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Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

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Abstract. Genome analysis is critical for tracking the spread of the CoVid-19 virus throughout Indonesia. Considering that Indonesia is an archipelago, the demographics of each province will create different virus variance results. The genome sequence, virus variants, and phylogenetic description of the SARS-CoV-2 strain from Manokwari, West Papua, are being determined urgently. The entire genome was sequenced using the Reagan Illumina COVIDSeq and the sequencing by synthesis (SBS) method. Annotation of the entire viral genome using the hCoV-19/Wuhan/Hu-1/2019 reference genome (NC 045512.2). Phylogenetic analysis was performed on 54 SARS-CoV-2 genomes obtained from GISAID in Indonesia and other countries. The virus strains found in Manokwari, West Papua, are Delta (B.1.617.2), B.1.466.2, and Omicron (BA.1, BA.1.15, and BA.1.13.1). The SARS-CoV-2 strain from Manokwari is classified into VOC and VUM variants based on the WHO variant classification. According to the results of genetic characterization analysis, the ORF1ab NSP3 gene, ORF3 gene, ORF7 gene, N gene, and S gene had the most mutations. The West Papua strain of SARS-CoV-2 Manokwari has ten clades, with the B.1.466.2 virus variant adjacent to the B.1.466.2 virus variant from Southeast Sulawesi and West Sumatra, according to the phylogenetic analysis of virus variants. The Manokwari West Papua SARS-CoV-2 virus strain has three variants: Delta (B.1.617.2), B.1.466.2, and Omicron (BA.1, BA.1.15 and BA.1.13.1).

Keywords: CoViD-19 · Genetic Characterization · Phylogenetic · Virus Variants

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1 Introduction

The severe acute respiratory syndrome coronavirus two (SARS-CoV-2) virus has spread and increased at an alarming rate since its discovery in mainland China. On March 2, 2020, this virus was detected and caused several reported cases of COVID-19 in Indonesia [1]. Since then, confirmed cases have continued to be investigated, despite several public health measures to stop the spread of COVID-19, such as the isolation of confirmed patients and the implementation of strictly enforced health protocols in the community. As of November 17, 2021, there were 255,103,209 confirmed cases and 5,126,415 deaths, with a COVID-19 incidence of 621,943 per 100,000 people. There are confirmed cases in Indonesia until November 17, 2021, with 4,251,945 people infected and 143,698 deaths [2].

West Papua Province in eastern Indonesia was not spared from the spread of the SARS-CoV-2. There were 23,135 confirmed positive patients until November 17, 2021, with 357 deaths. The rapid spread of SARS-CoV-2 in different regions resulted in mutation-driven evolution. Genomes with new variants emerged when the virus moved across different environmental conditions; thus, genomic surveillance of viral mutations is essential. SARS-CoV-2 mutations have spread faster than the previous strain until now. Furthermore, SARS-CoV-2 mutations are known to be caused by changes in genetic material (in the form of nucleotide bases), which result in phenotypic differences from the original virus [4]. Changes in the nature of the virus can affect how quickly it spreads, the severity of the existing complications, and the efficacy of vaccines, active ingredients, test equipment, and health measures. The phenotypic differences in SARS-CoV-2 caused by mutations are used to identify a variant. Active variants are currently being monitored globally to ensure that if significant amino acid substitutions are discovered, immediate action is taken to respond to these variants and prevent their spread [6]. The SARS-CoV-2 Evolution Working Group (SEWG) of the WHO established three levels of variants based on the level of alertness and the risk of decreased diagnostic, therapeutic, and vaccine efficacy, namely SARS-CoV-2, which has been shown to change in phenotypic properties. Because of mutations that have been identified and spread within a community or detected in multiple countries [6].

Based on Indonesian WGS data as of June 13, 2021 a total of 1,908 complete genome sequences were reported that the new SARS-CoV-2 virus variant in Indonesia was the Variant of Concern, consisting of Alpha (B.117) in 35 cases, Beta (B.1.351) in 5 cases, and Delta (B.1.617.2) in 50 cases. Variant of Interest, consisting of A.23.1 (6 cases), Eta (B.1.525) (4 cases), and B.1.617.3 (1 case). WGS data as of 17 November 2021 increased to 8,856 sequences, with a significant increase from VOC such as alpha (B.117) to 77 cases, beta (B.1.351) (22 cases), and delta (B.1.617.2) (4,977 cases). So far, variant mutations in Indonesia included B.1.621, Lamda, Gamma, Beta, Alpha, Delta, B.1.640, and Omicron (GISAID, 2021).

According to the WGS data, the number of SARS-CoV-2 sequences until November 2021 was still very low (0.2%) when compared to the number of confirmed COVID-19 patients. More variants will be confirmed if the SARS-CoV-2 genome is sequenced further. As a result, it is critical to conduct research on the complete genome sequence and phylogenetics of the SARS-CoV-2 virus collected in Manokwari, West Papua. The

goal of this study was to characterize the complete genome sequence and variants of the SARS-CoV-2 virus Manokwari, West Papua.

2 Methods

2.1 CoViD-19 Samples

All virus samples were obtained from SARS-CoV-2 patients' nasopharyngeal and oropharyngeal swabs at the West Papua Provincial General Hospital.

Viral RNA was extracted from nasopharyngeal samples using the taco extraction kit reagent and following the kit's instructions, which included pipetting 200μl of the sample and inserting it into the sample wells on the extraction plate for 45 min. Following the completion of the extraction process, 200μl of RNA is added to the sample stamp, and the RNA is ready for use.

