The Profile of SARS-Cov-2 Genome from Indonesia and Its Impact on Paxlovid™ in Treating Covid-19

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Abstract: Since April 2023, Paxlovid™ has been used to treat SARS-CoV-2 infection in Indonesia. This medication contains 2 active compounds, ritonavir (RTV) and nirmatrelvir (NTV) that act as human CYP3A4 and viral main proteinase or 3CL^{Pro} respectively. NTV is novel drug to inhibit SARS-CoV-2 progressivity. Mutations that decrease NTV effectiveness have been reported in several countries, however, the data from Indonesia is still limited. We are using SARS-CoV-2 genomic data from GISAID which were collected from Sept 1, 2022 – Oct 31, 2023, from Indonesia. Any mutation in 3CL^{Pro} was recorded and then proceeded to be analysed further. NTV-3CL^{Pro} complex was downloaded from the Protein data bank (7SI9), and separated in PDB format. The 3CL^{Pro} was mutated using FOLDX to generate the mutant variant. Docking simulation was performed using Autodock Vina which is integrated into PyRx.0.9.7 software with SARS-CoV-2 Wuhan-Hu isolate (NC_045512.2) as control. RMSD score was calculated using YASARA software and considered valid if the redocking score is <2.0 Å. In total, 13,345 genomes from COVID-19 patients were submitted in GISAID, and all of them were Omicron, with GRA clade and XBB subvariant being more predominant. We found out that 3CL^{Pro} encoding genes were relatively conserved with only P132H mutation motive (99.8%) identified. Therefore, we performed the simulation on mutant P184L which was found in the Delta variant. Docking simulation demonstrated that the binding affinity score between nirmatrelvir and control was -8.6 kcal/mol, and -8.5 kcal/mol on 3CL^{Pro} mutant. According to the visualization of NTV-3CL^{Pro} interaction, the mutant P132 and P184 showed no difference compared to the control. We found no mutation that potentially decreased the effectiveness of Paxlovid™ based on the activity of NTV in the SARS-CoV-2 genome collected from patients in Indonesia. Therefore, SARS-CoV-2 in Indonesia might be susceptible to Paxlovid™.

1 INTRODUCTION

SARS-CoV-2 infection causes coronavirus disease 2019 (COVID-19), which has caused approximately 6.7 million cases and over 160 thousand fatalities in Indonesia as of March 2023 (World Health Organization, 2023b). It remains a major public health threat globally and has been indicated that the virus will most likely remain as an established pathogen in humans and animals for a long time. On April 2023, Ministry of Health of The Republic of Indonesia has received a shipment of the oral antiviral medicine nirmatrelvir (NTV)/ritonavir (RTV) (PAXLOVID™) (World Health Organization, 2023a). It is drug cocktail that designed to stop COVID-19 from worsening by inhibiting the replication of SARS-CoV-2 in patients' body (Lin et al., 2023). It is suitable to treat early infection in order to stop the diseases progression. NTV is shown to be

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suppressing SARS-CoV-2 by binding and suppressing Main protease (Mpro) or known as 3CLpro as GC373 analog both in vitro and in vivo. However, it is metabolized mainly by cytochrome P450 3A4 (CYP3A4) which is responsible for the primary metabolism of about 50% of drugs. RTV boosts nirmatrelvir activity by inhibiting CYP3A (Lam & Patel, 2023).

In Coronaviridae genus, 3CLpro promotes the replication by cleaving polyprotein when the viral RNA enters the host cells. This protein exhibits >96% sequence identity with SARS-CoV, and the residues of its binding pocket are highly conserved. A study conducted in Canada using samples collected from 1 January 2020 – 12 January 2023 shows a very low-frequency (0.16 – 0,58%) variants at position linked to Paxlovid™ resistance genes (nsp5). Even though SARS-CoV-2 exhibit slower mutation rate (2.9×10^{-6} mutations/nt/cycle - 3.7×10^{-6} mutations/nt/cycle) (Gudiño León. et al., 2021), and 3CLpro binding pocket residues are highly conserved, the potential of drug resistant-associated mutation should not be neglected.

A study was conducted to identify mutation that naturally existed in the virus, and 100 mutations were found in 3CLpro encoding gene. These mutations located in NTV binding site and showed comparable enzymatic activity to the wild-type (kcat/Km <10-fold change) and resistance to NTV (Ki >10-fold increase). Another spot in SARS-CoV-2 genome for drug resistant is S144, M165, E166, H172, and Q192 which exhibit resistance 1.8 to 534.0-fold depending on the location of the mutation (Lee et al., 2022). Based on those data, it is important to identify the presence of NTV resistance-related mutation to understand the potential of drug-resistance. In this study we aim to identify the mutation associated with Paxlovid™ resistance, specifically NTV, and to analyse the interaction between NTV with 3CLpro mutant.

