Journal of Associated Medical Sciences 2025; 58(2): 58-64

JAMS
Journal of Associated Medical Sciences

Scopus Indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index



Detection and characterization of SARS-CoV-2 variants in Thailand from 2020-2023 using next-generation sequencing

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ARTICLE INFO

Article history:
Received 6 October 2024
Accepted as revised 5 January 2025
Available online 10 January 2025

Keywords:
SARS-CoV-2, genomic variants, Thailand, whole genome sequencing, COVID-19.

ABSTRACT

Background: The evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to multiple variants with significant public health implications.

Objective: This study aimed to detect and characterize SARS-CoV-2 genomic variants in Thailand using next-generation sequencing (NGS) from 2020 to 2023.

Materials and methods: A total of 2,592 oro/nasopharyngeal swab specimens with high viral loads (cycle threshold; Ct≤25.0) were collected from SARS-CoV-2-positive patients in Nonthaburi, Nakhon Phanom, and Tak Provinces. NGS was performed using Ion Torrent, Oxford Nanopore Technologies, and Illumina platforms to identify and analyze SARS-CoV-2 variants.

Results: The sequencing revealed various SARS-CoV-2 variants circulating in Thailand, with 2,592 lineages identified. The Omicron variant was predominant, accounting for 93.44% (2,422/2,592). The most common Omicron subvariants were BA.2 (54.86%, 1,422/2,592), BA.5 (20.22%, 524/2,592), and XBB (14.51%, 376/2,592). The Delta variant constituted 2.66% (69/2,592) of the cases, with AY.30 (1.20%, 31/2,592) and AY.85 (1.27%, 33/2,592) being the most frequent sub-lineages. The Alpha variant (B.1.1.7) was detected in 33 cases (1.27%), and the Beta variant (B.1.351) in 1 case (0.04%).

Conclusion: The findings reveal a significant shift in the SARS-CoV-2 variant landscape in Thailand, with Omicron becoming the dominant strain post-2022. Continuous genomic surveillance is critical for tracking viral evolution, informing public health strategies, and supporting pandemic control efforts in Thailand and Southeast Asia.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, has resulted in a global pandemic with significant public health implications. Since its emergence in December 2019, SARS-CoV-2 has undergone numerous mutations, leading to the emergence of various genomic variants with differing transmission rates, virulence, and impacts on vaccine efficacy.[1,2] Monitoring these variants is essential for understanding viral evolution guiding public health intervention.

Next-generation sequencing (NGS) has become a pivotal tool in identifying and tracking the spread of SARS-CoV-2 variants. By analyzing the complete viral genome, WGS provides detailed insights into the virus's genetic changes and evolutionary patterns.[3] This technique has been instrumental in detecting novel variants and

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doi: 10.12982/JAMS.2025.038
E-ISSN: 2539-6056

monitoring their spread across different regions.[4,5] World Health Organization (WHO) recommended using targeted NGS to determine SARS-CoV-2 variants, including Ion Torrent and Oxford Nanopore Technologies (ONT).[6]

In Thailand, the first confirmed case of COVID-19 was reported in January 2020. Since then, the country has faced multiple waves of infection, each associated with different SARS-CoV-2 variants. Understanding the genomic landscape of SARS-CoV-2 in Thailand is essential for developing targeted public health strategies and mitigating the impact of future outbreaks.[7-9]

Thailand is a significant area of study for several reasons. Its geographical location and status as a major travel hub in Southeast Asia mean it plays a crucial role in regional epidemiology. The high volume of international travel and trade through Thailand increases the likelihood of the importation and exportation of new SARS-CoV-2 variants, which can spread throughout Southeast Asia. Secondly, the country's diverse population and varied public health responses provide a unique opportunity to study how different factors influence the evolution and spread of the virus. Monitoring SARS-CoV-2 variants in Thailand can provide valuable insights into the virus's behavior in an epidemiologically and socioeconomically diverse region.[10,11]

