Whole-genome-scale analysis of circulating SARS-CoV-2 during the first COVID-19 wave in an international tourist destination, Bali, Indonesia

by Universitas Warmadewa Admin

Submission date: 14-Dec-2023 10:16AM (UTC+0700)
Submission ID: 2258432458
File name: document.pdf (669.71K)
Word count: 4508
Character count: 24017

ORIGINAL ARTICLE
Bali Medical Journal (*Bali MedJ*) 2023, Volume 12, Number 2: 1484-1489
P-ISSN.2089-1180, E-ISSN: 2302-2914

Whole-genome-scale analysis of circulating SARS-CoV-2 during the first COVID-19 wave in an international tourist destination, Bali, Indonesia

Sri Masyeni[1,2*], Duwi Sumohadi[1,3], Krisna Duta[1,3], Ida Ayu Mahayani[1,4], Komang Parwata[5], Agus Eka Darwinata[6], Harapan[7,8,9]

ABSTRACT

Background: The emergence of severe acute respiratory syndromes coronavirus 2 (SARS-CoV-2) variants such as B.1.17 (alpha), B.1351 (beta), and B.1.617.2 (delta) have caused a significant rise in coronavirus disease 2019 (COVID-19) cases worldwide. In response, the Indonesian government imposed restrictions on international and domestic travel, especially in areas with a high number of COVID-19 cases including Java Island and Bali. The aim of this study was to investigate the mutation patterns of indigenous SARS-CoV-2 strains in Bali.

Methods: We conducted whole-genome sequencing on isolates collected from COVID-19-confirmed patients at the end of the first wave (January – March 2021). The sequencing was carried out at the 1st BASE Pte Ltd Laboratory (Singapore) using Oxford Nanopore Platform (GridION) using IDT ARTIC nCoV-2019 V3 Panel. The phylogenetic tree was constructed on MEGA 7.0 with hCoV-19/Wuhan/WIV04/2019 as the genomic reference.

Results: Our data revealed that none of the isolates were variants of concern. Among the five lineages tracked, the viral isolates were most likely from B.1.466.2 (n=4). All viruses had D614G mutation in the spike protein with clade GH being most predominant (n=10) and followed by GR (n=1) and O (n=1). Additionally, mutations at NS3, NSP3, NSP12, and NSP6 were found.

Conclusion: At the end of the first COVID-19 wave in Bali, variants of concern were not detected which could be attributed to the heavy mobility restrictions.

Keywords: Bali, B.1.466.2, D614G, genomic surveillance, Indonesia.
Cite This Article: Masyeni, S., Sumohadi, D., Duta, K., Mahayani, I.A., Parwata, K., Darwinata, A.E., Harapan. 2023. Whole-genome-scale analysis of circulating SARS-CoV-2 during the first COVID-19 wave in an international tourist destination, Bali, Indonesia. *Bali Medical Journal* 12(2): 1484-1489. DOI: 10.15562/bmj.v12i2.4211

[1]Department of Internal Medicine, Faculty of Medicine and Health Sciences Universitas Warmadewa, Denpasar, Bali 80235, Indonesia;
[2]Department of Internal Medicine, Sanjiwani Hospital, Gianyar, Bali 80235, Indonesia;
[3]Tabanan Hospital, Tabanan, 82121, Indonesia;
[4]Mangusada Hospital, Mangusada, 80351, Indonesia;
[5]Klungkung Hospital, Semarapura, 80714, Indonesia;
[6]Department of Microbiology, Faculty of Medicine, Universitas Udayana, Denpasar, Bali 80232, Indonesia;
[7]Medical Research Unit, School of Medicine, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia;
[8]Tropical Disease Centre, School of Medicine, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia;
[9]Department of Microbiology, School of Medicine, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia;

*Corresponding author:
Sri Masyeni;
Department of Internal Medicine, Faculty of Medicine and Health Sciences Universitas Warmadewa, Denpasar, Bali 80235, Indonesia;
masyeniputu@yahoo.com