2.2 CoViD-19 Samples

Whole Genome Sequencing (WGS) was performed on 48 samples in VTM tubes that met the criteria at the Prof. Srie Oemijati National Laboratory, Health Development Policy Agency (BKPK) of the Ministry of Health in Jakarta. The Illumina COVIDSeq reagent and the sequencing by synthesis (SBS) method were used in the WGS process. The procedure begins with RNA extraction, followed by Anneal RNA using random hexamers to prepare for cDNA synthesis. To reverse transcribe the RNA fragments, cDNA synthesis was performed according to the manufacturer's instructions.

The target was amplified in two separate PCR reactions using CPP1 HT primer (COVIDSeq Primary Pool 1 HT) and CPP2 HT primer (COVIDSeq Primer Pool 2 HT). During this process, the preparatory library, assembling, and quantification are carried out step by step, and the amplification product is fragmented and tagged with adapters. The fragment that has been given an adapter will be amplified again in order to multiply the fragments, and the libraries will then be indexed and purified. The libraries were measured using the Qubit High Sensitivity dsDNA Quantification Kit with 4nM quantification requirements after purification. In the flow cell, sequencing libraries will assemble [3].

2.3 Genome Analysis

The hCoV-19/Wuhan/Hu-1/2019 (NC 045512.2) reference genome was used to annotate the full-length viral genome. 54 genomes of the SARS-CoV-2 virus from Indonesia and other countries were used for phylogenetic analysis. This information was obtained from gene Bank (GISAID). Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Kappa (B.1.617.2), Epsilon (B.1.427), Eta (B.1.525), Lota (B.1.526), Lambda (C.37), Mu (B.1.621), Omicron (BA.1), B.1.177, 19B, and B.1.466.2 are the viral variants used. The MAFFT program server (https://mafft.cbrc.jp/alignment/server/) was used for nucleotide sequence alignment. With a nucleotide length of 29,400, a phylogenetic tree is constructed. All analyses were carried out using the Geneious Prime software

(Kumar et al., 2018). The tree is rooted in the oldest virus, hCoV-19/Wuhan/Hu-1/2019 [2], because the goal of this phylogenetic analysis was to determine the evolutionary relationship between virus samples from the West Papua Provincial Hospital and other SARS-CoV-2 viruses.

3 Results

All virus samples were collected from nasopharyngeal and oropharyngeal swabs of patients who received SARS-CoV-2 testing at the West Papua Province General Hospital, with the sample criteria being people who had been vaccinated, had not been vaccinated against SARS-CoV-2 but were infected with COVID-19, and positive with a CT value of 25. RT-PCR testing was performed on the samples at the Molecular Laboratory of the Regional General Hospital of West Papua Province.

Based on the RT-PCR results, a total of 48 samples were used for Whole Genome Sequencing, which was performed at the Prof. Srie Oemijati Research Center, Health Development Policy Agency (BKPK), Ministry of Health in Jakarta. Whole genome sequencing analysis yielded 32 completely sequenced samples. There were 12 women and 20 men among the 32 samples. Meanwhile, there were 19 adults, 2 teenagers, 5 children, and 1 baby. There were 13 people with blood type O, 4 with blood type A, 7 with blood type B, and 3 with blood type AB, with the remaining 5 people having no known blood type. The vaccines used were generally the Sinovac vaccine for 19 people, the AstraZeneca vaccine for 5 people, and the unvaccinated vaccine for 8 people (Table 1).

Various points of mutation were discovered from the ORF1ab NSP3 gene region to the N gene using characterization analysis of the reference genome from Wuhan, China. Furthermore, the number of nucleotide base changes in the SARS-CoV-2 virus, Manokwari strain from West Papua, was determined. When the SARS-CoV-2 virus sequence from the Manokwari strain was compared to other sequences from Papua, several different mutation points are found.

The key mutation in the S gene for the B.1.466.2 virus variant is in the N439K region, while it is in the L452R and P681R regions for the Delta virus variant, and it is in the K417N, S477N, N501Y, and P681H regions for the omicron virus variant. The Delta Strain Manokwari virus variant did not have mutations in T95I, whereas four samples in G142D did, namely samples no 15, 20, 23, and 25. There were no mutations in the E156G and Del157/158 regions other than this Manokwar strain. Meanwhile, the Omicron virus variant (BA.1.13.1) lacks mutations in the DelN211 and G496S regions, and the Omicron virus variant (BA.1.15) lacks mutations in the DelN211 and G496S regions as well N440K and G446S.

Table 1. An investigation of the SARS-CoV-2 virus of Manokwari West Papua

No	ID Viruses	Sex	Age (y.o)	Ct Value	Blood Type	Vaccine Type	Symptoms
1	MKW_EPI_ISL_3151603	F	31	15,74	A	Sinovac	ODP
2	MKW_EPI_ISL_3151608	M	39	18,43	O	Sinovac	ODP
3	MKW_EPI_ISL_3151615	F	48	21,3	O	Sinovac	ODP
4	MKW_EPI_ISL_3151622	F	64	23,13	O	Sinovac	ODP
5	MKW_EPI_ISL_5022912	F	21	19,55	B	Sinovac	ODP
6	MKW_EPI_ISL_3151635	M	41	17,12	O	Sinovac	ODP
7	MKW_EPI_ISL_3151655	M	11	25,26	–	–	ODP
8	MKW_EPI_ISL_3151661	M	15	19,42	–	–	ODP
9	MKW_EPI_ISL_3278295	F	11	18,43	–	–	ODP
10	MKW_EPI_ISL_3151602	F	40	21,04	AB	AstraZeneca	OTG
11	MKW_EPI_ISL_3151607	F	35	19,23	O	Sinovac	OTG
12	MKW_EPI_ISL_3151614	M	38	23,01	O	AstraZeneca	ODP
13	MKW_EPI_ISL_3151621	M	21	19,23	B	Sinovac	OTG
14	MKW_EPI_ISL_3151628	F	30	20,15	A	Sinovac	OTG
15	MKW_EPI_ISL_3151634	M	15	16,19	–	–	OTG
16	MKW_EPI_ISL_3151641	M	35	18,25	O	Sinovac	ODP
17	MKW_EPI_ISL_3151642	M	43	19,47	B	AstraZeneca	OTG
18	MKW_EPI_ISL_3151647	F	14	20,19	–	–	ODP
19	MKW_EPI_ISL_3151648	M	28	23,17	O	Sinovac	ODP
20	MKW_EPI_ISL_3151653	F	18	22,19	O	–	ODP
21	MKW_EPI_ISL_3151654	F	54	20,79	B	Sinovac	ODP
22	MKW_EPI_ISL_3151659	M	35	25,21	AB	Sinovac	OTG
23	MKW_EPI_ISL_3151660	M	1	20,32	–	–	ODP
24	MKW_EPI_ISL_3151667	M	19	18,27	A	Sinovac	OTG
25	MKW_EPI_ISL_3151668	F	27	21,48	O	AstraZeneca	OTG
26	MKW_EPI_ISL_3151674	M	23	23,19	B	Sinovac	ODP
27	MKW_EPI_ISL-3151675	M	31	20,25	O	Sinovac	ODP

