access to resistance testing to guide optimal first-line antiretroviral treatment selection and to manage the growing HIV-1 epidemic in the Philippines. The development of effective strategies to prevent and manage drug resistance is crucial to ensuring the long-term success of HIV treatment programs in the country.

Keywords: HIV-1, Reverse Transcriptase Mutations, HIV-1 in the Philippines

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INTRODUCTION

The Philippines has experienced a significant increase in Human immunodeficiency virus-1 (HIV-1) infections in recent years, with the number of reported cases increasing from 4,300 in 2010 to over 12,000 in 2019.[1,2] The country is not alone in this trend, as the Asia-Pacific region has seen a steady increase in new HIV-1 infections, with an estimated 310,000 new cases reported by 2020. Several studies have reported that the major HIV-1 clades circulating in the Philippines are CRF01_AE and HIV-1 clade B.[2-4] Remarkably, CRF01_AE has become the predominant strain, accounting for up to 77% of HIV-1 infections in recent years.[2,4] The CRF01_AE strain is a part of the global epidemic of this recombinant form, which has spread throughout Southeast Asia, including countries such as Thailand, Vietnam, and Indonesia.[5] In contrast, HIV-1 clade B, while still present, makes up a smaller proportion of infections in the Philippines, comprising only approximately 13% of the strains in some studies.[6] Clade B is more commonly found in North America and Europe but has also spread to various regions worldwide.[6,7]

The increasing genetic diversity of HIV-1 in the Philippines has important implications for antiretroviral therapy (ART) and the development of drug resistance. Genetic variants can vary in their biological properties, evolutionary rates, disease progression, and resistance mutation profiles.[8-10] Genotyping is critical for assessing antiretroviral drug resistance, as certain mutations can confer resistance to various antiretroviral drugs.[8] Thus, there is a pressing need to analyze the trends in Nucleoside reverse transcriptase inhibitor (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) mutations in the Philippines, as these mutations may affect the efficacy of antiretroviral drugs. The emergence of drug-resistant HIV-1 strains can compromise the effectiveness of ART, leading to treatment failure and the increased transmission of resistant viruses. Furthermore, the spread of drug-resistant HIV-1 strains can limit the treatment options available to patients, making it essential to monitor the trend of NRTI and NNRTI mutations to inform treatment strategies and prevent the development of drug resistance.

This study aimed to analyze comprehensive data on common resistance mutations from 2009 to 2017, revealing an increasing trend in mutations observed in NRTI and NNRTI resistance among the predominant CRF01_AE strains. The findings of this study highlight the importance of comprehensive drug resistance surveillance and access to resistance testing to guide optimal first-line ART selection and manage the growing HIV epidemic in the Philippines.

METHODS

Data acquisition

The complete genome sequences of all HIV-1 were obtained as of 9th May 2024 from the HIV database using Geography Search Interface with the geographic distribution of subtyped sequences for the sample year (2009 to 2017). Data completeness and consistency were assessed using Microsoft Excel Version 16.86. The data extraction format included Accession, Subtype, Sampling Year, HXB2/MAC239 start, HXB2/MAC239 stop, and Sequence Length (Supplementary Table 1). All completed data were examined for clarity and consistency before the analysis.

To identify the most common major NRTI and NNRTI drug resistance mutations, we used a dataset of sequences from 2009 to 2017, with 2,174 sequences included in the HIV database. Records of HIV-1 subtype CRF 01_AE (n = 1,518) and HIV-1 clade B (n = 656) were retrieved. With the strict exclusion criteria of (a) no year on the data, (b) 2009 to 2017 year coverage, and (c) incomplete sequences that did not cover the pol gene, a total of 1,046 and 237 records were analyzed, respectively.

