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Ultra-Deep Sequencing Analysis on HIV Drug-Resistance-Associated Mutations Among HIV-Infected Individuals: First Report from the Philippines

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Abstract

A sharp increase in the number of people living with HIV has been documented in the Philippines. In response, the government has instituted antiretroviral therapy (ART) nationwide through HIV treatment hubs. However, no data presently exist on the status of ART drug-resistance-associated mutations (DRMs). In this study, we aim at analyzing DRM profiles in the Philippines and at providing comprehensive data on DRMs to guide treatment decisions and prevent viral failures. We conducted a cross-sectional study in 119 volunteers who tested positive for HIV from more than 8,000 participants screened for HIV across the nation through the 2013 Integrated HIV Behavioral and Serologic Surveillance (IHBSS) program. Amplicons were generated from plasma RNA by using primers designed to analyze diverse HIV-1 isolates targeting the reverse transcriptase region and sequenced on a 454 ultra-deep sequencing (UDS) platform to assess DRMs. DRMs were defined by using the Stanford HIV drug resistance database, and we found only 2 from 110 evaluable individuals with major HIV variants (>20% prevalence) that were highly resistant to the non-nucleoside reverse transcriptase inhibitor (NNRTI: efavirenz and nevirapine). However, a larger fraction of individuals harbored minority drug-resistant HIV variants (0.5%–20% prevalence) and they were highly resistant to NNRTI nevirapine (89/110), rilpivirine (5/110), and efavirenz (49/110). This study is the first report on the presence of HIV drug resistance in the Philippines and demonstrates the utility of UDS in assisting the detection of HIV minor variants. Monitoring for ART-DRMs will assist in improving HIV management strategies in curtailing the evolving epidemic in the Philippines.

Keywords: HIV, ARV drug resistance, ultra-deep sequencing, Philippines

Introduction

In the Philippines, after a period of low HIV national prevalence,[1,2] the number of people living with HIV (PLHIV) has grown exponentially, with a sharp increase observed since 2009. From January 1984 to January 2016, a total of 31,160 HIV-seropositive cases have been reported, with 804 new cases reported in the month of January 2016 only, representing a staggering 50% increase compared with the same period in the previous year.[1]

In response to the ongoing epidemic, the Government of the Philippines successfully initiated the Integrated HIV Behavioral and Serologic Surveillance (IHBSS) in 2005, conducted every 2 years, targeting populations at an elevated risk of HIV and other sexually transmitted infections (STI).[3] In addition to the national surveillance system, the government also provided WHO-recommended medications, enabling combination antiretroviral therapy (cART) to PLHIV through 40 treatment hubs and satellites nationwide. The country's HIV treatment program was initiated with the support from

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the Global Fund project in 2005, and as of January 2016, there were 12,335 PLHIV currently enrolled and accessing cART.[1] The cART regimen initiated is the most accepted first-line regimen worldwide, consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) (zidovudine [AZT] and lamivudine [3TC] as the main and tenofovir [TDF] and stavudine [d4T] as alternative drugs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) (nevirapine [NVP] as the main and efavirenz [EFV] as alternative drugs), with the two NRTIs and one protease inhibitor (lopinavir/ritonavir) as the second-line regimen.[4] The Therapeutics, Research, Education, and AIDS Training in Asia (TREAT Asia) study has documented the presence of multi-NRTI drug-resistance-associated mutations (DRMs) associated with first-line therapy failure in Asia[5]; however, data on DRMs focusing on PLHIV in the Philippines are not yet available.

Although cART has dramatically reduced HIV-related mortality and morbidity, the efficacy of cART can be compromised by the emergence of antiretroviral (ARV) drug-resistant HIV variants. Current genotypic-based assays to detect HIV variants resistant to ARV utilize the Sanger Sequencing platform, and although this platform has been validated and represents a major advancement in HIV management, its efficacy is limited to detecting HIV variants only if they comprise at least 20% of the total HIV population in an HIV-infected individual.[6,7] However, previous studies reported that the presence of ARV-resistant HIV variants, even if they comprise less than 20% of the total HIV population, can still potentially lead to treatment failure,[8–10] especially among NNRTI resistance-associated minority variants.[11–13] The introduction of ultra-deep sequencing (UDS) permits the analysis of minor HIV variants with a prevalence less than 20% of the total HIV population, and it provides a more thorough characterization of the viral population in HIV-infected individuals.

