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Original Article

Prevalence of major INSTI and HIV-1 drug resistance mutations in pre- and antiretroviral-treated patients in Indonesia

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Abstract

Indonesia has one of the highest HIV infection rates in Southeast Asia. The use of dolutegravir, an integrase strand transfer inhibitor (INSTI), as a first-line treatment underscores the need for detailed data on INSTI drug resistance mutations (DRMs). Currently, there is a lack of comprehensive data on DRMs INSTI and other HIV drug resistance in Indonesian patients, both pre- and post-treatment. The aim of this study was to identify the subtypes and drug resistance mutations of the protease, reverse transcriptase, and integrase genes in both treatment-naive and ARV-treated patients in Bandung, West Java, Indonesia. A cross-sectional study was conducted involving HIV-positive patients at Hasan Sadikin Hospital, Bandung, Indonesia, from September 2022 to January 2023. The patients were categorized into two groups: ARV-treated and pre-treatment patients. Peripheral blood mononuclear cells (PBMCs) were processed for DNA extraction, followed by amplification and sequencing of the pol gene to detect mutations and subtypes. The study found that the predominant subtype was CRF01_AE, accounting for 85.4% and 69% of pre-treatment and treated patients, respectively, followed by recombinant forms such as A1/CRF01_AE, CRF01_AE/CRF02_AG, subtype B, and other subtypes. Among ARV-treated/INSTI-naive patients, major INSTI DRMs R263K and Y143H were identified, while pre-treatment patients exhibited accessory integrase DRMs. The most common DRMs detected were non-nucleoside reverse transcriptase inhibitor (NNRTI) DRMs, with prevalences of 14.6% and 7% in pre-treatment and ARV-treated patients, respectively. In conclusion, CRF01_AE emerged as the predominant subtype in both pre-treatment and ARV-treated patients in Bandung, underscoring the necessity for ongoing surveillance of integrase DRMs, particularly given the presence of major INSTI DRMs in patients undergoing INSTI treatment.

Keywords: HIV-1, drug resistance, mutation, subtype, INSTI

Introduction

Human immunodeficiency virus (HIV) is the cause of acquired immune deficiency syndrome (AIDS), which remains a global concern [1]. The global HIV-infected population is projected to

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reach 39 million by the end of 2022, although this represents a significant 59% decrease from its peak in 1995 [1]. In Southeast Asia, Indonesia has one of the highest HIV infection rates [2,3], with Bandung in West Java Province being the third-most affected area in the country [4].

HIV-1 exhibits significant genetic diversity due to the low proofreading activity of reverse transcriptase, high replication mutation rates, and its capability for recombination, resulting in the formation of quasispecies within HIV-positive individuals [5,6]. Drug-resistant mutant viruses can enhance replication and become dominant variants selected under drug pressure [7]. These variants not only pose transmission risks but also can lead to treatment failure with antiretroviral therapy (ARV) due to drug resistance [5-8]. HIV drug resistance (HIVDR) is widespread globally, especially in regions or countries with more than four years of ARV use [10]. Most countries initiate treatment with non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine or efavirenz, with pre-treatment drug resistance (PDR) is notably higher among patients with prior ARV exposure (24%) compared to treatment-naive patients (7%) [11,12].

To the best of our knowledge, comprehensive information on HIV drug resistance among both experienced and treatment-naive HIV patients in Indonesia is limited. The monitoring system for HIV drug resistance is not widely accessible in the country, and most data beyond what is provided by the World Health Organization (WHO) primarily come from other studies [13-15]. As of 2021, Indonesia shows relatively low to moderate PDR prevalence, with resistance to NNRTIs, such as efavirenz and nevirapine being the most common at 6.5% [11,14]. Resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs remains low in Jakarta and Kepulauan Riau [15,16,17]. However, significant NNRTIs resistance rates exceeding 10% have been reported in North Sulawesi, Maumere, and West Papua [14,18-20].

WHO recommendation advocates for dolutegravir combined with two NRTIs as the initial HIV treatment regimen, highlighting the need to understand HIV drug resistance mutations in the integrase gene [23]. Indonesia began implementing dolutegravir as the primary regimen in 2022 [24]. Although acquired drug resistance to dolutegravir is generally limited due to its high genetic barrier, the study conducted between 2021 and 2022 outside Southeast Asia has reported resistance rates ranging from 3.9% to 19% [25]. Limited studies have been conducted on drug resistance mutations (DRMs) against integrase strand transfer inhibitors (INSTIs), including in Indonesia, underscoring the need for further investigation. The only study on INSTI was conducted in Surabaya, Indonesia, in 2012–2013 on treatment-naïve patients and found minor/secondary resistant integrase mutation [26]. The aim of this study was to identify HIV-1 subtypes and identify DRMs in the *pol* gene that encodes protease, reverse transcriptase, and integrase among ARV-experienced and treatment-naive or previously ARV-exposed patients in Bandung, West Java, Indonesia.

Methods

Study design and setting

A cross-sectional study was conducted involving HIV-positive individuals at HIV Outpatient Clinic, Hasan Sadikin Hospital, Bandung, West Java, Indonesia, from September 2022 to January 2023. The patients were categorized into two groups: ARV-treated and pre-treatment patients. The blood samples were collected from each patient within the two groups and had HIV drug resistance test and HIV-1 subtyping.

Sample and criteria

All HIV-positive patients who have met the inclusion were included as samples. The inclusion criteria for ARV-treated patients were people with HIV who had undergone ARV therapy for more than six months and had viral load data in the last eight months. The inclusion criteria for pre-treatment patients were HIV-positive individuals with naïve treatment who initiated ARV therapy or lost to follow-up patients more than three months who re-initiated ARV treatment [27]. The exclusion criteria were HIV patients with co-infection with tuberculosis who were undergoing oral tuberculosis treatment. Prior to conducting any study procedures, signed informed consent was obtained from all patients. The patients and blood samples were collected from the patients

based on a consecutive sampling strategy, in which patients who met inclusion criteria were selected in order of hospital visits.

