Global Health & Medicine. 2025; 7(2):151-160.
ORIGINAL ARTICLE
DOI: 10.35772/ghm.2025.01004

Large-scale screening of SARS-CoV-2 variants in Tokyo, Japan: A 3-year and 9-month longitudinal survey

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Abstract: Over nearly four years (March 10, 2021–December 31, 2024), we performed a comprehensive longitudinal analysis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants among patients in a single hospital in Tokyo, Japan. Using RT-qPCR and Sanger sequencing, complemented by whole-genome sequencing, we tested nasopharyngeal swab samples (n = 4,628) and tracked the emergence and evolution of variants of concern (VOCs). The findings demonstrate the utility of a hospital-based SARS-CoV-2 variant surveillance system for informing clinical decision-making and public health settings, including: *i*) serving as a reference for selecting appropriate treatments, *ii*) enabling early detection of VOCs, *iii*) contributing to the development of hospital infection control guidelines, *iv*) fostering cooperation with local governments, *v*) supporting cohort studies, and *vi*) identifying long-term SARS-CoV-2 infections. This work underscores the importance of real-time variant monitoring for mitigating the effects of pandemics and provides essential epidemiological and clinical data that can guide future outbreak management and policy development.

***Keywords*:** COVID-19, variant of concern (VOC), sequencing, epidemiology, clinical data

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19 (*1*), continues to mutate as the infection spreads. Since the Alpha variant (PANGO lineage B.1.1.7) (*2*) was designated a "Variant of Concern (VOC)" at the end of 2020, the Delta (B.1.617.2, AY sublineages) (*3*) and Omicron (B.1.1.529, BA sublineages) (*4*) variants were identified in May and November 2021, respectively. Subsequently, various sublineages of the Omicron variant, including BA.1, BA.2, BA.4, BA.5, and other recombinant strains, have been reported (*5*). When VOCs emerge independently, each may become regionally or globally dominant, replacing earlier variants. The replacement of these mutant strains has caused successive "waves" of infection, significantly burdening healthcare systems (*6,7*). By the end of 2024, Japan had faced eleven major SARS-CoV-2 epidemic waves. Each dominant variant exhibits unique clinical characteristics, including disease severity, immune evasion, transmissibility, and sensitivity to vaccines or therapeutics (particularly monoclonal antibodies) (*8*). In clinical settings, identifying the infecting variant can be crucial for determining treatment strategies for patients with underlying conditions or comorbidities, as well as for controlling the spread of infection within hospital wards (*9*).

The National Center for Global Health and Medicine (NCGM), Tokyo, one of four Designated Medical Institutions for Specified Infectious Diseases in Japan, has been involved in the COVID-19 response since the outbreak began. Activities included health checkups and RT-qPCR testing for returnees on chartered flights from Wuhan, China (*10*), and medical care for patients from the Diamond Princess cruise ship (*11*). In hospitals treating a large number of COVID-19 cases, it is particularly important to understand the circulating

variants with distinct virological characteristics in a clinical setting. During the early years of the pandemic, SARS-CoV-2 variant monitoring in Japan was documented using whole-genome sequencing-based (*12*) and Sanger sequencing-based methods (*13*). However, reports on the longitudinal surveillance of variants within a single medical institution remain limited.

This study aimed to develop a rapid and efficient SARS-CoV-2 variant monitoring system within a clinical setting and evaluate its impact on patient management and hospital infection control. Beginning on March 10, 2021, NCGM initiated SARS-CoV-2 variant testing using residual nasopharyngeal swab samples from COVID-19 patients with RT-qPCR-based kits or Sanger sequencing to monitor variant trends within the hospital. Additionally, we report findings from a large-scale screening of SARS-CoV-2 variants among hospitalized patients at NCGM over a period of three years and nine months, discussing how variant information was utilized in clinical settings. This study provides insights into the role of real-time variant surveillance in hospital settings, offering a model that could be applied to future infectious disease outbreaks.

Materials and Methods

Participants in the study

Between March 10, 2021, and December 31, 2024, we tested 4,628 residual nasopharyngeal swab samples from patients (both outpatients and inpatients) diagnosed with COVID-19 at NCGM in Tokyo, Japan.

RT-qPCR-based method

The VirSNiP SARS-CoV-2 Spike kits (Roche Diagnostics Corp., Tokyo, Japan) were used per the manufacturer's protocol.

Nucleic acid extraction and Sanger sequencing

Nucleic acid (50 µL) was extracted from 200 µL of nasopharyngeal swab samples using a KingFisher APEX System (Thermo Fisher Scientific, Waltham, MA, USA) and the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific). Subsequently, 15 µL of the extracted nucleic acid was used for cDNA synthesis using PrimeScript™ IV 1st Strand cDNA Synthesis Mix (Takara Bio, Shiga, Japan) with a random hexamer. A total of 1 µL of cDNA was amplified through 1st and 2nd PCR reactions using PrimeSTAR® Max DNA Polymerase (Takara Bio). The primer sets are listed in Table 1. These sets amplified approximately a 0.6 Kbp fragment of the receptor binding domain (RBD) of the *spike* (S) gene to identify SARS-CoV-2 variants (Figure 1A, Fragment 2). Since September 2024, an additional S gene region (Figure 1A, Fragment 1; N-terminal domain covering amino acid residues 1-70) has been analyzed to distinguish