Received: 2023-02-25
Accepted: 2023-04-19
Published: 2023-05-08

INTRODUCTION

In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged and have caused coronavirus disease 2019 (COVID-19) pandemic and associated with significant health problem in Indonesia.[1,2] In comparison to the previous two coronaviruses (MERS-CoV and SARS-CoV), the infectivity of SARS-CoV-2 is the highest but has a lower mortality rate.[1] The clinical spectrum of COVID-19 ranging from being asymptomatic to developing acute respiratory distress and systemic involvements.[3] Treatment of this disease was and remains a major challenge, attributed to the lack of hospital infrastructure and absence of specific antiviral.[4-6] Countries in tropical regions, such as Indonesia, is prone to COVID-19 coinfection with dengue increasing its health burden.[7] Hence, strict COVID-19 regulations coupled with genomic surveillance of SARS-CoV-2 are required to suppress the pandemic waves in Indonesia.

The first laboratory-confirmed COVID-19 cases in Indonesia were officially reported on March 2, 2020, transmitted from a Japanese traveler.[8] A few weeks later on March 20, 2020, the cases were no longer solely from importation, but also local transmission indicating the SARS-CoV-2 outbreak in Indonesia.[8] Despite these data, earlier importation of COVID-19 cases might occur since two cities in Indonesia, Jakarta and Denpasar, were found among global top high-risk cities which ranked 29th and 11th, respectively.[9] Denpasar is located in a tourism hotspot province of Indonesia, Bali, which receives intense frequency of international tourists.

Following the active and local human-to-human transmission of SARS-CoV-2, Indonesian government closed its border for foreign travelers while nationally imposing the COVID-19 restrictions to curb the pandemic.[10] These restrictions were then temporarily lifted. Steady rise of confirmed COVID-19 cases along with the emergence of new and more infectious SARS-CoV-2 strains, Indonesia closed its border once again from January to March 2022.[11]

Genomic epidemiology data could be used to assess the impact of government's responses toward the SARS-CoV-2 transmission. For example, a year of genomic surveillance in Africa revealed major importation of variants of concern (VOC) and variants of interest (VOI) was due to the lack of mobility restriction.[12] Moreover, whole genome sequencing could inform the mutation dynamics of SARS-CoV-2. Unfortunately, available published literatures reporting whole genome sequencing of SARS-CoV-2 in Indonesia only covered Java Island and Sulawesi Island, or immunocompromised population.[13-15] Herein, we performed whole-genome sequencing on SARS-CoV-2 positive samples collected from Bali from January to March 2021. The isolates were categorized into clades following the nomenclature Global Initiative on Sharing All Influenza Data (GISAID) proposed. The SARS-CoV-2 virus reference strain is assigned to clade L, while others were assigned to clades S, V, G, GH, GR, and GV.[16,17] Clade S members have L84S mutation at NS8, while clade V members have L37F mutation at NSP6 and G251 mutation at NS3.[16,17] As for SARS-CoV-2 strains with D614G mutation in the spike protein, they belong to clade G.[16,17] Additional mutations other than D614, namely NS3-Q57H, N-G204R and S-A222V categorize the strains into GH, GR and GV, respectively.[16,17] Genomes that could not be categorized in the foregoing major clades is included in clade O.[16,17] Phylogenetic tree analysis was also carried out to comprehend the mutation of the SARS-CoV-2 isolates in comparison with its ancestors.

METHODS

Sample selection

Oropharyngeal swab samples from confirmed COVID-19 based on real-time polymerase chain reaction (RT-PCR) using COVID-19 genesig® real-time PCR kit (Primerdesign Ltd., UK) were collected. The selection of the samples was conducted at four different centers: Biology Molecular Laboratory of Universitas Warmadewa, Tabanan Hospital, Mangusada Hospital, and Klungkung Hospital. Low cycle threshold (Ct) values on RT-PCR, indicating high viral load, were a considering factor of the sample inclusion. Written informed consent was obtained from all patients.

Whole genome sequencing

As much as 150 µL samples were treated for genomic RNA extraction using Viral Nucleic Acid Extraction Kit II (Geneaid, VR300). All samples were processed using ARTIC Network Protocols (nCoV-2019 sequencing protocol v3 (LoCost)). The RNA samples were directly used in cDNA synthesis using LunaScript™ RT SuperMix Kit (E3010L, New England BioLabs, Ipswich, MA, USA). The mixture contained 16 µL LunaScript SuperMix and 4 µL RNA samples.