The aim of this study was to analyze drug resistance profiles among HIV-infected individuals in the Philippines by using UDS to provide comprehensive data on ARV-DRMs, including the existence of minor HIV variant populations that otherwise cannot be detected by standard genotypic sequencing. With this study, we hope to provide the government of the Philippines and healthcare providers with knowledge of ARV-DRMs, improve treatment strategies, and contribute to halting this unique evolving epidemic.

Materials and Methods

Study participants and biological samples

The study participants in this cross-sectional study were recruited in the 2013 national IHBSS conducted by the Epidemiology Bureau of the Philippine Department of Health. The IHBSS for males who have sex with males used Time Location Sampling; the IHBSS for injecting drug users (IDU) used Respondent-Driven Sampling.

More than 8,000 individuals participated in the 2013 IHBSS. After the informed consent process, a face-to-face survey performed by trained interviewers, and 5 ml of whole blood were collected by venipuncture and delivered to the National HIV Reference Laboratory, San Lazaro Hospital–STD/AIDS Cooperative Central Laboratory (SLH-SACCL) for HIV screening assessment by an immunoassay (IA) approach. Reactive samples were further tested with a secondary IA: If the secondary IA was positive, specimens were labeled as reactive, and if negative, a third IA was performed. To be considered reactive/HIV seropositive, a two out of three reactive IA was necessary.

A total of 586 HIV-seropositive samples were identified, and, subsequently, RNA isolation was performed by using a QiAmp Viral RNA Mini Kit (Qiagen, Germany). Isolated RNA samples were then stabilized by using a GenTegra-RNA matrix (GenTegra) before shipment to Hawaii, and they were reconstituted in RNAse-free water according to the manufacturer's protocol on arrival. Reconstituted RNA was then used as a polymerase chain reaction (PCR) template for amplicon generation. Out of 586, 119 RNA samples had a detectable HIV *pol* region PCR band and, subsequently, underwent UDS. From these, 110 samples with an evaluable number of reads were generated and included in this study for final analysis.

This study was approved by the Department of Health, Republic of the Philippines Research and Ethics Review Board and the University of Hawaii's Committee on Human Studies.

Amplicon generation

Reverse transcriptase-PCR (RT-PCR) was conducted in a one-step protocol to synthesize first-strand cDNA followed by a subsequent PCR in a single reaction by using a MyTaq™ One-Step RT-PCR Kit (Bioline). Fusion primers designed to match the 454 Roche sequencing system were used, and these primers contained gene-specific sequences targeting part of the HIV *pol* region (HXB2 2748 to 3216) appended with 454-template ''adapter'' sequences, as well as a short sequence tag (known as a multiple identifier [MID]) for the identification of each amplicon. The assay was carried out in a final volume of 25 μl containing 12.5 μl of 2x MyTaq One-Step Mix, 1 μl each of 10 μM forward and reverse primers, 0.25 μl of reverse transcriptase, 0.5 μl of RNAse inhibitor, 1.75 μl of DEPC-H_2O, and 8 μl of RNA template. The RT-PCR condition was set as follows: 20 min of reverse transcription at 48°C and polymerase activation at 95°C for 1 min, followed by 40 cycles of denaturation (10 s at 95°C), annealing (10 s at 58°C), and extension (30 s at 72°C), with a final extension for 7 min at 72°C. To determine whether a PCR reaction for any given sample was successful, 1.5 μl of the PCR reaction was run on a 48-lane E-Gel Agarose Gel Electrophoresis system (Life Technologies) to visually confirm the presence or absence of the HIV *pol* amplicon band. PCR products were purified by using Agencourt AMPure XP beads (Beckman Coulter) and quantified by using Quant-IT PicoGreen dsAssay kit (Life Technologies), followed by subsequent normalization and pooling as a preparation for emulsion-based clonal amplification PCR (emPCR).