Table 1. Primer sets used in this study

Fragment 1 (for XEC)			
5'- to 3'-	**PCR**	**Direction**	**Period**
ATGTCATGCATGCAAATTACATATTTTGGA	1st	Forward	week 35 of 2024 ~
AATTCACAGACTTTAATAACAACATTAGTAGCG	1st	Reverse	
TTGTCTTCCTATTCTTTATTTGACATGAGT	2nd	Forward	
TCTAAAGTAGTACCAAAAATCCAGCCTC	2nd	Reverse	
Fragment 2 (for Receptor Binding Domain)			
5'- to 3'-	**PCR**	**Direction**	**Period**
ACTTGTGCCCTTTTGGTGAAGT	1st/2nd	Forward	week 17 of 2021 ~
TGCTGGTGCATGTAGAAGTTCA	1st/2nd	Reverse	
ACTTGTGCCCTTTTGRTGAAGT	1st/2nd	Forward	week 48 of 2021 ~
TGCTGGTGCATGTAGAAGTTCA	1st/2nd	Reverse	
TCCTTCACTGTAGAAAAAGGAATCTATCA	1st	Forward	week 43 of 2022 ~
GTCCACAAACAGTTGCTGGTG	1st	Reverse	
GATTTCCTAATATTACAAACTTGTGCC	2nd	Forward	
TGCTGGTGCATGTAGAAGTTCA	2nd	Reverse	
TCCTTCACTGTAGAAAAAGGAATCTATCA	1st	Forward	week 46 of 2023 ~
GTCCACAAACAGTTGCTGGTG	1st	Reverse	
GTTAGATTTCCTAATATTACAAACTTGTG	2nd	Forward	
TGCTGGTGCATGTAGAAGTTCA	2nd	Reverse	
CGTTGAAATCCTTCACTGTAGAAAAAGG	1st	Forward	week 41 of 2024 ~
TCCACAAACAGTTGCTGGTGC	1st	Reverse	
GAGTCCAACCAACAGAATCTATTGTTAGAT	2nd	Forward	
TCAAAAGAAAGTACTACTACTCTGTATGGTT	2nd	Reverse	

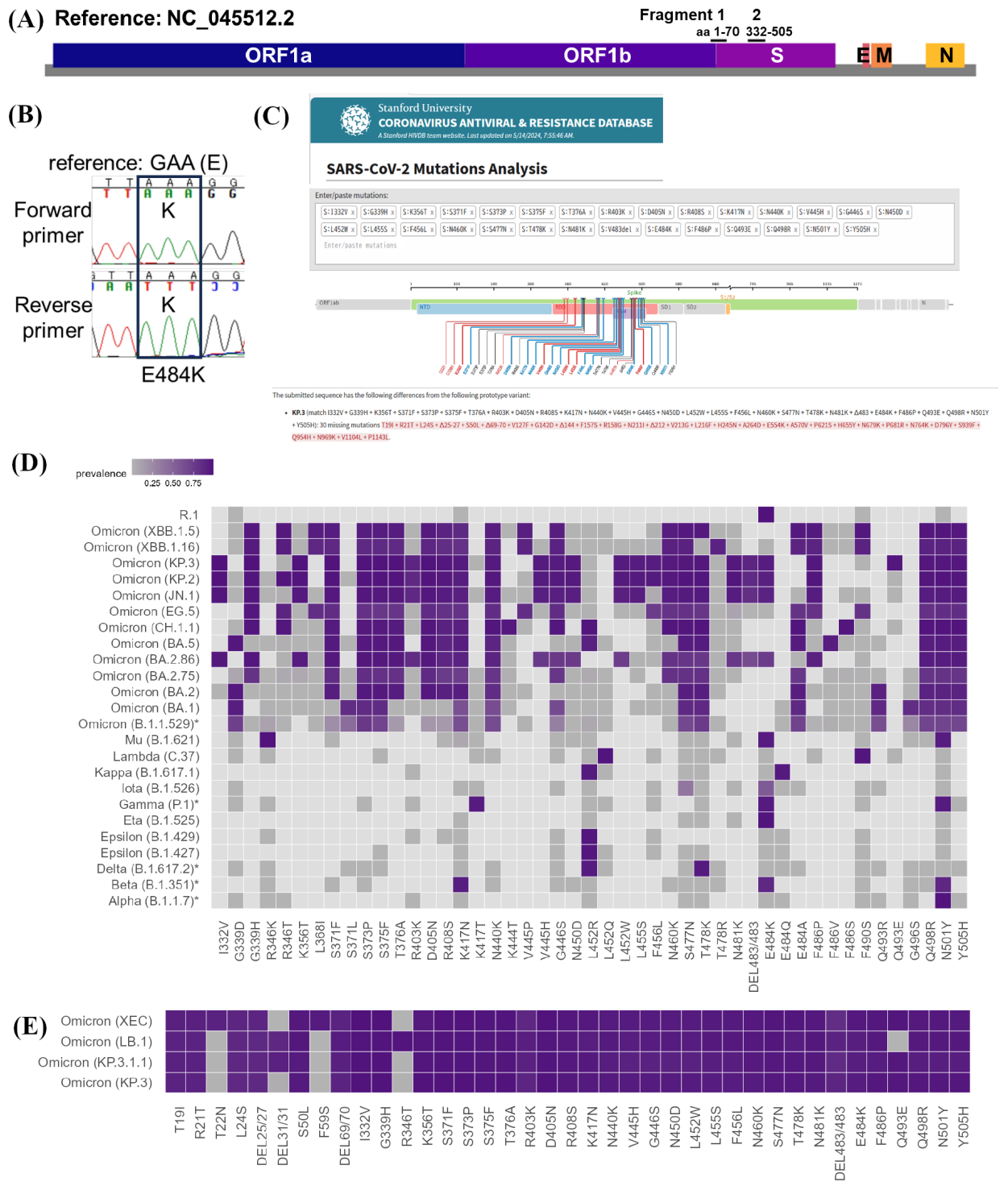

Figure 1. Inferring SARS-CoV-2 variants using Sanger sequencing. (A) RT-PCR amplification regions targeted in this study. Fragments 1 (covering amino acid residues 1–70) and 2 (covering amino acid residues 332–505) include key mutations that enable the identification of XEC and VOC, respectively. **(B)** The resulting sequence electropherograms were aligned with the Wuhan-Hu-1 reference sequence (NC_045512.2). **(C)** SARS-CoV-2 variants were inferred based on mutation patterns using the Stanford SARS-CoV-2 Mutations Analysis tool. **(D, E)** Prevalence of mutations within the sequenced S region for Variants of Concern (VOC), Variants of Interest (VOI), and the R.1 variant, one of the dominant variants during Japan's 4th wave, is shown. Mutations present in at least 75% of the sequences associated with each lineage are displayed. For VOC, an asterisk (*) is attached to each notation. Data were obtained from GISAID (accessed on 14 March 2025). The heatmap was generated using the R package outbreakinfo 0.2.0 (*42*). aa: amino acid residues.

between KP.3, KP.3.1.1, LB.1, and XEC variants (Figure 1E). Positive PCR products were validated *via* agarose gel electrophoresis, purified using a Millipore Multiscreen-HTS-PCR 96-well plate (Millipore, Billerica, MA, USA), and sequenced with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The resulting electropherograms were aligned to the Wuhan-Hu-1 reference sequence

(NC_045512.2) using Sequencher 5.4.6 (Hitachi Software Engineering Co., Ltd., Yokohama, Japan) (Figure 1B). Variant inference was based on mutation patterns using the Stanford SARS-CoV-2 Mutations Analysis tool (*14*) (Figure 1C).

