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SARS-CoV-2 genomic surveillance in Malaysia: displacement of B.1.617.2 with AY lineages as the dominant Delta variants and the introduction of Omicron during the fourth epidemic wave

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Objectives: This study reported SARS-CoV-2 whole genome sequencing results from June 2021 to January 2022 from seven genome sequencing centers in Malaysia as part of the national surveillance program.

Methods: COVID-19 samples that tested positive by reverse transcription polymerase chain reaction and with cycle threshold values <30 were obtained throughout Malaysia. Sequencing of SARS-CoV-2 complete genomes was performed using Illumina, Oxford Nanopore, or Ion Torrent platforms. A total of 6163 SARS-CoV-2 complete genome sequences were generated over the surveillance period. All sequences were submitted to the Global Initiative on Sharing All Influenza Data database.

Results: From June 2021 to January 2022, Malaysia experienced the fourth wave of COVID-19 dominated by the Delta variant of concern, including the original B.1.617.2 lineage and descendant AY lineages. The B.1.617.2 lineage was identified as the early dominant circulating strain throughout the country but over time, was displaced by AY.59 and AY.79 lineages in Peninsular (west) Malaysia, and the AY.23 lineage in east Malaysia. In December 2021, pilgrims returning from Saudi Arabia facilitated the introduction and spread of the BA.1 lineage (Omicron variant of concern) in the country.

Conclusion: The changing trends of circulating SARS-CoV-2 lineages were identified, with differences observed between west and east Malaysia. This initiative highlighted the importance of leveraging research expertise in the country to facilitate pandemic response and preparedness.

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Introduction

Since the declaration of COVID-19 as a pandemic by the World Health Organization in March 2020, COVID-19 continues to be an important health problem worldwide (Gao et al., 2021). The index case of COVID-19 was detected on December 1, 2019, in Wuhan City, Hubei Province, China (Helmy et al., 2020; Valencia, 2020). As of January 2022, there were 380 million COVID-19 cases, with a mortality rate of 1.5% (Worldometer, 2022). COVID-19 is caused by SARS-CoV-2, an enveloped, nonsegmented, positive-sense RNA β-coronavirus (Zhou et al., 2020). In January 2020, a complete reference genome of SARS-CoV-2 from the initial cluster in Wuhan, China was deposited into GenBank (NCBI Resource Coordinators, 2018; Zhu et al., 2020). The genome size of SARS-CoV-2 is ~30kb, encompassing the structural regions spike (S), envelope (E), membrane (M), nucleocapsid (N), and nonstructural regions; open reading frame 1a and 1b (ORF1ab), ORF3a, ORF6, ORF7b, and ORF8 genes (Hassan et al., 2020; Khailany et al., 2020). Over the progression of the pandemic, several novel variants of concern (VOCs), such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), have emerged that were more transmissible than their predecessor and better able to evade the host immune response (Yu et al., 2021).

Malaysia experienced the first wave of COVID-19 in late January 2020, which started with a Chinese tourist who arrived through Singapore and became the first COVID-19 case in Malaysia (Malaysia Ministry of Health, 2021). The first wave lasted for 3 weeks with a low number of cases and no fatalities. The second wave began on February 27, 2020, with >100 cases daily (Malaysia Ministry of Health, 2021; Ng et al., 2020). The government implemented a strict nationwide lockdown on March 18, 2020 to curb and control the virus transmission (Ng et al., 2020). The third wave began in October 2020, after 4 months with a low number of COVID-19 cases, >70% of cases were attributed to mass gatherings and an election in the State of Sabah (Lim et al., 2021). As of January 2022, a total of 2.8 million COVID-19 cases were reported in Malaysia, with a fatality rate of 1.2% (Worldometer, 2022).

As Malaysia was starting to reopen its international border for economic and tourism sectors, continuous genome surveillance became necessary to monitor the COVID-19 pandemic and alert the healthcare system of the emergence of novel VOCs. Many countries have initiated national programs for SARS-CoV-2 genomic surveillance to better understand evolutionary patterns, mutations, and genetic diversities to guide public health responses (Chen et al., 2022; Holshue et al., 2020; Robishaw et al., 2021). In June 2021, the Ministry of Science, Technology, and Innovation of Malaysia approved a nationwide study of SARS-CoV-2 genome surveillance to better monitor the circulating SARS-CoV-2 strains. Seven sequencing centers in different institutions, which included Universiti Kebangsaan Malaysia, Universiti Malaya, Universiti Teknologi MARA, Universiti Malaysia Sarawak, the Institute for Medical Research (Ministry of Health), and the Malaysia Genome and Vaccine Institute (Ministry of Science, Technology, and Innovation), collaborated in this genomic surveillance study. This study reported SARS-CoV-2 whole genome sequencing (WGS) results from seven genome sequencing centers in Malaysia from June 2021 to January 2022.

Materials and methods

Sampling procedures and real-time transcription quantitative polymerase chain testing

Samples were selected from all parts of Malaysia from June 2021 until January 2022. The swab samples in the viral transport medium were triple-sealed, transported using an unbroken cold chain, and stored under ultralow temperature conditions to avoid multiple freeze-thaw cycles. Viral RNA extraction and quantitative polymerase chain reaction (PCR) followed the standard operating procedure of the individual laboratories (Table 1). Samples with cycle threshold values of less than 30 were selected for sequencing.