emPCR and UDS

Three sequence runs were conducted by using a GS Junior 454 pyrosequencing (Roche 454 Life Science) system. Each run consisted of a different number of multiplexed samples to determine the number of samples that yielded the optimum read depths that covered the minority variants. emPCR was performed before sequencing to allow each strand of DNA produced in the HIV-1 region-specific PCR to be replicated clonally on a single bead by using emPCR Reagents and Kit (Roche 454 Life Science). The emPCR was performed first at

a ratio of 0.5 molecules per bead, and it was increased at the second and third runs to 0.55 and 0.6 molecules per bead to reach optimum DNA bead enrichment. emPCR protocol was carried out in a Gene Amp PCR System 9700 (Applied Biosystems) under the following conditions: 94°C for 4 min, followed by 51 cycles of denaturation (30 s at 94°C) and annealing/extension (10 s at 60°C) with final storage at 10°C. Beads were then recovered, and 500,000 enriched DNA beads were used to ensure the optimal picotiter plate loading. Amplicons were then individually sequenced with full amplicon processing protocol from both forward and reverse ends.

Data analysis

The sequencing reads were processed by using Integroomer (http://uh-bioinformatics.github.io/software/)[14]: Briefly, the reads were de-multiplexed by samples using the MID sequences and, subsequently, trimmed to discard adapters and low-quality sequences. The resulting sequences were then mapped by using custom scripts to the Stanford University HIV Drug Resistance database for the determination of subtypes and ARV-resistant-associated mutations. Mutation levels were categorized as susceptible (S), potential low (PL), low (L), intermediate (I), and high resistant (H); a read frequency between 0.5 and 20% from each sample was classified as a minor variant, and a read frequency >20% was classified as a major variant. The following ART compounds were considered for analysis that covered our gene-specific target primers: NRTI group: tenofovir (TDF), zidovudine (AZT), emtricitabine (FTC), stavudine (D4T), abacavir (ABC), lamivudine (3TC), and didanosine (DDI); from the NNRTI group, the following were considered: efavirenz (EFV), etavirine (ETR), nevirapine (NVP), and rilpivirine (RPV). All compounds are currently approved by the U.S. Food and Drug Administration (FDA).

Results

Study participants

The 110 evaluable HIV-seropositive participants were mostly men (94.5%) with a median age of 29.5 years (16 to 53 years); the majority were from the cities of Cebu ($n=70$; 64%) and Mandaue ($n=29$; 26%), with the rest ($n=11$; 10%) distributed in the cities of Quezon, Baguio, Caloocan, Manila, San Jose del Monte, and Angeles. The 2013 IHBSS focused on IDU, and on males having sex with males (MSM). The study participants' most common risk factor was IDU (91%), followed by MSM (9%). Out of 110 participants, only 1 (0.9%) were on ART treatment. From UDS reads, most samples were matched for subtype B (90%), followed by 5% of subtype E, and in 5 (4%) samples, we found two subtype alleles (B and CRF01_AE) (Table 1).

UDS amplicon analysis

We started with 119 samples sequenced in three separate GS Junior sequence runs: 25, 30, and 64 samples were included in runs 1, 2, and 3, respectively. Of the 119 samples, 7 samples were dropped during the first filtration process due to poor quality of the resultant reads, with an additional 2 samples excluded for having less than 50 reads generated from each sample with no minor variants detected from both samples. The remaining 110 samples generated at least 94 reads, with a median (interquartile range) number of reads of 979.5 (583–2,239) and a mean (95% confidence interval) sequence length of 333.6 (233.2–434.2) base pair (Table 2). In samples with the lowest number of reads (94), minor variants showing various degrees of resistance to ARV drugs were found in four out of seven NRTIs and in all NNRTIs.

Table 1. Subjects' Demographic Characteristic

Characteristics	
Age, years	29 (min 16, max 53)
Gender, *n* (%)	
Male	104 (94.5)
Female	6 (5.5)
Risk factor, *n* (%)	
IDU	100 (91)
MSM	10 (9)
Origin, *n* (%)	
Cebu	70 (64)
Mandaue	29 (26)
Baguio	3 (3)
Quezon	3 (3)
San Jose del Monte	2 (2)
Angeles	1 (1)
Caloocan	1 (1)
Manila	1 (1)
HIV subtype, *n* (%)	
B	99 (90)
CRF01_AE	6 (5)
B/01_AE	5 (4)

IDU, injecting drug users; MSM, males having sex with males.

ARV susceptibility

In all 110 participant samples evaluated, we found that the majority variants were susceptible to all NRTI drugs. In addition, the UDS detected 20 samples with minority variants that exhibited high resistance to FTC and 3TC (Table 3).