Whole-genome sequencing

Using nucleic acid from nasopharyngeal swab samples, cDNA synthesis, target amplification, and library preparation were performed according to the Illumina COVIDSeq Test Reference Guide (Illumina Inc., San Diego, CA, USA) with ARTIC primers (V3, 4, 4.1, or 5.3.2). SARS-CoV-2 genome sequencing was conducted on the Illumina iSeq 100 system, and results were analyzed using the Illumina DRAGEN COVID Lineage software.

Quantification of the virus

RT-qPCR testing was conducted using the SARS-CoV-2 Detection Kit -Multi- (NCV-403, TOYOBO CO., LTD., Osaka, Japan) and a LightCycler 96 System (Roche Diagnostics Corp.), following the manufacturer's instructions. Briefly, 10 μL of extracted nucleic acid was mixed with 40 μL of reaction mixture. The cycle quantification (Cq) values were obtained by amplifying two regions (N1 and N2 primer/probe sets) from the N gene.

Database analysis

The SARS-CoV-2 lineage distribution in Japan and worldwide during the study period was analyzed using data from the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (*15*) as of January 9, 2025. The following criteria were applied: "Collection date" between March 10, 2021, and December 31, 2024, or between January 1, 2020, and December 31, 2024. "Sequence length" ≥ 27,000, and "Passage details/history" is Original. For domestic sequences, "Location" was set to Japan.

Data visualization was performed using R 4.3.1, and statistical analyses were conducted using GraphPad Prism 9.3.0. (GraphPad Software Inc., San Diego, CA, USA).

Results

A total of 4,628 nasopharyngeal swab samples from COVID-19-diagnosed cases at NCGM were included in this study. Among these, the SARS-CoV-2 variants of 3,423 samples (74.0%) were inferred (Figure 2A). The World Health Organization (WHO) categorized certain variants as VOCs and variants of interest (VOIs) to enhance global monitoring (*16*). The Sanger sequencing protocol used in this study could distinguish the VOCs and VOIs, including those that did not spread domestically in Japan, except in cases where R.1 and Eta (B.1.525) could not be distinguished (Figure 1D). At NCGM, prior to the emergence of the Delta variant, variants for each sample were identified using RT-qPCR-based kits, such as the VirSNiP SARS-CoV-2 Spike kits (Roche Diagnostics Corp.). If the N501Y mutation was detected, the variant was classified as Alpha, while the presence of the E484K mutation identified it as R.1, one of the dominant variants during Japan's 4th wave. However, as the mutation patterns of the virus diversified, classifying variants based solely on a single mutation in the S gene became increasingly challenging (Figure 1D).

From May 2021 onward, when RT-qPCR-based kits could not determine variants, the Sanger sequencing protocol was adopted. On May 19, 2021, the first Delta variant case, initially detected in India on October 5, 2020 (*3*), was confirmed at NCGM. By late July 2021, just before the Tokyo Olympic and Paralympic Games, all samples at NCGM underwent Sanger sequencing. On December 2, 2021, the first Omicron variant case, originally identified in South Africa on November 25, 2021 (*5*), was detected at NCGM. Starting June 10, 2022, foreign tourists on package tours were allowed to enter Japan. Shortly thereafter, on June 14, 2022, the first BA.4/BA.5 Omicron sublineage was detected at NCGM.

The distribution of SARS-CoV-2 lineages in Japan during the study period was analyzed using all domestic SARS-CoV-2 genome data (≥27,000 nucleotides, n = 642,096) registered in the GISAID EpiCoV database (Figure 2B). While the data from NCGM primarily focused on hospitalized patients with severe cases, the data from GISAID comprises a broader range of data, including epidemiological surveys and quarantine data. Nevertheless, the trends in variant prevalence were consistent across both datasets. Relative fluctuations in the number of newly infected cases in Japan and Tokyo closely aligned with the sequencing data from NCGM and GISAID during Japan's 4th (Alpha) and 5th (Delta) epidemic waves (Figure 2, Figure 3). This is consistent with earlier reports indicating that the number of confirmed COVID-19 cases and the number of sequenced SARS-CoV-2 genomes were well correlated for the 1st to 5th (Delta) waves in Japan, with approximately 10% of confirmed cases being sequenced (*17*).

After the emergence of Omicron sublineages, the Sanger sequencing protocol used in this study could no longer differentiate between certain sublineages, such as BA.1 and BC.1, or BA.4/BA.5 and BF.x (*e.g.*, BF.5), due to the limited sequencing region (0.6 Kbps of the *spike* gene), unlike GISAID, which sequences the entire SARS-CoV-2 genome. For example, samples initially identified as BA.4/BA.5 through Sanger sequencing were later found to include BF.2 or BF.5

