

**SARS-CoV-2 VARIANTS GENOME ANALYSIS OF INDONESIAN ISOLATES AND THEIR RESPONSES TO AVAILABLE VACCINES****Kholis Abdurachim Audah<sup>1,2\*</sup>, Nasser Mohamed Ghassan Mohamed Adnan Shaikho<sup>1</sup>, Dyana Yurico<sup>1</sup>, Michael Jeremy<sup>1</sup>, Amalda Siti Anisa<sup>1</sup>**<sup>1</sup>Department of Biomedical Engineering, Faculty of Life Sciences and Technology, Swiss German University<sup>2</sup>Academic Research and Community Service, Swiss German University\*Email: [Kholis.audah@sgu.ac.id](mailto:Kholis.audah@sgu.ac.id)**ABSTRACT**

SARS-CoV-2 is a virus that initially appeared in Wuhan, China, at the end of 2019. In Indonesia, multiple variants of SARS-CoV-2 have been identified, as well as various local variants that are not yet considered to be 'variants of concern'. Therefore, this investigation is intended to understand the prevalence and epidemiology of the virus, along with detecting the mutations that occur in genes associated with whole-genome-sequences (WGS) isolated in Indonesia. Analyses were performed to investigate SARS-CoV-2 prevalence in Indonesia using data obtained from GISAID.org. Whole-genome sequencing was performed on random samples taken from GISAID.org utilizing the BLAST tool from NCBI. The variants identified in Indonesia are alpha, beta and delta variants, as well as local variants B.1.470 and B.1.466.2. In total there are 9,260 isolated genomes collected in GISAID were located in Indonesia. Using BLAST, the variants were compared with the Wild-Type from Wuhan NC.045512.2. Multiple mutations were observed in the samples. The results from whole-genome sequencing of variants isolated in Indonesia have found that multiple mutations have occurred in genes of the SARS-CoV-2 virus and it caused alterations in the characteristics of the virus and may affect vaccine efficacy.

**Keywords:** *GISAID.org, Indonesia, multiple mutations, SARS-CoV-2, virus***ABSTRAK**

SARS-CoV-2 merupakan virus yang pertama kali muncul di Wuhan, Tiongkok, pada akhir tahun 2019. Di Indonesia, telah teridentifikasi berbagai varian SARS-CoV-2, serta berbagai varian lokal yang belum dianggap sebagai virus. 'varian yang menjadi perhatian'. Oleh karena itu, penyelidikan ini dimaksudkan untuk memahami prevalensi dan epidemiologi virus, serta mendeteksi mutasi yang terjadi pada gen terkait seluruh genome-sequences (WGS) yang diisolasi di Indonesia. Analisis dilakukan untuk mengetahui prevalensi SARS-CoV-2 di Indonesia menggunakan data yang diperoleh dari GISAID.org. Pengurutan seluruh genom dilakukan pada sampel acak yang diambil dari GISAID.org menggunakan alat BLAST dari NCBI. Varian yang teridentifikasi di Indonesia adalah varian alpha, beta, dan delta, serta varian lokal B.1.470 dan B.1.466.2. Total ada 9.260 genom terisolasi yang dikumpulkan dalam GISAID yang berlokasi di Indonesia. Dengan menggunakan BLAST, varian tersebut dibandingkan dengan Tipe Liar dari Wuhan NC.045512.2. Beberapa mutasi diamati pada sampel. Hasil pengurutan seluruh genom varian yang diisolasi di Indonesia menunjukkan bahwa telah terjadi banyak mutasi pada gen virus SARS-CoV-2 dan menyebabkan perubahan karakteristik virus serta dapat mempengaruhi kemanjuran vaksin.

**Kata kunci:** *GISAID.org, Indonesia, multiple mutations, SARS-CoV-2, virus*

## INTRODUCTION

Towards the end of 2019, China reported its first case of a type of infectious disease in Wuhan. Since then, this disease, a type of coronavirus called COVID-19, has caused an ongoing global pandemic. Three bronchoalveolar lavage samples were collected from a patient with pneumonia with unknown etiology, and these samples were analyzed using polymerase chain reaction or PCR, showing a positive infection for pan-Betacoronavirus<sup>1</sup>. The very first whole genome sequence (WGS) of the virus was then obtained using Illumina and nanopore sequencing. This sequence is known as the Wild-Type (WT) WGS of SARS-CoV-2. Bioinformatics analysis using the phylogenetic tree showed that the virus had similar to with the coronavirus family and that the virus belonged to the Betacoronavirus 2B lineage (WHO 2020). Using sequence alignment, it was identified that the COVID-19 virus had resemblance with SARS for example, having many similarities with another coronavirus strain, BatCov RaTG13 (Clinic 2021).