In the NNRTI drug group, the majority variants in two samples were highly resistant to NVP and EFV; whereas among the minority variants, several samples evaluated were highly resistant to NVP ($n=89$), RPV ($n=5$), and EFV ($n=49$) (Table 3).

NRTIs-resistance-associated mutations

Of the total reads derived from the 110 individuals, the most common NRTI-DRM found was F77L (0.44%). Despite a lower than 0.5% prevalence, we found other mutations associated with major NRTI resistance, such as Y115F (0.024%), M184V (0.075%), M184I (0.267), and L210W (0.169).

Table 2. GS Junior 454 Sequence Run Profile

Sequence run	*No. of samples*	*Mean sequence length (bp)*	*Mean no. of filtered reads from each patient*
Run 1	25	365	2,276
Run 2	30	348	2,231
Run 3	64	288	715

Table 3. Prevalence Distribution of Patients' Susceptibility Level Toward Nucleoside Reverse Transcriptase Inhibitor and Non-Nucleoside Reverse Transcriptase Inhibitor

Susceptibility level	*NRTI*	*Prevalence*				*NNRTI*	*Prevalence*			
		<0.5%	*0.5–20%*	*>20%*	*Total*		*<0.5%*	*0.5–20%*	*>20%*	*Total*
H	TDF	0	0	0	0	NVP	17	89	2	108
I		0	0	0	0		5	102	1	108
L		26	0	0	26		38	0	2	40
PL		48	1	1	50		50	50	0	100
S		0	0	110	110		1	2	107	110
H	AZT	0	0	0	0	ETR	1	0	0	1
I		32	1	0	33		73	14	1	88
L		23	0	1	24		82	11	1	94
PL		60	40	0	100		3	105	1	109
S		0	0	110	110		1	1	108	110
H	FTC	76	20	0	96	RPV	22	5	0	27
I		0	0	0	0		53	50	2	105
L		0	0	0	0		4	104	1	109
PL		34	1	0	35		30	1	0	31
S		0	0	110	110		1	1	108	110
H	D4T	0	0	0	0	EFV	55	49	2	106
I		32	1	0	33		3	104	0	107
L		23	0	1	24		78	12	1	91
PL		60	40	0	100		43	57	1	101
S		0	0	110	110		1	3	106	110
H	ABC	0	0	0	0					
I		46	1	0	47					
L		75	21	0	96					
PL		56	44	1	101					
S		0	0	110	110					
H	3TC	76	20	0	96					
I		0	0	0	0					
L		0	0	0	0					
PL		34	1	0	35					
S		0	0	110	110					
H	DDI	0	0	0	0					
I		32	1	0	33					
L		5	0	0	5					
PL		21	86	1	108					
S		0	0	110	110					

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; D4T, stavudine; DDI, didanosine; EFV, efavirenz; ETR, etavirine; FTC, emtricitabine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir.

Susceptibility level: H, high resistant; I, intermediate resistant; L, low resistant; PL, potential low resistant; S, susceptible.

Figure 1A describes the proportion of wild type and mutated amino acids in each position on the RT gene.

Although we did not find any variants with NRTI-DRMs in greater than 20% prevalence, we detected minor variants, including M184V, L210W, and Q151L, in 8 (7.3%), 1 (0.9%), and 1 (0.9%) individuals, respectively (Table 4).

NNRTIs-resistance-associated mutations

Figure 1B describes the proportion of mutated amino acids associated with NNRTI-DRMs. The most common mutation identified was V106I (92.48%), and the other mutations found were L100F (0.843%), L100V (2.031%), K103E (6.416%), Y188C (0.7%), and V108I (0.618%). Although they comprise less than 0.5% of the HIV variant population, we also found NNRTI-DRMs, listed in Table 4.

Among the NNRTI-resistance-associated mutations observed, we detected three mutations, G190E, K101E, and Y188C, with a prevalence higher than 20%, each in 1 (0.9%) subject. We also detected minor variants E138G, K101E, K103N, L100I, V106M, V179E, Y181C, Y188C, and Y188H in 36 (32.7%), 11 (10%), 2 (1.8%), 4 (3.2%), 3 (2.7%), 1 (0.9%), 4 (3.2%, 4 (3.2%), and 5 (4.5%) subjects, respectively (Table 4).