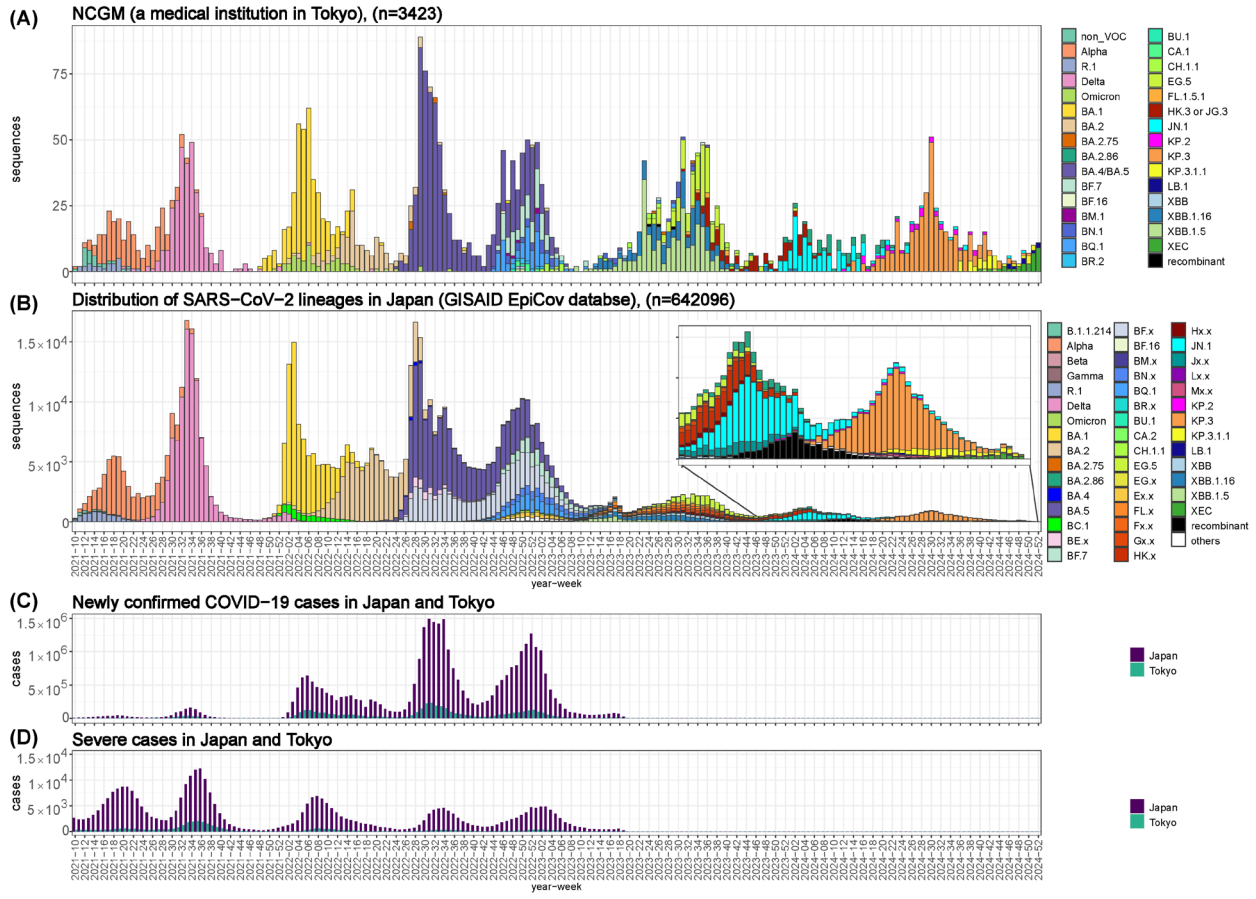

Figure 2. Weekly epidemiological distribution of SARS-CoV-2 variants between March 10, 2021, and December 31, 2024. **(A)** Data derived from patients diagnosed with COVID-19 at NCGM, Tokyo, Japan (n = 3,423). Sequences with no detectable single mutations were classified as "non_VOC". **(B)** All domestic samples (≥ 27,000 nucleotides) registered in the GISAID EpiCoV database (n = 642,096). A magnified view of the data from week 46 of 2023 onward is shown in the upper-right corner. **(C, D)** The number of new SARS-CoV-2 infections and severe cases in Japan and Tokyo based on open data from the Ministry of Health, Labour and Welfare (*43*).

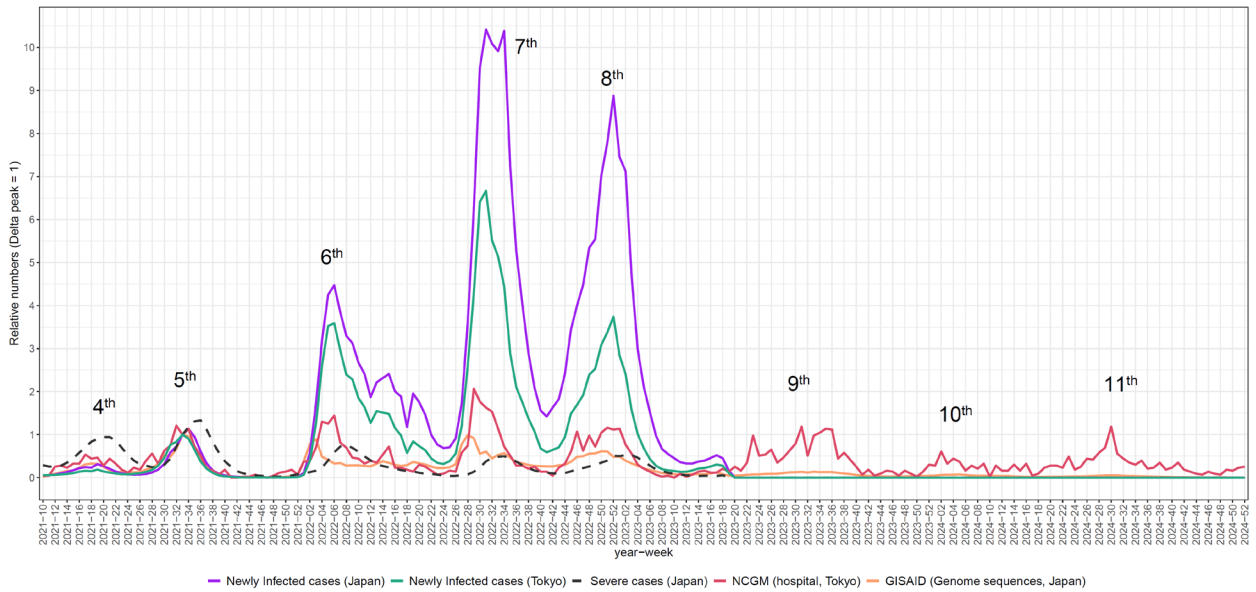

Figure 3. Relative numbers of newly infected cases in Japan (solid purple), in Tokyo (solid green), severe cases in Japan (dashed black), SARS-CoV-2 variants inferred at NCGM (solid red), and domestic genome sequences registered in the GISAID database (solid orange) are shown. The Y-value at the peak of the 5th wave (Delta peak, week 33 of 2021) is set to 1.

