//\J.Biomed.Transl.Res
ISSN: 2503-2178

Indexed by : DOAJ Google Crossref Dimension sinta GARUDA

JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

Available online at JBTR website: https://jbtr.fk.undip.ac.id

Original Research Article

Comparison of SARS-CoV-2 Variant Screening and Whole Genome Sequencing at an Indonesian Tertiary Hospital

Rebriarina Hapsari[1,2,3*], Irfan Kesumayadi[1], Desvita Sari[1,4], Dwi Utami Anjarwati[1,4,5], Nesia Hani Alfiyuliani[1,4], Mujahidah Mujahidah[1,4], Iva Puspita Sari[1,4], Purnomo Hadi[1,4]

[1]*Department of Microbiology, Faculty of Medicine, Universitas Diponegoro, Indonesia*
[2]*Microbiology Laboratory, Diponegoro National Hospital, Indonesia*
[3]*Microbiology Laboratory, KRMT Wongsonegoro Hospital, Indonesia*
[4]*Department of Clinical Microbiology, Kariadi Hospital, Indonesia*
[5]*Department of Microbiology, Faculty of Medicine, Universitas Jendral Soedirman, Indonesia*

Article Info
History
Received: 03 Jul 2023
Accepted: 31 Oct 2023
Available: 31 Dec 2023

Abstract

Background: The global COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), experienced a surge in cases with the emergence of the Omicron variant. Despite increasing vaccination coverage, Indonesia witnessed peaks in COVID-19 cases. Variant screening and whole genome sequencing (WGS) play a crucial role in identifying SARS-CoV-2 variants and monitoring their spread.

Objective: The objective of this study was to compare variant screening results with WGS data and assess the prevalence of subvariants.

Methods: Between November 7th and 18th, 2022, variant screening and WGS were conducted on samples with CT values below 30. Variant screening utilized the mBioCov-19+ VarScreen assay, while WGS was performed on the Oxford Nanopore Technologies (ONT) platform. Bioinformatics analysis was performed using epi2melabs. Demographic data were analyzed.

Results: Out of 89 subjects, all tested positive for the Omicron variant through variant screening. The variant screening identified two subvariants: Omicron BA.2 (64%) and Omicron B.1.1.529.1 (36%). WGS revealed that the XBB subvariant was the most dominant (52.8%), followed by BQ.1 (22.5%) and BA.5 (13.5%). When VarScreen indicated BA.2, the majority of WGS results showed XBB (82.5%), while for B.1.1.529.1, the majority of WGS results were BQ.1 (59.4%), followed by BA.5 (37.5%). XBB was the most prevalent variant in both females and males, while BQ.1 was more dominant in females (80%). No infections were detected among children aged 1-5 years.

Conclusion: Variant screening provides accurate and quick results for detecting the Omicron variant in laboratories without WGS capacity. However, it is important to continuously update the screening methodology based on the prevailing circulating variants. During the study period, XBB emerged as the predominant subvariant of the Omicron variant.

Keywords: *Variant screening; Whole Genome Sequencing; Omicron, XBB*
Permalink/ DOI: https://doi.org/10.14710/jbtr.v9i3.19147

INTRODUCTION

By the end of 2021, there was a significant increase in the daily worldwide cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), surpassing one million cases.

*Corresponding author:
E-mail: *r.hapsari@fk.undip.ac.id*
(Rebriarina Hapsari)

This surge was largely attributed to the emergence and spread of the Omicron variant, also known as B.1.1.529[1] Due to the lower fatality rate of the Omicron variant and the increasing number of vaccinated individuals, it is expected that the global pandemic will eventually reach to an end following the Omicron wave[2]. However, the number of COVID-19 cases in Indonesia continues to increase after the introduction of the Omicron variant, with three peaks occurring in 2022, specifically in February, August, and November, with the highest number of confirmed cases being ten of thousand cases.[1]

By the end of 2022, various SARS-CoV-2 variants, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.529) along with its subvariants, have been identified. Despite the availability of vaccines, certain subvariants have shown the ability to evade immunity, leading to infections in vaccinated individuals or reinfection in COVID-19 survivor.[3,4] Among all the variants, Omicron is believed to possess the highest potential for immune evasion due to its significant spike protein mutations. Therefore, the identification of SARS-CoV-2 variants through genome sequencing plays a crucial role in controlling the spread of the virus.[5]