Recent studies highlight the importance of variant monitoring in Thailand and its implications for Southeast Asia. The research conducted by Sooksawasdi Na Ayudhya et al. demonstrated the emergence and spread of multiple SARS-CoV-2 variants in Thailand, emphasizing the need for continuous genomic surveillance to track viral mutations and inform public health responses.[12] Furthermore, the study by Bunyong *et al*. underscored the role of WGS in identifying and characterizing new variants, which is crucial for effective vaccine deployment and containment strategies in the region.[13] Additionally, the research by Tongsima *et al*. provided insights into the regional dynamics of SARS-CoV-2 spread, highlighting the interconnectedness of Southeast Asian countries and the necessity of coordinated surveillance efforts.[14]

This study aims to detect and monitor SARS-CoV-2 variants in Thailand using NGS. The NGS was chosen for this study due to its high throughput, accuracy, and ability to provide comprehensive genomic data. These qualities make it an invaluable tool for tracking variants and understanding the dynamics of SARS-CoV-2 spread.[15,16] This study was intended to support global efforts against COVID-19 while providing data to enhance local surveillance and response strategies. This approach will help Thailand and neighboring Southeast Asian countries improve their public health responses and control measures against COVID-19.

Materials and methods

Specimen collection

Oropharyngeal or nasopharyngeal swab specimens were collected from 2,592 SARS-CoV-2-positive cases tested at the Bamrasnaradura Infectious Diseases Institute (Nonthaburi) and the Division of Global Health Protection, Thailand Ministry of Public Health - US CDC Collaboration, Thailand (Tak and Nakhon Phanom). The total number of specimens from each site corresponds to the number of SARS-CoV-2 sequencing submissions (N=2,592) in Table 1. These specimens, stored in a viral transport medium (VTM), were retrieved from storage and included in this study. All specimens originated from patients in the three sites from June 2020 to May 2023. We conducted a retrospective surveillance study of SARS-CoV-2 infections. The criteria for selecting SARS-CoV-2 positive specimen selection were based on a nucleocapsid protein (N Gene) cycle threshold (Ct) value of ≤ 25.0 on the qRT-PCR, with the average Ct value of 15 across all DNA samples. A Ct value of ≤ 25.0 on the qRT-PCR, corresponding to approximately 1,500 to 50,000 copies, was used to determine the copy number of a sample based on the known copy number in control reactions. Selecting the appropriate Ct value from real-time PCR is vital for obtaining high-quality SARS-CoV-2 genome data using the NGS platform.[17] The specimens were collected from patients with symptoms including cough, runny nose, sore throat, anosmia, tachypnea, shortness of breath, or difficulty breathing, and/or having a temperature ≥37.5 ^{o}C, or having traveled to/from a foreign country, or having contact with a confirmed COVID-19 case.

Table 1. The number of SARS-CoV-2 sequencing submissions (N=2,592)

Year	Submission month	Sequencing protocol	Nonthaburi	Nakhon Phanom	Tak
1 (N=1,349)	Jan-22	Ion AmpliSeq	-	10	15
	Feb-22	Ion AmpliSeq	54	-	-
	May-22	Ion AmpliSeq	76	23	16
		ARTIC-ONT	100	-	-
	Jun-22	Illumina COVID Seq	96	-	-
	Jul-22	Ion AmpliSeq	-	81	10
		Illumina MiniSeq RVOP	82	125	19
		Illumina COVID Seq	257	-	-
	Aug-22	Illumina MiniSeq RVOP	-	64	15
	Sep-22	Illumina MiniSeq RVOP	-	226	80
		Total	**665**	**529**	**155**
2 (N=538)	May-23	Ion AmpliSeq	-	218	36
	Jun-23	Ion AmpliSeq	-	150	42
	Jul-23	Ion AmpliSeq	-	39	25
	Aug-23	ONT Midnight	-	24	4
		Total	**-**	**431**	**107**
3 (N=705)	Sep-23	ONT Midnight	-	10	72
	Nov-23	ONT Midnight	-	269	146
	Dec-23	ONT Midnight	-	68	16
	Feb-24	ONT Midnight	-	52	17
	Jun-24	Ion AmpliSeq	-	15	40
		Total	**-**	**414**	**291**
		Total		**2,592**	