Symptoms from COVID-19 are fever, dry cough, and tiredness. Other symptoms include aches and pains, sore throat, diarrhea, headache, loss of taste or smell. Serious symptoms include shortness of breath or difficulty in breathing, chest pain or pressure, loss of speech and inability to move. These serious symptoms are indicators that the person should seek medical attention immediately. The fatality of this virus depends on various factors, such as age and whether a person has hereditary diseases. The fatality ratio increases as age increases.

In early March 2020, SARS-CoV-2 began to enter Indonesia, initially detected in Depok, West Java (Wiranti et al. 2020). As of November 2021, there are already seven SARS-CoV-2 variants worldwide, including the alpha variant, beta variant, delta variant, gamma variant, lambda variant, MU variant andOMICRON variant (Tracking SARS-CoV-2 variants, n.d). However, only three variants have been seen in Indonesia, alpha, beta, and delta variants. In addition, various local variants were also identified in Indonesia, such as B.1.470, B.1.466.2, B.1.1.398 and B.1.459, with both B.1.470

and B.1.466.2 enlisted as Indonesian lineages by PANGO (Fibriani et al. 2021). Both enlisted Indonesian lineages are not yet considered 'variants of concern', however the variant B.1.466.2 is currently being observed by World Health Organization enlisted as 'Alerts for Further Monitoring (Fibriani et al. 2021; WHO 2021).

## METHODS

The materials used in this study were obtained from GISAID.org. The search was filtered by selecting Indonesia as the location to obtain whole genome sequences of Indonesia isolates. In total there were 9,260 isolated genomes collected in GISAID were located in Indonesia, including specific variant such as Alpha, Beta, Delta, Local Variants and others. The Wild-Type WGS from Wuhan was obtained from NCBI. The WGS from Indonesia was then compared with the WGS Wild-Type from Wuhan. Afterward, the similarity between the mutations in the nucleotide sequences and the amino acids in the variants was obtained. The comparison was performed using the BLAST tool from NCBI. To obtain the prevalence in Indonesia, the data were obtained from GISAID.org.

## RESULTS AND DISCUSSION

To be able to deal with SARS-CoV-2, a better understanding of its structure and domains is important. The first part of an infection is viral entry, wherein the virus comes into contact with the host cell. For viral entry to occur, SARS-CoV-2 targets angiotensin-converting enzyme 2 (ACE2) receptors through the use of the receptor-binding domain (RBD), which is located in the spike protein of the virus (Mittal et al. 2020). By doing so, the virus is able to fuse to the host-cell membrane and start spreading.

The COVID-19 virus along with its variants, is a single-stranded RNA that is non-segmented and has a range of 26 to 32 kb (Mittal et al. 2020). Moreover, the virus is a spherical shape that is covered with spikes responsible for the transmission of the virus. The diameter of the virus ranges anywhere

from 60 nm to 140 nm, with spikes ranging from 9 nm to 12 nm (Mittal et al. 2020).

For the position of the structures for the sequence of SARS-CoV-2, the official reference sequence hCoV-19/Wuhan/WIV04/2019 (WIV04) isolated in Wuhan China will be utilized. For an elevated understanding, the nucleotide positions of each structure will be provided. The genome starts with ORF1a and ORF1b which overlap with one another, with ORF1a having positions 266 to 13468, and ORF1b having positions 266 to 21555 in the sequence (Gisaid, n.d.). The next structure is the S structure that corresponds to the spike protein of SARS-CoV-2, and it has the position of 21563 to 25384. Next in the sequence are ORF3a and ORF3b, which can be found at positions 25393 to 26220 for ORF3a and 25765 to 26220 for ORF3b respectively; ORF3a corresponds to nonstructural protein NS3a, whereas ORF3b corresponds to NS3b.

Next, the E structure corresponding to the envelope protein can be seen at positions 26245 to 26472. Another structure follows, structure M corresponding to the membrane protein and is positioned at 26523 to 27191. After that, ORF6 can be seen located at positions 27202 to 27387, which corresponds to nonstructural protein NS6. Then, ORF7a and ORF7b follow, with ORF7a at positions 27394 to 27759 and corresponding to nonstructural protein NS7a, and ORF7b at positions 27756 to 27887 and corresponding to nonstructural protein NS7b. ORF8 then follows, positioned at 27894 to 28259 and corresponding to nonstructural protein NS8. Next, the structure N, which corresponds to the nucleocapsid protein, can be seen at positions 28274 to 29533.