Genomic epidemiology has played a crucial role in the SARS-CoV-2 pandemic as it has proven to be a powerful technique for monitoring the emergence and transmission of new viruses, as well as assessing outbreaks.[6] The initial identification of SARS-CoV-2 occurred through whole genome shotgun sequencing of a pneumonia patient in Wuhan, China, in December 2019. Prior to this, six coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-NL63, HCoV-229E, SARS-CoV, and MERS-CoV) had been identified as infecting humans.[7] In comparison, SARS-CoV-2 shares approximately 94.4% of its genome sequence with SARS-CoV, and it was initially predicted to be a zoonotic infection originating from bats, given its 96.2% similarity to the genome sequence of SARSr-CoV RaTG13 and 97% similarity in the spike glycoprotein.[8,9]

Whole genome sequencing (WGS) is currently considered the most accurate method for identifying mutations in the SARS-CoV-2 virus. [10] However, due to limited resources and time-consuming, WGS is not commonly performed to identify SARS-CoV-2 variants. To address this, variant screening methods have been developed as alternatives to WGS. These methods include RT-PCR-based assays and Virus-Receptor-Based Electrical Biosensors, which can help identify variants before conducting WGS tests.[11,12] In Indonesia, we have developed an RT-PCR-based assay called mBioCov-19+ VarScreen, which can identify several SARS-CoV-2 variants, including Alpha (B.1.1.7), Beta (B.1.351)/Gamma (P1), Mu (B.1.621), Delta/Delta Plus (B.1.617.2), B.1.620, Lambda (C.37), Omicron (B.1.1.529.1), and BA.2. However, it is important to note that this variant assay may not be able to identify specific subvariants such as BA5, BQ.1, and XBB. In Asia, Omicron variant quickly replaced previous circulating strain after its introduction, and then followed by its subvariants, namely BA.1, BA.2, BA.4, BA.5, BQ.1, and XBB. Among those, XBB became the most predominating subvariants in October 2022.[13,14]

In our study, we conducted both variant screening and whole genome sequencing (WGS) tests. We examined the prevalence of subvariants identified through WGS and compared it with the results obtained from variant screening. Additionally, we investigated the correlation between these subvariants and demographic data.

MATERIALS AND METHODS

During 7th and 18th November 2022, we performed SARS-CoV-2 variant screening test and whole genome sequencing using the ONT platform (Oxford Nanopore Technologies, New York, NY, USA) on samples with CT value<30 (Bio Farma, Bandung, Indonesia).

SARS-CoV-2 variant screening was done using mBioCov-19+ VarScreen according to the manufacturer's instruction (Bio Farma, Bandung, Indonesia). mBioCov-19+ VarScreen kit was intended to identify Alpha (B.1.1.7), Beta (B.1.351)/Gamma (P1), Mu (B.1.621), Delta/Delta plus (B.1.617.2), B.1.620, Lambda (C.37), Omicron (B.1.1.529.1), and BA.2 variants. This kit incorporated two reactions targeting Helicase gene to detect SARS-CoV-2; and H69/V70, E484K/Q/A, N501Y, T478K, ORF1ab SGF3675-3677- mutations to determine variants and subvariants.

Whole genome sequencing was done using the PCR tiling of SARS-CoV-2 virus with rapid barcoding and Midnight RT PCR Expansion protocol on GridION (Oxford Nanopore Technologies, New York, NY, USA). Bioinformatics analysis, i.e., quality control check, ARTIC workflow run, and nextclade analysis, were performed by epi2melabs.[15] Gender and age were collected on this study and analyzed based on the group of SARS-CoV-2 variants.

This study has been approved by the Institutional Review Board of the Dr. Kariadi Hospital, Semarang, Indonesia (Number 1363/EC/KEPK-RSDK/2022).

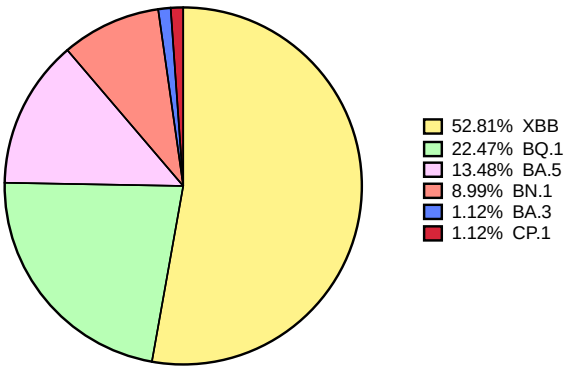

Figure 1. Distribution frequency of Omicron subvariants based on WGS

RESULTS

A total of 89 subjects were enrolled during the study period. Variant screening revealed that all the subjects were infected by the Omicron variant. Among them, 57 (64%) were identified as Omicron BA.2, whereas 32 (36%) were identified as Omicron B.1.1.529.1 by mBioCov-19+ VarScreen. Based on WGS and lineage assignment, XBB was the most dominant subvariant, accounting for 47 (52.81%), followed by BQ.1 and BA.5, which accounted for 20 (22.47%) and 20 (13.48%),