ODP: People in monitoring of CoViD-19
OTG: People without symptoms

Table 2. SNP and variant analysis of the SARS-CoV-2 virus strain Manokwari, West Papua

No	ID Virus	SNP	MISSENSE	SILENT	Varian
1	MKW_EPI_ISL_3151603	25	32	21	B.1.466.2
2	MKW_EPI_ISL_3151608	26	37	21	B.1.466.2
3	MKW_EPI_ISL_3151622	26	33	23	B.1.466.2
4	MKW_EPI_ISL_3151655	34	43	38	B.1.466.2
5	MKW_EPI_ISL_3151661	24	37	22	B.1.466.2
6	MKW_EPI_ISL_3278295	30	48	21	B.1.466.2
7	MKW_EPI_ISL_3151602	28	42	22	B.1466.2
8	MKW_EPI_ISL_3151621	29	42	26	B.1466.2
9	MKW_EPI_ISL_3151641	26	37	22	B.1.466.2
10	MKW_EPI_ISL_3151642	27	37	19	B.1.466.2
11	MKW_EPI_ISL_3151647	26	37	20	B.1.466.2
12	MKW_EPI_ISL_3151653	27	41	24	B.1.466.2
13	MKW_EPI_ISL_3151659	26	41	20	B.1.466.2
14	MKW_EPI_ISL_3151660	26	4	23	B.1.466.2
15	MKW_EPI_ISL_5022912	43	60	25	Delta (B.1.617.2)
16	MKW_EPI_ISL_3151635	31	49	9	Delta (B.1.617.2)
17	MKW_EPI_ISL_3151615	39	65	17	Delta (B.1.617.2)
18	MKW_EPI_ISL_3151607	38	66	16	Delta (B.1.617.2)
19	MKW_EPI_ISL_3151614	38	61	13	Delta (B.1.617.2)
20	MKW_EPI_ISL_3151628	33	40	15	Delta (B.1.617.2)
21	MKW_EPI_ISL_3151634	38	60	16	Delta (B.1.617.2)
22	MKW_EPI_ISL_3151648	43	71	22	Delta (B.1.617.2)
23	MKW_EPI_ISL_3151654	43	71	18	Delta (B.1.617.2)
24	MKW_EPI_ISL_3151667	41	65	20	Delta (B.1.617.2)
25	MKW_EPI_ISL_3151668	42	71	20	Delta (B.1.617.2)
26	MKW_EPI_ISL_3151674	36	61	13	Delta (B.1.617.2)
27	MKW_EPI_ISL-3151675	34	43	18	Delta (B.1.617.2)
28	MKW_EPI_ISL-11994142	58	62	23	Omicron (BA.1.13.1)
29	MKW_EPI_ISL-11994116	57	60	28	Omicron (BA.1)
30	MKW_EPI_ISL-11994140	58	62	23	Omicron (BA.1)
31	MKW_EPI_ISL-11994141	57	63	26	Omicron (BA.1.15)
32	MKW_EPI_ISL-11994143	57	61	24	Omicron (BA.1)