The samples for sequencing were selected based on several criteria (i) cases from the area under close surveillance for high transmission or with significant increase in cases over a short period of time consisting of different age groups, different severity from mild to severe or fatal cases, or different locality within the state; (ii) children in the area with an increased incidence of pediatric cases; (iii) severe cases among those aged more than 60 years and without any underlying comorbidity or severe cases among younger patients without underlying comorbidities; (iv) patients with unusual manifestations, e.g., patients who deteriorate quickly and in those in the intensive care unit; (v) cases suspected with reinfection; (vi) health care workers and some from the community with breakthrough infections after vaccinations; (vii) cases with negative or significantly weaker positive S-gene result in multiplex real-time transcription PCR (RT-PCR) assays, with positive results for the other targets; (viii) clinical cases that appear with symptoms similar to COVID-19 yet consistently produce negative RT-PCR results; (ix) international points of entries; and (x) any deaths or brought-in-death cases with RT-PCR SARS-CoV-2 positive results. All the epidemiological data are collected with the samples as anonymized national data, collected routinely by the Malaysia Ministry of Health. These samples, except those from Sarawak, were sent to the Institute for Medical Research and then distributed to the sequencing laboratories. The samples from Sarawak were sent to the laboratory at Universiti Malaysia Sarawak.

WGS of SARS-CoV-2

Three different platforms for the genomic surveillance WGS were used, namely Illumina Miseq (Illumina Inc, CA, USA), Oxford Nanopore Technology MinION Mk1C and GridION (Oxford Nanopore Technology, Oxford, UK), and Ion Torrent Ion GeneStudio™ S5 Prime System (Thermo Scientific, CA, USA), as summarized in Table 1. The FASTQs generated from the Illumina platforms were processed and analyzed using the Illumina COVID Lineage (Illumina Inc, CA, USA) (Washington et al., 2021). Variant calling and consensus sequence generation were performed for samples with a minimum of 90% SARS-CoV-2 virus amplicon target. For the Oxford Nanopore platform, raw fast5 file was base-called using Guppy version 5.0.13 (Oxford Nanopore Technology, Oxford, UK). Output FASTQ files were uploaded to either EpI2Me Lab Launcher version 2020.11-01 or PoreCov, and the quality of sequencing result were assessed (Brandt et al., 2021). For the Ion Torrent platform, genome assembly was conducted using the IMG-AREport v1.2.1.0 (Thermo Scientific, CA, USA). All the sequences were preprocessed, aligned, and mapped to the SARS-CoV-2 reference genome (GenBank accession no NC_045512) to generate variant and consensus sequences. The lineages were defined using the Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin) tool version v3.1.11, v3.1.14, v3.1.16, and v3.1.17 (O’Toole et al., 2021).

Reporting

All the results were verified by each sequencing center before submission to a centralized reporting center at the Ministry of Health. All the sequence files were uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) (Khare et al., 2021).
Table 1
Summary of the kit used in the viral RNA extraction, qRT-PCR, library preparation and whole genome sequencing in each sequencing center of the consortium.