Table. 1 Crosstabulation of VarScreen and WGS

VarScreen	Whole Genome Sequencing Lineages					
	XBB	BQ.1	BA.5	BN.1	BA.3	CP.1
BA.2	47 (82.5%)	1 (1.8%)	0 (0%)	8 (14%)	1 (1.8%)	0 (0%)
Omicron B.1.1.529.1	0 (0%)	19 (59.4%)	12 (37.5%)	0 (0%)	0 (0%)	1 (3.1%)

Table. 2 Demographic among Omicron Lineages

	XBB	**BQ.1**	**BA.5**	**BN.1**	**BA.3**	**CP.1**
Gender						
Male	18 (52.9%)	4 (11.8%)	7 (20.6%)	5 (14.7%)	0 (0%)	0 (0%)
Female	29 (52.7%)	16 (29.1%)	5 (9.1%)	3 (5.5%)	1 (1.8%)	1 (1.8%)
Age, years (Mean±SD)	44.64±22.71	39.33±23.68	32.63±20.2	58.88±21.74	31*	34*
≤1 years	6 (66.7%)	2 (22.2%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)
1-5 years	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
6-18 years	0 (0%)	1 (33.3%)	2 (66.7%)	0 (0%)	0 (0%)	0 (0%)
18-60 years	29 (52.7%)	12 (21.8%)	8 (14.5%)	4 (7.3%)	1 (1.8%)	1 (1.8%)
>60 years	12 (54.5%)	5 (22.7%)	1 (4.5%)	4 (18.2%)	0 (0%)	0 (0%)

*Data obtained only from one subject

respectively. The less frequent variants were BN.1, BA.3, and CP.1, which accounted for 8 (8.99%), 1 (1.12%), and 1 (1.12%), respectively (Figure 1).

According to the variant screening results, the BA.2 subvariant was primarily comprised of XBB and BN.1, accounting for 47 (82.5%) and 8 (14%), respectively. On the other hand, the variant screening results for Omicron B.1.1.529.1 indicated that the dominant subvariants were BQ.1 and BA.5, accounting 19 (59.4%) and 12 (37.5%), respectively. Interestingly, there was one subject where the BQ.1.1 subvariant was detected within the BA.2 variant screening. A cross-tabulation between variant screening and WGS is shown in Table 1.

Demographics among Omicron subvariants

Based on the data presented in Table 2, we can conclude that XBB was the most dominant variant observed in both females and males across different age groups during the study period. Among females, BQ.1 was more dominant, accounting for 16 (80%), compared to males where it accounted for 4 (20%). No infections were detected among children aged 1-5 years in our study.

DISCUSSION

Variant screening for SARS-CoV-2 refers to the process of detecting and identifying genetic variant groups or mutations of the virus. Some SARS-CoV-2 variants may be associated with increased virulence, or increased transmissibility, or decreased efficacy of current diagnostics, vaccinations, or treatments, the variants which are known as variants of concern. To date, there have been five variants of concern of SARS-CoV-2 i.e., Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.529). Variant screening has helped in monitoring the spread and prevalence of different SARS-CoV-2 variants (especially during the Delta variant outbreak and early Omicron outbreak). It provided valuable information for public health interventions and the implementation of control measures. By understanding the prevalence and characteristics of different variants, public health authorities can make informed decisions to mitigate the spread of the virus and adapt response efforts accordingly.[16,17]

Significant mutations in the SARS-CoV-2 virus leading to highly transmissible virus were identified in November 2021, in which the resulting strain has been called as Omicron variant (B.1.1.529) and considered as a variant of concern by the World Health Organization. The first confirmed case was reported in South Africa, and subsequent cases were identified globally, leading to the displacement of the Delta variant in many countries.[18] Despite extensive mutations in the spike protein, Omicron appears to have lower pathogenicity due to alterations in the non-RBD portion of its S protein, affecting cellular tropism. Compared to previous variants, the Omicron variant is suspected to enter cells via an endosomal entry route in the upper respiratory tract (where TMPRSS2 expression is low), as opposed to a plasma membrane entry route in lung tissue with high

TMPRSS2 expression. This altered entry route is believed to contribute to its high transmissibility. [19,20]

Before the Omicron outbreak occurred, Indonesia experienced 4 months with relatively few cases. However, when Omicron struck, there was a sudden surge in positive COVID-19 cases in Indonesia, accompanied by an increase in hospitalizations and deaths. VarScreen testing plays a role in the early stages of the Omicron outbreak due to the limited availability of WGS facilities. Health authorities require rapid epidemiological data on the spread of the SARS-CoV-2 virus to understand its transmission and effectively address it.