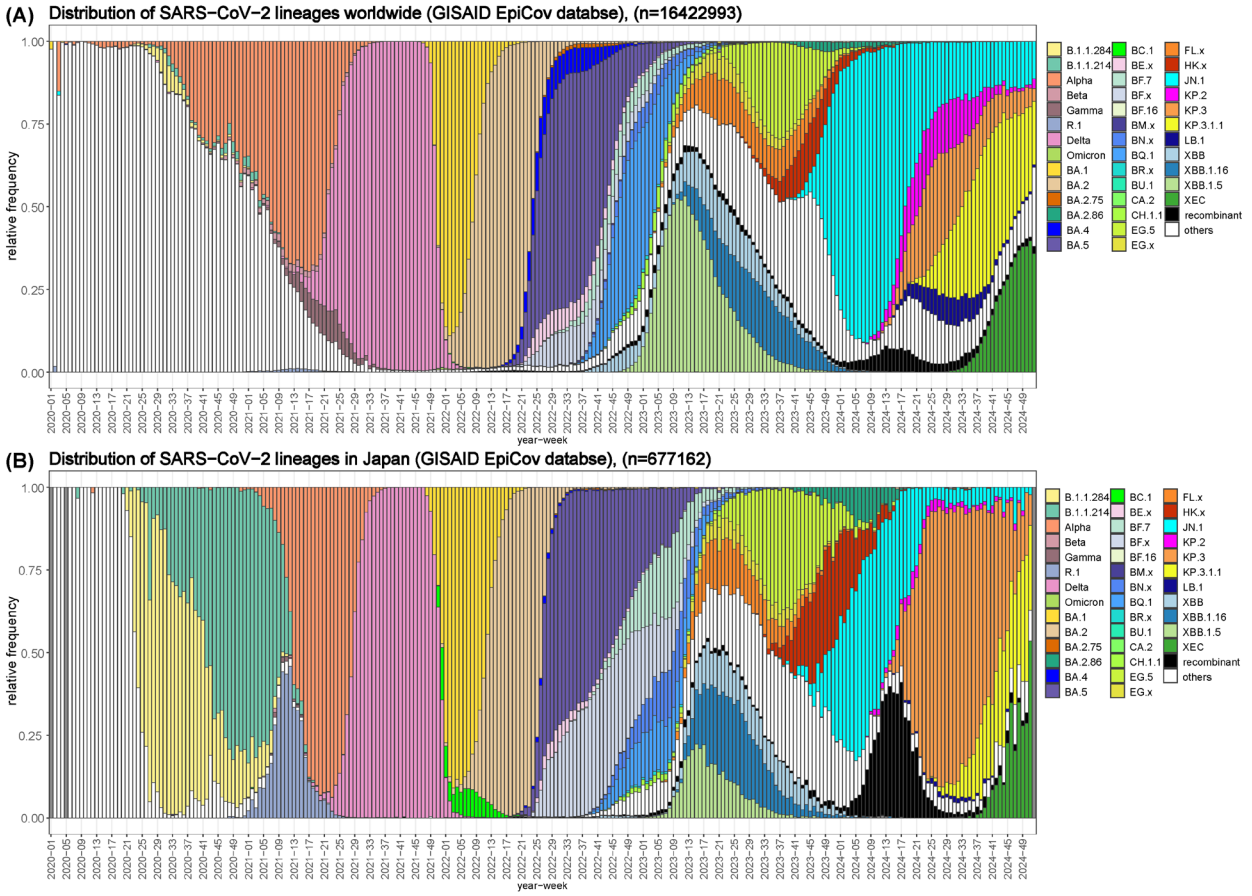

Figure 4. Weekly epidemiological relative frequency of SARS-CoV-2 variants between January 1, 2020, and December 31, 2024. (A) All worldwide (*n* = 16,422,993) and **(B)** domestic (*n* = 677,162) samples (≥ 27,000 nucleotides) registered in the GISAID EpiCoV database.

upon whole-genome sequencing. Nonetheless, NCGM data successfully captured the overall trends of SARS-CoV-2 variants.

On May 8, 2023 (week 19 of 2023), the Japanese government reclassified COVID-19 as a "Class 5 disease" under the Infectious Diseases Control Law, equating it to seasonal influenza (*18,19*). Consequently, COVID-19 surveillance in Japan transitioned from a notifiable system to a sentinel-based system (*20*), and reporting all detected COVID-19 cases was no longer mandatory. As a result, the proportion of sequences registered in GISAID decreased after the 9th wave (Omicron XBB.1.5/EG.5.1) compared to NCGM data. In NCGM data, the peak case numbers of the 8th and 9th waves were nearly identical. However, in GISAID data, the peak of the 9th wave was reduced to one-quarter of that of the 8th wave, suggesting that while both NCGM and GISAID captured domestic outbreak waves, GISAID had a lower capture rate compared to NCGM.

Next, regarding a comparison of trends between Japan and global data, Figure 4 presents the distributions of SARS-CoV-2 lineages between January 1, 2020, and December 31, 2024. Several differences were noted: *i*) detection of region-specific minor strains and other VOCs, such as the Beta (B.1.351) and Gamma (P.1) variants, during the pre-Delta period; and *ii*) variations in the frequency of Omicron sublineages. Conversely, transition to the Delta variant and subsequent spread of the Omicron BA.1 and BA.2 variants followed consistent patterns worldwide.

Finally, both NCGM and GISAID (Japan and worldwide) datasets demonstrated that when variants with higher transmissibility or basic reproduction numbers (R_0) emerged, their replacement of previous variants was clearly observable, as documented in prior studies (*21*). For example, the transmission advantage of BA.1 (170%) is approximately double that of Delta (85%), and more recent Omicron variants, such as XBB (280%), exhibit significantly greater transmissibility.

To investigate the underlying cause of amplification failure in some samples during the 2nd PCR step of Sanger sequencing, we quantified the viral load of 60 nasopharyngeal swab samples using RT-qPCR testing. All of these samples were derived from the same epidemic wave (8th wave) and amplified using the same primer sets. Of these, 30 were sequentially extracted from the period with low amplification efficiency (3/30, weeks 8-10 of 2023), while the remaining 30 were sequentially extracted from the period with high amplification efficiency (27/30, weeks 47-48 of 2022).