Within the range of the N structure, ORF9a and ORF9b can be found at positions 28284 to 28577 and 28734 to 28955 respectively; ORF9a corresponds to the nonstructural protein NS9a, whereas ORF9b corresponds to nonstructural protein NS9b. Finally, ORF10 is positioned at 29558 to 29674, and corresponds to the nonstructural protein NS10; currently there is no evidence to suggest that this protein plays a role in the transmission or replication of the

virus. The structure of the SARS-CoV-2 virus is a 5'-capped and 3'-polyadenylated sequence, and within this sequence, various open reading frames (ORFs) can be found along with 2 untranslated regions (UTRs) (Mittal et al. 2020). These ORFs are responsible for encoding proteins. Moreover, the arrangement of the genome starts with 5'-UTR—replicase genes (ORF1ab) structure proteins S, E, M, and N, as well as accessory proteins—noncoding 3'-UTR (Mittal et al. 2020). ORF1a and ORF1ab, located at the 5'-end of the genome of SARS-CoV-2, are responsible for 15 nonstructural proteins (Wu et al. 2020). These overlapping ORFs produce 2 polypeptides due to ribosomal frameshifting that occurs, pp1a and pp1ab, and the genome encodes the proteases PL2pro along with 3CLpro to cleave these polypeptides to produce 15 nsps (non-segmented proteins), with some of these enzymes being highly important for transcription as well as the replication of viral RNA (Mittal et al. 2020). Among the structures of SARS-CoV-2, E is the smallest and is responsible for targeting the endoplasmic reticulum and Golgi complex within host cells. The M protein, is responsible for viral assembly, being at the center of it. The N protein is responsible for viral genome packaging (Mittal et al. 2020). Finally, the S protein is responsible for viral attachment and entry into a host-cell.

In this study, the differences between the variants were analyzed using blast from NCBI BLAST. Using this, the identical percentage was then measured, as well as the location of the mutations between the different genome sequences. The variants isolated in Indonesia were selected randomly from the GISAID website. Alpha variants with the accession ID EPI\_ISL\_3138832, beta Variant (EPI\_ISL\_2434175), and delta variant (EPI\_ISL\_5462140) of Sars-CoV2 whole genome sequences in Indonesia were obtained from the GISAID database, while the original genome sequence of Sars-CoV2 isolated from Wuhan, China was obtained from the NCBI database (NCBI NC\_045512.2). The local variants not yet considered 'variants of concern' were also obtained from the GISAID database; B.1.470 Indonesian local variant

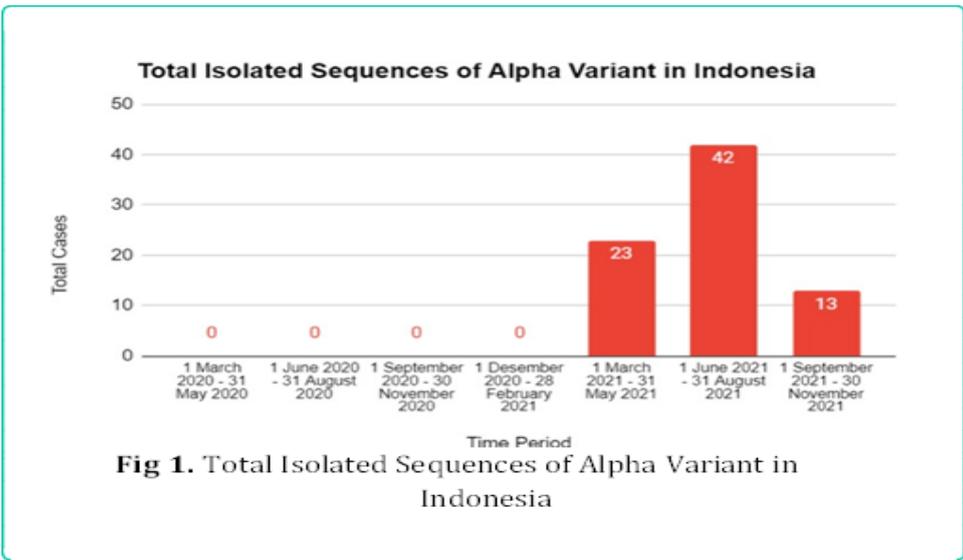
(EPI\_ISL\_2854771) and B.1.466.2 Indonesian local variant (EPI\_ISL\_6827341).