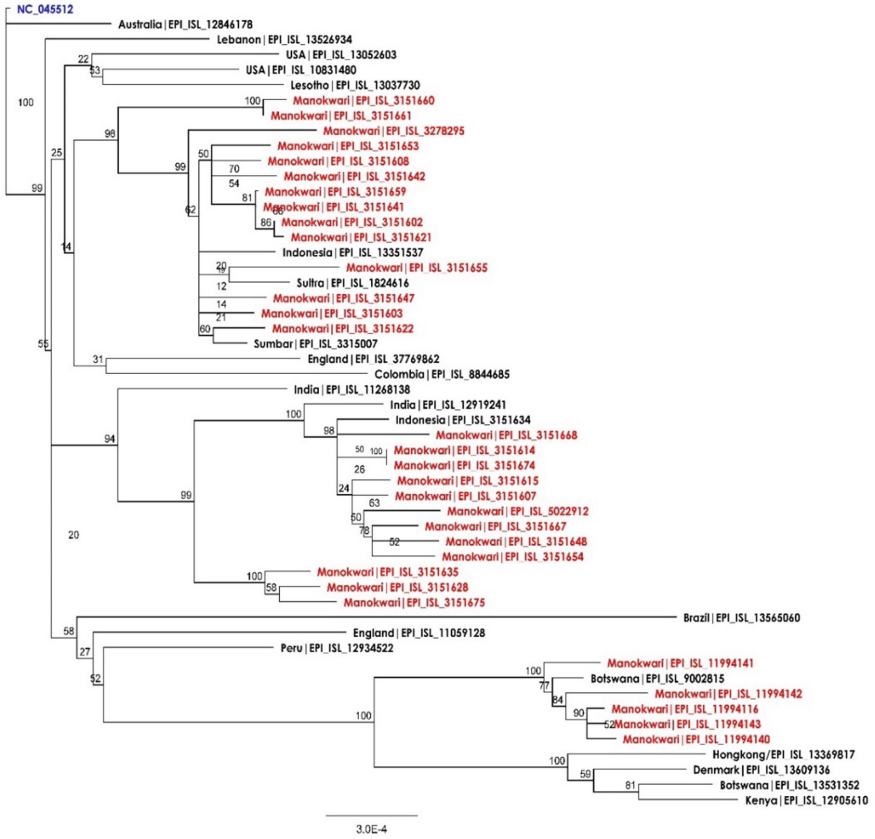

Fig. 1. The West Papua Manokwari Strain SARS-CoV-2 Virus Phylogenetic Tree was reconstructed in Genious Prime software using the Neighbor-Joining method

3.1 Single Nucleotide Polymorphism (SNP) and Virus Variants

SNP and variant analysis was performed using the data bank available at the link (https://usegalaxy.org/welcome/new) and Pangolin version 1.3.1.7. Based on SNP analysis and variance, the SARS-CoV-2 virus sample from Manokwari, West Papua contained three variants, such as Delta (B.1.617.2), B.1.466.2, and Omicron (BA.1, BA.1.15, and BA.1.13.1). According to WHO classifying of variants, the SARS-CoV-2 virus of Manokwari is categorized as VOC and VUM (Table 2).

3.2 Phylogenetic Analysis

GISAID as gene bank provided phylogenetic analysis and a collection of data from 54 genomes of the SARS-CoV-2 virus in Indonesia and several other countries. Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Kappa (B.1.617.2), Epsilon (B.1.427), Eta (B.1.525), Lota (B.1.526), Lambda (C.37), Mu (B.1.621), Omicron (BA.1), B.1.177, 19B, and B.1.466.2 are the viral variants used. The tree is rooted

in the oldest virus, NC 045512.2, and was analyzed using a nucleotide length of 29,903 (Fig. 1).

According to the phylogenetic tree in Fig. 2, the West Papua Manokwari strain's SARS-CoV-2 virus variant consists of 10 clades, with the B.1.466.2 virus variant adjacent to the B.1.466.2 virus variants from Southeast Sulawesi and West Sumatra [1].

4 Discussions

Each life's genetic information is stored in its genome, and annotations are the first step in interpreting its sequence. The SARS-CoV-2 genome is approximately 29,903 kb in length. It also contains 5 structural proteins and 16 non-structural proteins. In this study, we worked to determine the characteristics of the SARS-CoV-2 virus's complete genome sequence in Manokwari, West Papua, from 48 samples that were subjected to whole genome sequencing analysis, 16 of which were not properly sequenced. This was influenced by the lengthy storage period. The delivery procedure.

The West Papua Manokwari virus strain had three variants in the analysis of variance, namely Delta (B.1.617.2), B.1.466.2, and Omicron (BA.1, BA.1.15 and BA.1.13.1). The Delta variant was discovered in India in October 2020, while the B.1.466.2 variant was discovered in Indonesia in November 2020, and the Omicron variant was discovered in Botswana, Africa in early November 2021. (WHO, 2021). According to the WHO variant classification, the SARS-CoV-2 virus, the Manokwari stain, is classified as a Variant of Concern (VOC) and a Variant Under Monitoring (VUM). VOC variants have increased transmission and caused negative changes in the epidemiology of SARS-CoV-2 infection, as well as increased virulence or clinical disease presentation. These variants are classified as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.6172), and Omicron (B.1.6172) (BA.1). The SARS-CoV-2, VUM variant is a variant with genetic changes that are thought to affect the virus's characteristics, with indications that it may pose a risk in the future, but the evidence for a phenotypic or epidemiological impact is currently unclear, it is still being studied, and more research is needed. B.1.466.2, B.1.429, B.1.1.523, B.1.619, B.1.620, B.1.526, B.1.525, B.1.617.1, B.1.318, C.1.2, B.1.640, AV.1, AT.1, P.2, P.3, R.1, B.1.1.519, C.36.3, B.1.214.2, B.1.427, and B.1630 are the VUM variants (WHO, 2021).

Furthermore, there are 1166 SNP, 602 missense, and 668 silent variants (Table 3). The Omicron variant virus sample contained more than 56 SNPs, whereas the B.1.466.2 variant virus sample contained the fewest, namely 26. This finding is consistent with that of (covariants.org, 2022), who discovered that the Omicron variant had the same mutations. More common than the Delta variant, and B.1.466.2 contains over 60 mutations. Following a characteristic analysis with the reference genome from Wuhan, China, it was discovered that the ORF1ab NSP3 gene region contained the most mutations in the ORF3 gene, ORF7 gene, N gene, and S gene. Cytosine-thymine 552 and Guanine-thymine 147 are the most common mutations. Both Cytosine - Thymine and Thymine - Cytosine, are synonyms but Guanine - Thymine mutations can change amino acids [4].