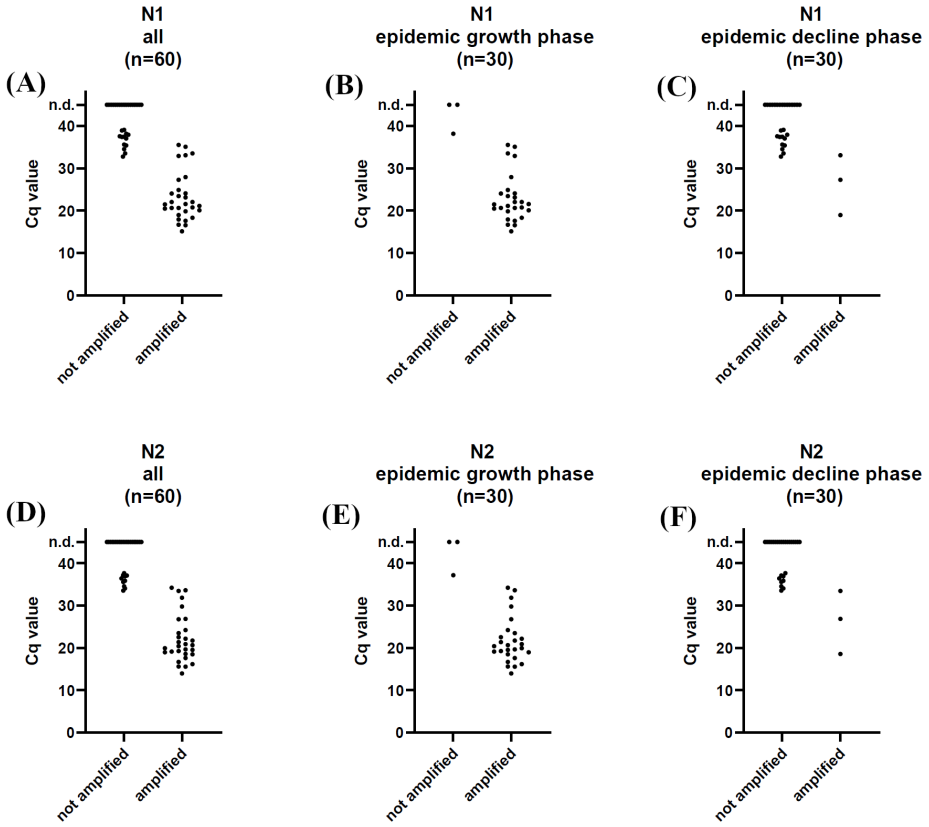

Figure 5. (A, D) Comparison of Cq values between successfully amplified (n = 30) and not amplified (n = 30) samples during the 2nd PCR. Among the 60 samples, **(B, E)** samples collected during the epidemic growth phase of the 8th wave (weeks 47-48 of 2022) and **(C, F)** samples collected during the epidemic decline phase (weeks 8-10 of 2023) are shown separately. Cq values were quantified using the SARS-CoV-2 Detection Kit -Multi- (TOYOBO) by amplifying two regions (N1 and N2 primer/probe sets) derived from the N gene. n.d.: not detected.

As expected, samples with lower Cq values (indicating higher viral loads) were more likely to be amplified (Figure 5). Among the non-amplified samples, 17 out of 30 for N1 and 20 out of 30 for N2 were below the detection limit. Median Cq values for N1 and N2 in the non-amplified samples were 37.42 (interquartile range [IQR]: 34.98-38.06) and 36.14 (IQR: 34.41-37.13), respectively, which were significantly higher than those of successfully amplified samples, whose medians were 21.54 (IQR: 19.62-25.49) for N1 and 20.55 (IQR: 18.55-24.85) (both $p < 0.0001$).

Interestingly, among the 60 samples, those collected during the epidemic's growth phase (weeks 47-48 of 2022) exhibited higher viral loads (Figure 5B, E), while those collected during the decline phase (weeks 8-10 of 2023) showed lower viral loads (Figure 5C, F). This observation aligns with previous findings that the early phase of an epidemic wave often involves a higher proportion of recently infected individuals with higher viral loads, whereas the late phase sees a greater proportion of individuals with relatively older infections and lower viral loads (*22*). Our data further confirmed differences in viral copy numbers between samples collected during the epidemic expansion and contraction phases.

Discussion

At NCGM, variant information was provided in real-time as a reference for the Center Hospital, the Laboratory Testing Department, and the Infection Control Team. Additionally, upon request, this information was supplied to local governments. Representative applications include the following: *i*) Since the Omicron variant and its sublineages are resistant to monoclonal antibody treatments (*23-25*), variant information was used as a reference for selecting appropriate treatments; *ii*) The variant information facilitated the early detection of Delta and Omicron cases in Japan (*26-28*); *iii*) In cases of COVID-19 outbreaks within the Center Hospital, suspected samples were analyzed in detail, including whole-genome sequencing and phylogenetic analysis. These findings were shared with the Infection Control Team to trace virus transmissions within wards, contributing to the development of hospital infection control guidelines; *iv*) At the request of local governments, whole-genome sequencing of samples from severe COVID-19 cases was performed and registered in the GISAID database; *v*) Some data were utilized in cohort studies of breakthrough infections,

particularly in relation to vaccination or prior infection among NCGM staff (*29-31*); *vi*) Long-term SARS-CoV-2 infection cases were identified as part of variant surveillance efforts (*32*). On a global scale, examining the genetic changes in SARS-CoV-2 has significantly enhanced public health responses. A notable example is the development of mRNA vaccines. The Delta variant, for instance, demonstrated increased transmissibility and virulence, which was associated with higher morbidity rates (*33,34*). These findings prompted intensified vaccination efforts. In countries such as the United Kingdom and the United States, vaccination campaigns were accelerated to mitigate severe COVID-19 outcomes (*35*). Similarly, Japan successfully implemented the primary COVID-19 vaccination series (first and second doses), achieving 75% coverage by the end of November 2021 (*36,37*). The effectiveness of the primary series of COVID-19 mRNA vaccines against symptomatic infection in Japan was reported at 89.8% during the Delta wave. In contrast, during the Omicron wave, the effectiveness dropped to 21.2%, but with the administration of a third dose, it rose to 71.8% (*38*). These findings underscore the necessity of booster doses, and Japan began administering the third dose in December 2021. However, the effectiveness of this dose waned over time, particularly against Omicron sublineages (*39*). In response to the reduced vaccine effectiveness, bivalent mRNA vaccines (containing Spike-encoding mRNA of both the original SARS-CoV-2 strain and Omicron-BA.1 or -BA.4/5) and additional Omicron-XBB.1.5 or -JN.1 monovalent mRNA vaccines have been developed (*39,40*).