Multiplex PCR was performed using the cDNA samples. Two PCR reactions (Pool 1 and Pool 2) were used per sample using primers from IDT ARTIC nCoV-2019 V3 Panel as described elsewhere.[18] PCR conditions were as follows; 12.5 µL Q5 Hot Start High-Fidelity 2x Master Mix (M0493L, New England BioLabs, Ipswich, MA, USA), 4 µL V3 Pool 1 (10uM) (Pool 1)/V3 Pool 2 (10µM) (Pool 2), 6 µL Nuclease-free water, and 2.5 µL cDNA. PCR cycling conditions were: 98°C for 30s followed by 35 cycles of 98°C for 15 s and 65°C for 5 min.

The two PCR products were combined for a total volume of 50 µL. The combined sample then proceeded to PCR clean-up using 50 µL Agencourt AMPure XP (A63881, Beckman Coulter, Indianapolis, IN, USA). The cleaned-up samples were then quantified using Qubit dsDNA HS kit (Q32851, ThermoFisher, Carlsbad, CA, USA) and 100 ng was taken forward to library preparation using SQK-LSK109 with EXP-NBD104 (1-12) & EXP-NBD114 (13-24) for multiplexing samples using different barcode. Details protocols can be found elsewhere.[18]

Phylogenetic analysis

Data of SARS-CoV-2 sequences available until March 2021 were downloaded from GISAID, where they were subsequently aligned on MAFFT v.7.309, as implemented in Geneious v.10.1.3.[19,20] Phylogenetic tree was then constructed to depict the relationship between the SARS-CoV-2 viruses from Bali and other places with the maximum likelihood-based phylogenetic tree was estimated using Randomized Axelerated Maximum Likelihood (RAxML) v.7.2.8.[21,22] Reconstructions of phylogenetic tree were performed on MEGA 7.0. visualization using FigTree v1.4.3 was used for the resultant phylogenies.

RESULTS

SARS-CoV-2 obtained from all samples along with mutations are presented in Table 1. Among the 12 patients, 3 patients were above 60 years old, and one patient was 4 years old, with male being the most predominant (n=10). Most of the samples were collected in March (n=9) then in February (n=1) or January (n=2). From the most to the least frequent, the viruses were found to be in these following clades: GH (n=10), GR (n=1), and O (n=1). As many as 4 viruses were from B.1.466.2 lineage, whilst the others were from B.1.470 (n=2), B.1.375 (n=2), B.1.1.269 (n=1), B.1.468 (n=1), and B.1 (n=1). All viruses had mutations on the D614G protein. Other common mutations were found in NSP3, NSP6, NSP12, and NS3. None of the viruses was indicated to have VOC mutations.

The phylogenetic tree among the isolated viruses is presented in Figure 1. The viruses had established a distant relationship with the overall common ancestor suggesting a rapid mutation of this virus. The reference genome of Wuhan isolate (hCoV-19/Wuhan/WIV04/2019)

Table 1. **SARS-CoV-2 viruses from samples collected from January to March 2021 in Bali.**