**Total Variant Prevalence in Indonesia**

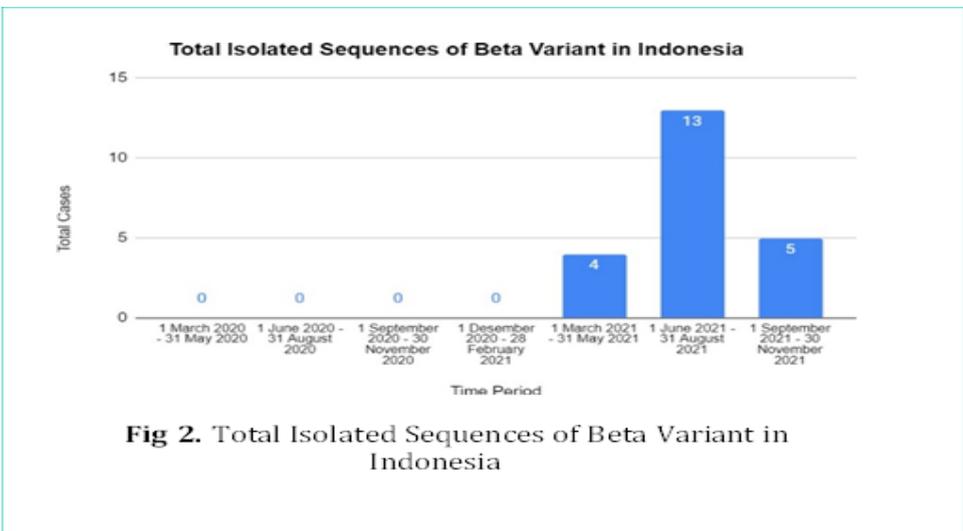
Figure 1, Figure 2, Figure 3 show total cases of variant isolates that were sequenced in Indonesia with a period of time of every three months, starting from March 1, 2020 until November 30, 2021. It can be concluded from the 2021 h above, that from March 1, 2020 until February 28, 2021, no variant isolated were sequenced in Indonesia. Figure 1 shows the alpha variant isolates that were sequenced starting in early March 2021, with 23 isolates being sequenced. An increase in alpha variant isolates was then observed in the months that followed, from June 2021 until August 2021 with a total of 42 isolates sequenced, with isolate sequencing showing a decrease in the months to follow, from September 2021 until November 30, 2021 with a total of 13 isolates sequenced.

In Figure 2, the sequencing for beta variants can be observed. The graph shows that the sequencing of the beta variant in Indonesia started in early March, 2021. From March 1, 2021 until May 30, 2021, 4 isolated sequences for the beta variants were recorded, and this number started increasing in June 2021 until 31 August 2021 with 13 isolated sequences, and later decreased in September 2021 until November 30, 2021 with 5 isolated sequences.

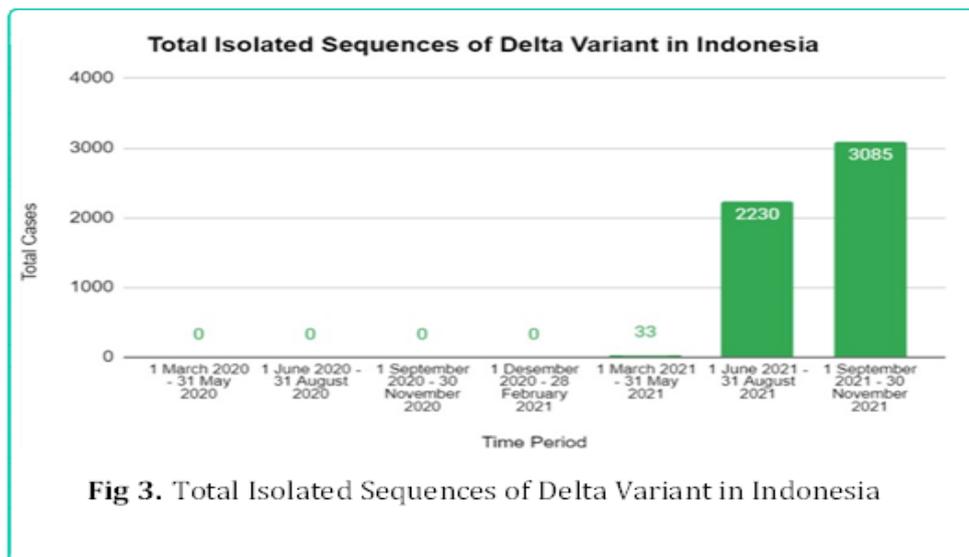
Figure 3 shows delta variants isolated sequences in Indonesia that started being sequenced in Indonesia with 33 isolates during the month of March 2021 until May 2021. This number increased drastically in June 2021 until August 2021 with a total of 2,230 isolated sequences and continued to increase in September 2021 until November 30, 2021 with 3,085 more isolated variants. Table 1 showcases the percentage of all variants prevalence in Indonesia based on data acquired from GISAID.org.



