



# Subtype Distribution and Drug Resistance Patterns Among HIV-1 Strains Prevalent in Makassar, Indonesia

Khairunisa, Siti Qamariyah ; Megasari, Ni Luh Ayu ; Ueda, Shuhei ; Kotaki, Tomohiro ; Hidayati, Afif Nurul ; Nasronudin ; Kameoka,...

---

(Citation)

AIDS Research and Human Retroviruses, 39(3):124-129

(Issue Date)

2023-03

(Resource Type)

journal article

(Version)

Accepted Manuscript

(Rights)

This is the accepted version of the following article: [Subtype Distribution and Drug Resistance Patterns Among HIV-1 Strains Prevalent in Makassar, Indonesia. Siti Qamariyah Khairunisa, Ni Luh Ayu Megasari, Shuhei Ueda, Tomohiro Kotaki, Afif Nurul Hidayati, Nasronudin, and Masanori Kameoka. AIDS Research and Human Retroviruses 202...

(URL)

<https://hdl.handle.net/20.500.14094/0100479373>



**Sequence Note**

**Subtype distribution and drug resistance patterns among HIV-1 strains prevalent in Makassar, Indonesia**

Siti Qamariyah Khairunisa,<sup>1,2</sup> Ni Luh Ayu Megasari,<sup>2</sup> Shuhei Ueda,<sup>2,3,4</sup> Tomohiro Kotaki,<sup>5</sup>

Afif Nurul Hidayati,<sup>1,6</sup> Nasronudin,<sup>1,6</sup> Masanori Kameoka<sup>2,4</sup>

<sup>1</sup>Doctoral Program - Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

<sup>2</sup>Indonesian-Japan Collaborative Research Center for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

<sup>3</sup>Center for Infectious Diseases, Kobe University Graduate School of Medicine, Hyogo, Japan.

<sup>4</sup>Department of Public Health, Kobe University Graduate School of Health Sciences, Hyogo, Japan.

<sup>5</sup>Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

<sup>6</sup>Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

18

19 Running head: HIV-1 epidemiology in South Sulawesi, Indonesia.

20

21 **Corresponding authors:**

22 Nasronudin, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. E-mail:

23 [nasronudindr@yahoo.com](mailto:nasronudindr@yahoo.com). Tel.: +62-811-3440-774

24 Masanori Kameoka, Division of Global Infectious Diseases, Department of Public Health,

25 Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku,

26 Kobe, Hyogo 654-0142, Japan. E-mail: [mkameoka@port.kobe-u.ac.jp](mailto:mkameoka@port.kobe-u.ac.jp). Tel.: +81-78-796-

27 4594, Fax: +81-78-796-4509.

28

## 29    **Abstract**

30    Human immunodeficiency virus type 1 (HIV-1) is characterized by a large degree of  
31    genetic variability because of high rates of recombination and mutation, sizable  
32    population sizes, and rapid replication. Therefore, the present study investigated HIV-1  
33    subtype distribution and the appearance of drug resistance mutations (DRMs) in viruses  
34    that are prevalent in Makassar, South Sulawesi, Indonesia. The HIV-1 *pol*, *env*, and *gag*  
35    genes were amplified from 63 infected individuals and sequenced for a subtyping analysis.  
36    CRF01\_AE was identified as the predominant HIV-1 circulating recombinant form (CRF)  
37    in Makassar, South Sulawesi, Indonesia. Subtype B and recombinant viruses containing  
38    CRF01\_AE, CRF02\_AG, and/or subtype B gene fragments were also detected. Several  
39    major DRMs against non-nucleoside reverse transcriptase inhibitors were found among  
40    antiretroviral therapy (ART)-experienced subjects, while ART-naïve subjects did not  
41    possess any transmitted drug resistance. The prevalence of DRMs was very high among  
42    ART-experienced subjects; therefore, further surveillance is required in this region.