Blood sample collection and processing

Each patient underwent intravenous blood sample collection, with a total of 8 mL whole blood collected in vacutainer tubes containing EDTA and in less than four hours, the whole blood was processed to peripheral blood mononuclear cells (PBMC). Blood plasma was separated by centrifugation at 2000 rpm for 10 minutes and stored at -80°C. PBMCs were isolated using Ficoll Paque (Sigma-Aldrich, Darmstadt, Germany) and stored at -30°C until it was analyzed. The DNA isolation procedure from the PBMC samples was performed using the QIAmp Viral DNA Blood Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

HIV-1 DNA amplification and sequencing

HIV-1 *pol* gene, encoding protease, reverse transcriptase, and integrase, was amplified using GoTaq Green Master Mix (Promega, Madison, USA) and a nested PCR method with primers based on the HIV-1 reference strain HXB2 (GenBank accession number K03455) [20]. For protease gene amplification, the set *protease I* primers DRPR05 and DRPR02L were used for the initial amplification, while DRPR01M and DRPR06 were used for the nested PCR [19]. When the fragment failed to be amplified, the set protease II primers, outer 5prot1AS, and outer 3prot1S, were used in the first amplification of the protease gene, and the primers inner 5prot2AS and inner 3prot2S were used for nested PCR [28]. The set reverse transcriptase primer, RT18 and RT21, were used in the first amplification of the reverse transcriptase gene, and the primers, RT7L and DRRT6L, were used for nested PCR [16,23]. The set integrase primer, INPS1 and INPR8 were used in the first amplification, and the primers INPS3 and INPR9 were used for nested PCR of integrase gene [28]. The set primers used in the study are listed in **Table 1**.

Table 1. Set primers used to amplify and sequence the protease, reverse transcriptase, and integrase genes of HIV-1

Primers	Amplicon	Set primers and sequence 5' - 3'
Protease I	465 base pair (nucleotide 2148–2612)	DRPR05 = F-AGACAGGYTAATTTTTTAGGGA DRPR02L = R-ATGGATTTTCAGGCCCAATTTTTGA DRPR01M = F-AGAGCCAACAGCCCCACCAG DRPR06 = R-ACTTTTGGGCCATCCATTCC
Protease II	515 base pair (nucleotide 2136–2650)	Outer 3prot1AS = F-GCAAATACTGGAGTATTGTATGGATTTCAGG Outer 5prot1S = R-TAATTTTTTAGGGAAGATCTGGCCTTCC Inner 3prot2AS = F-AATGCTTTTATTTTTTCTTCTGTCAATGGC Inner 5prot2S= R-TCAGAGCAGACCAGAGCCAACAGCCCCA
Reverse transcriptase	888 base pair (nucleotide 2485–3372)	RT18 = F-ATGATAGGGGGAATTGGAGGT TT RT 21 = R-CTGTATTTCTGCTATTAAGTCTTTTGATGGG RT7L = F-GACCTACACCTGTCAACATAATTGG DRRT6L = R-TAATCCCTGGGTAAATCTGACTTGC
Integrase	723 base pair (nucleotide 4371–5093)	INPS1 = F-AG TAG CCA GCT GTG ATA AAT GTC INPR8 = R-TTC CAT GTT CTA ATC CTC ATC CTG INPS3 = F-GAA GCC ATG CAT GGA CAA G INPR9 = R-ATC CTC ATC CTG TCT ACT TGC C

F: forward; R: reverse

The PCR products were separated using 1% agarose gel electrophoresis with GelRed Nucleic Acid Stain dye (Biotium, Fremont, CA, USA) and visualized using GelDoc (Bio-Rad, CA, USA). The sequencing of DNA templates was performed using AmpliTaq Gold 360 Master Mix (Applied Biosystem, Foster City, CA, USA) with ExoSAP-IT Express PCR Product Clean Up (ThermoFisher, Santa Clara, CA, USA). Purified amplification products were sequenced using BigDye X Terminator (ThermoFisher, Vilnius, Lithuania) and analyzed via capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were analyzed using SnapGene 7.0.1 software (GSL Biotech LLC, Boston, MA, USA) and transformed into FASTA format for further processing.

Analysis for drug resistance mutations (DRMs) and HIV-1 subtyping

DRMs were classified using the Stanford HIV Database (Stanford HIVdb) version 9.5.1 (https://hivdb.stanford.edu/hivdb/by-sequences/) [11]. DRMs classification includes major and accessory/minor mutations for the protease gene, NRTI and NNRTI mutations for the reverse transcriptase gene, and major and accessory/minor mutations for the integrase gene. The major mutation is DRM, which contributes significantly to a reduced sensitivity to one or more ARVs, whereas a minor or accessory mutation is DRM, which contributes to a decreased susceptibility to ARV when combined with a major DRM. Non-DRMs or other mutations refer to changes that do not impact drug resistance in these genes. HIV subtypes for protease, reverse transcriptase, and integrase genes were determined using the Stanford HIVdb subtyping tool [11,25]. In cases of subtype or circulating recombinant form (CRF) discrepancies among the three genes, the viral strain was considered recombinant [17,18,26,27].

The phylogenetic analysis included samples from various regions in Indonesia and other countries as reference sequences from the Stanford HIVdb (https://hivdb.stanford.edu/page/hiv-subtyper/), the HIV Sequence Database (https://www.hiv.lanl.gov/components/sequence/HIV/search/search.html), and GenBank. Phylogenetic trees were constructed using MEGA version 11 (Tamura K, Stecher G, and Kumar S 2021) [32], with neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×.

Study variables

Patients' characteristics including age, sex (male, female), marital status (unmarried, married, and divorced), occupation (civil servant, private employee, and unemployed), and risk of transmission (men who have sex with men, injection drug user, heterosexual, bisexual, and others) were independent variables in this study. Other variables included duration of ARV treatment, viral load (undetected <40 copies/mL or ≥40 copies/mL), ARV regimens (combination of NRTI+NNRTI, NRTI+PI, or NRTI+INSTI) of ARV-treated patients, pre-treatment status (naive treatment or lost to follow-up), and the duration of lost to follow-up were also collected. Viral load data was collected from the last viral load results (the viral load was measured in less than eight months) on ARV-treated patients. In addition, subtypes, a list of ARV regimens of ARV-treated and previous ARV regimens of lost to follow-up patients were also collected. The major DRMs and list of ARVs with drug resistance were assessed. The dependent variables were ARV-treated and pre-treatment patients.

Statistical analysis

Continuous data were presented as mean and standard deviation (for normally distributed data) and median (minimum-maximum) for non-normally distributed data. Categorical data were presented as frequency and percentages. The Shapiro-Wilk test was utilized to assess data normality. The Pearson Chi-squared test or Fisher's exact test and Student t-test were used to analyze associations between independent and dependent variables as appropriated. SPSS version 28.0 software (IBM SPSS, Chicago, Illinois, USA) was employed for data analysis, with $p \leq 0.05$ was considered statistical significance.

Results

Characteristics of the patients

Out of 150 patients, only 112 (71 ARV-treated and 41 pre-treatment patients) were included in this study due to the successful sequencing of the protease, reverse transcriptase, and integrase genes of ARV-treated patients. Among the ARV-treated patients, the majority were male (80.3%), with a mean age of 37.8±8.23 years. A total of 46.5% were single and 67.6% worked in the private sector (**Table 1**). The average duration of ARV medication was 6 years (range: 1 to 19 years). Approximately 95% of these patients had undetectable viral load RNA in the last 8 months, while the remaining had RNA levels above 40 copies/mL. NNRTI-based regimens were used by 90.1% of patients, 8.5% used INSTI-based regimens, and PI-based regimens in 1.4% of patients (**Table 1**).