Table 3. Changes in the Nucleotide Bases of the SARS-CoV-2 Virus Manokwari strain, West Papua

No	ID Virus	**Base changes (SNPs)**											
		A-C	A-G	A-T	C-A	C-G	C-T	G-A	G-C	G-T	T-A	T-C	T-G
1	MKW_EPI_ISL_3151603	0	1	0	1	1	20	0	0	1	0	1	0
2	MKW_EPI_ISL_3151608	0	1	0	1	1	18	0	0	3	0	1	0
3	MKW_EPI_ISL_3151615	0	4	0	1	2	17	3	0	9	0	2	1
4	MKW_EPI_ISL_3151622	0	1	0	1	1	20	0	0	2	0	1	0
5	MKW_EPI_ISL_5022912	0	5	0	1	2	19	4	0	9	0	2	1
6	MKW_EPI_ISL_3151635	0	4	1	1	2	12	1	0	4	0	4	1
7	MKW_EPI_ISL_3151655	0	2	1	1	1	21	1	0	2	0	5	0
8	MKW_EPI_ISL_3151661	1	3	0	1	0	15	0	0	3	0	1	0
9	MKW_EPI_ISL_3278295	0	1	0	1	1	19	0	1	4	0	2	1
10	MKW_EPI_ISL_3151602	0	2	0	2	1	18	0	0	3	0	2	0
11	MKW_EPI_ISL_3151607	0	5	0	1	2	14	2	0	8	0	2	4
12	MKW_EPI_ISL_3151614	0	5	0	1	2	17	2	0	8	0	2	1
13	MKW_EPI_ISL_3151621	0	2	0	2	1	18	0	0	3	0	3	0
14	MKW_EPI_ISL_3151628	0	4	0	1	2	13	3	0	6	0	3	1
15	MKW_EPI_ISL_3151634	0	4	0	1	2	17	3	0	8	0	2	1
16	MKW_EPI_ISL_3151641	0	2	0	1	1	17	0	0	3	0	2	0
17	MKW_EPI_ISL_3151642	0	1	0	1	1	19	0	0	3	0	2	0
18	MKW_EPI_ISL_3151647	0	1	0	1	1	19	1	0	2	0	1	0
19	MKW_EPI_ISL_3151648	0	5	0	2	2	17	5	0	9	0	2	1
20	MKW_EPI_ISL_3151653	0	1	0	1	1	18	2	0	2	1	1	0
21	MKW_EPI_ISL_3151654	0	6	0	2	2	15	4	1	8	0	4	1
22	MKW_EPI_ISL_3151659	0	2	0	1	1	18	0	0	3	0	1	0
23	MKW_EPI_ISL_3151660	1	4	0	1	0	16	0	0	3	0	1	0
24	MKW_EPI_ISL_3151667	0	7	0	2	2	16	3	0	8	0	2	1
25	MKW_EPI_ISL_3151668	1	2	0	1	2	16	4	0	10	0	3	1
26	MKW_EPI_ISL_3151674	0	5	0	1	2	17	2	0	8	0	2	1
27	MKW_EPI_ISL-3151675	0	5	0	1	2	14	2	0	5	0	4	1
28	MKW_EPI_ISL-11994142	2	7	3	6	1	19	9	1	2	1	4	3
29	MKW_EPI_ISL-11994116	2	7	3	6	1	18	9	1	2	1	4	3
30	MKW_EPI_ISL-11994140	2	7	3	6	1	19	9	1	2	1	4	3
31	MKW_EPI_ISL-11994141	2	8	3	6	1	18	7	1	2	1	6	2
32	MKW_EPI_ISL-11994143	2	7	3	6	1	18	9	1	2	1	4	3

This finding is similar to the findings of Fibriani et al. (2021) in Bandung, who discovered mutations in the Delta variant (B.1.617.2), variant B.1.466.2, and variant B.1.470, with the most mutations in the genomic regions of spike protein, NSP3, nucleocapsid, NSP12, and ORF3a. A new mutation occurs in the E region of the gene for the Omicron variants (BA.1, BA.1.15, and BA.1.13.1). Because of this new mutation, the virus is no longer recognized by the majority of N-terminal domain (NTD) antibodies and receptors. RBDs are RNA-binding domains [5]. The envelope membrane protein (E) is a group of relatively small viral proteins that aid in the morphogenesis, assembly, and release of virions [6]. The Omicron variant has a much higher presentation in Indonesia than the Delta variant, according to data on (GISAID, 2022) on 23 May 2022, the Omicron variant reported in Indonesia is 11,283 while the Delta variant is 8,689, which is consistent with what was predicted by [7] in his research, which stated that the SARS-CoV-2 Omicron variant quickly replaced the Delta variant and spread throughout the world. 11,283 while the Delta variant is 8,689, which is consistent with what was predicted by [7] in his research, which stated that the SARS-CoV-2 Omicron variant quickly replaced the Delta variant and spread throughout the world, When compared to previous virus variants, the clinical symptoms of the Omicron variant were milder, as evidenced by a lower rate of hospitalization at the Hospital [8].

This finding is similar to the findings of Fibriani et al. (2021) in Bandung, who discovered mutations in the Delta variant (B.1.617.2), variant B.1.466.2, and variant B.1.470, with the most mutations in the genomic regions of spike protein, NSP3, nucleocapsid, NSP12, and ORF3a. A new mutation occurs in the E region of the gene for the Omicron variants (BA.1, BA.1.15, and BA.1.13.1). Because of this new mutation, the virus is no longer recognized by the majority of N-terminal domain (NTD) antibodies and receptors. RBDs are RNA-binding domains [5]. Random mutation followed by selection and recombination can cause genetic variation in the SARS-CoV-2 genome [9]. The high number of mutations in the S gene, particularly in the receptor binding domain (RBD) and the N-terminal domain (NTD), is most likely due to substitution selection that promotes viral adaptation, namely, changes in the structure of the spike protein that result in an increase in the host receptor or not being recognized. Thanks to antibodies [9]. Deletions may also occur as a result of selection for resistance to neutralizing antibodies [9]. Selection also causes the emergence of new mutations in other genes; for example, mutations in the E gene have been linked to increased immune shedding via suppression of the host's innate immune response [10].