Virus name	Accession ID	Date of sample collection	Patient's Characteristics		Clade	Lineage	Mutations
			Age (year)	Sex			
hCoV-19/Indonesia/BA-Unwar-36493/2021	EPI_ISL_8919637	8 January 2021	65	Male	GH	B.1.466.2	Spike D614G, Spike N439K, Spike V6F, N S2P, N T205I, NS3 Q57H, NSP3 E177D, NSP3 P822L, NSP3 T350I, NSP12 P323L
hCoV-19/Indonesia/BA-Unwar-KL20/2021	EPI_ISL_8919269	16 January 2021	24	Female	GR	B.1.1.269	Spike D614G, N G204R, N R203K, N T141I, NSP3 T779I, NSP12 P323L, NSP15 T33I
hCoV-19/Indonesia/BA-Unwar-KL23/2021	EPI_ISL_8918713	10 February 2021	43	Male	GH	B.1.466.2	Spike D614G, Spike N439K, Spike P681R, N T205I, NS3 Q57H, NSP3 P822L, NSP3 S126L, NSP3 T350I, NSP6 L75F, NSP12 P323L, NSP13 S259L
hCoV-19/Indonesia/BA-Unwar-49352/2021	EPI_ISL_8880974	16 March 2021	47	Male	GH	B.1.375	Spike D614G, N T205I, NS3 D155H, NS3 Q57H, NS3 S40L, NS3 S220I, NS7b E39stop, NSP2 L217V, NSP3 A1711V, NSP6 F108del, NSP6 G107del, NSP6 L260F, NSP6 S106del, NSP12 P227L, NSP12 P323L
hCoV-19/Indonesia/BA-Unwar-TB17/2021	EPI_ISL_8919645	16 March 2021	62	Male	GH	B.1.470	Spike D614G, Spike T22I, N T205I, NS3 D155H, NS3 I37T, NS3 Q57H, NS3 S220I, NS7a L102F, NS7b E39stop, NSP1 L92P, NSP2 L217V, NSP3 S1039L, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P227L, NSP12 P323L, NSP13 R339H
hCoV-19/Indonesia/BA-Unwar-TB16/2021	EPI_ISL_8919644	16 March 2021	48	Male	GH	B.1.468	Spike D614G, N D144Y, N T148A, NS3 Q57H, NS8 E59V, NS8 V62L, NSP3 A58T, NSP3 L1244F, NSP3 S370L, NSP5 V247F, NSP12 P323L, NSP13 A123S, NSP13 R248G
hCoV-19/Indonesia/BA-Unwar-49362/2021	EPI_ISL_8919640	16 March 2021	52	Male	GH	B.1.375	Spike D614G, Spike Y28H, N T205I, NS3 D155H, NS3 Q57H, NS3 S220I, NS7b E39stop, NS8 V62L, NSP2 L217V, NSP3 A1711V, NSP3 V473I, NSP6 A128V, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P227L, NSP12 P323L, NSP14 K349N
hCoV-19/Indonesia/BA-Unwar-TB15/2021	EPI_ISL_8919642	16 March 2021	52	Female	GH	B.1	Spike D215Y, Spike D614G, Spike G832C, Spike S494P, N T205I, NS3 Q57H, NS7b E39stop, NSP2 F498L, NSP4 G257C, NSP12 P323L
hCoV-19/Indonesia/BA-Unwar-49356/2021	EPI_ISL_8919639	16 March 2021	78	Male	O	B.1.466.2	Spike D614G, Spike N439K, Spike P681R, N T205I, NS3 Q57H, NSP3 P822L, NSP3 S126L, NSP3 T350I, NSP6 A41del, NSP6 A46del, NSP6 A51del, NSP6 A54del, NSP6 A56L, NSP6 A65L, NSP6 A76S, NSP6 A79S, NSP6 E39K, NSP6 F42del, NSP6 F55Y, NSP6 F59W, NSP6 F66L, NSP6 F70L, NSP6 G48del, NSP6 H62del, NSP6 H64del, NSP6 I49del, NSP6 I50del, NSP6 ins79M, NSP6 K61del, NSP6 K63del, NSP6 L37F, NSP6 L43M, NSP6 L72Q, NSP6 M47del, NSP6 M52del, NSP6 M57L, NSP6 M58L, NSP6 M83V, NSP6 N40del, NSP6 N82S, NSP6 P73stop, NSP6 S53del, NSP6 S74C, NSP6 T77N, NSP6 T206I, NSP6 V78I, NSP6 V84C, NSP6 Y38M, NSP6 Y80H, NSP6 Y85F, NSP12 P323L, NSP13 S259L, NSP16 K160R