In pre-treatment patients, 75.6% were ARV-naive and 24.4% had re-initiated ARV after being lost to follow-up (**Table 1**). The median age was 35.26±9.57 years; 43.9% were married and 58.5% were employed in the private sector. Among those who re-initiated ARV after lost to follow-up, the median duration of discontinuation was eight months (range: 4–97 months), with 60% receiving NNRTI-based treatment and the remainder receiving INSTI-based treatment (**Table 1**).

Table 2. Comparison of patients' characteristics between antiretroviral (ARV)-treated and pre-treatment

Characteristics	ARV-treated (n=71)	Pre-treatment (n=41)	*p*-value
	n (%)	n (%)	
Age (years), mean±SD	37.8±8.23	35.26±9.57	0.142 [a]
Sex			
Male	57 (80.3)	33 (80.5)	0.979 [b]
Female	14 (19.7)	8 (19.5)	
Marital status			
Unmarried	33 (46.5)	16 (39.0)	0.737 [b]
Married	28 (39.4)	18 (43.9)	
Divorced	10 (14.1)	7 (17.1)	
Occupation			
Civil servant	7 (9.9)	1 (2.4)	0.090 [c]
Private employee	48 (67.6)	24 (58.5)	
Unemployed	16 (22.5)	16 (39.0)	
Risk of transmission			
Men who have sex with men	17 (23.9)	11 (26.8)	0.023 [c]
Injection drug user	17 (23.9)	3 (7.3)	
Heterosexual	17 (23.9)	6 (14.6)	
Bisexual	2 (2.8)	0 (0)	
Others	18 (25.4)	21 (51.2)	
Duration of ARV treatment (years), median (min-max)	6 (1–19)	NA	NA
Viral load (copies/mL)			
Undetected (<40 copies/mL)	68 (95.8)	NA	NA
≥40 copies/mL	3 (4.2)	NA	
ARV regimen			
NRTI+NNRTI	64 (90.1)	NA	NA
NRTI+PI	1 (1.4)	NA	
NRTI+INSTI	6 (8.5)	NA	
Pre-treatment status			
Naive treatment	NA	31 (75.6)	NA
Lost to follow-up	NA	10 (24.4)	
Duration of loss to follow-up (months), median (min-max)	NA	8 (4–97)	NA

ARV: antiretroviral; INSTI: integrase strand transfer inhibitor; NA: not applicable; NNRT: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor
[a] Analyzed using Student t-test
[b] Analyzed using Chi-squared test
[c] Analyzed using Fisher's exact test

HIV subtyping

All samples from both the pre-treatment and ARV-treated groups that were successfully sequenced underwent HIV-1 subtype analysis using Stanford HIVdb subtyping tools. The subtyping results from Stanford HIVdb were consistent with those obtained from phylogenetic analysis (**Figures 1–6**). In the pre-treatment group, CRF01_AE was the most prevalent subtype (85.4%), followed by recombinants between A1 and CRF01_AE (4.9%), subtype B (2.4%), CRF01_AE and B (2.4%), CRF01_AE and CRF02_AG (2.4%), and CRF53_02B and CRF01_AE (2.4%). Similarly, the ARV-treated group predominantly had CRF01_AE subtype (69%), followed by recombinants between CRF01_AE and CRF02_AG (22.5%), CRF01_AE and A1 (4.2%), CRF02_AE and B (2.8%), and recombinants between F2, CRF01_AE, and CRF02_AG (1.4%). The data indicated that CRF01_AE remains the predominant subtype in Indonesia; however, several recombinants between subtypes/CRFs have also emerged.