For the SARS-CoV-2 virus variant, there are several key point mutations in the Spike protein; for the B.1.466.2 virus variant, it is located at N439K. Mutations in this region are linked to fitness and virulence, and they may confer a higher binding affinity to the human ACE2 receptor, allowing antibody-mediated immunity to escape [11]. The key mutation point in the Delta variant is at L452R, which is associated with increased binding to ACE2 and neutralizing antibody resistance, resulting in faster virus replication and increased virus transmission [9]. A Delta virus variant with mutations in the T19R, G142D, E156G region, and two deletions of F157 and R158 within the NTD has altered monoclonal antibody (mAb) recognition sites recognized by loops N1 and N3, allowing the virus to evade the antibody response [12]. In RBD, there are 11 mutations in the Omicron variant: G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A,

Q493R, Q498R, and N501Y. This mutation causes the virus to contribute significantly to changing the spectrum of the SARS-CoV-2 host in avoiding the immune response, as well as making the virus more infectious than the Delta variant [13]. While Omicron has mutations in the S375F, N501Y, P681H, D796Y, N764K, and N969K regions, resulting in a lower binding affinity for Spike-ACE2 compared to the Delta variant, Omicron (BA.2) has a strong binding affinity for Spike-ACE2 [14].

Although the SARS-CoV-2 mutation appears in the same gene, it indicates the virus's evolution to the vaccination program. In studies [15] and [16], the efficacy of two doses of BNT162b2 vaccine and chadox1 ncov-19 vaccine against Omicron was significantly lower than Delta; however, booster doses of mRNA vaccines have been shown to partially restore protection against Omicron, according to studies of neutralizing antibodies [9]. According to the phylogenetic tree in Fig. 9, the West Papua Manokwari strain of the SARS-CoV-2 virus has ten clades. Based on the virus variant, the West Papua Manokwari strain of the SARS-CoV-2 virus is divided into three large clades. The first clade is a variant of the B.1.466.2 virus in which the sample isolates with the ID EPI ISL 3151660 and EPI ISL 3151661 are identical, with a 100% bootstrap value. Based on this, those infected with EPI ISL 3151660 are the same as those infected with EPI ISL 3151661, and isolate samples with ID EPI ISL 3151602 and EPI ISL 3151621 have the same bootstrap value of 86%. When looking at the transmission in this first clade, which the epidemiological path of the virus that infects Manokwari, West Papua, generally passes through West Sumatra and Southeast Sulawesi.

The second clade is a Delta virus variant (B.1.617.2) in which the sample isolates have the same ID, EPI ISL 3151614 and EPI ISL 3151674, with a bootstrap value of 100%. Based on this, those infected with EPI ISL 3151614 are the same as those infected with EPI ISL 3151674, with the second clade still adjacent to the Indian Delta virus variant. The third clade is an Omicron virus variant in which samples of the West Papua Manokwari strain Omicron virus variant are still similar to the Botswana Omicron virus variant [17].

According to the guidelines of the Minister of Health Decree No HK.01.07/MENKES/4842/2021, it is necessary to continue identifying and characterizing the SARS-CoV-2 virus variant in a more widespread and consistent manner, both in terms of quantity and time of implementation.

5 Conclusion

The Manokwari West Papua virus SARS-CoV-2 strain has three variants such as Delta (B.1.617.2), B.1.466.2, and Omicron. The regions with the most mutations were the ORF1ab NSP3 gene region, the ORF3 gene, the ORF7 gene, the N gene, and the S gene, while the Omicron variant had a new mutation in the E gene.

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Authors' Contributions. Conceptualization: MK and MF; Methodology: MK, MF, HH, AP, and RU, Formal analysis and validation: MK, MF, AS, EM; Preparation: NCA, NT; Writing: MK, MF, and RD.

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AUTHORS

211 authors

Aisah, Siti
The Duration of Using ARV Therapy and the Incidence of Anemia in People Livin with HIV/AIDS (PLWHA)

Alfina, Eka Maulidya
The Effectiveness of Project-Based Learning Injection Skill Towards Self-confidence of First Level Students at Stikes Hang Tuah Surabaya

Allenidekania
Application of Katherine Kolcaba's Comfort Theory in Overcoming Fatigue in Children with Cancer Undergoing Chemotherapy

Amini, Puji Prastyaning
The Effeck PRIMA Supervision as a More Humanist and Professional Approach in Improving Nurse Performance

Amir, Nurul Cahyani
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Andini, Ratna Dylla
Effectiveness of E-Health Emergency Neonates on Increasing Mother Awareness

Anggraini, Novita Nining
Psychoeducation Effectiveness of Parenting Patterns in Stunting Prevention: A Literature Review

Anggraini, Novita Nining
Overview of Sources of Information and Knowledge About Self-Breast Examination (Sadari) and Ingenious Programs in Non-communicable Disease (PMT) Cadres

Anissa, Sasa
Spiritual Caring of Nurse's at Patient Departemen in the Pandemi Covid 19 of RSI Sultan Agung Semarang

Antari, Arlita Leniseptaria
Analysis of Tumor Necrosis Factor-Alpha Levels and Nasal Mucociliary Clearance Time between "Biosmart and Safe Bus" and Regular Bus Passengers

Aprilia, Zesi
Application of Katherine Kolcaba's Comfort Theory in Overcoming Fatigue in Children with Cancer Undergoing Chemotherapy

Arief, Yuni Sufyanti

Factors Influencing Nutritional Practice of Mothers with Stunted Children

Arman, Stefan
Analysis of Tumor Necrosis Factor-Alpha Levels and Nasal Mucociliary Clearance Time between “Biosmart and Safe Bus” and Regular Bus Passengers

Aryani, Hasna Try
Psychoeducation Effectiveness of Parenting Patterns in Stunting Prevention: A Literature Review

Astuti, Nur Muji
Diabetic Foot Deformity Examination in Diabetics at Surabaya, Indonesia