43

44    **Key words:** HIV-1 subtype, CRF01\_AE, HIV drug resistance, Makassar, Indonesia

45

46     **Text**

47             Human immunodeficiency virus type 1 (HIV-1) infection remains a significant  
48     global health issue. HIV-1-infected individuals receive antiretroviral therapy (ART),  
49     which is highly effective and recommended for all individuals diagnosed with HIV-1  
50     irrespective of their CD4<sup>+</sup> T-lymphocyte count. The significant development of  
51     antiretrovirals for ART has transformed HIV-1 from an almost uniformly fatal infectious  
52     disease into a manageable chronic disease. First-line ART drug regimens consist of a  
53     combination of two groups of reverse transcriptase (RT) inhibitors; nucleoside RT  
54     inhibitors (NRTI), such as zidovudine and lamivudine (3TC), and non-nucleoside RT  
55     inhibitors (NNRTI), including efavirenz (EFV) and nevirapine (NVP), are prescribed in  
56     Indonesia.<sup>1</sup>

57             Indonesia is a country with the largest number of HIV-infected cases in Southeast  
58     Asia. Based on data reported by the Indonesian Ministry of Health, South Sulawesi was  
59     one of the ten provinces with the highest numbers of HIV-infected cases in 2019.  
60     Makassar, Pare-Pare, and Kabupaten Janeponto had the highest prevalence of HIV in  
61     South Sulawesi province.<sup>2</sup> Makassar is the capital city of South Sulawesi, the fifth largest  
62     metropolitan city after Jakarta, Surabaya, Bandung, and Medan in Indonesia, and has the

highest HIV infection rate in South Sulawesi. As a result of the HIV epidemic and its integral roles as a metropolitan city, HIV-1 epidemiology needs to be investigated in Makassar. Previous studies identified CRF01\_AE as the most prevalent HIV-1 circulating recombinant form (CRF) across the majority of Indonesian cities, including Medan (North Sumatra), Kepulauan Riau, Pontianak (West Kalimantan), Manado (North Sulawesi), Jakarta, Surabaya (East Java), Bali, and Maumere (West Nusa Tenggara).<sup>3</sup> The prevalence of subtype B was reported to be high in West Papua and Papua.<sup>4,5</sup> Nevertheless, data on HIV-1 epidemiology in Makassar remains limited.

ART has improved the quality of life of infected individuals and has also decreased mortality and morbidity associated with HIV-1 infection. The emergence of acquired drug resistance (ADR) among ART-experienced subjects and transmitted drug resistance (TDR) among ART-naïve subjects are major issues associated with ART. The prevalence of drug resistance mutations (DRMs) amongst ART-naïve HIV-positive pregnant women was estimated to be 2.3–25%, and was recently found to be 24% in freshly-infected juveniles,<sup>6</sup> while the prevalence of TDR in Surabaya was less than 5%.<sup>7</sup> However, data on HIVDR in Makassar remains limited; therefore, surveillance for the emergence of DRMs is needed in the area.

Sixty-three HIV-1-infected individuals were recruited and enrolled in the Voluntary Counseling and Testing program in Makassar Hospital. The present study was approved by the Institutional Ethics Committees of Universitas Airlangga (approval number: 25-995/UN3.14/PPd/2013) and the Kobe University Graduate School of Medicine (approval number: 784). Written informed consent was acquired from all study participants before the execution of this study. Inclusion criteria for recruiting participants to this study were adults older than 18 years, HIV infections confirmed by three diagnostic methods, and ART-experienced for more than one year or ART-naïve. Exclusion criteria were individuals younger than 18 years and pediatric patients.