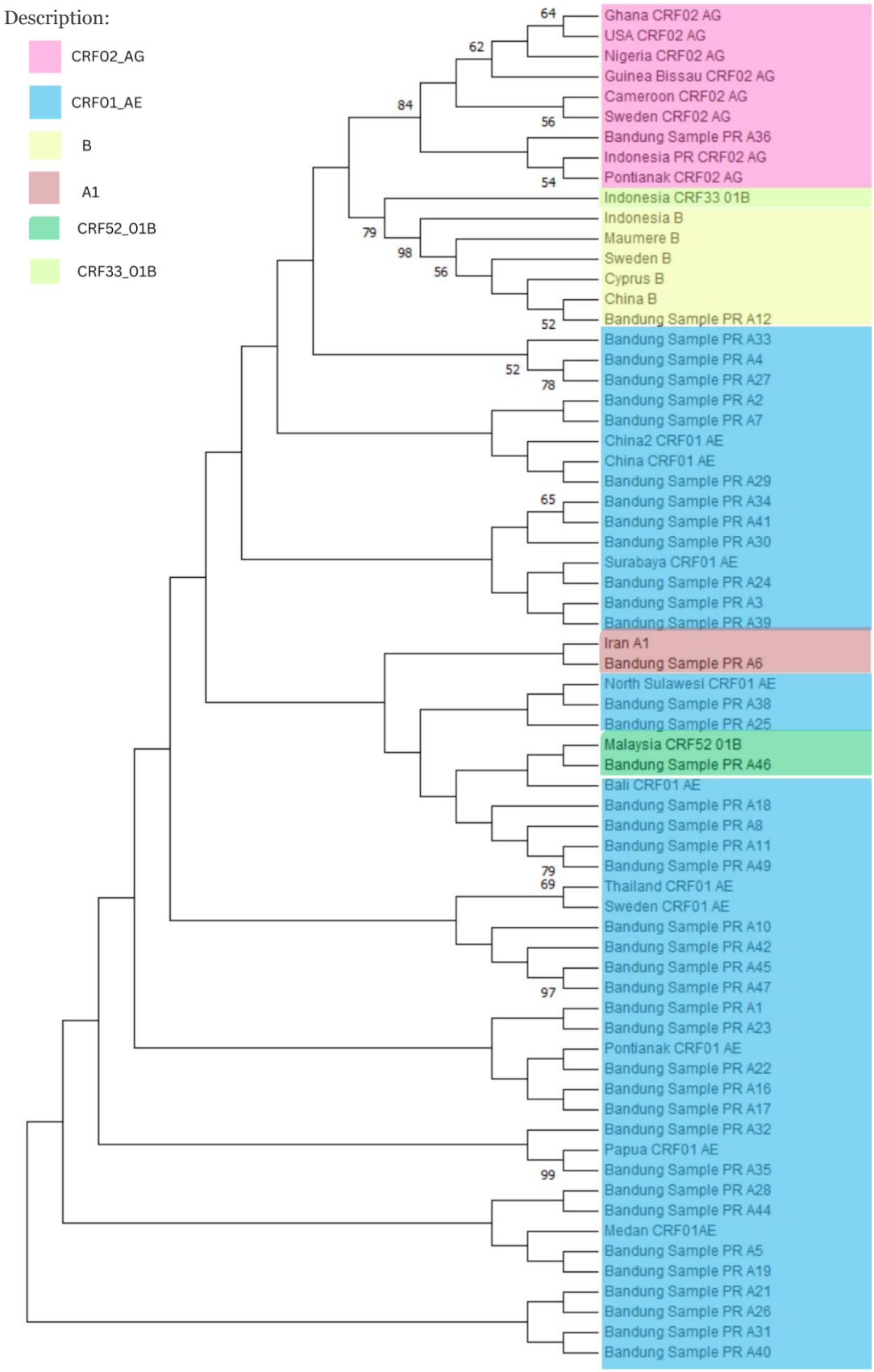

Figure 1. Phylogenetic analysis of HIV-1 protease gene in pre-treatment HIV generated from the study (n=41) together with corresponding viral gene of reference HIV-1 strains (n=26). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample PR A(number).

Figure 2. Phylogenetic analysis of HIV-1 protease gene in ARV-treated HIV patients generated from the study (n=71) together with corresponding viral gene of reference HIV-1 strains (n=24). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample PR B(number).

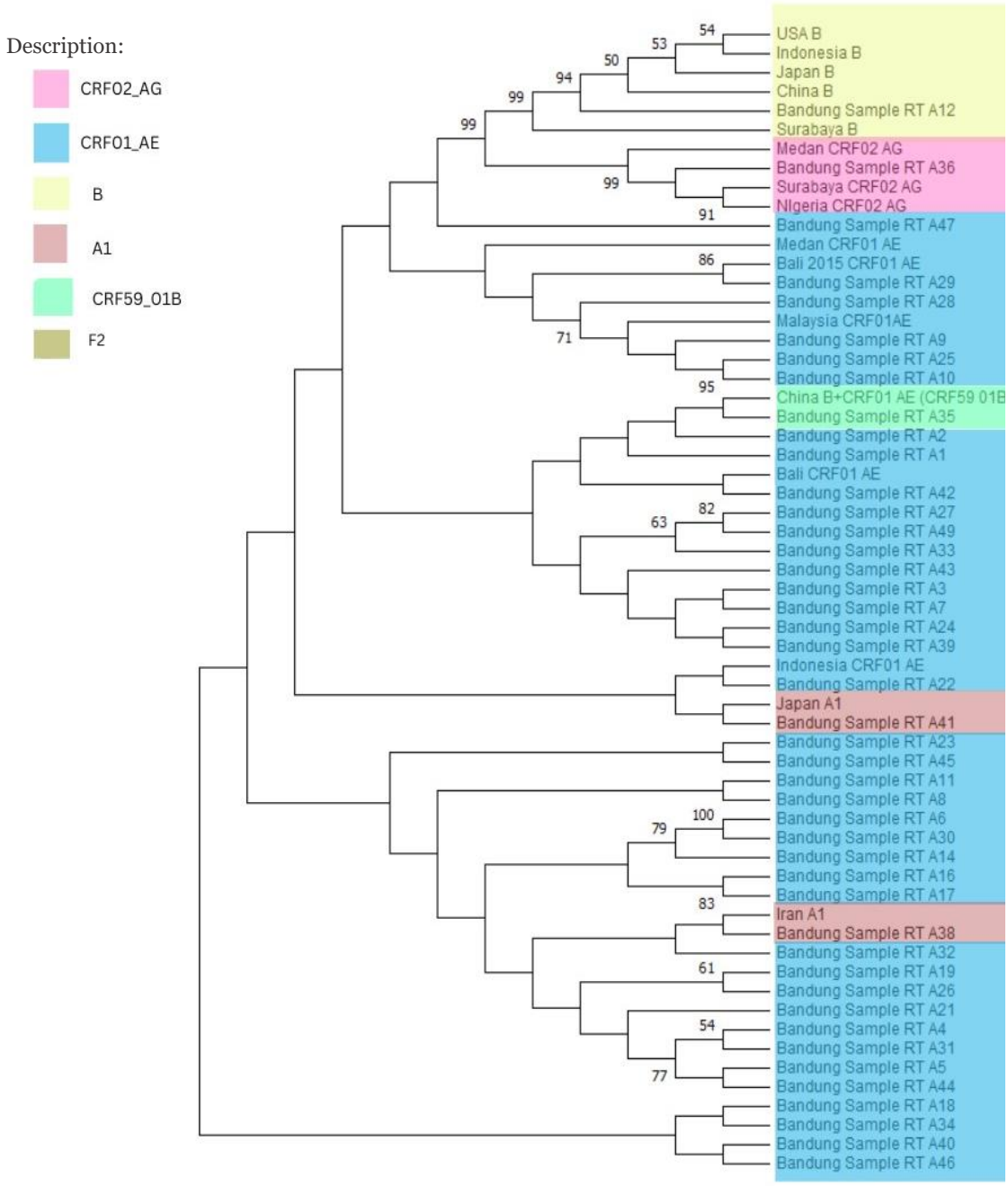

Figure 3. Phylogenetic analysis of HIV-1 reverse transcriptase gene in pre-treatment HIV patients generated from the study (n=44) together with corresponding viral gene of reference HIV-1 strains (n=16). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample RT A(number).

Analysis of subtype and drug resistance mutations in protease, reverse transcriptase, and integrase genes

In ARV-treated patients, DRM rates were 12.7% for both reverse transcriptase and integrase genes, and 7% for the protease gene. Among pre-treatment patients, the reverse transcriptase gene had the highest DRMs rate at 17.1%, followed by 9.8% for the integrase gene and 4.9% for the protease gene. DRMs NNRTI was predominantly found in both groups: 14.6% in pre-treatment patients and 7% in ARV-treated patients. In the ARV-treated group, 2.8% had major DRMs, and 2.8% had a combination of major and accessory DRMs, whereas all DRMs in pre-treatment patients were accessory DRMs.