2 METHODS

2.1 Analysis of SARS-CoV-2 Genome Profile

This was a case series study, with population was COVID-19 patients who were eligible for SARS-CoV-2 genome isolation according to Ministry of Health genomic surveillance policy. We downloaded full genome sequences submitted between Sept 1, 2022 – Oct 31, 2023 from the GISAID EpiCoV database (https://www.gisaid.org/). We excluded the genome if it was incomplete, and contains >5% unidentified nucleotide. We extracted nsp5 genes from the genomes and aligned the sequences using MEGA X software and used SARS-CoV-2 Wuhan-Hu isolate (NC_045512.2) as control. The data such as patients' gender and age, and virus' data such as clade lineage, and mutations were recorded from meta data of the genome.

2.2 Molecular Docking of NTV-3CLpro

PyRx 0.9.7 integrated Autodock Vina was utilized to perform molecular docking between NTV and 3CLPro. We were using a computer with the following configuration ASUS ROG GL553 VE, 8GB RAM. NTV-3CLPro complex was obtained from PDB (PDB:7SI9) and separated using saved in PDB format. The mutant form of 3CLPro was generated using FOLDX. Docking validation was performed by redocking NTV to 3CLPro, and the docking procedure was considered as valid if RMSD value was < 2.0 Å.

3 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Molecular Epidemiology of SARS-CoV-2

There were 15,753 genomes submitted between September 1, 2022 – October 31, 2023 and all of them were identified as Omicron variant of SARS-CoV-2. Among them, 14,835 genomes were having complete genes composition, and 13,345 genomes were having <5% of unidentified nucleotides. These genomes were extracted from nasopharynx swab of patients who were receiving treatment in hospital and from genomic surveillance majority in Java Island. Majority of them were female with age 19-45 years old (median of age was 39 years old) (Table 1).

Table 1: Demographic and SARS-CoV-2 characteristics, and samples distribution

Characteristics	**Frequency** (n=13,345)
Age, Median (median, SD)	39±21
Age group, n (%)	
0-18	1,553 (11.6)
19-45	5,631 (42.2)
46-65	3,127 (23.4)
>65	1,799 (13,5)
Missing	1,255 (9.3)

Gender, n (%)	
Female	6,494 (48.7)
Male	5,654 (42.4)
Missing	1,197 (8.9)
Clade, n (%)	
GH	1 (0.0)
GK	2 (0,0)
GR	7 (0,1)
GRA	13,334 (99.9)
O	1 (0,0)
Lineages, n (%)	
B.1	1,760 (13.2)
BA.1	651 (4.9)
BA.5	4,075 (30.5)
EG	1,117 (8.4)
FL	787 (5,9)
XBB	4,159 (31.2)
Others	796 (6.0)
Patients' status, n (%)	
Survive	2,717 (20.4)
Deceased	73 (0,5)
Unknown	10,555 (79.1)
Samples' distribution, n (%)	
Sumatera	1,861 (13.9)
Java	9,908 (74.3)
Bali	624 (4.7)
Kalimantan	604 (4.5)
Sulawesi	207 (1.6)
Nusa Tenggara	67 (0.5)
Maluku	27 (0.2)
Papua	47 (0.3)

Clade GRA was predominating which caused almost 100% the infection. In overall, subvariant XBB was prevalent in Java, Bali, Nusa Tenggara, and Papua while Sumatera, Kalimantan, Sulawesi, and Maluku were dominated by BA.5 (table 2), however the fluctuation was observed across subvariants (figure 1).

Table 2: Samples' distribution by lineage dominance

Characteristics	**Frequency** (n=13,345)	**Dominant lineage (%)**
Samples' distribution, n(%)		
Sumatera	1,861 (13.9)	BA.5 (47.0)
Java	9,908 (74.3)	XBB (33.2)
Bali	624 (4.7)	XBB (39.7)
Kalimantan	604 (4.5)	BA.5 (31.0)
Sulawesi	207 (1.6)	BA.5 (57.0)
Nusa Tenggara	67 (0.5)	XBB (27.0)
Maluku	27 (0.2)	BA.5 (81.4)
Papua	47 (0.3)	XBB (42.6)

On September 2022, the BA.5 subvariant was predominant and causing 90.4% of the SARS-CoV-2 infection.