Discussion

The results presented here are the first to comprehensively study HIV drug resistance profiles among HIV-infected individuals in the Philippines. The HIV epidemic in the Philippines occurred relatively recently compared with its neighbors, and it spread rapidly, especially among IDU.[2,15] Low and stable HIV prevalence rates (0%–0.52%) were reported between 2002 and 2007; however, more recent survey data from the Philippine National HIV surveillance suggest that the HIV epidemic exhibited exponential growth in two

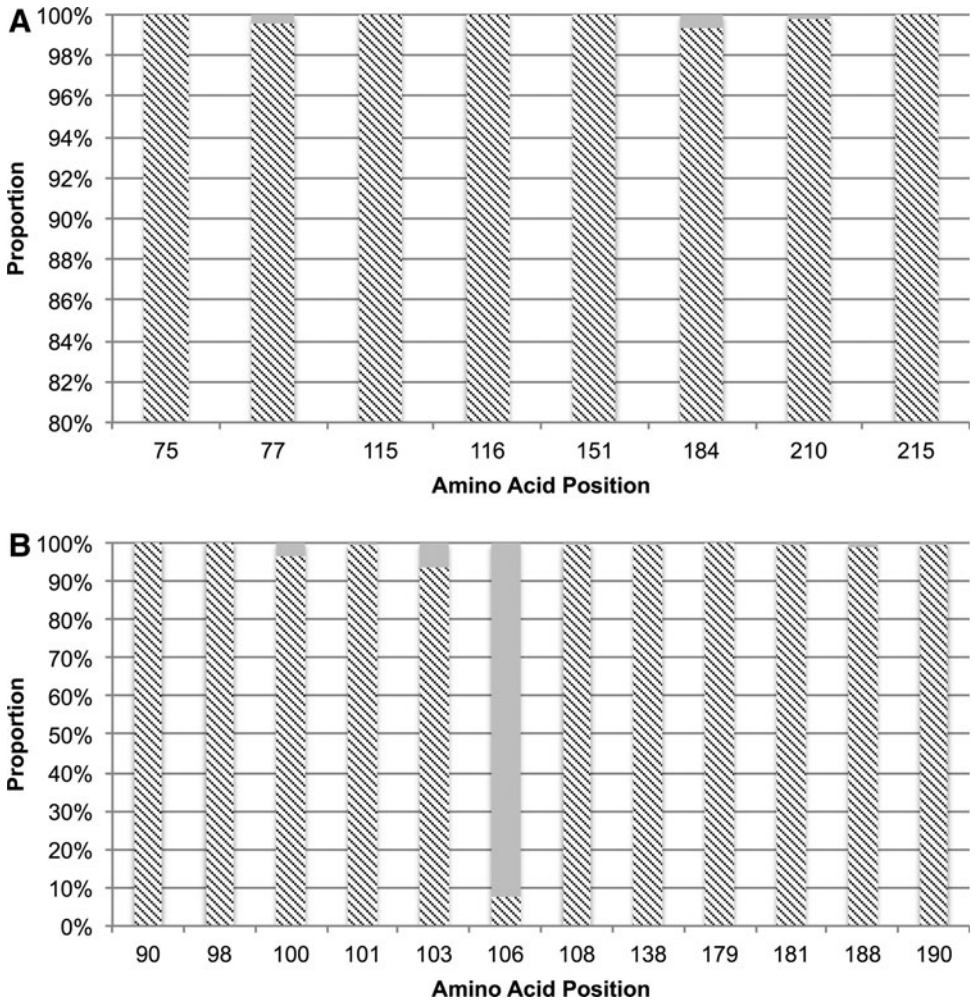

FIG. 1. The proportion of wild type and mutated amino acids in each **(A)** NRTI- and **(B)** NNRTI-resistance-related position found in 110 patients. The wild type amino acid is represented by *dashed lines*, whereas the mutated amino acid is indicated by *solid gray*. The NRTI-resistance-associated mutations were compared with the mutation interest list from the Stanford University HIV Drug Resistance database. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

TABLE 4. PREVALENCE OF MAJOR NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR AND NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-RELATED AMINO ACID MUTATIONS AMONG 110 SAMPLES