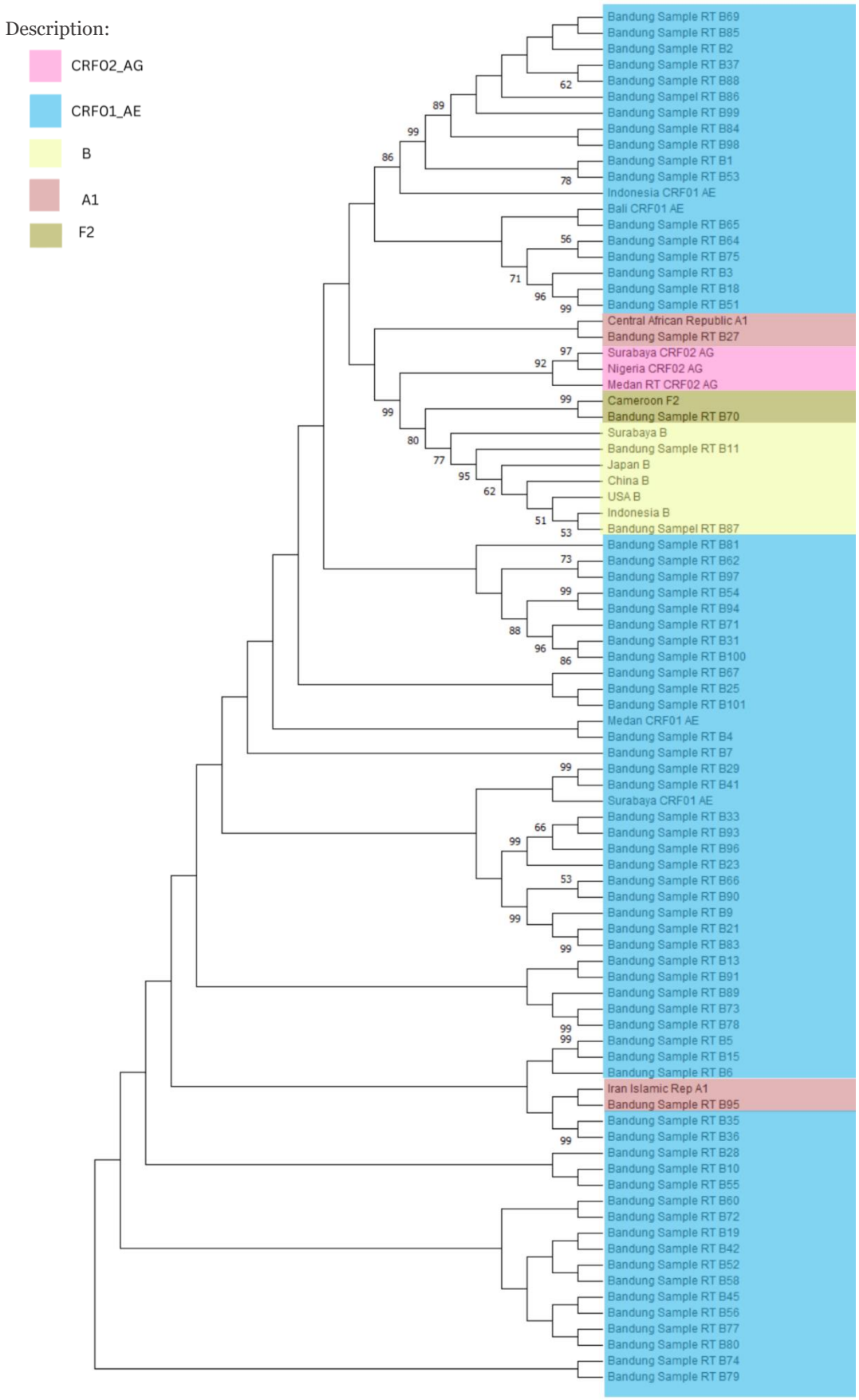

Figure 4. Phylogenetic analysis of HIV-1 reverse transcriptase gene in ARV-treated HIV patients generated from the study (n=71) together with corresponding viral gene of reference HIV-1 strains (n=15). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample RT B(number).

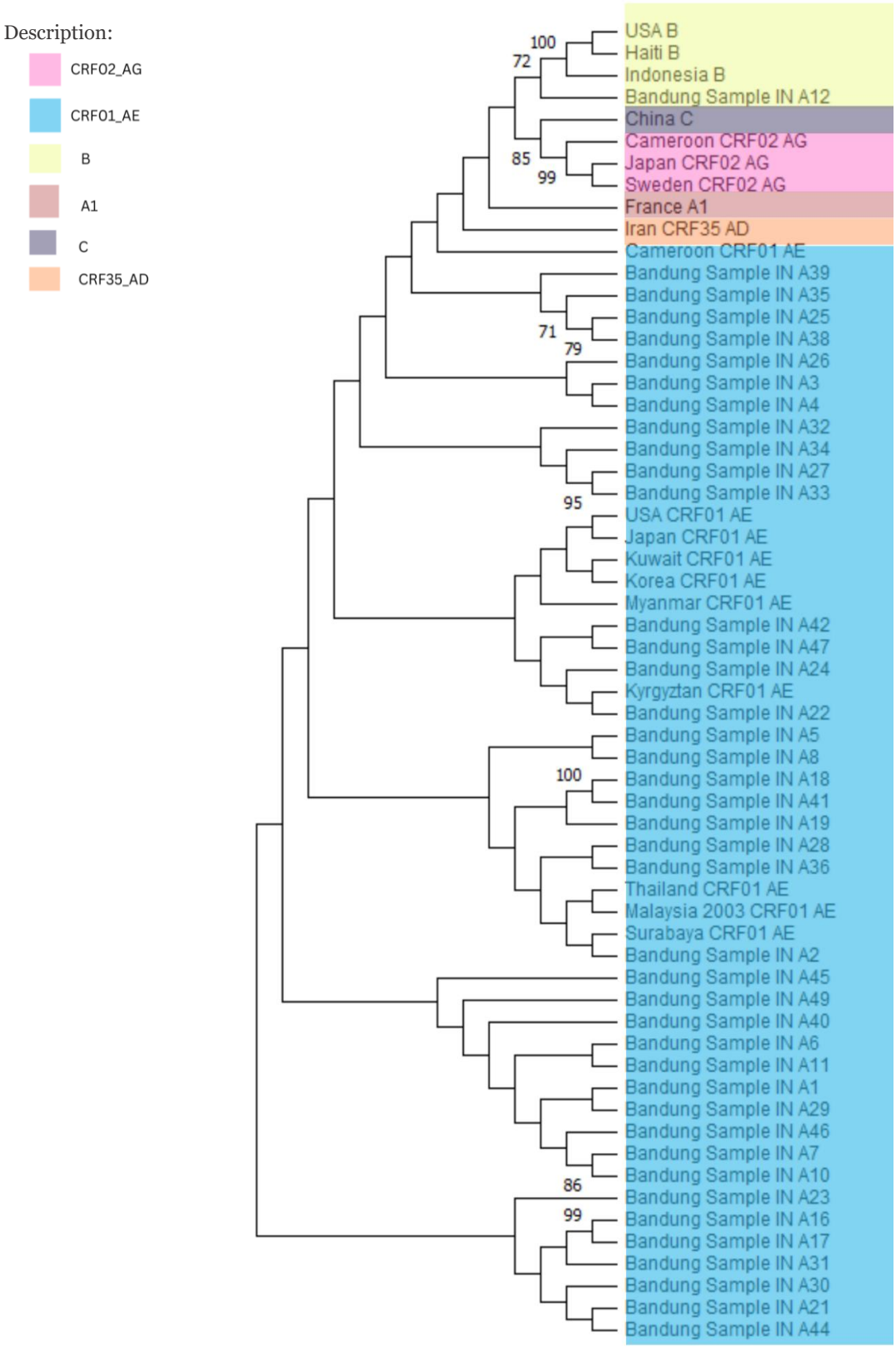

Figure 5. Phylogenetic analysis of HIV-1 integrase gene in pre-treatment HIV patients generated from the study (n=41) together with corresponding viral gene of reference HIV-1 strains (n=19). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample IN A(number).