| Sequencing center | Viral RNA extraction | qRT-PCR | Library preparation | Sequencing | Platform |
|-------------------|----------------------|---------|---------------------|-------------|----------|
| Institute of Medical Research (IMR) | • MagMAX™ Viral Pathogen Nucleic Acid Isolation kit (Thermo Fisher, MA, USA) • Invitrogen Turbo DNA-free kit (Ambion, Darmstadt, Germany) | • Real-time Fluorescent RT-PCR kit for detecting SARS-CoV-2 (BGI Genomics, China) | • TruSeq Stranded Total RNA (Metagenomics) (Illumina Inc, CA, USA) • Midnight protocol (Oxford Nanopore Technology, Oxford, UK) | • NextSeq 500/500 High Output Kit v2.5 (Illumina Inc, CA, USA) • Rapid barcoding kit 96 (Oxford Nanopore Technologies) • Flow cell R9.4.1 (Oxford Nanopore Technologies) | • Illumina Miseq (Illumina Inc, CA, USA) • Oxford Nanopore GridION (Oxford Nanopore Technology, Oxford, UK) |
| Malaysia Genome and Vaccine Institute | • NuclDeal spin RNA Virus isolation kit (Macherey-Nagel, Dueren, Germany) • GF-1 Viral Nucleic Acid extraction kit (Vivantis, Malaysia) | • Thunderbird Probe One-Step qRT-PCR kit (Toyobo, Osaka, Japan) • DUAL-Labeled Probe, 5FAM/3 bHQ-1 | • Truseq Nano library prep kit (Illumina Inc, CA, USA) • COVIDSeq (Illumina Inc, CA, USA) | • Miseq reagent V4 (2 × 150 bp) (Illumina Inc, CA, USA) | • Illumina Miseq (Illumina Inc, CA, USA) |
| Universiti Kebangsaan Malaysia (UKM) Medical Molecular Biology Institute | • Qiagen Viral RNA (QIAGEN Inc, Valencia, USA) • MagMAX Viral Pathogen Nucleic Acid Isolation kit (Thermo Fisher, MA, USA) | • LifeRiver Novel Coronavirus (2019-nCOV) Real-time Multiplex RT-PCR kit (LifeRiver Bio-tech, Shanghai, China) | • COVIDSeq (Illumina Inc, CA, USA) • NEBNext ARTIC SARS-CoV-2 Companion Kit for Oxford Nanopore Technologies (New England Biolabs, MA, USA) | • Miseq reagent V3 (2 × 76 bp) (Illumina Inc, CA, USA) • Nanopore Sequencing Ligation Kit, SQK LSK109 (Oxford Nanopore Technology, Oxford, UK) | • Illumina Miseq (Illumina Inc, CA, USA) • Oxford Nanopore GridION (Oxford Nanopore Technology, Oxford, UK) |
| Faculty of Medicine, Universiti Malaya | • Qiagen Viral RNA (QIAGEN Inc, Valencia, USA) | | • Midnight protocol (Oxford Nanopore Technology, Oxford, UK) | • Rapid Barcoding Sequencing Kit 96; SQK-RBK110.96 (Oxford Nanopore Technology, Oxford, UK) | • Oxford Nanopore MinION (Oxford Nanopore Technology, Oxford, UK) |
| Tropical Infectious Disease Research & Education Centre, Universiti Malaya | • MagMAX Viral Pathogen Nucleic Acid Isolation kit (Thermo Fisher, MA, USA) | • Allplex™ 2019-nCoV assay (Seegene, South Korea) | • Ion AmpliSeq SARS-CoV-2 Research panel • Ion AmpliSeq SARS-CoV-2 Insight Research Assay • Midnight protocol (Oxford Nanopore Technology, Oxford, UK) | • Ion S10™ & Ion 520™ & Ion 530™ Kit Chef (Thermo Fisher, MA, USA) • Ion 540™ Kit Chef (Thermo Fisher, MA, USA) | • Ion GeneStudio S5 Prime System (Thermo Scientific, CA, USA) • Oxford Nanopore GridION (Oxford Nanopore Technology, Oxford, UK) |
| Integrative Pharmacogenomics Institute, Universiti Teknologi MARA | • Qiagen Viral RNA (QIAGEN Inc, Valencia, USA) | • COVID-19 Real-Time Multiplex RT-PCR kit (Laboratory Diagnostic Oy, VANTAA Finland) | • COVIDSeq (Illumina Inc, CA, USA) • Midnight protocol (Oxford Nanopore Technology, Oxford, UK) | • Miseq reagent V4 (2 × 150 bp) (Illumina Inc, CA, USA) | • Illumina Miseq (Illumina Inc, CA, USA) • Oxford Nanopore MinION (Oxford Nanopore Technology, Oxford, UK) |
| Institute of Health and Community Medicine, Universiti Malaysia Sarawak | • Qiagen Viral RNA (QIAGEN Inc, Valencia, USA) | | | | • Oxford Nanopore MinION (Oxford Nanopore Technology, Oxford, UK) |

qRT-PCR, quantitative real-time transcription polymerase chain reaction.
Figure 1. SARS-CoV-2 sequences in Malaysia during the fourth epidemic wave from June 2021 to January 2022 (n = 6163). (a) Map of Malaysian states with the number of SARS-CoV-2 sequences as of January 2022. (b) Molecular epidemiology of SARS-CoV-2 that circulated in Malaysia and the top 20 most prevalent circulating lineages based on the Pango nomenclature. (c) SARS-CoV-2 strains that circulated in Malaysia categorized by month. (d) Molecular epidemiology of SARS-CoV-2 and COVID-19-related death cases and the total number of sequenced cases categorized by month. (e) SARS-CoV-2 lineage and the total number of COVID-19-related death cases sequence categorized by the patient's age group. The B.1.617.2 and AY lineages belonged to the Delta VOC, while B.1.1.529 and BA-lineages belonged to the Omicron VOC. VOC, variant of concern

Phylogenetic and mutational analysis

We retrieved the FASTA files from the GISAID database for Malaysian isolates from June 2021 to January 2022. Clade assignment, mutation calling, and sequence quality control score were performed using the open-source Nextclade pipelines comprising the phylodynamic analysis component Augur and the interactive visualization component Auspice (https://clades.nextstrain.org) (Aksamentov et al., 2021). The sequences were aligned pairwise using the Multiple Alignment using Fast Fourier Transform alignment program (Kath and Stanley, 2013). A maximum likelihood tree was constructed with the IQ-TREE server using the general time-reversible model with proportion of invariable sites and gamma rate variation across sites (Nguyen et al., 2015). The final dataset was displayed using the Interactive Tree of Life (Letunic and Bork, 2021). The phylogeny tree was outgroup rooted on the earliest lineage B sequence from Wuhan (GenBank accession no NC_045512). The frequency of identified mutation for the variants was determined and annotated using the mutation list as listed in the COV-Glue (https://cov-glue.cvr.gla.ac.uk) (Singer et al., 2020).