Virus name	Accession ID	Date of sample collection	Patient's Characteristics		Clade	Lineage	Mutations
			Age (year)	Sex			
hCoV-19/Indonesia/BA-Unwar-B138/2021	EPI_ISL_8919646	18 March 2021	4	Male	GH	B.1.466.2	Spike D614G, Spike I844V, Spike N439K, N T205I, NS3 L108F, NS3 Q57H, NS3 V29F, NS7b S31L, NSP3 P822L, NSP3 T350I, NSP8 T148I, NSP12 P323L, NSP15 R257C
hCoV-19/Indonesia/BA-Unwar-KL43/2021	EPI_ISL_8919634	23 March 2021	35	Male	GH	B.1.466.2	Spike D614G, Spike N439K, Spike V6F, N S2P, N T205I, NS3 Q57H, NSP3 E177D, NSP3 P822L, NSP3 S1314G, NSP3 T350I, NSP12 P323L, NSP12 V335F
hCoV-19/Indonesia/BA-Unwar-B139/2021	EPI_ISL_8919647	24 March 2021	61	Male	GH	B.1.470	Spike D614G, Spike V1176F, N T205I, NS3 D155H, NS3 Q57H, NS3 S220I, NS7b E39stop, NSP2 L217V, NSP2 T149I, NSP3 S1039L, NSP3 T181I, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P227L, NSP12 P323L

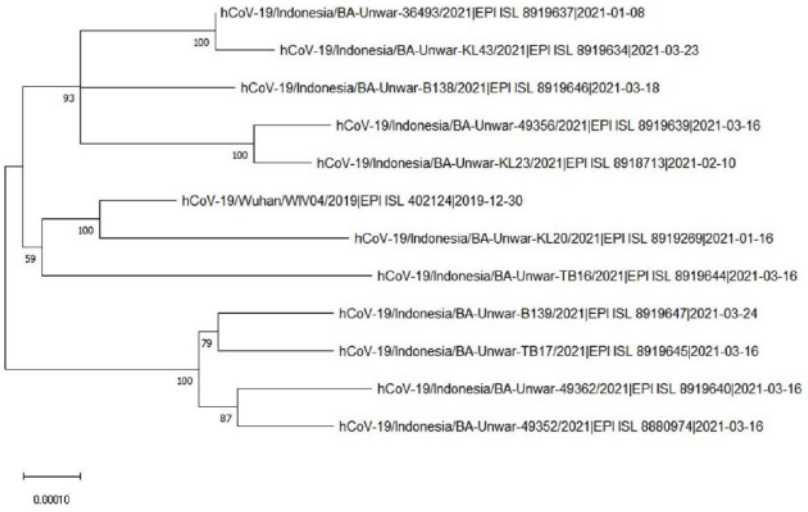

Figure 1. Figure 1. Phylogenetic tree of SARS-CoV-2 from samples collected in Bali at the end of the first COVID-19 wave (January to March 2021).

had the closest relationship with the ancestor and grouped along with two Bali isolates (hCoV-19/Indonesia/BA-Unwar-KL20/2021 and hCoV-19/Indonesia/BA-Unwar-TB16/2021). The isolate collected on 8 January 2021 had the least mutation, meanwhile the isolate with the highest number of mutations was from sample collected later on 16 March 2021 (Figure 1).

DISCUSSION

In Indonesia, the isolates appeared to be included in clades L, G, GR, GH, and O.[13] Herein, the results revealed that high prevalence of SARS-CoV-2 belonged to clade GH, where only one that belonged to clade GR. We also found one isolate that could not be included in the seven major clades (G, GH, GR, GV, S, V, and L), hence included into clade O. In a continental scale, based on a report published on 19 April 2021, Asia was reported to be predominated by clade GR which happened to similarly occur on South America, Africa, and Oceania.[23] In a more specific region, a study analyzing samples from four patients in Java Island, Indonesia, revealed the predominance of GH (n=3).[14] A study from a neighboring country, Malaysia, employing 1000 participants using data deposited until July 19, 2021, revealed the similar predominance of clade GH (n=311).[24] Meanwhile in Singapore, within January to March 2021, clades G and GH were predominant compared with others.[25] Furthermore, in this present study, the most dominant tracked linage was B.1.466.2 of the Balinese isolates. In a nationwide scale, the lineage occupied 50% of daily infection, where Java and Sumatra had been suggested as its epicenters of transmission.[26] This SARS-CoV-2 lineage was also observed in Malaysia and Singapore, but not in other ASEAN countries.[27] These suggest that the prevalence of SARS-CoV-2 clades is more likely to be influenced by the nearby cities or countries, and the clade distribution could be significantly different when distantly located countries are included in the analysis.