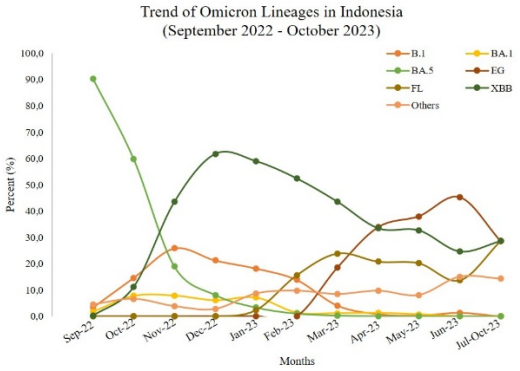


Figure 1: The trend of Omicron lineages in Indonesia during the study. BA.5 lineage was dominating the infection in the beginning of the observation, and then replaced by EG lineage in the end of the observation.

The BA.5 infection was decreased constantly until undetectable on January 2023. While BA.5 infection was decreasing, XBB subvariant was increasing and became dominant and reach its peak on December 2022. The XBB infection remained dominant for at least a month and slowly decreased while EG subvariant was increasing. On April 2023, the proportion of XBB and EG subvariant was the same. The XBB was undetected since July 2023, however subvariant EG was taking over the infection until October 2023.

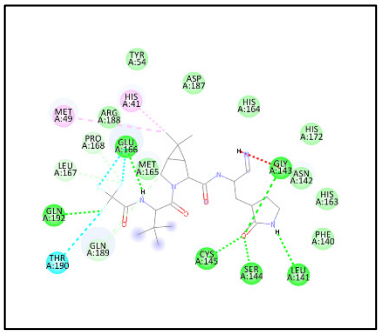
3.1.2 Mutation Identification and Molecular Docking

We found that 3CLpro encoding genes (nsp5) were relatively conserved with only P132H mutation motive (99.8%) identified. We performed molecular docking on 3CLpro mutant P132H to NTV. In order to obtain better understanding on the potential of mutation to NTV susceptibility, we used 3CLpro mutant P184H which was common in Delta variant of SARS-CoV-2. The binding affinity of NTV-3CLpro wild type was -8.6 kcal/mol, and the mutant P132H and P184L were slightly weaker compared to wild type (-8.5 kcal/mol) (Table 3).

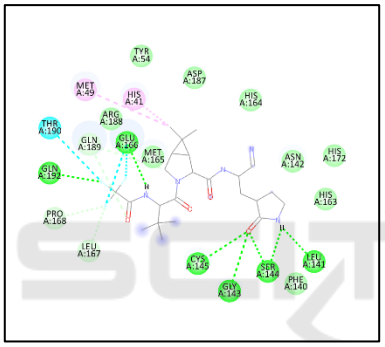
Table 3: Docking result on NTV-3CLpro wild type and mutants

Reseptor	Binding Affinity (kcal/mol)	Protein stability (kcal/mol)
Wild type	-8.6	59.16
P132H	-8.5	59.9
P184L	-8.5	60.19

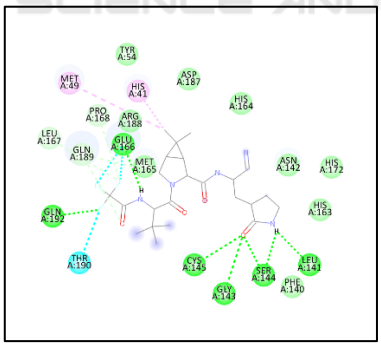
The binding affinity between 3 models was insignificantly different, and this result was supported by the visualization of the interaction. The hydrogen bond was formed in the same position, which was in amino acid LEU141, GLY143, SER144, CYS145, GLU166, GLN192.



Wild type



3CL-Pro mutant P132H



3CL-Pro mutant P184L

Figure 3: Comparison of hydrogen bond position in 3CLPro wild type and mutant P132H in Omicron and P184L in Delta.

3.2 Discussion

This study depicted the profile of SARS-CoV-2 and COVID-19 patients based on metadata obtained from GIS database. In this study, majority of the patients (74.3%) was from Java Island, the centre of Indonesia with highest population density (Dsikowitzky et al., 2018). All of the patients were infected with Omicron variants of SARS-CoV-2, majority with XBB. Study showed that proportion of asymptomatic and mild patients was higher in Omicron group than Delta and Beta groups, however those with Omicron had more throat soreness and less headache (Yang et al., 2022). Majority of the patients was between 19-49 years old, this was consistent with previous studies which showed that greater proportion of Omicron infection was found in younger age group, while Delta was found in older age group of patients (Miao et al., 2023; Yang et al., 2022).