Phylogenetic analysis

Philippine HIV pol gene sequences representative of CRF 01_AE and clade B HIV-1 were obtained from the HIV database (Supplementary Table 2). These sequences were aligned using MAFFT v.7.520 with default parameters.[11] Following multiple sequence alignments, poorly aligned regions were trimmed using TrimAl v.1.2.[12] A maximum likelihood (ML) phylogenetic tree of the genome was inferred using IQTree v.2.2.2.7

with the GTR+F+I+R3 substitution model (based on best-model testing), with 1000 bootstrap iterations.[13] Sequence alignment was performed using AliView v.1.28,[14] and then edited using FigTree v.1.4.4.[15]

Mutation classification

The Stanford HIV Drug Resistance Database (HIVDB) (https://hivdb.stanford.edu/hivdb/by-sequences/) was used to identify NRTI and NNRTI resistance mutations in a dataset of sequences retrieved from the HIV database. The HIVDB has an online genotypic resistance interpretation program to help clinicians and laboratories interpret HIV-1 genotypic resistance tests. The program accepts complete or incomplete (pol region) genome sequences and returns a list of interpretations of drug resistance.

RESULTS

HIV-1 Diversity in the Philippines

In the HIV-1 dataset retrieved, 1,518 sequences were identified as HIV-1 subtype CRF01_AE, whereas only 657 sequences were from HIV-1 clade B, clearly illustrating the current dominance of CRF01_AE. Figure 1a presents the HIV-1 phylogenetic analysis from

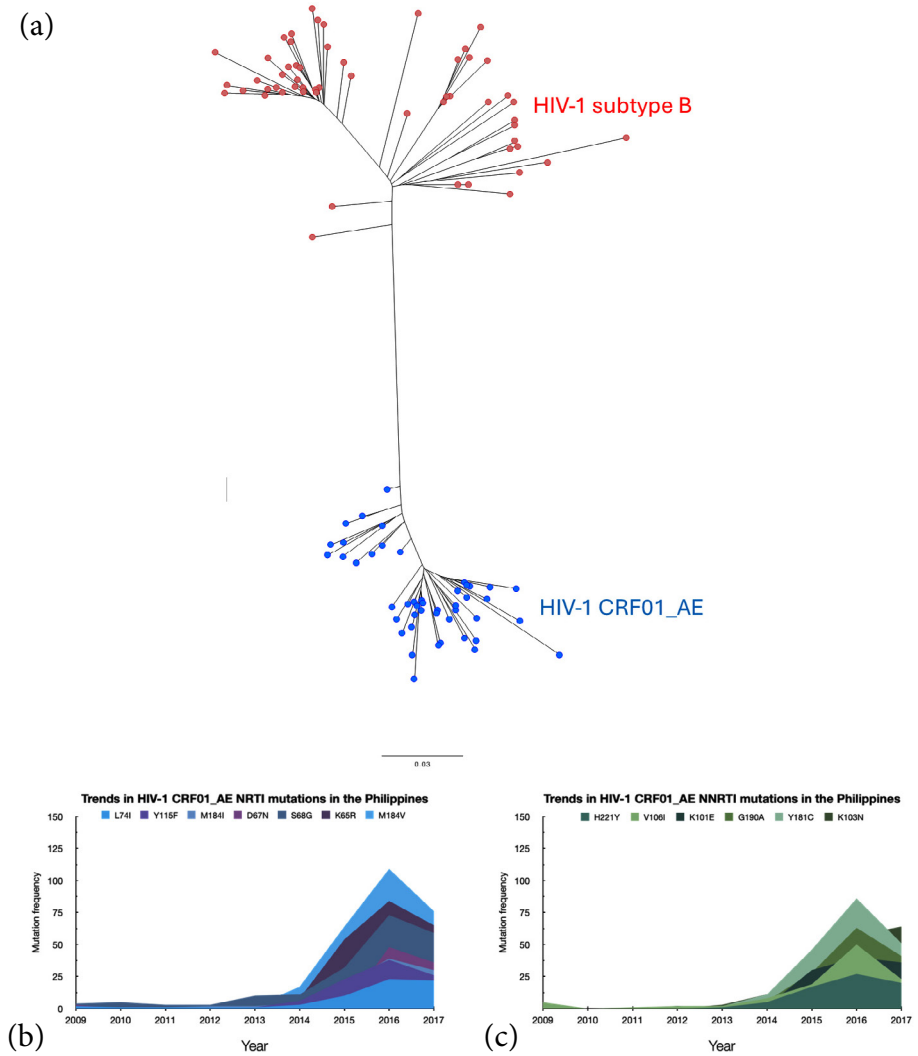

Figure 1. The HIV-1 phylogenetic analysis from representative of HIV-1 subtype CRF 01_AE and B (a), and the trends in HIV-1 subtype CRF 01_AE NRTI (b) and NNRTI (c) mutations in the Philippines

representatives of HIV-1 subtypes CRF01_AE and B, likely demonstrating the genetic relationships and diversity within these two major clades.