Data analysis

The descriptive data were analyzed using SPSS software (version 24.0, IBM, Chicago, IL, USA), whereas graphs were constructed using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

Results

From June 2021 to January 2022, a total of 6163 samples from the local cases were sequenced by the consortium, representing 0.3% of the reported COVID-19 cases during this period (Figure 1a). The SARS-CoV-2 genomic variants in Malaysia during the fourth epidemic wave were diverse. From the 6163 samples, 5907 (95.8%) were identified as the Delta variant, followed by the Omicron variant (n = 144, 2.4%), non-VOC variants (n = 95, 1.5%), and the Beta variant (n = 19, 0.3%). From the 93 samples with the non-VOC variants, 64.5% were within the AU lineage (n = 60), followed by B.1.524 lineage (n = 15, 16.1%), B.1.466.2 lineage (n = 10, 10.8%), B.1.3616 lineage (n = 6, 6.5%), B.1.1.519 lineage (n = 1, 1.1%), and B.1 lineage (n = 1, 1.1%). Based on the Pango nomenclature, the Delta variant was the predominant variant circulating with the AY.23 lineage being the most common (n = 2289, 35.1%), followed by the original Delta variant, B.1.617.2 lineage (n = 1812, 29.4%), AY.59 lineage (n = 715, 11.6%), AY.79 lineage (n = 552, 70.0%), and AY.4 lineage (n = 132, 2.1%; Figure 1b; Supplementary Table 1). Within Omicron, BA.1.1 was the most common lineage circulating in Malaysia (n = 84, 1.4%; Figure 1b; Supplementary Table 1). From June to July 2021, the B.1.617.2 lineage was the dominant circulating lineage, and this was gradually replaced by the Delta variant AY.23 lineage in August 2021 (Figure 1c; Supplementary Table 2). From October to December, the Delta variant AY.59 and AY.79 lineages replaced AY.23 lineage to become the dominant circulating Delta lineages detected. From December 2021 onward, the Omicron variant BA.1 and BA.1.1 lineages became the predominant lineage, replacing the Delta variant as the major circulating SARS-CoV-2 variant.

From the 6163 samples sequenced during the surveillance period, 423 (6.9%) samples were from COVID-19-related death cases. Of these, 58.6% were male, 52.0% were patients aged >60 years (n = 220), 51.8% were unvaccinated patients (n = 219), and 80.1% were brought-in-dead cases (n = 339) (Table 2). Most COVID-19-related death cases were associated with the Delta variant.
Table 2
Distribution and demographic data of the sequenced samples of COVID-19-related death in patients categorized by variants and lineages, n = 423.

| Characteristics          | SARS-CoV-2 variant | SARS-CoV-2 lineage |
|--------------------------|--------------------|--------------------|
|                          | Delta, n (%)       | Omicron, n (%)     | Non-VOC, n (%) | B.1.617.2, n (%) | AV.59, n (%) | AV.23, n (%) | AV.79, n (%) | AV.4, n (%) | Others, n (%) |
| Total                    | 423 (100.00)       | 419 (99.05)        | 1 (0.24)       | 3 (0.71)         | 252 (59.57) | 52 (12.29) | 29 (6.86)   | 25 (5.91)   | 17 (4.02)    | 48 (11.35) |
| Sex                      |                    |                    |                |                 |             |             |             |             |              |            |
| Male                     | 248 (58.63)        | 245 (58.47)        | 0 (0.00)       | 3 (100.00)       | 147 (58.33) | 31 (59.62) | 16 (55.17)  | 16 (64.00)  | 8 (47.06)    | 30 (62.50) |
| Female                   | 175 (41.27)        | 174 (41.53)        | 1 (100.00)     | 0 (0.00)         | 105 (41.67) | 21 (40.38) | 13 (44.83)  | 9 (36.00)   | 9 (52.94)    | 18 (37.50) |
| Type of death            |                    |                    |                |                 |             |             |             |             |              |            |
| Brought in dead          | 339 (80.14)        | 336 (80.19)        | 1 (100.00)     | 2 (66.67)        | 213 (84.52) | 30 (57.69) | 25 (86.21)  | 14 (56.00)  | 13 (76.47)   | 44 (91.67) |
| In-hospital death        | 84 (19.86)         | 83 (19.81)         | 0 (0.00)       | 1 (33.33)        | 39 (15.48)  | 22 (42.31) | 4 (13.79)   | 11 (44.00)  | 4 (23.53)    | 4 (8.33)   |
| Age group, years         |                    |                    |                |                 |             |             |             |             |              |            |
| <1                       | 3 (0.71)           | 3 (0.72)           | 0 (0.00)       | 0 (0.00)         | 2 (0.79)    | 1 (1.92)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| 1-20                     | 5 (1.18)           | 5 (1.19)           | 0 (0.00)       | 0 (0.00)         | 4 (1.59)    | 1 (1.92)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| 21-40                    | 53 (12.53)         | 53 (12.65)         | 0 (0.00)       | 0 (0.00)         | 42 (16.67)  | 2 (3.85)   | 3 (10.44)   | 1 (4.00)    | 1 (5.88)     | 4 (8.33)   |
| 41-60                    | 138 (32.62)        | 136 (32.46)        | 0 (0.00)       | 2 (66.67)        | 88 (34.92)  | 17 (32.69) | 5 (17.24)   | 8 (32.00)   | 6 (35.29)    | 14 (29.17) |
| >60                      | 220 (52.01)        | 218 (52.03)        | 1 (100.00)     | 1 (33.33)        | 112 (44.44) | 31 (59.62) | 21 (72.41)  | 16 (64.00)  | 10 (58.82)   | 30 (62.50) |
| Not Available            | 4 (0.95)           | 4 (0.95)           | 0 (0.00)       | 0 (0.00)         | 4 (1.59)    | 0 (0.00)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| Vaccination status       |                    |                    |                |                 |             |             |             |             |              |            |
| Unvaccinated             | 219 (51.77)        | 216 (51.55)        | 1 (100.00)     | 2 (66.67)        | 122 (48.41) | 28 (51.85) | 23 (79.31)  | 8 (32.00)   | 12 (70.59)   | 26 (54.17) |
| Incomplete vaccination, 1 dose | 40 (9.46)       | 40 (9.55)          | 0 (0.00)       | 0 (0.00)         | 28 (11.11)  | 2 (3.85)   | 2 (6.90)    | 4 (16.00)   | 0 (0.00)     | 4 (8.33)   |
| Complete vaccination, 2 dose | 95 (22.46)       | 94 (22.43)         | 0 (0.00)       | 1 (33.33)        | 49 (19.44)  | 17 (32.69) | 4 (13.79)   | 11 (44.00)  | 1 (58.82)    | 13 (27.08) |
| Not available            | 69 (16.31)         | 69 (16.47)         | 0 (0.00)       | 0 (0.00)         | 53 (21.03)  | 5 (9.02)   | 2 (8.00)    | 4 (23.53)   | 5 (10.42)    |            |
| Medical history          |                    |                    |                |                 |             |             |             |             |              |            |
| None                     | 174 (41.13)        | 174 (41.53)        | 0 (0.00)       | 0 (0.00)         | 94 (37.30)  | 24 (46.15) | 11 (37.93)  | 12 (48.00)  | 10 (58.82)   | 23 (47.92) |
| Diabetes                 | 17 (4.02)          | 16 (3.82)          | 0 (0.00)       | 1 (33.33)        | 11 (4.37)   | 3 (5.77)   | 1 (3.45)    | 1 (4.00)    | 0 (0.00)     | 1 (2.08)   |
| Hypertension             | 54 (12.77)         | 54 (12.89)         | 0 (0.00)       | 0 (0.00)         | 29 (11.51)  | 7 (13.46)  | 9 (31.03)   | 4 (16.00)   | 2 (11.76)    | 3 (6.25)   |
| Obesity                  | 5 (1.18)           | 5 (1.19)           | 0 (0.00)       | 0 (0.00)         | 4 (1.59)    | 1 (1.92)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| Diabetes & hypertension | 69 (16.31)         | 67 (15.99)         | 1 (100.00)     | 1 (33.33)        | 37 (14.68)  | 6 (11.54)  | 4 (13.79)   | 7 (28.00)   | 1 (5.88)     | 14 (29.17) |
| Diabetes & obesity       | 1 (0.24)           | 1 (0.24)           | 0 (0.00)       | 0 (0.00)         | 1 (0.40)    | 0 (0.00)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| Diabetes, hypertension & obesity | 4 (0.95)         | 4 (0.95)           | 0 (0.00)       | 0 (0.00)         | 3 (1.19)    | 1 (1.92)   | 0 (0.00)    | 0 (0.00)    | 0 (0.00)     | 0 (0.00)   |
| Not available            | 99 (23.40)         | 98 (23.39)         | 0 (0.00)       | 1 (33.33)        | 7 (2.78)    | 10 (19.23) | 4 (13.79)   | 1 (4.00)    | 4 (23.53)    | 7 (14.58) |