Our study revealed that all subjects enrolled during the study period were infected with the Omicron variant, as indicated by both the Variant Screening and WGS results. Initially, the Omicron variant has been categorized into BA.1 and BA.2 sub-lineages. As time passes, mutations continue to occur, leading to the discovery of other major sub-lineages, including BA.3, BA.4, BA.5, and XBB. In our study, the most prevalent sub-lineage of the Omicron variant was XBB, followed by BQ.1. During the study period, when VarScreen results showed BA.2, the majority of WGS results were XBB (82.5%), and for Omicron B.1.1.529.1 in VasScreen, the majority of WGS results were BQ.1 (59.4%), followed by BA.5 (37.5%).

A previous study documented that using a RT-PCR based SARS-CoV-2 variant screening assays can quickly provide the probable variant of SARS-COV-2, but it requires careful quality control and interpretation.[21] Previously, the assay in our study has been carefully evaluated against Omicron variant by using he spike (S)- gene target failure (SGtF) and S-gene target positive (SGtP) with the principle of the single nucleotide polymorphism (SNP)-probe test. Here in our study, we did practical analysis in performing both variant screening and WGS examination.

The XBB subvariant of Omicron is considered a recombinant strain originating from the BA.2 lineage. It is formed through recombination of two specific sublineages, namely BA.2.10.1 and BA.2.75. This recombinant strain is characterized by several mutations in the Spike protein, which play a crucial role in the virus's infectivity and interaction with the host cells.[22] BQ.1 is a sublineage derived from BA.5, and it is characterized by specific spike mutations occurring in significant antigenic sites, such as K444T and N460K. Furthermore, the BQ.1.1 sublineage carries an extra spike mutation in another crucial antigenic site, namely R346T.[22,23] BQ.1 was previously noted to be more prevalent in the United States. It has been proposed that the virus has the ability to evolve uniquely in each geographic area, resulting in the emergence of variants that are better adapted to specific local communities.[24] Our study also showed that XBB is equivalently distributed among sex and ages. Meanwhile, BQ.1 is more dominant in female. There is no current study describe the association of BQ.1 and female sex, thus it might be coincidence.

The current VarScreen has limited effectiveness in today's (July 2023 when this article is prepared) context due to the predominant presence of mutations originating from XBB sublineages and not from others. With the emergence of the Omicron subvariants, there is a pressing need to enhance the capabilities of VarScreen to accurately detect and identify the strains currently circulating. This adaptation is crucial to provide timely and relevant information for effective public health interventions. By updating and modifying VarScreen to target the specific genetic markers and mutations associated with the Omicron subvariants, we can improve its sensitivity and specificity in identifying the currently circulating strains. This would enable healthcare professionals and public health authorities to quickly identify cases, implement appropriate isolation measures, and conduct contact tracing to prevent further transmission.

CONCLUSION

Variant screening provides accurate and quick result of Omicron variant in the laboratories with no WGS capacity. During the study period, a BA.1.1.529.1 found in the variant screening would be more likely to be a BQ.1, while BA.2 would be more likely to be an XBB variant. Variant screening reagents should be continuously updated based on the predominating circulating variants.

ACKNOWLEDGMENTS

We thank the Agency for Health Policies Development, the Ministry of Health of the Republic of Indonesia for providing reagents and systems to perform SARS-CoV-2 whole genome sequencing and VarScreen in our laboratories. This study was partially funded by the research grant of the Faculty of Medicine Universitas Diponegoro (4509/UN7.5.4.2.1/PP/PM/2020).

REFERENCES

1. Covid WHO. Dashboard. Geneva: World Health Organization. 2020;2020. https://covid19.who.int/region/searo/country/id
2. Murray CJL. COVID-19 will continue but the end of the pandemic is near. The Lancet. 2022;399(10323):417-9. https://doi.org/10.1016/s0140-6736(22)00100-3
3. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IATM, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature. 2021;599(7883):114-9. https://doi.org/10.1038/s41586-021-03944-y
4. Lazarevic I, Pravica V, Miljanovic D, Cupic M. Immune Evasion of SARS-CoV-2 Emerging Variants: What Have We Learnt So Far? Viruses. 2021;13(7). https://doi.org/10.3390/v13071192
5. Ke H, Chang MR, Marasco WA. Immune Evasion of SARS-CoV-2 Omicron Subvariants. Vaccines. 2022;10(9):1545. https://doi.org/10.3390/vaccines10091545
6. Gardy JL, Loman NJ. Towards a genomics-informed, real-time, global pathogen surveillance system. Nat Rev Genet. 2018;19(1):9-20. https://doi.org/10.1038/nrg.2017.88

7. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol. 2016;24(6):490-502. https://doi.org/10.1016/j.tim.2016.03.003
8. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. https://doi.org/10.1038/s41586-020-2012-7
9. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virol. 2020;94(7). https://doi.org/10.1128/jvi.00127-20
10. Ong DSY, Koeleman JGM, Vaessen N, Breijer S, Paltansing S, de Man P. Rapid screening method for the detection of SARS-CoV-2 variants of concern. J Clin Virol. 2021;141:104903. https://doi.org/10.1016/j.jcv.2021.104903
11. Park S, Kim H, Woo K, Kim JM, Jo HJ, Jeong Y, et al. SARS-CoV-2 Variant Screening Using a Virus-Receptor-Based Electrical Biosensor. Nano Lett. 2022;22(1):50-7.
12. Camp JV, Buchta C, Jovanovic J, Puchhammer-Stöckl E, Benka B, Griesmacher A, et al. RT-PCR based SARS-CoV-2 variant screening assays require careful quality control. J Clin Virol. 2021;141:104905. https://doi.org/10.1021/acs.nanolett.1c03108.s001
13. Singapore MoH. Update on COVID-19 situation and measures to protect healthcare capacity 2022 [Cited 2023 Sept 24]. Available from: https://www.moh.gov.sg/news-highlights/details/update-on-covid-19-situation-and-measures-to-protect-healthcare-capacity.
14. Nextstrain. Genomic epidemiology of SARS-CoV-2 with subsampling focused on Asia over the past 6 months . Available from: https://nextstrain.org/ncov/gisaid/asia/6m?dmax=2022-11-17&dmin=2022-01-01&l=scatter&p=full.
15. SARS-Cov-2 Analysis Workflow [Cited 2023 March 24]. Available from: https://labs.epi2me.io/notebooks/SARS_CoV_2_Analysis_Workflow.html.
16. Boudet A, Stephan R, Bravo S, Sasso M, Lavigne JP. Limitation of Screening of Different Variants of SARS-CoV-2 by RT-PCR. Diagnostics (Basel). 2021;11(7). https://doi.org/10.3390/diagnostics11071241
17. Zhou Y, Zhang L, Xie YH, Wu J. Advancements in detection of SARS-CoV-2 infection for confronting COVID-19 pandemics. Lab Invest. 2022;102(1):4-13. https://doi.org/10.1038/s41374-021-00663-w
18. Fan Y, Li X, Zhang L, Wan S, Zhang L, Zhou F. SARS-CoV-2 Omicron variant: recent progress and future perspectives. Signal Transduct Target Ther. 2022;7(1):141. https://doi.org/10.1038/s41392-022-00997-x
19. Meng B, Abdullahi A, Ferreira IATM, Goonawardane N, Saito A, Kimura I, et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. Nature. 2022;603(7902):706-14. https://doi.org/110.1038/s41586-022-04474-x
20. Peacock TP, Brown JC, Zhou J, Thakur N, Newman J, Kugathasan R, et al. The SARS-CoV-2 variant, Omicron, shows rapid replication in human primary nasal epithelial cultures and efficiently uses the endosomal route of entry. BioRxiv. 2022:2021-12. https://doi.org/10.1016/j.jcv.2021.104905
21. Camp JV, Buchta C, Jovanovic J, Puchhammer-Stöckl E, Benka B, Griesmacher A, et al. RT-PCR based SARS-CoV-2 variant screening assays require careful quality control. Journal of Clinical Virology. 2021;141:104905.
22. Focosi D, McConnell S, Casadevall A. The Omicron variant of concern: Diversification and convergent evolution in spike protein, and escape from anti-Spike monoclonal antibodies. Drug Resist Updat. 2022;65:100882. https://doi.org/10.1016/j.drup.2022.100882
23. WHO. TAG-VE statement on Omicron sublineages BQ.1 and XBB 2022 [Cited 2023 March 24]. Available from: https://www.who.int/news/item/27-10-2022-tag-ve-statement-on-omicron-sublineages-bq.1-and-xbb.
24. Ngiam JN, Al-Mubaarak A, Maurer-Stroh S, Tambyah PA. Does the COVID-19 XBB Omicron subvariant signal the beginning of the end of the pandemic? Singapore Med J. 2022. https://doi.org/10.4103/singaporemedj.smj-2022-180