NRTI and NNRTI Resistance Mutations in CRF01_AE

Analysis of NRTI resistance mutations in HIV-1 CRF01_AE circulating in the Philippines has revealed several frequent mutations. Figure 1b presents the most frequent NRTI mutations that include M184V (n = 277, 26.48%), K65R (n = 214, 20.46%), S68G (n = 200, 19.12%), D67N (n = 87, 8.32), M1814I (n = 99, 9.46%), Y115F (n = 94, 8.99%). Figure 1c presents the most frequent NNRT mutations, including K103N (n = 167, 15.97%), Y181C (n = 199, 19.02%), G190A (n=143, 13.67%), K101E (n = 110, 10.52%), V106I (n = 110, 10.52%), and H221Y (n = 70, 6.69%). There was also an observable increase in this trend from 2014 to 2017 for both the NRTI and NNRTI mutations. On the other hand, due to the limited sequences of subtype B, the trend was not plotted, but the list of frequent mutations is listed in Table.

Table. Most frequent HIV DR mutations in HIV-1 Clade B from 2009-2017 (n = 377)

NRTI Mutations	Frequency (%)
M184V	96 (24.49)
K65R	45 (11.48)
S68G	26 (6.63)
K70R	24 (6.12)
T215F	23 (5.87)
A66V	22 (5.61)
L74I	21 (5.36)
D67N	19 (4.85)
Y115F	19 (4.85)
K219E	16 (4.08)
M41L	15 (3.83)
L210W	11 (2.81)
NNRTI Mutations	
V106I	114 (29.08)
K103N	64 (16.33)
Y181C	54 (13.78)
G190A	31 (7.91)
V108I	26 (6.63)
K101E	22 (5.61)
V179D	19 (4.85)
H221Y	18 (4.59)
L100I	18 (4.59)

DISCUSSION

Emerging evidence from recent studies[3,6,16-18] have highlighted a notable shift in the genetic diversity of HIV-1 strains circulating in the Philippines. These findings indicate a growing predominance of the CRF01_AE strain accompanied by a significant decline in the incidence of subtype B infections. Our retrospective genomic analysis aligns with and corroborates these important observations, providing further evidence for the evolving landscape of HIV-1 in the country. Our data confirmed that there are two major subtypes circulating in the Philippines, with CRF01_AE and subtype B being the dominant strains. Although our analysis did not provide detailed information on the relative proportions of these subtypes, it underscores the importance of continued genomic surveillance to track the trends and shifts in the HIV-1 epidemic. Moreover, changes in the HIV-1 genetic landscape have important implications for the ongoing fight against the epidemic in the Philippines, as the predominance of CRF01_AE has been associated with higher viral loads, faster immunological decline, and higher rates of transmitted drug resistance compared to other subtypes.

Genotyping is critical because different genetic variants can vary in their biological properties, evolutionary rates, disease progression, and resistance mutation profiles.[19] This study analyzed comprehensive data on common resistance mutation patterns from 2009 to 2017, revealing an increasing trend in mutations observed in NRTI and NNRTI resistance among the predominant CRF01_AE strains. However, this trend is inconsistent across Asia. The WHO's 2021 HIV Drug Resistance Report indicated that drug resistance to efavirenz or nevirapine in Southeast Asia was 5.3%, lower than that in the Americas (16.7%) and Africa (15.4%).[20] In the Philippines, the most common NRTI mutations are M184V, K65R, and S68G, which are observed in both CRF01_AE and HIV-1 clade B strains.