VOC, variant of concern.
(n = 419, 99.1%), followed by non-VOC variants (n = 3, 0.7%; consisted of B.1.466.2 (n = 2, 0.5%) and B.1.36.16 [n = 1, 0.2%]) and the Omicron variant (n = 1, 0.2%; Table 2). The result suggested that Delta VOC was the most virulent SARS-CoV-2 variant compared with the Omicron VOC and non-VOC during the fourth epidemic wave. About 52.0% of COVID-19 death cases infected with Delta variant were patients aged more than 60 years. A total of 51% of the COVID-19 death cases infected with Delta variant were unvaccinated patients. In the unvaccinated patients, most of the COVID-19-related death cases were associated with the B.1.617.2 lineage (n = 122, 55.7%), followed by AY.59 lineage (n = 28, 12.8%), AY.23 lineage (n = 23, 10.5%), AY.4 lineage (n = 12, 5.5%), and others (n = 11, 5.0%). The B.1.617.2 lineage was associated with most COVID-19-related death cases from June to August 2021 (Figure 1d). However, from September 2021, most of the COVID-19-related death cases were associated with AY.59 and AY.79 lineages. In elderly individuals (aged >60 years), most of the COVID-19-related death cases were associated with the B.1.617.2 lineage (Figure 1e).

Molecular epidemiology of SARS-CoV-2 in Peninsular and east Malaysia

State-level analysis was performed to determine SARS-CoV-2 lineages that circulated in east and west (Peninsular) Malaysia from June 2021 to January 2022 (Figure 1a). Results revealed that SARS-CoV-2 strains that circulated in the states and federal territories located in the west (Peninsular) Malaysia region (Johor, Kelantan, Kedah, Kuala Lumpur, Melaka, Pahang, Perak, Perlis, Penang, Putrajaya, Selangor, and Terengganu) differed from those of east Malaysia (Sabah, Sarawak, and Labuan; Figure 2a; Supplementary Table 3). In Peninsular Malaysia, the B.1.617.2 lineage was the predominant circulating lineage; nevertheless, in east Malaysia, the AV.23 lineage was predominant.