Nucleic acid extraction

Following the manufacturer's protocol, total nucleic acid was extracted from the oropharyngeal or nasopharyngeal swab specimens using the automated MagNA Pure 96 platform (Roche, Switzerland). Then, a QC step is performed by use the Qubit dsDNA High Sensitivity Quantification Assay (Thermo Fisher Scientific, USA).[18]

Library preparation and whole genome sequencing

Total nucleic acid was reverse transcribed into RNA, followed by amplification of the cDNA targets and barcode tagging using a specific reagent kit. The barcoded samples were pooled and sequenced. Sequencing libraries were prepared using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay for the Ion Torrent platform and loaded onto Ion 540 Chips (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.[19] Sequencing was performed on the Ion Torrent S5 System (Thermo Fisher Scientific, USA). For the Oxford Nanopore Technologies (ONT) platform: (1) ARTIC libraries were prepared using a ligation-based sequencing kit (SQK-LSK109), and (2) Midnight libraries were prepared using the Midnight RT PCR Expansion (EXP-MRT001) kit. Both were sequenced on the MinION Mk1B and Mk1C (Oxford Nanopore Technologies, UK) following the manufacturer's protocol.[20] For the Illumina platform, sequencing was performed using the COVIDSeq Assay and Respiratory Virus Oligo Panel (RVOP) according to the manufacturer's instructions.[21,22]

Variant calling and data processing

The sequence was interpreted using package software, including variantCaller and SARS-CoV-2_coverage analysis. The collected FASTA data were uploaded to two online databases, including Pangolin COVID-19 Lineage (https://pangolin.cog-uk.io)[23] and Nextclade (https://clades.nextstrain.org).[24] The findings of this investigation were then examined using descriptive statistics, which included the percentage of variants detected in all samples. Confidence interval for a proportion was calculated by Sample Size Calculator (https://sample-size.net/confidence-interval-proportion).[25]

Ethical considerations

The study was approved by the Institutional Review Board of the Bamrasnaradura Infectious Disease Institute (approval ID: BIDI S033h/63_ExPD) and the National Institute of Health in Thailand (approval ID: DMSc-EC002).

Results

Between January 2020 and May 2023, 2,592 oro/nasopharyngeal swab specimens were collected from SARS-CoV-2-positive patients in Nonthaburi, Nakhon Phanom, and Tak, Thailand. These specimens were tested

using Next-Generation Sequencing (NGS) platforms, including Ion Torrent, Oxford Nanopore Technologies, and Illumina. A retrospective surveillance study of SARS-CoV-2 infections was conducted, with specimens selected based on a nucleocapsid protein (N Gene) cycle threshold (Ct) value ≤ 25.0, indicating a high viral load.

The sequencing results revealed a wide variety of SARS-CoV-2 variants circulating in Thailand during the study period, with a total of 2,592 SARS-CoV-2 lineages identified. These lineages were distributed across several variants, including Alpha, Beta, Delta, and Omicron, as shown in Table 2 and Figure 1. The Omicron variant emerged as the predominant strain, accounting for 93.44% (2,422/2,592) of the total sequenced samples. Within the Omicron lineage, BA.2 was the most common subvariant, representing 54.86% (1,422/2,592) of the cases, followed by BA.5 (524/2,592, 20.22%) and XBB (376/2,592, 14.51%).

The Delta variant contributed 2.66% (69/2,592) of the cases, with AY.30 (31/2,592, 1.20%) and AY.85 (33/2,592, 1.27%) as the most frequently detected sublineages. The Alpha variant, represented by B.1.1.7, was detected in 33 cases (1.27%), while the Beta variant (B.1.351) was found in only 1 case (0.04%).

These findings suggest a significant shift in the SARS-CoV-2 variant landscape in Thailand, with the Omicron lineage becoming the dominant strain after early 2022. The NGS platform, combined with careful specimen collection and variant calling using the GISAID database, provided a comprehensive overview of the circulating SARS-CoV-2 variants and their distribution in these three regions of Thailand.