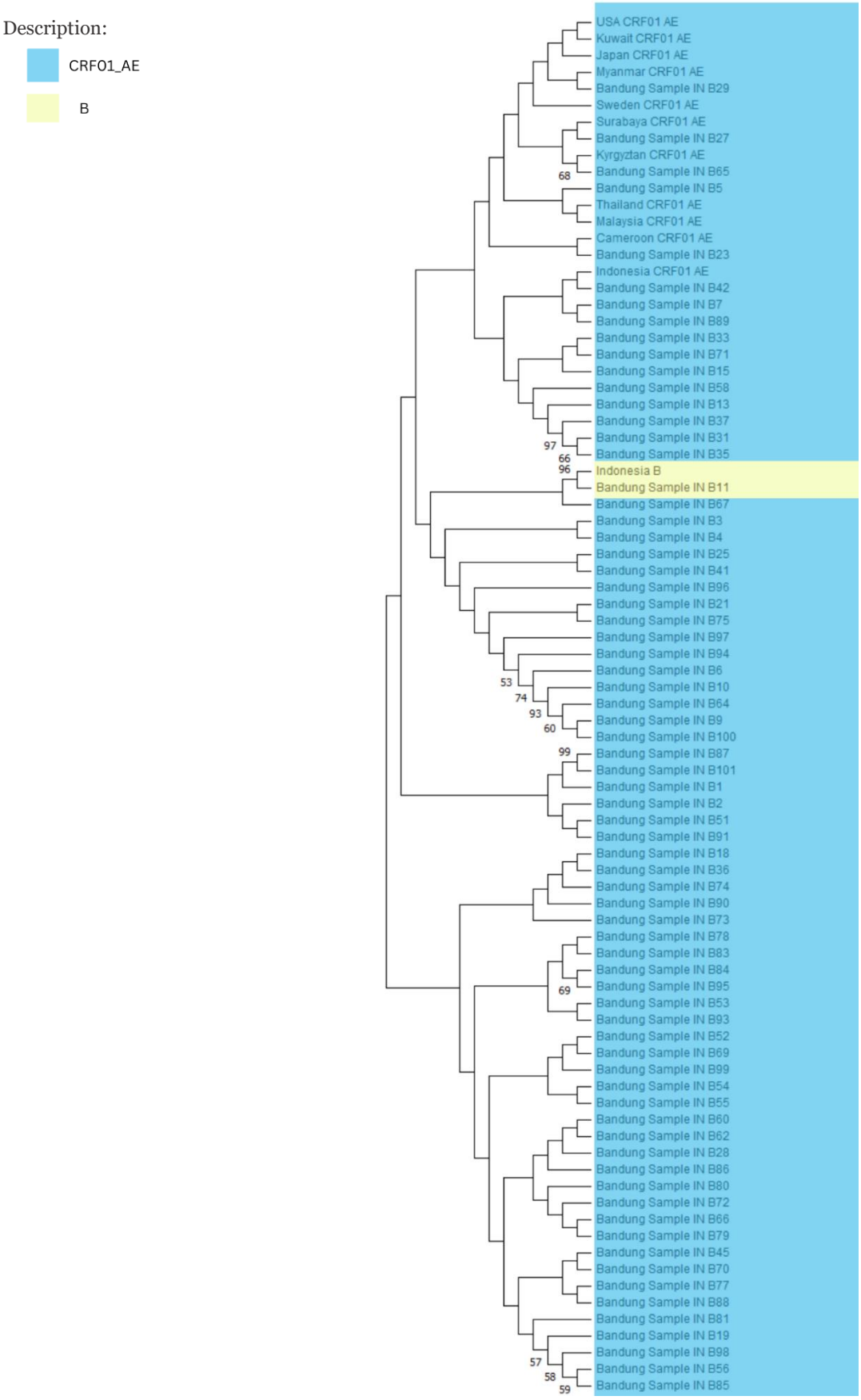

Figure 6. Phylogenetic analysis of HIV-1 integrase gene in ARV-treated HIV patients generated from the study (n=71) together with corresponding viral gene of reference HIV-1 strains (n=12). The phylogenetic tree was constructed using neighbor-joining method, Kimura 2 model parameters, and bootstrap replication of 1000×. The newly generated sequences are labeled with Bandung Sample IN B(number).

In ARV-treated patients, 22 types of DRMs were identified: four in protease, 14 in reverse transcriptase, and four in integrase genes (**Figure 7**). Among patients with CRF01_AE, the most frequently observed DRMs were accessory IN Q95K (five occurrences), followed by R263K (three occurrences), and D232N, NNRTI K103N, and protease L10F (two occurrences each). In pre-treatment patients, 11 distinct types of DRMs were identified: two in protease, one in NRTI, four in NNRTI, and four in integrase genes (**Figure 7**). The most prevalent DRMs in pre-treatment patients with CRF01_AE were NNRTI K103N and V179D. Several DRMs were common in both groups and were exclusively subtypes of CRF01_AE: protease L33F and G73S, reverse transcriptase K103N, E138A, and V179D, and integrase D232N and Q95K (**Figure 7**).