Whole peripheral blood samples from 63 individuals (60 ART-experienced and 3 ART-naïve individuals) were collected into ethylenediaminetetraacetic acid-treated vacutainer tubes. Plasma was separated by centrifugation at 2,000 rpm for 10 minutes. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO, USA). Cellular DNA was isolated from PBMCs using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany). The HIV-1 *pol* gene encoding RT (the RT gene) and protease (the PR gene) as well as the viral *env* and *gag* genes were then amplified using the GoTaq Green

Master Mix (Promega, USA) and a pair of specific primers corresponding to the target genes. Primers for amplification and sequencing were the same as those previously described<sup>3</sup> and information is available upon request. Sequencing data collection and alignment were performed using Genetyx software version 10 (Genetyx, Tokyo, Japan). Viral RT, PR, env, and/or gag genes were successfully amplified and sequenced from 48 subjects. The nucleotide sequences collected in the present study were registered to the GenBank database with the accession numbers ON244098 - ON244133 (PR genes), ON244134 - ON244168 (RT genes), ON244206 - ON244242 (*env* genes), and ON244169 - ON244205 (*gag* genes).

HIV-1 subtyping was performed using the recombinant identification program (RIP) available at the HIV sequence database website (<http://www.hiv.lanl.gov/>) and jumping profile Hidden Markov Model (jpHMM)-HIV ([http://jphmm.gobics.de/submission\\_hiv](http://jphmm.gobics.de/submission_hiv)) on successfully sequenced PR, RT, *gag*, and *env* genes. If the subtype or CRF amongst these genes was inconsistent, the viral gene was considered to be derived from a recombinant virus. In addition, the generation of neighbor-joining (NJ) trees with a Kimura two-parameter model was conducted by MEGA X software. Subtypes A1, A2, B, C, D, and G as well as CRF01\_AE and



114 CRF02\_AG, as major pandemic subtypes and CRFs of HIV-1, and 3 CRF01\_AE/subtype  
115 B-recombinants, CRF15\_01B, CRF33\_01B, and CRF34\_01B, as recombinants  
116 frequently found in Indonesia, were included in the phylogenetic tree analysis. Sequence  
117 information on the representative reference strains of subtypes and CRFs were retrieved  
118 from the website  
119 ([https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/RefSeqs2005/RefSeqs05.ht](https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/RefSeqs2005/RefSeqs05.html)  
120 [ml](https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/RefSeqs2005/RefSeqs05.html)). In addition, the identification of DRMs in the PR and RT genes of ART-naïve and  
121 ART-experienced subjects was performed manually in accordance with the International  
122 Antiviral Society-United States (IAS-USA) guidelines.<sup>8</sup>

123 The demographic characteristics of 63 study participants are shown in Table 1. Of  
124 these, 73.0% were male and 27.0% were females. The median age of all patients was 34.2  
125 years (range between 21 and 47 years). In addition, 36.5% of patients were of Bugis and  
126 Makasar ethnicity. Among these individuals, intravenous drug use and heterosexual  
127 intercourse were the two main transmission routes, accounting for 34.9 and 30.2% of  
128 infections in the study population. Among 63 individuals, 60 had received ART for longer  
129 than one year, while the remaining three were ART-naïve. Medication history showed that  
130 the majority of ART-experienced individuals had been treated with the combination of

131 3TC, tenofovir, and EFV (49.2%) with an average treatment duration of more than three  
132 years.

133 Thirty-eight PR (296 bp), 36 RT (762 bp), 38 *env* (383 bp), and 39 *gag* (369 bp)  
134 gene fragments were successfully sequenced from 48 samples and subjected to a  
135 phylogenetic analysis. According to the phylogenetic tree, RIP, and jpHMM analyses, the  
136 distribution of each subtype and CRF were as follows: 33/48 (68.8%) were CRF01\_AE,  
137 6/48 (12.5%) were recombinant viruses containing CRF01\_AE and CRF02\_AG genomic  
138 fragments, 4/48 (8.3%) were subtype B, 4/48 (8.3%) were recombinant viruses containing  
139 CRF01\_AE and subtype B genomic fragments, and 1/48 (2.1%) was a recombinant virus  
140 containing CRF02\_AG and subtype B genomic fragments (Fig. 1). In the present study  
141 CRF01\_AE was the predominant CRF in Makassar, Indonesia, similar to other provinces  
142 in Indonesia, including Medan, Manado, Surabaya, Jakarta, and Bali.<sup>3</sup>