As demonstrated, the capability to analyze SARS-CoV-2 variants within a hospital and the establishment of a collaborative team to share real-time information can be critical for optimizing treatment strategies, controlling the spread of infections in healthcare settings, understanding regional variant trends, and advancing epidemiological and clinical research. However, this study has several limitations. First, since it was conducted at a single medical institution in Tokyo, Japan, the findings on SARS-CoV-2 variant trends may not be generalizable to other regions or countries. Second, the testing methods changed throughout the study. Initially, the RT-qPCR method was used, but from May 2021, it was replaced with the Sanger method. Third, the Sanger method used in this study only covers approximately 0.6 Kbps of the S gene, which limits the resolution of lineage identification. While lineage estimation is possible, precise identification requires next-generation sequencing (NGS)-based full-genome analysis for more detailed information.

In conclusion, our study highlights the utility of RT-qPCR and Sanger sequencing, complemented by whole-genome sequencing, in screening SARS-CoV-2 variants, both in clinical settings and for gaining epidemiological and medical insights. By tracking nearly all patient samples from a Tokyo hospital over three years and nine months, we acquired valuable insights into the turnover of variants in symptomatic patients, including those with severe cases. The WHO declared a Public Health Emergency of International Concern for COVID-19 on January 30, 2020, which was officially lifted on May 5, 2023. However, the WHO emphasized that emergence of new variants still poses a potential risk of renewed surges in cases and deaths (*41*). Our findings improve the understanding of SARS-CoV-2 variant trends in Tokyo, Japan, and will assist in detection of emerging variants in the future.

Acknowledgements

We thank Yumiko Kito, Nozomi Ariyoshi, Ataru Moriya, Ryoko Tamura, and Yurika Tanaka for their technical support.

Funding: This study was supported by the National Center for Global Health and Medicine Novel Coronavirus Infection Response Fund (Grant Number 22K900C).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Wu F, Zhao S, Yu B*, et al.* A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579:265-269.
2. Volz E, Mishra S, Chand M, *et al.* Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature. 2021; 593:266-269.
3. Mlcochova P, Kemp SA, Dhar MS, *et al.* SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature. 2021; 599:114-119.
4. Viana R, Moyo S, Amoako DG, *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature. 2022; 603:679-686.
5. Dhawan M, Saied AA, Mitra S, Alhumaydhi FA, Emran TB, Wilairatana P. Omicron variant (B.1.1.529) and its sublineages: What do we know so far amid the emergence of recombinant variants of SARS-CoV-2? Biomed Pharmacother. 2022; 154:113522.
6. Haileamlak A. The impact of COVID-19 on health and health systems. Ethiop J Health Sci. 2021; 31:1073-1074.
7. Fatani M, Shamayleh A, Alshraideh H. Assessing the disruption impact on healthcare delivery. J Prim Care Community Health. 2024; 15:21501319241260351.
8. Carabelli AM, Peacock TP, Thorne LG, Harvey WT, Hughes J; COVID-19 Genomics UK Consortium; Peacock SJ, Barclay WS, de Silva TI, Towers GJ, Robertson DL. SARS-CoV-2 variant biology: immune escape, transmission and fitness. Nat Rev Microbiol. 2023; 21:162-177.
9. Ferdinand AS, Kelaher M, Lane CR, da Silva AG, Sherry NL, Ballard SA, Andersson P, Hoang T, Denholm JT, Easton M, Howden BP, Williamson DA. An