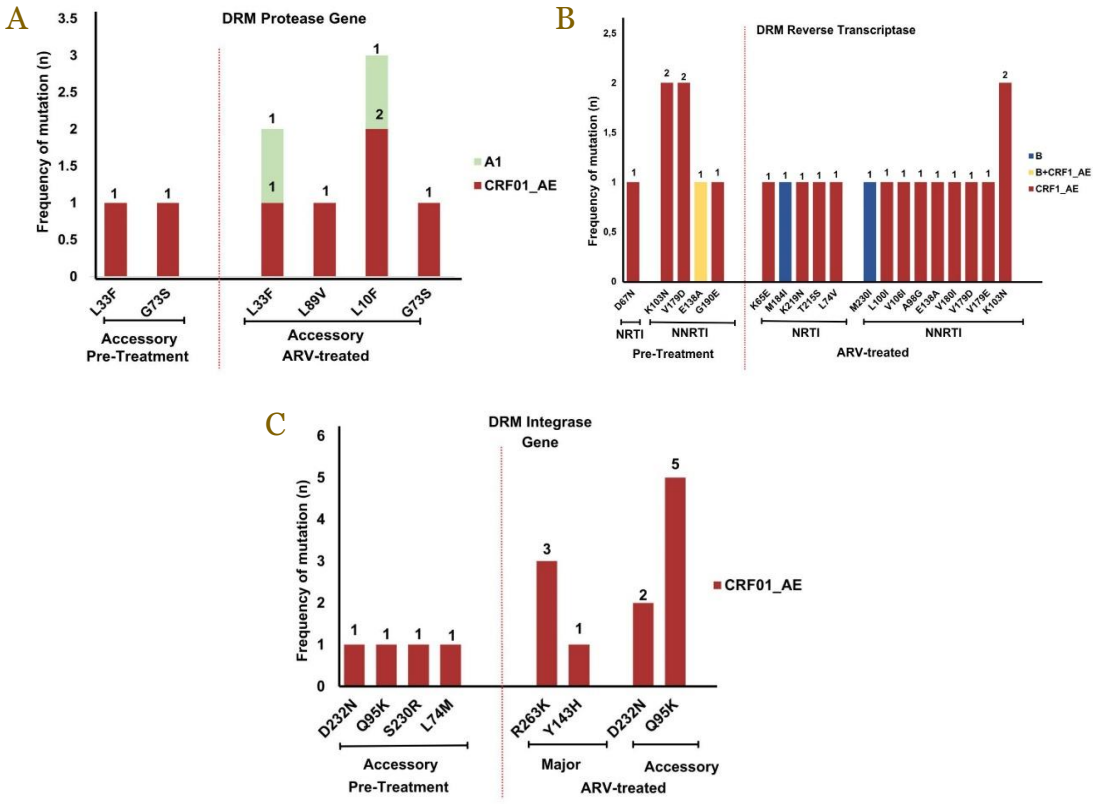

Figure 7. Analysis of drug resistance mutations and subtypes in (A) the protease gene, (B) the reverse transcriptase gene, and (C) the integrase gene for pre-treatment and ARV-treated patients. DRM: drug resistance mutation.

As presented in **Table 3**, among ARV-treated patients, four (5.6%) showed major DRMs integrase, including two patients (2.8%) with a combination of major R263K and accessory D232N, one patient (1.4%) with major Y143H, and one patient (1.4%) with major R263K. All of them were never treated with INSTI (INSTI naïve). Most patients used TDF+3TC as NRTI backbone (with one using AZT+3TC) and EFV as NNRTI. Additionally, two patients (2.8%) had combined NRTI and NNRTI DRMs: the first with major M184I (NRTI) and major M230I (NNRTI) while on TDF+3TC+EFV, and the second with major L74V (NRTI) and major L100I+ K103N (NNRTI) while on AZT+3TC+EFV. One patient (1.4%) had major NNRTI K103N while on TDF+3TC+DTG. According to Stanford DRMs analysis, two patient samples showed DRMs likely due to hypermutation activity of APOBEC host enzymes against HIV: reverse transcriptase M184I and M230I in patient B11, and integrase R263K and D232N in patient B25 (**Table 3**).

In pre-treatment patients, DRMs were detected in four patients (9.6%), comprising one (2.4%) treatment-naive patient with NNRTI K103N DRM, and three (7.3%) lost to follow-up patients with DRMs: NNRTI K103N, NRTI D67N, and G190E (**Table 3**). All lost follow-up patients had previously taken NRTI and NNRTI (EFV or NVP) before discontinuation of treatment. There were no major DRM proteases found in either ARV-treated or pre-treatment patients in the present study.

Table 3. Demographic patients' characteristics and major drug resistance mutations (DRMs) in reverse transcriptase and integrase genes among ARV-treated and pre-treatment HIV patients

Treatment status	ID	Sex	Age (years)	Risk of transmission	HIV-1 subtype	ARV-treated/ previous ARV (lost to follow-up) regimen	Major drug resistance mutation				Drug resistance
							Reverse transcriptase gene		Integrase gene		
							NRTI	NNRTI	Major	Accessory	
ARV-treated	B11	Male	40	Other	CRF01_AE and B	TDF+3TC+EFV	M184I	M230I	_	_	ABC, ddI, FTC, 3TC, DOR, EFV, ETR, NVP, RVP
ARV-treated*	B25	Male	30	Men who have sex with men	CRF01_AE	AZT+3TC+EFV	_	_	R263K	D232N	BIC, CAB, DTG, EVG, RAL
ARV-treated*	B41	Male	41	Men who have sex with men	CRF01_AE	TDF+3TC+EFV	_	_	R263K	D232N	BIC, CAB, DTG, EVG, RAL
ARV-treated	B58	Male	41	Injection drug user	CRF01_AE	AZT+3TC+EFV	L74V, K219N	L100I, K103N, V106I	_	_	ABC, AZT, d4T, ddI, DOR, EFV, ETR, NVP, RVP
ARV-treated*	B66	Male	28	Other	CRF01_AE	TDF+3TC+EFV	_	_	Y143H	_	CAB, EVG, RAL
ARV-treated*	B98	Male	24	Other	CRF01_AE and CRF02_AG	TDF+3TC+EFV	_	_	R263K	_	BIC, CAB, DTG, EVG, RAL
ARV-treated	B101	Male	45	Other	CRF01_AE and CRF02_AG	TDF+3TC+DTG	_	K103N	_	_	EFV, NVP
Treatment-naive	A1	Male	37	Other	CRF01_AE	_	_	K103N	_	_	EFV, NVP
Lost to follow-up	A10	Male	41	Injection drug user	CRF01_AE	TDF+3TC+EFV	_	K103N	_	_	EFV, NVP
Lost to follow-up	A39	Male	29	Men who have sex with men	CRF01_AE	TDF+3TC+EFV	D67N	_	_	_	AZT, d4T
Lost to follow-up	A47	Male	44	Injection drug user	CRF01_AE	AZT+3TC+NVP	_	G190E	_	_	DOR, EFV, ETR, NVP, RPV

3TC: lamivudine; ABC: abacavir; ARV: antiretroviral; AZT: zidovudine; BIC: bictegravir; CAB: cabotegravir; ddI: didanosine; DOR: doravirine; DTG: dolutegravir; EFV: efavirenz; ETR: etravirine; EVG: elvitegravir; FTC: emtricitabine; NNRT: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NVP: nevirapine; RAL: raltegravir; RVP: rilpivirine

*INSTI naïve

Discussion

In the present study, CRF01_AE was the predominant subtype identified in both pre-treatment and ARV-treated patients. This aligns with global surveillance data from WHO and findings from various studies, indicating its prevalence in Indonesia and Southeast Asia [3,11,16,18,29,30]. CRF01_AE is widely distributed across Southeast Asia, including countries such as Thailand, Philippines, Singapore, Vietnam, and Indonesia. It is recognized as the most prevalent circulating recombinant form globally [34]. Its initial spread in Asia originated from major outbreaks in nations such as Thailand in the mid-1980s, followed by its dissemination throughout the region [3].