143 As discovered by the online Genotypic Resistance Interpretation Algorithm  
144 (<https://hivdb.stanford.edu/hivdb/by-mutations/>) and in accordance with IAS-USA  
145 guidelines,<sup>8</sup> DRMs were identified in the RT and PR genes. Information on DRMs  
146 corresponding to antiretrovirals is listed in Table 2. In RT genes, the percentage of  
147 subjects with major and minor DRMs was 5/36 (13.9%). However, ADR in the RT genes

was identified in five subjects who were ART-experienced individuals. Only NRTI-associated, not NNRTI-associated DRMs were detected in RT genes. The main DRMs found in RT genes were K103N and E138A, which confer resistance to EFV, NVP, ETR, and RPV. Our previous studies on Pontianak and several provinces in Indonesia revealed the emergence of K103N and E138A among 28.6% (2/7) and 20.0% (8/40) of RT genes derived from ART-treated individuals, respectively.<sup>3,6</sup> In addition, minor DRMs detected in RT genes were V90I and V179D, which confer resistance to ETR. Moreover, major and minor DRMs were detected in two PR genes, MK10 and MK4. The main mutations found were D30N and M46I, which confer resistance to nelfinavir and indinavir/ritonavir.

In contrast, no TDR was identified among ART-naïve individuals. Minor DRMs, including M36I (33/38, 86.8%), L89M (32/38, 84.2%), K20R/I (27/38, 71.1%), and I93L/M (19/38, 50.0%), were repeatedly detected in PR genes. The aforementioned mutations were discovered to be natural polymorphisms amid CRF01\_AE viruses.<sup>8</sup> These results are similar to previous findings showing the presence of minor DRMs affiliated with PR inhibitors in several regions across Indonesia.<sup>3</sup> Nevertheless, treatment outcomes were largely unaffected by the large number of natural polymorphisms found in CRF01\_AE.<sup>9</sup>

Based on the present results, CRF01\_AE was identified as the dominant HIV-1 CRF in Makasar, Indonesia, similar to other regions in Indonesia. In addition, major and minor DRMs conferring resistance to NRTI were frequently found in RT genes derived from ART-experienced subjects. We consider continuous monitoring for the emergence of HIVDR to be necessary in order to maintain the efficiency of ART and reduce TDR in Indonesia.

## **Sequence Data**

Nucleoside sequences are available under GenBank accession numbers ON244098 - ON244205.

## **Acknowledgments**

The authors would like to express their appreciation to all study participants and staff at the Voluntary Counseling and Testing program in Makassar Hospital. Their most sincere gratitude is extended to J-GRID, AMED and ITD – Universitas Airlangga who facilitated this study (130/UN3.9.4/PT/2022). This manuscript was proofread by Medical English Service, Kyoto, Japan.

182

183    **Author Contributions Statement**

184    S.Q.K., Nasronudin, and M.K. conceived the study. S.Q.K., N.L.A.M., and S.U.

185    performed the experiments. S.Q.K., N.L.A.M., T.K., A.N.H. and M.K. analyzed the data.

186    S.Q.K. drafted the manuscript. All authors reviewed the manuscript.

187

188    **Author Disclosure Statement**

189    There were no competing financial interests in this study.

190

## References

1. WHO. Indonesia: Summary Country Profile for HIV/AIDS Treatment Scale-Up: HIV/AIDS Data Hub for the Asia-Pacific Region; 2005. Available from: <https://www.aidsdatahub.org/sites/default/files/resource/indonesia-antiretroviral-therapy-target-declared.pdf> [last accessed: September 15, 2022]
2. Kemenkes RI. Infodatin HIV AIDS. Kementerian Kesehat Republik Indones 2020;1–8. Available from: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/infodatin%202020%20HIV.pdf> [last accessed September 15, 2022]
3. Khairunisa SQ, Megasari NLA, Ueda S, et al. 2018-2019 Update on the molecular epidemiology of HIV-1 in Indonesia. *AIDS Res Hum Retroviruses* 2020;36(11):957–963; doi: 10.1089/aid.2020.0151.
4. Witaningrum AM, Kotaki T, Khairunisa SQ, et al. Genotypic characterization of human immunodeficiency virus type 1 derived from antiretroviral therapy-naïve individuals residing in Sorong, West Papua. *AIDS Res Hum Retroviruses* 2016;32(8):812–817; doi: 10.1089/AID.2016.0054.
5. Yunifiar MQ, Kotaki T, Witaningrum AM, et al. Sero- and molecular epidemiology

208 of HIV-1 in Papua Province, Indonesia. *Acta Med Indones* 2017;49(3):205–214.