This present study revealed that all Balinese isolates had D614G mutation. Other mutations occurred predominantly at NS3, NSP3, NSP6, and NSP12. Previously, the number of D614G mutations were only occurred among 36/60 and 23/40 isolates, collected in 2020. Later findings (January-April 2021) from Sulawesi Island revealed 100% D614G mutations, which is similar with the results herein. The foregoing studies in Java and Sulawesi Islands also found the mutations at NS3, NSP3, and NSP12.[13-15] Mutations at NSP3 were also observed in high frequency by a study using GISAID metadata of B.1.466.2 strains isolated from

Indonesia.[26] It is also worth-noting that the isolate from a 78-years-old male patient in this present study had the most frequent mutations with NSP6 being the major mutation site. Mutation locations have been found to be linked with the disease severity and viral replication and stability, suggesting the importance of monitoring SARS-CoV-2 mutations especially during the pandemic.[28,29] Moreover, phylogenetic tree herein highlights that circulating SARS-CoV-2 strains in Balinese have sequence variations compared with the early ancestors.

In regards with VOCs, Indonesia has reported alpha and beta variants in January 2021.[27] However, in early 2021, among South-east Asian countries, Indonesian COVID-19 cases were dominated by non-VOCs.[27] In this present study, we reported the absence of VOCs at the end of the first COVID-19 wave based on the whole genome sequencing of 12 patients from January to March 2021. This is in line with the government's report that a single case of alpha variants was found only in the beginning of the second wave (June 2021).[30] During January to March 2021, a strict lockdown policy was imposed by the government due to a significant increase of COVID-19. The policy mostly covered restrictions on people's mobility and face-to-face social interaction; such as but not limited to, 75% working office activities should be performed from home, online learning for all schools, and 50% limit for worship house occupancy. At the same time, Indonesian government also imposed domestic and international travel bans.[31] Taken altogether, the COVID-19 restrictions had successfully prevent the entry of VOCs into Bali during the first wave of the pandemic.

CONCLUSIONS

All collected isolates from Bali had multiple mutations from their early ancestors. No VOCs were found from the Balinese isolates at the end of the first COVID-19 wave in Indonesia which could indicate the effectiveness of COVID-19 restrictions. As the restrictions have been loosened in Bali, we recommend the continuation of genomic surveillance on SARS-CoV-2 mutations.

ETHICS APPROVAL

The study protocol was approved by the Universitas Warmadewa Ethical Review Board (No. 65/Unwar/FKIK/EC-KEPK/VII/2021).

ACKNOWLEDGMENT

We would like to all patients participate in this present study.

DISCLOSURE OF CONFLICTS OF INTEREST

No conflict of interest.

FUNDING

This study was funded by Faculty of Medicine and Health Sciences, Universitas Warmadewa.

AUTHORS CONTRIBUTION

The authors contributed to the research process, including preparation, conceptualization, data collection and analysis, drafting, and approval to publish.