The M184V mutation, associated with high-level resistance to lamivudine (3TC) and emtricitabine (FTC), was found to be highly prevalent (0.5-20%) among treatment-naïve patients, potentially increasing the risk of virological failure in 3TC-containing regimens.[21] A study in the Philippines found a high prevalence of M184V minority variants (0.5-20%) in treatment-naïve patients, which could increase the risk of virological failure in 3TC-containing regimens.[2] The K65R mutation confers resistance to multiple NRTIs including tenofovir, abacavir, didanosine, and stavudine. A study in the Philippines reported unexpectedly high rates of K65R tenofovir resistance among patients who failed first-line ART.[16] This concern was also observed in other Asian countries, such as Kazakhstan, where high levels of drug resistance were found with differential DRM frequencies between HIV-1 subtypes.[22] The S68G accessory NRTI mutation can contribute to resistance when present with other NRTI mutations, such as M184V and K65R,[23] and its increasing prevalence among CRF01_AE and subtype B strains in the Philippines is concerning.

Recent data from Zhengzhou City, China, provides additional insights into NRTI resistance patterns. Among ART-experienced people living with HIV (PLWH) with low-level viremia (LLV), the most common NRTI mutations were M184V/I (18.27%), followed by M41 (7.69%) and K65R (7.69%).[24] The overall prevalence of NRTI resistance to NRTIs was 23.08% in this population. Notably, resistance to specific NRTI drugs varied, with 21.15% showing low- or high-level resistance to lamivudine (3TC), 15.38% to tenofovir (TDF), and 7.69% to zidovudine (AZT). Moreover, in Vietnam, a study of HIV-infected pregnant women reported genetic resistance to NRTIs in 8.20% (95% CI: 2.72-18.10%) of patients.[25] This lower prevalence, compared to other drug classes, suggests that NRTI resistance may be less common in certain populations or regions.

The variability in HIV-1 drug resistance patterns, particularly for NRTIs, across different Asian countries underscores the need for continued surveillance and tailored antiretroviral strategies in the region. This also highlights the importance of regular resistance testing to guide treatment decisions and prevent the spread of resistant strains.

The most common mutations observed in CRF01_AE were K103N, Y181C, and G190A, whereas the predominant NNRTI mutations in HIV-1 clade B were V106I, K103N, and Y181C. Studies have shown that the presence of K103N before starting the WHO first-line regimen of tenofovir/lamivudine/efavirenz is associated with an increased risk of virological failure.[26] The Y181C mutation has been linked to high-level resistance to nevirapine and efavirenz,[27] whereas the G190A mutation appears to increase virological failure with nevirapine and efavirenz.[28] Interestingly, the prevalence of NNRTI-resistance mutations varies geographically, with higher rates reported in regions with longer histories of NNRTI use.[25] Furthermore, recent studies have highlighted the potential of next-generation NNRTIs, such as doravirine, to maintain efficacy against viruses harboring some of these common resistance mutations, offering new treatment options for patients with NNRTI-resistant HIV-1.[29,30]

The predominance of CRF01_AE, which is associated with poor clinical outcomes and rapid disease progression, further complicates HIV-1 management in the Philippines. Comprehensive drug resistance surveillance and access to resistance testing are critical for guiding optimal first-line ART selection and for managing the growing HIV-1 epidemic in the country. The limitations of this study include the lack of clinical data, as the data were retrieved from the database. This limitation highlights the need for future studies that incorporate clinical data to better understand the impact of genetic diversity on treatment outcomes. Future studies should incorporate clinical data to better understand the effects of genetic diversity on the treatment outcomes. Comprehensive drug resistance surveillance and access to resistance testing are critical to guide optimal first-line ART selection and to manage the growing HIV-1 epidemic in the Philippines. The development of effective strategies to prevent and manage drug resistance is crucial for ensuring the long-term success of HIV-1 treatment programs in the Philippines.

CONCLUSION

The findings of this study emphasize the importance of comprehensive drug resistance

surveillance and access to resistance testing to inform optimal first-line ART selection and to mitigate the risks associated with the CRF01_AE strain. Future studies should incorporate clinical data to better understand the effect of genetic diversity on treatment outcomes. By developing effective strategies to prevent and manage drug resistance, we can ensure the long-term success of HIV treatment programs in the Philippines and ultimately improve the health and well-being of those affected by the disease.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.18.4.18

Additional file: Additional Table S1-S2.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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