**Fig 1.** Total Isolated Sequences of Alpha Variant in Indonesia



**Fig 2.** Total Isolated Sequences of Beta Variant in Indonesia



**Fig 3.** Total Isolated Sequences of Delta Variant in Indonesia

**Table 1.** Percentage of variants in Indonesia: (PREVALENCE)

Database	Variant	Total	Percentage
GISAID	Delta Variant	5,348	57.75%
	Alpha Variant	78	0.84%
	Beta Variant	22	0.24%
	Gamma Variant	0	0%
	Lambda Variant	0	0%
	MU Variant	0	0%
	Local Variant B.1.470	572	6.18%
	Local Variant B.1.466.2	1,833	19.79%
	Local Variant B.1.1.398	219	2.37%
	Local Variant B.1.459	160	1.73%
	Wild-Type	1,028	11.10%
Total Isolated WGS in Indonesia			9,260

**Comparison of WGS using NCBI Blast**

Using NCBI BLAST, we were able to align sequences to one another for comparison, with the differences between the genome sequences being obtained. Using NCBI BLAST, the reference genome obtained from NCBI NC\_045512.2 The Wuhan genome was compared with the Wild-Type in Indonesia and the variants that were present in Indonesia (as of November 30, 2021) mentioned in the previous section. When the Indonesian alpha Variant EPI\_ISL\_3138832

obtained from GISAID was compared with the reference genome, the sequence alignment showed some mutations and changes in the amino acids between the two sequences. The identical percentage between them was 99.82%. This was performed for the beta, delta, and local variants. Table 2 shows the common mutations that occur in alpha, beta, delta and local variants. There are no number of percentages for the local variants because no previous study was conducted for these variants.

**Table 2.** Detectable Mutations

Alpha (B.1.1.7)		Beta (B.1.351)		Delta (B.1.617.2)		Indonesian Local Variant	
Mutation	%	Mutation	%	Mutation	%	Mutation	%
H69del	94	D80A	87	K77T	0.8	D614G	
V70del	95	D215G	85	T95I	41	P323L	
Y144del	94	L241-	77	G142D	54	Q57H	
E484K	0.2	L242-	78	E156G	88	T77A	
S494P	0.3	L243-	77	F157-	89	T350I	

Alpha (B.1.1.7)		Beta (B.1.351)		Delta (B.1.617.2)		Indonesian Local Variant	
Mutation	%	Mutation	%	Mutation	%	Mutation	%
N501Y	97	P384L	1.2	R158-	88	S126L	
A570D	98	K417N	88	W258L	0.2	G142D	
		E484K	87	K417N	0.4	P681R	
		N5017	87	L452R	97	N439K	
		E516Q	0.3				

### Sequence Comparison between Alpha Variant Isolate in Indonesia and WT from Wuhan

The alpha variant of SARS-CoV-2 B.1.1.7 was first identified in the UK. The BLAST NCBI was used to compare the alpha variant EPI\_ISL\_3138832 with the WT from Wuhan. From this comparison, it was seen that the alpha variant EPI\_ISL\_3138832 from Indonesia had accumulated approximately 20 mutations

which can be seen in Table 4 in the Appendix. Of the mutations, four mutations have the most potential to influence the characteristics of the virus. These four mutations include I68-H69del, V70I, N501Y, and P681H. The mutation N501Y was observed to occur as nucleotide A changes to T. According to (Starr et al. 2020), the N501Y mutation increases the affinity of the spike protein to bind to the hACE2 receptor and thereby increases the transmission rate of the virus.