REFERENCES

1. Fahriani M, Anwar S, Yufika A, Bakhtiar B, Wardani E, Winardi W, et al. Disruption of childhood vaccination during the COVID-19 pandemic in Indonesia. Narra J. 2021;1(1). Available from: http://dx.doi.org/10.52225/narraj.v1i1.7
2. Winardi W, Wahyuni H, Hidayat M, Wirawan A, Uddin MN, Yusup M. Challenges on tuberculosis care in health care facilities during COVID-19 pandemic: Indonesian perspective. Narra J. 2022;2(2). Available from: http://dx.doi.org/10.52225/narra.v2i2.80
3. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. N Engl J Med. 2020;383(25):2451–60. Available from: http://dx.doi.org/10.1056/nejmcp2009575
4. Masyeni S, Santoso MS, Widyaningsih PD, Asmara DW, Nainu F, Harapan H, et al. Serological cross-reaction and coinfection of dengue and COVID-19 in Asia: Experience from Indonesia. Int J Infect Dis. 2020/10/25. 2021;102:152–4. Available from: https://pubmed.ncbi.nlm.nih.gov/33115680
5. Ghayda RA, Lee KH, Han YJ, Ryu S, Hong SH, Yoon S, et al. Estimation of global case fatality rate of coronavirus disease 2019 (COVID-19) using meta-analyses: Comparison between calendar date and days since the outbreak of the first confirmed case [Internet]. Cold Spring Harbor Laboratory; 2020. Available from: http://dx.doi.org/10.1101/2020.06.11.20128959
6. Sharun K, Tiwari R, Yatoo MI, Natesan S, Megawati D, Singh KP, et al. A comprehensive review on pharmacologic agents, immunotherapies and supportive therapeutics for COVID-19. Narra J. 2022;2(3):e92. Available from: http://dx.doi.org/10.52225/narra.v2i3.92
7. Masyeni S, Iqhrammullah M, Frediansyah A, Nainu F, Tallei T, Emran T Bin, et al. Molnupiravir: A lethal mutagenic drug against rapidly mutating severe acute respiratory syndrome coronavirus 2-A narrative review. J Med Virol. 2022/04/02. 2022;94(7):3006–16. Available from: https://pubmed.ncbi.nlm.nih.gov/35315098
8. Mudatsir M, Keam S, Winardi W, Yufika A, Rabaan AA, Rodriguez-Morales AJ, et al. Early transmission dynamics of SARS-CoV-2 in Indonesia. Trends Infect Glob Heal. 2021;1(1):1–6. Available from: http://dx.doi.org/10.24815/tigh.v1i1.20134
9. Lai S, Bogoch I, Ruktanonchai N, Watts A, Lu X, Yang W, et al. Assessing spread risk of Wuhan novel coronavirus within and beyond China, January-April 2020: a travel network-based modelling study. medRxiv Prepr Serv Heal Sci. 2020;2020.02.04.20020479. Available from: https://pubmed.ncbi.nlm.nih.gov/32511631
10. Katsnelson A. Coronavirus entry points could explain its spread. C&EN Glob Enterp. 2020;98(17):4. Available from: http://dx.doi.org/10.1021/cen-09817-scicon9
11. Wilson D. "Pre-Omicron", COVID-19 cases peaked mid-2021 in India, Indonesia and Japan… [Internet]. Organisation for Economic Co-Operation and Development (OECD); 2022. Available from: http://dx.doi.org/10.1787/7d18b6be-en
12. Heguy A. Review 2: "A year of genomic surveillance reveals how the SARS-CoV-2 pandemic unfolded in Africa" [Internet]. MIT Press - Journals; 2021. Available from: http://dx.doi.org/10.1162/2e3983f5.3d1fbfc5
13. Nidom R V, Indrasari S, Normalina I, Nidom AN, Afifah B, Dewi L, et al. An Updated Investigation Prior To COVID-19 Vaccination Program In Indonesia: Full-Length Genome Mutation Analysis Of SARS-CoV-2 [Internet]. Cold Spring Harbor Laboratory; 2021. Available from: http://dx.doi.org/10.1101/2021.01.26.426655
14. Gunadi, Hendra W, Marcellus, Mohamad SH, Edwin WD, Ludhang PR, et al. Full-length genome characterization and phylogenetic analysis of SARS-CoV-2 virus strains from Indonesia. Research Square. 2020.
15. Massi MN, Abidin RS, Farouk A-E, Halik H, Soraya GV, Hidayah N, et al. Full-genome sequencing and mutation analysis of SARS-CoV-2 isolated from Makassar, South Sulawesi, Indonesia. PeerJ. 2022;10:e13522–e13522. Available from: https://pubmed.ncbi.nlm.nih.gov/35707124
16. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22(13):30494. Available from: https://pubmed.ncbi.nlm.nih.gov/28382917