Baidhowy, Arief Shofyan
Nursing Care in Acute Decompensated Heart Failure (ADHF) Based on Levine’s Conservation Model: A Case Study

Bakar, Abu
Development of an Android-Based Wise (Wound Internet Assessment) Application for Assessing the Condition Status of Diabetic Ulcer Patients

Bangun, Mediana
Application of Katherine Kolcaba’s Comfort Theory in Overcoming Fatigue in Children with Cancer Undergoing Chemotherapy

Bashori, Khoiruddin
The Role of Continuing Education on Elderly Memory: Islamic Perspective

Basuki, Rochman
The Role of Continuing Education on Elderly Memory: Islamic Perspective

Budiarti, Astrida
The Relationship Between Spirituality, Coping, and Quality of Family Life in Caring for a Schizophrenic Patient at Menur Psychiatric Hospital

Budiarti, Astrida
The Relationship Between Communication Patters and Parental Closeness with Adolescent Sexual Behavior at Privat Vocational School in Sidoarjo

Damayanti, Fitriani Nur
The Comparison of the Service Gap in Midwife Practice Between Village and City

Damayanti, Fitriani Nur

Psychoeducation Effectiveness of Parenting Patterns in Stunting Prevention: A Literature Review

Damayanti, Fitriani Nur
Overview of Sources of Information and Knowledge About Self-Breast Examination (Sadari) and Ingenious Programs in Non-communicable Disease (PMT) Cadres

Darmawati, Sri
Analysis of Neonatal Hyperbilirubinemia Caused by Variations in the G6PD Gene Sequence at RSIA Qurrata A'yun Samarinda

Darmawati, Sri
Trend of Wound Healing Research Across in Indonesia Medical Plasma Activated Natural Substance as Wound Healing: A Systematic Review

Dewi, Maria Ulfah Kurnia
Effectiveness of E-Health Emergency Neonates on Increasing Mother Awareness

Dewi, Ratnasari
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Dhamanik, Reina
Psychological Intervention for Lactation Period in Postpartum Mother: Systematic Review

Dhani, Eno Noventa Rahma
Effectiveness of E-Health Emergency Neonates on Increasing Mother Awareness

Endarti, Dwi Wahyu
The Relationship Between Communication Patters and Parental Closeness with Adolescent Sexual Behavior at Privat Vocational School in Sidoarjo

Ernawati, Ernawati
The Duration of Using ARV Therapy and the Incidence of Anemia in People Living with HIV/AIDS (PLWHA)

Erwin
Coping Among Married Couple with Cancer Survivor Partner

Erwin
Nursing Care in Acute Decompensated Heart Failure (ADHF) Based on Levine’s Conservation Model: A Case Study

Fani, Rifatul
Supportive Educative Model Based on Theory of Planned Behavior to Adolescent Sedentary Life

Farida, Imroatul
Diabetic Foot Deformity Examination in Diabetics at Surabaya, Indonesia

Fazri, Muhamad
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Fitryasari, Rizki
Relationship Between Family Functioning and Burden Among Family Caregivers of Schizophrenia Patients in Menur Mental Hospital Surabaya Indonesia

Hambali, Anthony Sevan
Analysis of Tumor Necrosis Factor-Alpha Levels and Nasal Mucociliary Clearance Time between “Biosmart and Safe Bus” and Regular Bus Passengers

Hapsari, Hana
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Harti, Agnes Sri
The Effectiveness of Galenic Cream of Seromucoid of Snail and Chitosan for Chronic Wound Treatment

Hartiti, Tri
Spiritual Caring of Nurse’s at Patient Departemen in the Pandemi Covid 19 of RSI Sultan Agung Semarang

Hastuti, Apriyani Puji
Factors Influencing Nutritional Practice of Mothers with Stunted Children

Hastuti, Apriyani Puji
Supportive Educative Model Based on Theory of Planned Behavior to Adolescent Sedentary Life

Hastuti, Puji
The Relationship Between Communication Patters and Parental Closeness with Adolescent Sexual Behavior at Privat Vocational School in Sidoarjo

Herawati, Tuti

Nursing Care in Acute Decompensated Heart Failure (ADHF) Based on Levine's Conservation Model: A Case Study

Hidayati, Eni
Theory Analysis: Dorothy E. Johnson (Behavioral Theory) According to the Chinn and Kramer Model

Hidayati, Eni
Caregivers' Experiences: Psychosocial Problem of the Elderly in Nursing Home-A Qualitative Study

Huda, Nurul
Coping Among Married Couple with Cancer Survivor Partner

Ihsannudin, Muhammad
The Effect of Deep Breath Relaxing and Reading the Qur'an on the Stress Level of Adolescents in Karanganyar, Indonesia

Imamah, Nur Fithriyanti
Husband and Healthcare Provider Support Toward the Prenatal Anxiety in Indonesia

Indari
Factors Influencing Nutritional Practice of Mothers with Stunted Children

Indrawan, Alfian Bayu
Development of an Android-Based Wise (Wound Internet Assessment) Application for Assessing the Condition Status of Diabetic Ulcer Patients

Indrawati, Nuke Devi
Midwives' Knowledge About Antenatal Care Services During the Covid-19 Pandemic

Indrawati, Nuke Devi
Effectiveness of E-Health Emergency Neonates on Increasing Mother Awareness

Irmawati, Alfita Ayu
The Comparison of the Service Gap in Midwife Practice Between Village and City

Ismail, Suhartini
Theory Analysis: Dorothy E. Johnson (Behavioral Theory) According to the Chinn and Kramer Model

Ismiwiranti, Ratri
Differences in Anxiety and Pain Levels in Endoscopic Patients Before and After the Provision of Information Control

Istiana, Siti
The Impact of Pregnancy with Covid-19 on Ashpyxia in Newborn Infant: A Litera Review