**Table 3.** Isolates under study and results obtained by BLAST NCBI

Accession ID from GISAID	Location	Date	Mutation Detected	Associated Variant
EPI_ISL_3138832	Indonesia	30 July 2021	T1001I, A1708D, 12330T, SGF3675-3677del, P4619L H69del, V70I, N501Y, A570D, D614G, P681H, T719I, S982A, D1118H ( <b>Spike</b> ) Q27stopm R52I, K68stop, Y37C, ( <b>ORF38</b> )	Alpha variant
EPI_ISL_5462140	Indonesia	25 October 2021	P380L, A1306S, T1496I, P2287S, V2930I, T3255I, K3678R, P4715L, G5063S, P5401L, C6568Y ( <b>ORF1ab</b> ) T19R, L452R, T478K, D614G, D950N ( <b>Spike</b> ) S26L ( <b>ORF3a</b> ) I82T ( <b>M</b> ) V82A, T120I ( <b>ORF7a</b> ) T401 ( <b>ORF7b</b> ) G215C, D377Y ( <b>N</b> )	Delta variant
EPI_ISL_2434175	Indonesia	07 Juni 2021	S2517F, K3353R, K3365R, SGF3675-3677del, M3733V, P4715L, T265I, T6500I ( <b>ORF1ab</b> ) D215G, D614G, A701V, L241-, L242-, K417N ( <b>Spike</b> ) Q57H, A72S ( <b>ORF3a</b> ) Q9H, A90S ( <b>N</b> ) H155Y ( <b>M</b> ) S944L, P1640L, L3644F, P4715L ( <b>ORF1ab</b> ) D614G, N439K, P681R ( <b>spike</b> ) S206T, T205I ( <b>N</b> ) Q57H ( <b>ORF3a</b> )	Beta Variant

Accession ID from GISAID	Location	Date	Mutation Detected	Associated Variant
EPI_ISL_6827341	Indonesia	29 November 2021	I2N ( <b>NS7b</b> ) P822L, S126L, T350I ( <b>NSP3</b> ) L75F ( <b>NSp6</b> ) P323L ( <b>NSP12</b> ) S259L ( <b>NSP13</b> )	Local Variant B.1.466.2

The P681H was also observed. This mutation occurs due to the change of nucleotide C to A. This mutation occurs at the proteolytic cleavage site S protein where it increases the cleavage the S1/S2 junction (Socher et al. 2021). However, it is still unclear whether this affects the infectivity and membrane fusion rate. Another mutation that was of significance and was the nucleotide C changes to A. This mutation induces significant conformational destabilization of the virus (Socher et al. 2021).

Another mutation observed was in the 982nd position where the amino acid S changed to A due to a change in nucleotide from T to G. The effect of this substitution results in the intermolecular hydrogen bonding potential between S protein subunits being missing in the alpha variant (Ostro 2021). The D1118H mutation is another mutation that was observed, which occurs in the alpha variant due to the change of nucleotide G to C. This mutation is present in the S2 subunit in the S protein (Chrysostomou et al. 2021). It has been suggested that this mutation can alter the stability and dynamics of trimer assembly (Zhao et al. 2021). These mutations majorly occur within the S protein. Mutations that occur in the gene encoding the S protein can change how the virus interacts with the hACE2 receptor in humans, thereby increasing the infection rate. However, to date, the alpha variant appears to have little impact on the effectiveness of vaccines.

### Sequence Comparison between Delta Variant Isolate in Indonesia and WT from Wuhan

The SARS-CoV-2 B.1.617.2 delta variant was first identified in India. This delta variant contains 3 sub-lineages, B.1.617.1, B.1.617.2, and B.1.617.3. From the comparison between the WT from Wuhan, it can be seen that the Delta variant contains several mutations throughout its genome, which

can be seen in Table 5 in the Appendix. The identical percentage between the Delta variant and the reference variant was 99.90%. Of all the mutations, some mutations observed raise concerns, including D614G, T478K, L452R and P681R. A specific mutation in the nucleotide from C to G changed the amino acid from proline (P) to arginine (R), P681R. This specific mutation found within the furin cleavage site of the Delta variant causes an enhancement of Delta variant replication through an increase in S1/S2 cleavage (Liu et al. 2021).

Another mutation can be seen at L452R in the Delta variant when the nucleotide changes from T to G. This mutation causes a higher affinity for the S protein towards the ACE2 receptor, and causes a decrease in the recognition capability of the immune system (Mohammadi et al. 2021). Another mutation from A to G caused the amino acid to change from D to G in 614, D614G. The final concerning mutation in the Delta variant is the mutation from C to A which causes the change from T to K in T478K. All these mutations could explain how the Delta variant becomes increasingly more easily spread and becomes the highest number of variants that infect people in Indonesia.

### Sequence Comparison between Beta Variant Isolate in Indonesia and WT from Wuhan

The beta variant was initially discovered in South Africa. The comparison of the beta variant EPI\_ISL\_2434175 from Indonesia isolate with the WT from Wuhan as the reference genome showed multiple mutations across various domains in the genome.