implementation science approach to evaluating pathogen whole genome sequencing in public health. Genome Med. 2021; 13:121.
10. Hayakawa K, Kutsuna S, Kawamata T, *et al*. SARSCoV-2 infection among returnees on charter flights to Japan from Hubei, China: A report from National Center for Global Health and Medicine. Glob Health Med. 2020; 2:107-111.
11. Tsuboi M, Hachiya M, Noda S, Iso H, Umeda T. Epidemiology and quarantine measures during COVID-19 outbreak on the cruise ship Diamond Princess docked at Yokohama, Japan in 2020: A descriptive analysis. Glob Health Med. 2020; 2:102-106.
12. Ode H, Nakata Y, Nagashima M, *et al*. Molecular epidemiological features of SARS-CoV-2 in Japan, 2020-1. Virus Evol. 2022; 8:veac034.
13. Ko K, Takahashi K, Nagashima S, *et al.* Mass screening of SARS-CoV-2 variants using sanger sequencing strategy in hiroshima, Japan. Sci Rep. 2022; 12:2419.
14. Tzou PL, Tao K, Pond SLK, Shafer RW. Coronavirus Resistance Database (CoV-RDB): SARS-CoV-2 susceptibility to monoclonal antibodies, convalescent plasma, and plasma from vaccinated persons. PLoS One. 2022; 17:e0261045.
15. GISAID. The Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database. *https://gisaid.org* (accessed January 9, 2025).
16. World Health Organization. Tracking SARS-CoV-2 variants. *https://www.who.int/activities/tracking-SARS-CoV-2-variants/* (accessed March 3, 2025).
17. Nakagawa S, Katayama T, Jin L, Wu J, Kryukov K, Oyachi R, Takeuchi JS, Fujisawa T, Asano S, Komatsu M, Onami JI, Abe T, Arita M. SARS-CoV-2 HaploGraph: visualization of SARS-CoV-2 haplotype spread in Japan. Genes Genet Syst. 2023; 98:221-237.
18. Kitahara K, Nishikawa Y, Yokoyama H, Kikuchi Y, Sakoi M. An overview of the reclassification of COVID-19 of the Infectious Diseases Control Law in Japan. Glob Health Med. 2023; 5:70-74.
19. The Ministry of Health, Labour and Welfare. Response to COVID-19 after the classification change. *https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000164708_00079.html* (accessed March 3, 2025).
20. Arima Y, Takahashi T, Kasamatsu A, Arashiro T, Kobayashi Y, Otsuka M, Takahara O, Shimbashi R, Komase K, Kamigaki T, Suzuki M. Sentinel surveillance of COVID-19: The importance of epidemiologic concepts and reasoning. J Epidemiol. 2025; 35:106-107.
21. Lee B, Quadeer AA, Sohail MS, Finney E, Ahmed SF, McKay MR, Barton JP. Inferring effects of mutations on SARS-CoV-2 transmission from genomic surveillance data. Nat Commun. 2025; 16:441.
22. Hay JA, Kennedy-Shaffer L, Kanjilal S, Lennon NJ, Gabriel SB, Lipsitch M, Mina MJ. Estimating epidemiologic dynamics from cross-sectional viral load distributions. Science. 2021; 373:eabh0635.
23. Focosi D, McConnell S, Casadevall A, Cappello E, Valdiserra G, Tuccori M. Monoclonal antibody therapies against SARS-CoV-2. Lancet Infect Dis. 2022; 22:e311-e326.
24. Imai M, Ito M, Kiso M, Yamayoshi S, Uraki R, Fukushi S, Watanabe S, Suzuki T, Maeda K, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Halfmann PJ, Kawaoka Y. Efficacy of antiviral agents against Omicron subvariants BQ.1.1 and XBB. N Engl J Med. 2023; 388:89-91.
25. VanBlargan LA, Errico JM, Halfmann PJ, Zost SJ, Crowe JE Jr, Purcell LA, Kawaoka Y, Corti D, Fremont DH, Diamond MS. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat Med. 2022; 28:490-495.
26. Okumura N, Saito S, Takamatsu Y, Takeuchi JS, Asai Y, Sanada M, Iwamoto N, Maeda K, Mitsuya H, Ohmagari N. Antibody titers and neutralizing activity in cases of COVID-19 after a single dose of vaccination. J Infect Chemother. 2022; 28:1704-1706.
27. Okumura N, Tsuzuki S, Saito S, Hattori SI, Takeuchi JS, Saito T, Ujiie M, Hojo M, Iwamoto N, Sugiura W, Mitsuya H, Ohmagari N. Neutralising activity and antibody titre in 10 patients with breakthrough infections of the SARS-CoV-2 Omicron variant in Japan. J Infect Chemother. 2022; 28:1340-1343.
28. Okumura N, Tsuzuki S, Saito S, Saito T, Takasago S, Hojo M, Iwamoto N, Ohmagari N. The first eleven cases of SARS-CoV-2 Omicron variant infection in Japan: A focus on viral dynamics. Glob Health Med. 2022; 4:133-136.
29. Yamamoto S, Matsuda K, Maeda K, Oshiro Y, Inamura N, Mizoue T, Konishi M, Takeuchi JS, Horii K, Ozeki M, Sugiyama H, Mitsuya H, Sugiura W, Ohmagari N. Omicron BA.1 neutralizing antibody response following Delta breakthrough infection compared with booster vaccination of BNT162b2. BMC Infect Dis. 2023; 23:282.
30. Yamamoto S, Matsuda K, Maeda K, *et al.* Protection of Omicron bivalent vaccine, previous infection, and their induced neutralizing antibodies against symptomatic infection with Omicron XBB.1.16 and EG.5.1. Open Forum Infect Dis. 2024; 11:ofae519.
31. Yamamoto S, Matsuda K, Maeda K, *et al.* Preinfection neutralizing antibodies, Omicron BA.5 breakthrough infection, and long COVID: A propensity score-matched analysis. J Infect Dis. 2023; 228:1652-1661.
32. Iwasaki M, Hashimoto M, Takeuchi JS, Kusaba Y, Kimura M, Terada-Hirashima J, Sugiura W, Hojo M. Relapse of COVID-19 and viral evolution in a patient with Good syndrome: A case report. Cureus. 2024; 16:e52592.
33. Saito A, Irie T, Suzuki R, *et al*. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature. 2022; 602:300-306
34. Ward IL, Bermingham C, Ayoubkhani D, Gethings OJ, Pouwels KB, Yates T, Khunti K, Hippisley-Cox J, Banerjee A, Walker AS, Nafilyan V. Risk of covid-19 related deaths for SARS-CoV-2 omicron (B.1.1.529) compared with delta (B.1.617.2): Retrospective cohort study. BMJ. 2022; 378:e070695.
35. Alhamlan FS, Al-Qahtani AA. SARS-CoV-2 variants: Genetic insights, epidemiological tracking, and implications for vaccine strategies. Int J Mol Sci. 2025; 26:1263.
36. Kayano T, Ko Y, Otani K, Kobayashi T, Suzuki M, Nishiura H. Evaluating the COVID-19 vaccination program in Japan, 2021 using the counterfactual reproduction number. Sci Rep. 2023; 13:17762.
37. The Ministry of Health, Labor and Welfare. Number of doses to receive the new corona vaccine. *https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/kenkou/kekkaku-kansenshou/yobou-sesshu/syukeihou_00002.html* (accessed March 3, 2025). (in Japanese)
38. Mimura W, Ishiguro C, Maeda M, Murata F, Fukuda H. Effectiveness of messenger RNA vaccines against infection with SARS-CoV-2 during the periods of Delta

and Omicron variant predominance in Japan: The Vaccine Effectiveness, Networking, and Universal Safety (VENUS) study. Int J Infect Dis. 2022; 125:58-60.
39. Song S, Madewell ZJ, Liu M, Miao Y, Xiang S, Huo Y, Sarkar S, Chowdhury A, Longini IM Jr, Yang Y. A systematic review and meta-analysis on the effectiveness of bivalent mRNA booster vaccines against Omicron variants. Vaccine. 2024; 42:3389-3396.
40. Happle C, Hoffmann M, Kempf A, Nehlmeier I, Stankov MV, Calderon Hampel N, Witte T, Pohlmann S, Behrens GMN, Dopfer-Jablonka A. Humoral immunity after mRNA SARS-CoV-2 omicron JN.1 vaccination. Lancet Infect Dis. 2024; 24:e674-e676.
41. World Health Organization. *https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing---5-may-2023* (accessed March 4, 2025).
42. Gangavarapu K, Latif AA, Mullen JL, *et al.* Outbreak.info genomic reports: Scalable and dynamic surveillance of SARS-CoV-2 variants and mutations. Nat Methods. 2023; 20:512-522.
43. The Ministry of Health, Labour and Welfare. Visualizing the data: information on COVID-19 infections. *https://covid19.mhlw.go.jp/en/* (accessed March 3, 2025).

Received January 20, 2025; Revised March 23, 2025; Accepted April 1, 2025.

Released online in J-STAGE as advance publication April 9, 2025.

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