Istiana, Siti
4T Risk Factors on Pregnancy and Labor

Kamaruddin, Mudyawati
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Kamaruddin, Mudyawati
Analysis of Neonatal Hyperbilirubinemia Caused by Variations in the G6PD Gene Sequence at RSIA Qurrata A'yun Samarinda

Kamaruddin, Mudyawati
Trend of Wound Healing Research Across in Indonesia Medical Plasma Activated Natural Substance as Wound Healing: A Systematic Review

Kariasa, I. Made
Roy Adaptation Model in Perioperating Nursing Management of Patients with HNP L5-S1 Post Microdisectomy Operation: A Case Study

Khasanah, Umi
The Knowledge of Midwives About Poedji Rochjati’s Scorecard

Khasanah, Umi
The Effect of Deep Breath Relaxing and Reading the Qur’an on the Stress Level of Adolescents in Karanganyar, Indonesia

Khasanah, Uzlifatul Khisbiyatul
Diabetic Foot Deformity Examination in Diabetics at Surabaya, Indonesia

Khiaokham, Lawitra
Psychoeducation Effectiveness of Parenting Patterns in Stunting Prevention: A Literature Review

Kurnia, Anna
Roy Adaptation Model in Perioperating Nursing Management of Patients with HNP L5-S1 Post Microdisectomy Operation: A Case Study

Kurniati, Ika Dyah
Risk Factors Associated of Pediculosis Capitis Among Elementary School, Semarang City, Indonesia

Kurniawan, Ardhiles Wahyu
Supportive Educative Model Based on Theory of Planned Behavior to Adolescent Sedentary Life

Kurniawati, Ninuk Dian
Anxiety and Community Compliance of the Covid-19 Health Protocol: Implementation in Ponorogo Regency

Kusumawati, Erna
The Impact of Pregnancy with Covid-19 on Ashpyxia in Newborn Infant: A Literature Review

Kusumawati, Erna
4T Risk Factors on Pregnancy and Labor

Latief, Hilman
The Role of Continuing Education on Elderly Memory: Islamic Perspective

Latifah, Hamnah
The Importance of Mother-in-law in Mental Health of Infertile Women During Fertility Treatment Period

Lutfitasari, Ariyani
Maternal Risk Factors for Low Birth Weight

Lutfitasari, Ariyani
The Effect of Deep Breath Relaxing and Reading the Qur'an on the Stress Level of Adolescents in Karanganyar, Indonesia

Machmudah
Psychological Intervention for Lactation Period in Postpartum Mother: Systematic Review

Machmudah, Machmudah
Husband Support Improves Maternal Fetal Attachment

Mahmudah
The Effectiveness of Project-Based Learning Injection Skill Towards Self-confidence of First Level Students at Stikes Hang Tuah Surabaya

Mariyam
The Effect of Building Block Therapeutic Play Program on Preschooler's Anxiety Levels in Indonesia

Mariyam, Mariyam
Parents' Role in Optimizing Preschool-Age Children Vision

Maryani, Era
Analysis of Genetic Characterization and Phylogenetic of SARS-CoV-2 from Manokwari West Papua

Meivitaningrum, Rahmawati Nur
Risk Factors Associated of Pediculosis Capitis Among Elementary School, Semarang City, Indonesia

Mubin, Mohammad Fatkul
Caregivers' Experiences: Psychosocial Problem of the Elderly in Nursing Home-A Qualitative Study

Mufarokhah, Hanim
Supportive Educative Model Based on Theory of Planned Behavior to Adolescent Sedentary Life

Mulyanti, Lia
Maternal Risk Factors for Low Birth Weight

Mulyanti, Lia
Psychoeducation Effectiveness of Parenting Patterns in Stunting Prevention: A Literature Review

Mulyatsih, Enny
Roy Adaptation Model in Perioperating Nursing Management of Patients with HNP L5-S1 Post Microdisectomy Operation: A Case Study

Munika, Siska
Anxiety and Community Compliance of the Covid-19 Health Protocol: Implementation in Ponorogo Regency

Muniroh, Muflihatul
Analysis of Tumor Necrosis Factor-Alpha Levels and Nasal Mucociliary Clearance Time between "Biosmart and Safe Bus" and Regular Bus Passengers

Muslimah
Analysis of the Quality of Life of Stroke Patients with the EQ-5D-5L Method at the Tlogosari Kulon Health Center

Mustika, Dian Nintyasari

The Importance of Mother-in-law in Mental Health of Infertile Women During Fe Treatment Period

Mustika, Dian Nintyasari
Midwives' Knowledge About Antenatal Care Services During the Covid-19 Pandemic

Mustofa, Khalid
Trend of Wound Healing Research Across in Indonesia Medical Plasma Activated Natural Substance as Wound Healing: A Systematic Review

Mutiasari, Hana
Nursing Care in Acute Decompensated Heart Failure (ADHF) Based on Levine's Conservation Model: A Case Study

Mutyah, Diyan
The Effectiveness of Project-Based Learning Injection Skill Towards Self-confidence of First Level Students at Stikes Hang Tuah Surabaya

Nasriati, Ririn
Anxiety and Community Compliance of the Covid-19 Health Protocol: Implementation in Ponorogo Regency

1 2 3 >

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Dear Mudyawati Kamaruddin

Congratulation!

We are pleased to inform you that your abstract with entitled "**Characterization of Genome Sequences of The SARS-CoV-2 From Manokwari, West Papua Indonesia**" have been successfully accepted in the 1st Lawang Sewu International Symposium on Health Sciences 2022 organized by Universitas Muhammadiyah Semarang, Indonesia. Further information will be given through email.
Should you have any other inquiries related to this confrence, please do not hesitate to contact us.

Regards,

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