In Peninsular Malaysia, the B.1.617.2 lineage was the predominant lineage from June to September 2021 (Figure 2b; Supplementary Table 4). However, AY.59 and AY.79 lineages became predominant and replaced B.1.617.2 lineage in October and remained dominant until December 2021. Due to the lack of samples sequenced in the Peninsular Malaysia region in January 2022, the genomic profile of SARS-CoV-2 circulating for that month could not be determined. In east Malaysia, the AU.2 and AV.23 lineages were the predominant lineages in June 2021 (Figure 2c; Supplementary Table 5). However, the AU.2 lineage was soon replaced by the B.1.617.2 and AV.23 lineages from July 2021. Between August and December 2021, AV.23 became the predominant lineage in the east Malaysia region before being displaced by the BA.1 and BA.1.1 lineages in January 2022.

Molecular epidemiology of COVID-19 imported cases

Based on the Malaysia COVID-19 open-source database (COVID-NOW), a total of 5083 imported COVID-19 cases were detected at the international points of entry of Malaysia, including the Kuala Lumpur International Airport and Johor Bahru Customs, Immigration and Quarantine Complex from June to December 2021 (Figure 3a). The number of imported cases increased after December 2021 and coincided with the resumption of international travel and vaccinated travel lanes on October 18 and November 29, respectively. The number of imported cases increased to 679% in December 2021 (n = 3859) compared with the number of imported cases reported in November 2021 (n = 568). From 5083 imported cases, WGS testing was requested for 2276 (44.8%) cases. Of the 2276 cases, 48.6% (n = 1105) were imported cases with a history of travel to Saudi Arabia, followed by the United Arab Emirates (UAE; n = 96, 4.2%; Supplementary Table 6). From the 2276 cases, genome sequencing was performed on 16.7% of these cases (n = 379), whereas the remaining 83.6% of the samples either had cycle threshold value >30 or failed to produce sufficient sequences. From the 379 sequenced samples, 271 (71.5%) were identified as the Omicron variant, followed by the Delta variant (n = 107, 28.2%), and a non-VOC variant (n = 1, 0.3%). COVID-19 imported cases from Saudi Arabia, the United Kingdom, Turkey, Qatar, and UAE were classified as either Delta or Omicron variants during the surveillance period (Figure 3b; Supplementary Table 7). The first imported case of Omicron variant BA.1 lineage was identified in the samples collected in November 2021. From the 379 samples sequenced, a total of 271 (71.5%) were found to be BA.1 lineage (n = 219, 57.8%), BA.1.1 lineage (n = 50, 18.5%), and BA.2 lineage (n = 2, 0.5%) (Figure 3d; Supplementary Table 8). From the 271 imported COVID-19 cases identified as BA.1 lineage, 56.8% (n = 154) of these cases’ origin country is unknown. A total of 43 (15.9%) cases were imported from Saudi Arabia, followed by Qatar (n = 11, 4.1%), Singapore (n = 3, 1.1%), United Kingdom (n = 3, 1.1%), UAE (n = 2, 0.7%), Turkey (n = 2, 0.7%), and Netherlands (n = 1, 0.4%). From the 50 imported COVID-19 cases identified as BA.1.1 lineage, 48 (96.0%) cases were imported from Saudi Arabia, followed by Turkey (n = 1, 2.0%) and the United Kingdom (n = 1, 2.0%).

Approximately 107 (28.2%) imported COVID-19 cases were found to be the Delta variant, with 21 cases infected with the B.1.617.2 lineage and the remaining cases infected with Delta AY lineages (Figure 3b and c; Supplementary Table 7). Only one imported case of the B.1.1 lineage was detected during this surveillance period. Delta variant AY.114, AY.109, AY.124, AY.125, AY.39, and AY.4.8 lineages were not detected in samples collected in community transmission. Nonetheless, there remained a possibility that these lineages circulated in the community but were eventually outcompeted by the other Delta lineages.

Phylogenetic analysis of SARS-CoV-2 genomes in Malaysia

A total of 6851 Malaysian SARS-CoV-2 sequences were retrieved from the GISAID database based on the samples collected from June 2021 to January 2022. From 6851 sequences, 3706 sequences of samples were isolated from Peninsular Malaysia, 2548 sequences were isolated from east Malaysia, and 597 sequences imported to Malaysia were either detected at the international entry point (Kuala Lumpur International Airport Health Office) or were declared by the originating laboratory as COVID-19 imported cases. Based on the phylogenetic tree, Delta was the predominant variant during the surveillance period (Figure 4a). Interestingly, the AU.2 lineage was circulating exclusively in east Malaysia (marked with yellow strips). Most of the isolates from Peninsular and east Malaysia were well separated, with the exceptions of a few cases of interstate imported cases. The majority of the Omicron variant cases detected during the surveillance period were isolated from the imported cases (marked with red strips); however, the Omicron variant was also detected in the isolates obtained from local cases (marked with blue and yellow strips) (Figure 4b). The first case of imported Omicron variant was detected in the sample collected on November 19, 2021 (hCOV-19/Malaysia/IMR_WC606483), whereas the first local case of Omicron VOC was detected in the sample collected on December 14, 2021 (hCOV-19/Malaysia/UNIMAS_HBTU489/2021). This result suggested that the Omicron variant could have been introduced to Malaysia through imported cases during the fourth epidemic wave, and almost 1 month of cryptic undetected transmission between the first imported and the first local cases took place.
Mutation analysis of SARS-CoV-2 isolates in Malaysia