The present study found no major DRMs for INSTIs in pre-treatment patients who were lost to follow-up or treatment-naive. The observed prevalence of 5.6% among ARV-treated patients with INSTI naïve is consistent with a study in Taiwan, which reported a DRMs prevalence of 1.7% in ARV-treated but INSTI-naive patients using combinations of ABC/AZT/TDF + 3TC and an NNRTI [35]. The prevalence of INSTI DRMs in pre-treatment/INSTI-naive patients in Southeast Asia remains very low (1.1%) [36]. In Indonesia, dolutegravir was introduced as a third agent in initial ARV combination therapy around 2022, typically used in combinations with TDF, 3TC, and dolutegravir [24].

R263K and G118R mutations are primarily found in INSTI-naive patients [34, 35]. The R263K mutation in the integrase gene results in moderate resistance to dolutegravir and reduces viral fitness in vitro [36,37]. DTG-RESIST study has shown that R263K is the most frequently observed DRMs in HIV patients with viremia undergoing dolutegravir-based therapy [25]. R263K, along with G118R and Q148H/R, are commonly associated with virological failure during dolutegravir treatment [34,35].

Y143H mutation, along with Y143C/R, confers significant resistance to the first-generation INSTI raltegravir, but causes only minimal resistance to other INSTIs unless accompanied by additional INSTI DRMs [39,40]. Y143H is less common than Y143C/R and may act as a transitional mutation between the wild-type Y and R forms [42]. Furthermore, Y143H does not confer resistance to dolutegravir and bictegravir, but it can lead to low-level resistance to cabotegravir and elvitegravir [43].

The major INSTI DRMs identified in the present study may indicate transmitted drug resistance (TDR), as the patient had never been exposed to ARV INSTIs. Unlike patients currently undergoing treatment, who often develop resistance due to selective drug pressure, DRMs occurrence in treatment-naive patients is rare and usually involves a fitness cost [42,43], suggesting the possibility of superinfection from other HIV patients who are undergoing treatment with major INSTI DRMs [42,44]. Additionally, Q95K accessory DRM found in the present study confers low-level resistance to raltegravir and elvitegravir. When combined with the major N155H DRM, Q95K acts as a compensatory mutation, enhancing viral fitness and contributing to resistance against both raltegravir and elvitegravir [45,46].

In pre-treatment patients, NNRTI DRMs were the most common type of DRMs. A meta-analysis of PDR in low- and middle-income countries has documented an increasing trend in NNRTI resistance after 15 years of ARV therapy for HIV [49]. The prevalence of PDR against efavirenz and nevirapine in Indonesia is 6.5%. [11]. In the present study, K103N and V179D were identified as the most prevalent NNRTI mutations in pre-treatment patients, consistent with global statistics from WHO surveys indicating a prevalence of 3.8% for NNRTI K103H/N/S/T DRMs and 4.1% for NNRTI V179D/E DRMs [11].

The K103N mutation was identified in the present study in both pre-treatment and ARV-treated patients. It is a clinically significant NNRTI DRM, associated with 20–50 times higher resistance to nevirapine and efavirenz, but it does not affect sensitivity to rilpivirine, etravirine, or doravirine. K103N is a commonly transmitted drug resistance mutation listed in WHO surveillance of drug resistance mutations [48,49]. It typically emerges under selective pressure from nevirapine or efavirenz treatment, significantly reducing their effectiveness [48,49]. In one patient in the present study, K103N was accompanied by NNRTI DRMs L100I, which further enhances resistance to nevirapine and efavirenz by over 50-fold [52]. Despite its low genetic barrier and minimal impact on viral fitness, K103N persists detectably over extended periods [53].

The K65E mutation identified in this study is an uncommon NRTI DRM. It alters amino acid residues in HIV reverse transcriptase, affecting the binding of NRTI ARVs to viral DNA by discriminating against the dideoxy-NRTI terminator strand over host cellular deoxynucleotide triphosphate (dNTP) [54], suggesting that K65E may severely impair viral fitness, contributing to its infrequent occurrence [55].

In the present study, a patient with the T215S mutation was treated with a regimen of tenofovir, lamivudine, and efavirenz. Additionally, this patient also exhibited the V179E NNRTI DRM, which may confer potential low-level resistance to efavirenz. Further research is necessary to fully understand its impact on NNRTIs [56]. The T215S mutation indicates reduced susceptibility to zidovudine and may suggest the previous presence of the T215Y mutation, which confers resistance to zidovudine, stavudine, and other drugs [55,56,57].

In the present study, two samples exhibited DRMs associated with Apolipoprotein B mRNA editing catalytic polypeptide (APOBEC) enzyme activity. Clinical experts hold conflicting views on the relevance of such DRMs [51]. Some suggest excluding sequences with hypermutations when interpreting resistance testing [60]. However, further research is needed to assess the therapeutic implications of these hypermutation DRMs [60].

There are some limitations of the study that need to be discussed. This study provides comprehensive coverage of ARV-treated and pre-treatment patients with *pol* gene, with a particular focus on INSTI DRM, which is still rarely studied in Indonesia. However, the analysis in our study used Sanger's sequencing, which, although a standard genotype for HIV drug resistance testing, might not detect minor variants below 20% of the viral population [61] and may underestimate the prevalence of DRM in this study. Additionally, the relatively small number of research samples suggests the need for future research with a greater sample to gain a more comprehensive understanding of the subtype and DRM of INSTI, as well as other DRMs in Indonesia.

Conclusion

CRF01_AE emerged as the predominant subtype in both pre-treatment (85.4%) and ARV-treated patients (69%). Various recombinants, including CRF02_AE and subtype B, were also identified. Some ARV-treated patients not previously exposed to INSTIs showed major resistance mutations in the integrase gene (R263K and Y143H), whereas only accessory integrase DRMs were found in pre-treatment patients. NNRTI DRMs were most prevalent in both groups (14.6% in pre-treatment and 7% in ARV-treated patients), while accessory protease DRMs were common across both groups. These findings underscore the importance of ongoing surveillance of ARV resistance mutations to effectively manage HIV/AIDS in Indonesia and Southeast Asia.

Ethics approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, No. 405/UN6.KEP/EC/2022.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request. The nucleotide sequences are available under GenBank accession number PQ155077, PQ177473 - PQ177477, PQ238076 - PQ238099, PQ238100 - PQ238199, PQ238200 - PQ238297, PQ261012 - PQ261023, and PQ273282 - PQ273380.

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