COVID-19 medication was mostly involving immunological intervention by using antibody such as tocilizumab and other anti-SARS-CoV-2 monoclonal antibody products, however, high variation in spike of the virus is major obstacle to keep the medication works as the way it is (Treatment Guidelines Panel, 2023). The discovery of 3CLpro inhibitors represents a major breakthrough in COVID-19 treatment (Zhu et al., 2020). In this study, all the samples collected were identified as Omicron variant of SARS-CoV-2, with 353 sub-lineages, and 35% of them were XBB, and followed by BA.5 (34%). Omicron variant is more transmissible and can evade immune system better compared to previous variants of SARS-CoV-2 (Veltri et al., 2023). The Omicron variant have dominated epidemiologic landscape of SARS-CoV-2 infection globally. It also had evolved remarkably in diversity by forming over 1,000 sub-lineages with the standards lineages are BA.1, BA.2, BA.3, BA.4, and BA.5, which share many mutations, but also significantly different (Velavan et al., 2023; Veltri et al., 2023).

In this study, on September 2022 we observed that BA.5 was identified in majority of the samples. BA.5 was first detected in South Africa, and then in Belgium, France, China, Botswana, Portugal, Germany and Australia. The most recent ancestor of BA.5 is estimated most closely related to BA.2. There has been reported that BA.2 and BA.5 are more transmissible and resistant to immunity generated by previous variants and most monoclonal antibodies. BA.5 dominance was dropping on October 2022 and replaced by XBB variant. XBB was likely originated via BA.2 descendant recombination. It was first detected in India on October 2022 and immediately became predominant globally. WHO declared that XBB as Omicron subvariant under monitoring on October 28, 2022 (Lee et al., 2022).

EG started to replace XBB since April 2023 until October 2023. Limited information about EG.2 is available; it is one of many subvariants that has F456L in spike which makes it harder for many existing antibodies to recognize the viral particles. If

this virus also has L455F, it might have the ability of immune-evading and tighter binding to the ACE2 protein, which is likely to enhance cell entry. The EG.2 variant is radically different from the others, and Q52H and F456L mutations make this variant more similar to the original Omicron which has greater binding affinity compare to the other XBB descendants (Veltri et al., 2023)

Our study showed that mutation that has potential in developing drug resistance remains rare, however P132H was identified in almost all samples (99.8%). This result is in line with current global situation where this mutation is the most prevalent globally. P132H mutation is exclusively associated with Omicron because this mutation was identified in >98% of Omicron (Sacco et al., 2022). On 2022, P132H was most prevalent in UK (44%), and it was found in 98% of Omicron subvariant. P132H mutation is localized at about -22 Å away from the catalytic site and not in direct contact with any of the residues of allosteric pocket. Study showed that P132H got 3CLpro thermal stability compromised (Lee et al., 2022). A study demonstrated that P132H mutation alone related to a decreased stability of the enzyme in vitro. Another study also revealed the crystal structures shows that the mutations does not give rise any significant changes of the protein around the binding pocket or the site of the mutation (Greasley et al., 2022).

The effect of the P132H mutation on Omicron 3CLpro remains unclear. We found that the binding affinity of P132H mutant was not significantly different compared to the wild type. This result was supported by visualization of hydrogen bond position, we observed the same position of hydrogen bond across the mutations. Our result is consistent with previous study which showed that P132H might not reduce enzymatic activity and inhibitor binding. Study showed that P132H decrease thermal stability of 3CLpro and may cause the increasing protein flexibility which might broaden substrate profile substrate profile or to alter ligand binding (Sacco et al., 2022).

Another study showed that P132H will give effect to thermal stability only if the proline at position 108 3CLpro is also replaced by other amino acid. Another impact of P132H was observed by Chen et al., Omicron with P132H mutation permitted charged L-Lys and the enzyme activity is increased toward L-Trp and L-Tyr compared to the wild type. Catalytic efficiency was observed on the double mutant K90 and P132H.(Chen et al., 2023) In this study we observed that P132H alone without other mutation along with it, therefore P132H alone is considered to be non-major changes related to the chemical characteristic of 3CLpro because this mutation does not play role whether in the active site or the allosteric binding site of the protein (Ullrich et al., 2022).

4 CONCLUSIONS

NTV is still potent to be used as oral antiviral to treat COVID-19 in Indonesia, however routine genomic surveillance is necessary to anticipate the appearance of mutations that have been proven to be associated with NTV resistances.

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