The mutations observed can be seen in Table 6 in the Appendix. The identical percentage between them was 99.89%. Utilizing BLAST NCBI, a comparison of the beta variant isolated from Indonesia and the WT

from Wuhan was conducted to analyze mutations. Out of all the mutations observed, four mutations detected have the most potential to influence the characteristics of the virus, D215G, L241-, L242-, and K417N. The first mutation to be discussed is D215G, which occurs as nucleotide A changes to G. This mutation, located in the N-terminal domain of the spike protein, results in a change in the amino acid produced, from aspartic acid (D) to glycine (G) (Tegally et al. 2021). The next mutation observed is L241-. Here, nucleotides TACTT are deleted in the beta variant, resulting in the deletion of the amino acid leucine (L). Similar to L241-, L242- has the nucleotide TACTT deleted, once again resulting in the deletion of the amino acid leucine (L).

Both L241- and L242- were also observed in the spike protein, located in the N-terminal domain (Tegally et al. 2021). Finally, the last mutation of concern observed was K417N. This mutation once again occurs in the spike protein, and it occurs when the G nucleotide becomes T, resulting in a change in the amino acid from lysine (K) to asparagine (N). K417N has more potential to influence the virus due to the substitution being at key residues of the receptor binding domain of the spike protein, and this region can be used for the binding of neutralizing antibodies (Tegally et al. 2021). Moreover, K417N results in the tightening of the interactions of the receptor binding domain (RBD) with the cellular receptor angiotensin-converting enzyme 2 (ACE2) (Sanches et al. 2021). This mutation at 417 results in an increase in the interaction of ACE2 with the amino acid substitution asparagine (N).

### **Sequence Comparison between Local Variant Isolates in Indonesia and WT from Wuhan**

Table 7 in the supplementary files shows the comparison between the local variant in Indonesia Isolate EPI\_ISL\_2854771 that is identified B.1.470 with the reference genome WT from Wuhan. The identical percentage between them was 99.87%. The Indonesian local variant had 12 synonymous mutations, and 18 nonsynonymous mutations. The spike protein had 3 mutations: S12F, L242F and D614G. The

D614G mutation was also found in this Indonesian isolate caused by the mutation of nucleotides A to G. This mutation, which can also be found in the delta and beta variants, increases infectivity and enhances the replication of SARS-CoV-2 Virus in the upper respiratory tract, induce conformational change in the S1 subunit of S protein, as well as in the SD2 domain where they enhance the cleavage of furin at the S1/S2 domain junction.

Another observed mutation is S12F, present on the hydrophobic residues in the signal peptide sequence. This mutation occurs due to a change in the nucleotide sequence from C to T, and has been theorized to affect the entry of the spike glycoprotein into the endoplasmic reticulum (Timmers et al. 2021). Another observed mutation in the spike protein is L242F due to the change in nucleotides from C to T. T2051 was another mutation seen in the nucleocapsid protein due to a change from C to T. The ORF3a protein was seen to have some mutations as well, including D155H, Q57H, and S220I. This genome was also seen to contain the deletion of SGF in the ORF1a/ORF1ab polyprotein, which was also present in the alpha variant.

The last comparison made in this review paper was the comparison between the local variant in Indonesia Isolate EPI\_ISL\_2854771, which is identified as B.1.466.2, with the reference genome WT from Wuhan which can be seen in Table 8 in the Appendix. The identical percentage between them was 99.83%. The D614G mutation was also found in this isolate, similar to the other variants. Another key mutation observed was N439K. This mutation is able to produce a salt bridge with Glu329 of the hACE2 receptor, with the salt bridge prompting a greater electrostatic complementarity. This mutation also creates a weak salt bridge with Asp442 of the RBD. Another mutation, N439K, was observed. N439K bound to the hACE2 receptor with a higher affinity than the Wild-Type, which may lead to more infection. In addition, this mutation causes resistance to the SARS-CoV-2 neutralizing antibody REGN10987. This may lead to the failure of neutralization (Zhou et al. 2021). Another mutation found in this local variant was P681R which was also previously found

in the delta variant. All these mutations were located in the spike protein. Other mutations found in this variant were S206T, T250I, Q57H, I2N, P822L, S126L, and T350I. A summary of all blasts conducted can be seen in Table 3.

### Responses to Available Vaccines

As of writing this paper, there are 6 approved COVID-19 vaccines, Pfizer-BioNTech (BNT162b2), Moderna (mRNA-1273), Oxford/AstraZeneca (AZD1222), J&J/Janssen (Ad26.COV2.S), Novavax (NVX-CoV2373), Sinovac, and Sinopharm. These vaccines differ from one another in what type of vaccine they are; Pfizer-BioNTech and Moderna are mRNA vaccines, Oxford.AstraZeneca and J&J/Janssen are viral vector vaccines, Novavax is a recombinant spike vaccine, and Sinovac and Sinopharm are inactivated vaccines (Cevik et al. 2021). All of these vaccines were based on the Wild-Type virus sequenced early on during the pandemic (in January 2020) and as such their efficacy and effectiveness towards the newer variants is of utmost importance.