209 6. Khairunisa SQ, Megasari NLA, Indriati DW, et al. Identification of HIV-1 subtypes  
210 and drug resistance mutations among HIV-1-infected individuals residing in  
211 Pontianak, Indonesia. *Germes* 2020;10(3):174–183; doi:  
212 10.18683/germs.2020.1203.

213 7. Kotaki T, Khairunisa SQ, Witaningrum AM, et al. HIV-1 transmitted drug  
214 resistance mutations among antiretroviral therapy-naïve individuals in Surabaya,  
215 Indonesia. *AIDS Res Ther* 2015;12(1):5; doi: 10.1186/s12981-015-0046-y.

216 8. Wensing AM, Calvez V, Ceccherini-Silberstein F, et al. 2019 update of the drug  
217 resistance mutations in HIV-1. *Top Antivir Med* 2019;27(3):111-121.

218 9. Sun Z, Ouyang J, Zhao B, et al. Natural polymorphisms in HIV-1 CRF01\_AE  
219 strain and profile of acquired drug resistance mutations in a long-term combination  
220 Treatment cohort in Northeastern China. *BMC Infect Dis* 2020;20(1):178; doi:  
221 10.1186/s12879-020-4808-3.

222

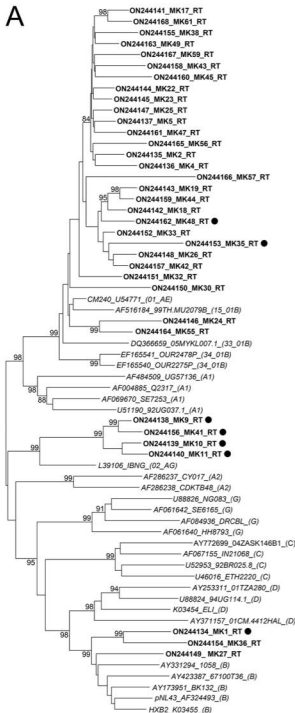
## Figure legends

**FIG. 1.** Phylogenetic tree analysis of HIV-1 RT, PR, *env*, and *gag* genes collected in Makassar, Indonesia.

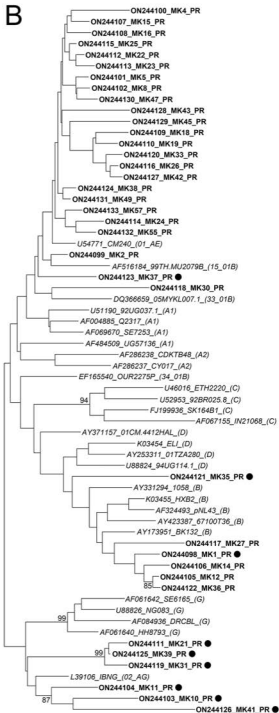
Phylogenetic trees were constructed for the HIV-1 RT (A), PR (B), *env* (C), and *gag* genes newly sequenced in the present study (D). The corresponding viral genes of reference HIV-1 strains representing subtypes A1, A2, B, C, D, and G as well as CRF01\_AE (01\_AE), CRF02\_AG (02\_AG), CRF15\_01B (15\_01B), CRF33\_01B (33\_01B), and CRF34\_01B (34\_01B) were included in analyses (shown in *Italic letters*). Sequence IDs are presented as a GenBank accession number, sample ID, or the ID of the reference HIV-1 strain and the subtype or CRF of the reference strain (shown in parentheses) in that order. New sequences identified in the study are highlighted by bold letters, while those derived from recombinant viruses are denoted with circles. Bootstrap values were shown if they were >70.