NRTI-related AA mutations	*Prevalence*			*Total*	*NNRTI-related AA mutations*	*Prevalence*			*Total*
	<0.5%	*0.5–20%*	*>20%*			*<0.5%*	*0.5%–20%*	*>20%*	
Y115F	25	0	0	25	E138G	64	36	0	100
M184V	78	8	0	86	E138K	51	0	0	51
M184I	55	0	0	55	E138Q	4	0	0	4
L210W	19	1	0	20	G190A	1	0	0	1
T215G	1	0	0	1	G190E	59	0	1	60
Q151L	32	1	0	33	G190K	1	0	0	1
					G190S	1	0	0	1
					K101E	76	11	1	88
					K103N	42	2	0	44
					K103S	1	0	0	1
					L100I	18	4	0	22
					V106A	8	0	0	8
					V106M	78	3	0	81
					V179D	20	0	0	20
					V179E	0	1	0	1
					V179F	3	0	0	3
					Y181C	74	4	0	78
					Y188C	74	4	1	79
					Y188H	77	5	0	82

metropolitan areas of Manila and Cebu, inflecting sharply upward trends since 2009.[2,3,15]

In 2005, the IHBSS began involving three key affected populations: IDU, MSM, and female sex workers (FSWs), at an elevated risk of HIV and STI. The IHBSS was mandated by Republic Act 8504 (AIDS Law), was initiated by the Philippines Department of Health through the Epidemiology Bureau, and has been conducted every 2 years to measure the magnitude of the rapidly evolving HIV epidemic in the Philippines and to guide prevention and treatment strategies based on epidemic structure and behavioral factors. The 2013 IHBSS had more than 6,000 participants, which included IDU, MSM, and FSW populations.[3]

Our study consisted mostly of male participants with IDU identified as the most common risk factor. In the 2009 and 2010 IHBSS, Metro Cebu was reported to have a rapid spread of HIV, among IDU,[15] and there is a continuing high-level HIV prevalence among IDU in Cebu Province (adjusted HIV prevalence was 51.5% for men and 35% for women among IDU in Cebu City) and expanding epidemics among MSM (11% prevalence in Quezon City, 10.5% in Cebu City, and 6.3% in Manila) reported from 2013 IHBSS.[3] In our current study, most HIV-positive samples that were confirmed by PCR and subsequently with UDS came from individuals recruited in Cebu, followed by Mandaue, a city located next to Cebu city. These data indicate that HIV transmission among IDU is still occurring in the Cebu region and highlight a critical need for targeted interventions.

By using the 454 pyrosequencing platform, we were able to detect minority HIV-1 mutations at frequencies down to 0.1% at some nucleotide positions.[16] We selected >0.5% as the threshold for detecting minority variant populations and >20% for detecting majority variant populations based on previous reports, suggesting that sequence variants obtained by 454 at frequencies below 0.5% may result from 454 sequencing error in HIV-1 genomes.[17–19] In the evaluation of multiple samples using combinatorial MIDs per sequence run, we were able to successfully detect HIV minority variants in multiple amino acid positions for both NRTI- and NNRTI-resistance-associated mutations, including the run with 64 samples, which produced the least number of reads per sample. The presence of ARV-resistant minor variants has been associated with reduced treatment efficacy.[7,8] Previous studies suggest that low-abundance NNRTI-resistant variants in chronically HIV-infected treatment-naïve patients were associated with a higher risk of viral failure.[9,11,20] Moreover, the pre-existing minority variants could increase the risk of viral failure in treatment-naïve patients receiving first-line NNRTI-based ART.[21,22] In view of these results, we conclude that in our study, we detected HIV minority variants in our subjects who might require continuous monitoring as it might potentially increase their risk of viral failure in the future.

TREAT Asia, a study evaluating HIV drug resistance collectively from 12 clinical sites in Thailand, Hong Kong, Indonesia, Malaysia, and Philippines, reported a high prevalence of multi-NRTI DRMs among patients failing first-line ART.[5] Although this study collectively reported HIV drug resistance in Southeast Asia, data on drug resistance focusing on PLHIV in the Philippines were not available. The government program providing first-line ART regimen to PLHIV and the presence of multi-NRTI DRMs in Southeast Asia rendered the current study essential for HIV management in the Philippines. In addition, in a limited-resources setting, a sensitive baseline drug resistance database might be important to help guide therapies for treatment-naïve patients where there is no treatment history, including avoiding ARV, that are likely to cause viral failure in patients with certain ARV-resistant minor variants.