It must be noted that the efficacy of different vaccines cannot truly be compared to one another since the testing conducted depends on the clinical environment, studied population, geographical location, among others (Cevik et al. 2021). Additionally, the real-time effectiveness of vaccines may prove to be challenging due to various factors, such as variant transmissibility and population vaccination status. However, another aspect to consider is the initial antibody responses, which may help with the overall picture of understanding the efficacy and effectiveness of the various vaccines to the various variants of SARS-CoV-2 (Cevik et al. 2021). Finally, it may prove difficult to assess the efficacy and effectiveness as T cells play a major role in protecting the body from infections (Cevik et al. 2021).

To understand the efficacy and effectiveness of the vaccines towards variants of SARS-CoV-2, one must take the structures of the virus into consideration. One structure that is particularly important is the S structure responsible for the spike proteins that is responsible for the virus's ability to enter host cells and is considered the main target of most vaccines (Cevik et al. 2021). Despite

the S structure being the main target, it is not the only target, as vaccines are produced in such a way to incorporate various antibodies and T cells to reduce the risk of a single mutation-, drastically reducing the efficacy and effectiveness of the vaccine (Cevik et al. 2021).

It is worth noting that immunity towards the vaccine should theoretically take a long time. This is mainly because the vaccine acts to prevent infection rather than treating it (Cevik et al. 2021). Regarding the interactions of the vaccine with the various variants, certain amino acid substitutions that occur due to mutations within the S structure receptor-binding domain and well N-terminal domain could negatively affect the effectiveness of the vaccines in regard to antibody neutralization (Cevik et al. 2021).

First, the deletion of the N-terminal domain (NTD) H69/V70, according to a study conducted by Rees-Spear et al., does not affect the neutralization potency of most mAbs (cross-neutralizing monoclonal antibodies) (Rees-Spear et al. 2021). However, the N501Y mutation, which is shared across multiple variants, removes almost all neutralizing activity of mAbs.

Another mutation that is similar to N501Y and could negatively affect the effectiveness and efficacy of vaccines is E484K, which occurs in beta and gamma variants. For this mutation, the reduction for the beta variant was seen to be the biggest, with gamma being only moderately affected. It is worth noting that alpha variants do not have this E484K mutation, which could possibly explain why vaccine efficacy and effectiveness are higher for this variant; the alpha variant shows a slight decrease when tested for antibody neutralization in in-vitro utilizing Oxford/AstraZeneca and Pfizer-BioNTech<sup>22</sup>. The delta variant, similar to the alpha variant, does not contain the E484k mutation, and as such shows a slight reduction in antibody neutralization when tested against Oxford/AstraZeneca and Pfizer-BioNTech. However, it seems that inactivated vaccines (such as Sinovac and Sinopharm) are capable of neutralizing the E484K and N501Y mutations (Tregoning et al. 2021).

Overall, it seems that variants with the mutation N501Y, as well as E484K, result in

a lower antibody neutralization, and that those with both see the most reduction in efficacy and effectiveness, as seen in Beta and Gamma when compared to the other variants (Cevik et al. 2021; Lucas et al. 2021).

## CONCLUSION

In the alpha variant, four mutations need to be considered because they affect the characteristics of the virus, causing an increase in cleavage of S1/S2 and in turn increasing the infection rate. However, the alpha variant does not drastically reduce the efficacy of the vaccines. Moreover, the delta variant mutations can reduce immunity and increase replication, making it easier to spread which could explain why it is the most common variant in Indonesia. Despite this, the efficacy and effectiveness of the vaccine is only slightly reduced by delta. For the beta variant, it was observed that the virus's mutations can bind to the neutralizing antibody, affecting the work of the antibodies and thus reducing the efficacy of the vaccines at a higher level than the other variants.

The local variants B.1.470 and B.1.466.2 were seen to have several shared mutations with other the variants (alpha, beta, and delta variants), with these mutations increasing the infection rate and enhancing the replication of the virus. However, one mutation that can be observed in the B.1466.2 variant may cause more infections, and that mutation is N439K. This mutation can bind to hACE2 with higher affinity than the Wild-Type and can also neutralize antibodies of the vaccines.

Finally, the isolates utilized in this paper were acquired as early as the end of October 2021 until the end of November 2021. It should be noted that the classification of the isolates is not set in stone and is constantly changing with new findings as many isolates are constantly being reclassified on GISAID.org.

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