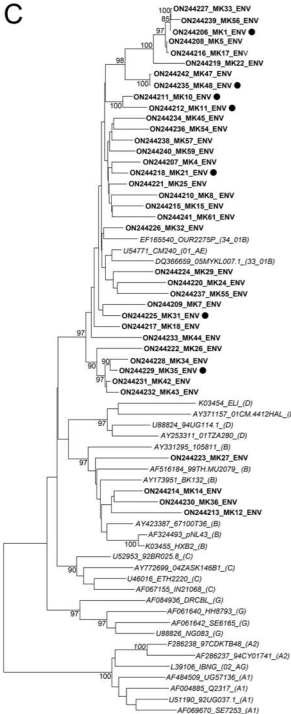
A



B



C



D

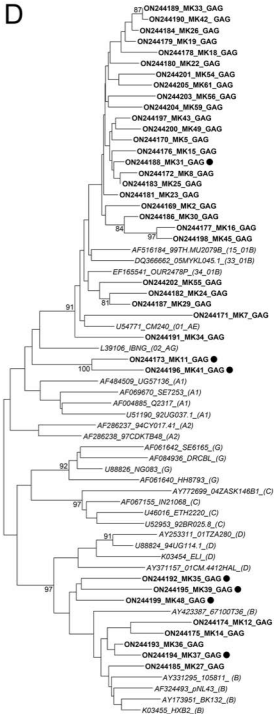


Table 1. Demographic characteristics of study participants

Characteristics	Value (n = 63)
Age, mean years (SD)	34.2 (6.3)
Sex, n (%)	
Male	46 (73.0%)
Female	17 (27.0%)
Marital status, n (%)	
Married	33 (52.4%)
Single	30 (47.6%)
Ethnicity, n (%)	
Makasar	23 (36.5%)
Bugis	23 (36.5%)
Jawa	5 (7.9%)
Toraja	2 (3.2%)
Batak	1 (1.6%)
Other	9 (14.3%)
Transmission risk category, n (%)	
Heterosexual intercourse	19 (30.2%)
Homosexual intercourse	12 (19.0%)
Intravenous drug use	22 (34.9%)
Commercial sex worker	7 (11.1%)
Unidentified	3 (4.8%)
Types of ART used, n (%)	
3TC+AZT+NVP	13 (20.6%)
3TC+AZT+EFV	15 (23.8%)
3TC+TDF+EFV	31 (49.2%)
3TC+TDF+NVP	1 (1.6%)
Naïve	3 (4.8%)
Opportunistic infection, n (%)	
No	49 (77.8%)
Yes	51 (22.2%)

Table 2. Drug resistance mutations and HIV-1 subtypes/CRFs detected in viral genes derived from HIV-1-infected individuals receiving ART

Sample ID	Type of ART; Subtype	Drug Resistance Mutations*			Conferred Resistance to
		NRTI	NNRTI	PI	
MK9	3TC, AZT, EFV; CRF01_AE/CRF02_AG	-	V90I	-	ETR
MK10	3TC, TDF, EFV; CRF01_AE/CRF02_AG- recombinant	-	<b>K103N</b>	G16E K20I <b>D30N</b> M36I <b>M46I</b> L63P L89M I93L	<b>EFV, NVP</b>
MK26	3TC, TDF, EFV; CRF01_AE	-	<b>E138A</b>	G16E K20R M36I V77I L89M I93L	ETR, <b>RPV</b>
MK27	3TC, AZT, NVP; subtype B	-	V179D	L33V I64V I93L	ETR
MK41	3TC, AZT, EFV; CRF01_AE/CRF02_AG- recombinant	-	V90I	G16E K20I <b>D30N</b> M36I <b>M46I</b> I62V L63P V77I L89M	ETR

\* Mutations associated with high resistance according to the guidelines published by the International AIDS Society United States (IAS-USA) are shown. Major mutations are shown in bold.