State-level analysis revealed that among the isolates detected from Peninsular Malaysia, the most common mutation found among the samples was a mis-sense mutation of a segment of ORF1ab encoding the RNA-dependent RNA polymerase (P323L; Figure 5a; Supplementary Table 9). P323L mutation affected the RdRp and resulted in the change of amino acids C14408T. In addition, the nonsynonymous mutations in the S protein D614G and T478K were the second and third most common mutations detected, respectively. D614G and T478K affected the S protein, respectively, resulting in the change of amino acids A23403G and C22995A. The most common mutations related to immune escape in the Peninsular Malaysia samples were L452R (n = 2806, 90.3%),
Figure 3. Molecular epidemiology of COVID-19 imported cases in Malaysia (a) Number of COVID-19 imported cases in Malaysia reported from June to December 2021. (b) Map of the world with the number of COVID-19 imported cases to Malaysia and the SARS-CoV-2 variant detected in the samples. (c) Percentage of SARS-CoV-2 variant detected in the samples. (d) Top ten most prevalent SARS-CoV-2 lineages in imported COVID-19 cases and their originating countries, n = 379.

Figure 4. A maximum likelihood phylogenetic tree of SARS-CoV-2 isolates in Malaysia from June 2021 to January 2022 based on the Global Initiative on Sharing Avian Influenza Data submission. (a) A maximum likelihood phylogenetic tree of the circulating SARS-CoV-2 categorized by region (Peninsular vs east Malaysia) inferred using the general time-reversible + proportion of invariable sites + gamma rate variation model in the IQ-TREE and outgroup rooted on the earliest lineage B sampled from Wuhan (GenBank accession no. NC_045512) using iTOL software. (b) Phylogenetic tree of Omicron variant categorized by the type of cases. The blue strip indicates isolates from Peninsular Malaysia, the yellow strip indicates isolates from east Malaysia, and the red strip indicates isolates from COVID-19 imported cases to Malaysia. VOC, variant of concern
followed by K417N (n = 175, 5.6%), E484Q (n = 11, 0.3%), and E484K (n = 10, 0.3%). The N501Y mutation was found in 228 (7.3%) isolates.

Among the samples collected in east Malaysia, the most common mutation found was a miss-sense mutation of a segment of ORF1ab encoding the RNA-dependent RNA polymerase (P323L), with 99.9% of the samples carrying this mutation, followed by the nonsynonymous mutation in the S protein, D614G (99.9%), and synonymous mutation in predicted phosphoesterase papain-like proteinase (NSP3) mutation, F106F (99.5%; Figure 5b; Supplementary Table 10). In addition, the F106F mutation in NSP3 resulted in the change of amino acid C3037T. The most common mutations related to immune escape in the Peninsular Malaysia isolates were L452R (n = 2793, 92.7%), followed by K417N (n = 135, 4.5%), E484K (n = 7, 0.2%), and E484Q (n = 6, 0.2%). In addition, the N501Y mutation was detected in 135 samples (4.5%).

Discussion

The national genomic surveillance for SARS-CoV-2 was successfully initiated in June 2021 to track VOC transmission in this country. In the fourth epidemic wave starting in April 2021, the genomic profiles of SARS-CoV-2 isolated from the west (Peninsular) and east Malaysia differed. In the early fourth epidemic wave, the original Delta variant B.1.617.2 lineage was the dominant circulating lineage detected in June 2021 throughout Malaysia. However, Delta variant AY.59 and AY.79 lineages outcompeted the B.1.617.2 lineage, becoming the dominant circulating lineage in November 2021 in Peninsular Malaysia. The AY.59 and AY.79 lineages were first reported in December 2020 and February 2021, respectively (Tsung et al., 2022). In east Malaysia, the Delta variant AY.23 lineage displaced the B.1.617.2 lineage from August 2021. In January 2022, the Omicron variant BA.1 lineage displaced the Delta variant to be the predominant variant in east Malaysia. Over the surveillance period from June to December 2021, the Delta variant was the dominant circulating variant, and the Omicron variant eventually displaced the Delta variant by January 2022. In addition, the number of COVID-19 imported cases increased after the relaxing of the border restriction and commencement of the vaccinated travel lane, which subsequently aided the Omicron introduction into Malaysia.

The differences in genomic profiles of SARS-CoV-2 isolated from Peninsular and east Malaysia could be caused by the source of the strains, geographical location, and relative proximity of the regions, in addition to COVID-19 disease management. From June to September 2021, the government enforced the National Recovery Plan phase I and II that prohibited cross-state travel, thus resulting in fewer numbers of interstate imported cases and segregation of isolates obtained in Peninsular and east Malaysia during that period. During this surveillance period, AU.2 lineage was exclusively detected in east Malaysia, suggesting transmission profiles unrelated to Peninsular Malaysia. Further evidence of unique and unrelated transmission dynamics between west and east Malaysia was supported by detecting the AY.23 lineage as the predominant lineage in east Malaysia, whereas AY.59 and AY.79 were the predominant lineages in west Malaysia during this surveillance period. The displacement of the AU.2 lineage by the AY.23 lineage in east Malaysia could have been driven by the T478K mutation in the S protein of the AY.23 lineage. T478K mutation confers greater ACE2 binding and enhances antibody escape, subsequently increasing the transmissibility of this variant (Cherian et al., 2021; Di Giacomo et al., 2021). Interestingly, the neighboring country, Indonesia, also reported that AY.23 was the predominant lineage during the Delta period in 2021 (Cahyani et al., 2022). Because east Malaysia borders the Indonesian region of Kalimantan on the Island of Borneo, active migration across the border may have facilitated the transmission of AY.23 lineage to east Malaysia.