In our current study, we found 20 subjects with HIV minority variants (0.5%–20% prevalence) exhibiting high resistance to FTC and 3TC. The high prevalence of minority variants toward 3TC might be related to Hepatitis B, which is highly endemic in the Philippines.[23] In the NNRTI drug group, we found two subjects with a more than 20% variant that was highly resistant to NVP and EFV (Table 3). From the minority variant data of the NNRTI drug group, we found that 89 subjects were highly resistant to NVP, 49 to EFV, and 5 to RPV. The high prevalence of ARV-DRM minority variants, combined with the fact that there was only one participant with a history of ART treatment, suggests the presence of circulating mutated strains among treatment-naïve HIV patients in the Philippines and the potential of these patients developing viral failure toward first-line NNRTI-based ART.

Among 110 samples, NNRTI-related G190E, K101E, and Y188C were the only majority variants identified. These DRMs were reported as nonpolymorphic mutations selected by NVP and EFV, and G190E was also related to a highly reduced susceptibility to RPV and ETR.[24] However, we found minority variants harboring E138G, K101E, K103N, L100I, V106M, V179E, Y181C, and Y188H in NNRTI-related mutations, and also M184V, L210W, and Q151L in NRTI-related mutations. In NNRTI-related mutations, the identified DRMs were reported to be associated with the contribution to reduce phenotypic susceptibility to one or more NNRTIs, and although these DRMs were identified as minority variants, they may reduce response to treatment when paired with other mutations.[25,26] Among NRTI-related DRMs, although there were no major variants identified, we found minority variant M184V, and considering that 3TC is widely used in the Philippines, it is important to monitor the development of M184V prevalence among HIV-infected individuals in the Philippines. It is also worth noting the presence of Q151L in this study. Although it did not appear to reduce NRTI susceptibility, Q151L was reported as an extremely rare nonpolymorphic NRTI-selected mutation representing a transition between wild type and Q151M.[27]

Our study focused exclusively on RT-associated DRMs, as most first-line ARVs available in the Philippines are from NRTI and NNRTI groups. Limitations to our study include having a large number of HIV-seropositive plasma RNA samples that could not be amplified successfully. Several factors might have contributed to the low amplification rate: First, the 454 pyrosquencing platform has a higher sequencing error rate associated with A- and T-rich homopolymers compared with other platforms such as Ilumina sequencing.[28] Second, the samples were obtained during a surveillance program from relatively healthy individuals with possible low plasma viral loads. The low amplification yield can also be due to the blood sampling process where the samples were obtained at the surveillance site where RNAse contamination and stable temperature was difficult to control, resulting in poor RNA quality due to RNAse degradation and rapid temperature changes. In addition, as with all self-reported studies, some of the subjects with DRMs may have

had previous exposure to ART and may not have disclosed this during the interview.

The IHBSS program continues to be a unique surveillance system with great potential to conduct continuous surveillance of the ongoing epidemic. Even with the low RNA amplification rate, we were still able to detect HIV minor variants that were highly resistant to ARV, and based on our results the integration of UDS utilization might help optimize drug resistance screening and reduce the overall management costs in the long run.

In summary, from our data, it is evident that the higher sensitivity of UDS will enable a better characterization of ARV-resistant-associated mutations. The high prevalence of ARV-DRM minority variants among patients not exposed to ART strengthens the need for a combination of a nationwide surveillance program and a low-cost, high-sensitivity method to detect and monitor the presence of ARV DRMs, which will further improve existing HIV management strategies in the evolving epidemic in the Philippines.

Sequence Data

GenBank Sequence Reads Archive (SRA) accession number of the bulk sequence dataset used in this article is SRP075904, and the accession numbers of the nucleotide sequences representing the bulk sequence of each patient are: KX247148, KX247257.

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Authors' Contributions

Conceived and designed the experiments: I.N.S.B. and L.C.N. Performed the experiments: I.N.S.B. Analyzed the data: I.N.S.B., L.C.N., M.B., and D.S. Provided samples and data: G.S., N.S., E.T., and S.L. Contributed reagents/material/analysis tools: I.N.S.B., L.C.N., C.M.S, H.C.-Y, and T.H. Wrote the article: I.N.S.B., L.C.N., C.S., N.S., G.S., and M.B.

Author Disclosure Statement

No competing financial interests exist.

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