17. Islam O, emran H Al, Hasan M, Anwar A, Jahid M, Hossain M. Emergence of European and North American mutant variants of SARS-CoV-2 in Southeast Asia [Internet]. Authorea, Inc.; 2020. Available from: http://dx.doi.org/10.22541/au.159112172.23549094
18. Freed N, Silander O. nCoV-2019 sequencing protocol (RAPID barcoding, 1200bp amplicon) v3 [Internet]. ZappyLab, Inc.; 2020. Available from: http://dx.doi.org/10.17504/protocols.io.bgggjttw
19. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013/01/16. 2013;30(4):772–80. Available from: https://pubmed.ncbi.nlm.nih.gov/23329690
20. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012/04/27. 2012;28(12):1647–9. Available from: https://pubmed.ncbi.nlm.nih.gov/22543367
21. Stamatakis A, Hoover P, Rougemont J. A Rapid Bootstrap Algorithm for the RAxML Web Servers. Syst Biol. 2008;57(5):758–71. Available from: http://dx.doi.org/10.1080/10635150802429642
22. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006;22(21):2688–90. Available from: http://dx.doi.org/10.1093/bioinformatics/btl446
23. Hamed SM, Elkhatib WF, Khairalla AS, Noreddin AM. Global dynamics of SARS-CoV-2 clades and their relation to COVID-19 epidemiology. Sci Rep. 2021;11(1):8435. Available from: https://pubmed.ncbi.nlm.nih.gov/33875719
24. Zainulabid UA, Mat Yassim AS, Hussain M, Aslam A, Soffian SN, Mohd Ibrahim MS, et al. Whole genome sequence analysis showing unique SARS-CoV-2 lineages of B.1.524 and AU.2 in Malaysia. PLoS One. 2022;17(2):e0263678–e0263678. Available from: https://pubmed.ncbi.nlm.nih.gov/35213571
25. Yingtaweesittikul H, Ko K, Abdul Rahman N, Tan SYL, Nagarajan N, Suphavilai C. CalmBelt: Rapid SARS-CoV-2 Genome Characterization for Outbreak Tracking. Front Med. 2021;8:790662. Available from: https://pubmed.ncbi.nlm.nih.gov/34970567
26. Zhu M, Zeng Q, Saputro BIL, Chew SP, Chew I, Frendy H, et al. Tracking the molecular evolution and transmission patterns of SARS-CoV-2 lineage B.1.466.2 in Indonesia based on genomic surveillance data. Virol J. 2022;19(1):103. Available from: https://pubmed.ncbi.nlm.nih.gov/35710544
27. Hoan NX, Pallerla SR, Huy PX, Krämer H, My TN, Tung TT, et al. SARS-CoV-2 viral dynamics of the first 1000 sequences from Vietnam and neighbouring ASEAN countries. IJID Reg. 2022/01/20. 2022;2:175–9. Available from: https://pubmed.ncbi.nlm.nih.gov/35721434
28. Nagy Á, Pongor S, Győrffy B. Different mutations in SARS-CoV-2 associate with severe and mild outcome. Int J Antimicrob Agents. 2020/12/23. 2021;57(2):106272. Available from: https://pubmed.ncbi.nlm.nih.gov/33347989
29. Alam ASMRU, Islam OK, Hasan MS, Islam MR, Mahmud S, Al-Emran HM, et al. Dominant clade-featured SARS-CoV-2 co-occurring mutations reveal plausible epistasis: An in silico based hypothetical model. J Med Virol. 2021/11/01. 2022;94(3):1035–49. Available from: https://pubmed.ncbi.nlm.nih.gov/34676891
30. Wise J. Covid-19: Delta variant doubles risk of hospital admission compared with alpha variant, study shows. BMJ. 2021;n2152. Available from: http://dx.doi.org/10.1136/bmj.n2152
31. Alkayyali T, Ochuba O, Srivastava K, Sandhu JK, Joseph C, Ruo SW, et al. An Exploration of the Effects of Radiofrequency Radiation Emitted by Mobile Phones and Extremely Low Frequency Radiation on Thyroid Hormones and Thyroid Gland Histopathology. Cureus. 2021;13(8):e17329–e17329. Available from: https://pubmed.ncbi.nlm.nih.gov/34567874

Whole-genome-scale analysis of circulating SARS-CoV-2 during the first COVID-19 wave in an international tourist destination, Bali, Indonesia

ORIGINALITY REPORT

4%	3%	2%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

★ Submitted to Udayana University

Student Paper

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		