The Delta variant was introduced to Malaysia by travelers returning from the country with high community transmissions, such as India, Sri Lanka, and the United Kingdom (Ministry of Health Malaysia, 2021a). At the end of May, the Malaysia Ministry of Health reported that 6% of the sequenced SARS-CoV-2 samples collected in May 2021 were the B.1.617.2 lineage (Ministry of Health Malaysia, 2021b). However, the number of Delta variant cases rapidly increased to >80% of the sequence samples by the end of June 2021, and the Delta variant displaced the B.1.524 lineage to become the dominant circulating variant in Malaysia. The concurrent mutation of D614G and P323L in the S protein could have driven the displacement of B.1.524 by the Delta vari-
ant. The P323L mutation provided epidemiological advantages because these mutations enhance the infectivity of the virus and allow transmission by asymptomatic or presymptomatic individuals (Iljmárv et al., 2021). Another common mutation to the Delta variant, the C23604G (P681R) mutation in the S protein, which improves viral fusogenicity by facilitating S protein cleavage, resulted in a more efficient viral infiltration into respiratory epithelial cells and hence, greater transmissibility (Cherian et al., 2021).

The introduction of the Omicron variant into Malaysia was attributed to travelers and Malaysians arriving from countries with high numbers of Omicron cases. Although the Malaysian Government had imposed travel restrictions to countries with known high numbers of Omicron, most of the imported Omicron cases were travelers and pilgrims returning from the Middle East and Asia, such as Saudi Arabia, Qatar, and the UAE. The first original Omicron variant case, B.1.1.529, was detected in an international student who arrived in Malaysia on November 19, 2021, after returning from South Africa through Singapore (Malaysia Ministry of Health, 2021). Nevertheless, all close contacts of this patient tested negative, and no local transmission was reported for this B.1.1.529 lineage. Due to the concern about the spread of the Omicron variant, the Malaysia Ministry of Health required all COVID-19-positive cases detected from November 11 to November 28 at the international entry points to be sequenced (Malaysia Ministry of Health, 2022). The first imported cases of the Omicron variant BA.1 lineage arrived through travelers from the Middle East at the end of November 2021. Despite public health measures, such as compulsory quarantine and mandatory SARS-CoV-2 testing for travelers, this did not prevent the spread of the Omicron variant and subsequently the introduction of the Omicron variant into the community. In the same month, the first community transmission of Omicron variant BA.1 lineage in east Malaysia in a patient with no history of traveling was detected in December 2021 (TheStar, 2021). By January 2022, BA.1 and BA.1.1 lineages had become the predominant variants in the east Malaysian region, accounting for more than 80% of the community transmission cases (Malaysia Ministry of Health, 2022).

As Malaysia is preparing for the transition to endemicity, apart from strengthening the vaccination program, establishing the national SARS-CoV-2 genome surveillance program will aid in rapid notification of new VOC introduction into the country and circulating variants in the local community, and this will facilitate public health management in future waves of SARS-CoV-2. Moving forward, strengthening and upsampling the sequencing capacity, with the addition of more sequencing centers and active surveillance of SARS-CoV-2 in wastewater, will help in preparing for future pandemics. Further sequence and epidemiological data are needed from Malaysia and other countries in the region to understand the full extent of the spread of the Delta, Omicron, and future variants.

In conclusion, we observed spatiotemporal dynamics of lineage turnover in the west and east Malaysia, culminating in the dominance of the Omicron variant, which continues to be dominant worldwide at the time of writing this manuscript. SARS-CoV-2 circulating in Peninsular Malaysia had distinct genetic profiles from those in Sabah and Sarawak. We found that the isolates obtained from the west and east Malaysia were well segregated, and few imported cases between states were detected. Therefore, it is possible that the restriction of movement and the close border policy could have affected the virus transmission within Malaysia. However, relaxing the border restriction could have facilitated the introduction of the Omicron variant in Malaysia. Moving forward, this top-down government initiative and continuous genome surveillance are vital for a more efficient pandemic response and preparedness plan for future outbreaks.

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**Ethical approval**

No ethical approval was required because the data used in this study were anonymized national data collected routinely by the Ministry of Health.

**Author contributions**

NAMA, DP, RT, SAB, ICS, MZS, MNMI, and KKT analyzed and interpreted and wrote the original draft. NSAM, WKA, JS, YFC, TLK, and GA collected, analyzed, and interpreted the data. ZBMR, JCCJ, KAK, JYT, OKR, LWF, NAJ, MI, RIMY, MIA, JEW, JYLF, MNFN, ISS, MFMM, NAAMS, KNAG, SNHMY, and YMIN performed the experiments and collected data. DP, RT, ICS, MZS, MNMI, and RJ provided reagents and materials. NAMA prepared the visualization and data presentation. RJ designed, supervised, and wrote and revised the manuscript, and acquired funding with NAMA. All authors approved the final version of the manuscript.

**Declaration of competing interest**

The authors have no competing interests to declare.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.10.044.

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