Table 2. The number of 2,537 SARS-CoV-2 lineage sequenced by NGS platform in Nonthaburi, Nakhon Phanom and Tak during January 2020 to May 2023.

SARS-CoV-2 variants and lineages		Number of detections	95% CI
	A	**35**	1.35 (0.94-1.87)
	B	**32**	1.23 (0.85-1.74)
Delta	AY.125	1	0.04 (0.0-0.22)
	AY.30	31	1.20 (0.81-1.69)
	AY.79	3	0.12 (0.02-0.34)
	AY.85	33	1.27 (0.88-1.78)
	B.1.617.2	1	0.04 (0.0-0.22)
	Total	**69**	
Alpha	B.1.1.7	**33**	1.27 (0.88-1.78)
Beta	B.1.351	**1**	0.04 (0.0-0.22)
Omicron	BA.1	80	0.007 (0.025-0.038)
	BA.2	1422	0.019 (0.539-0.578)
	BA.4	20	0.003 (0.004-0.011)
	BA.5	524	0.016 (0.188-0.219)
	XBB	376	0.013 (0.118-0.145)
	Total	**2422**	
Total		**2592**	

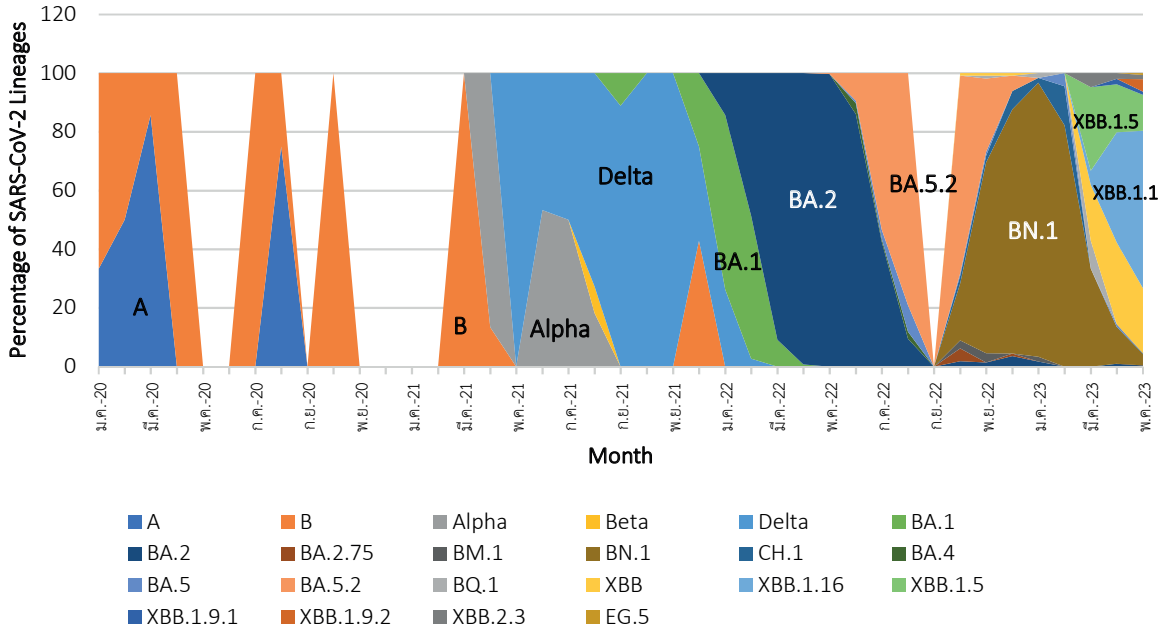


Figure 1. *The distribution percentage of 2,592 SARS-CoV-2 lineage in Nonthaburi, Nakhon Phanom, and Tak from January 2020 to May 2023. A: A, A.6, B: B, B.1, B.1.1, B.1.1.142, B.1.524, B.41, B.1.36.6, Delta: AY.125, AY.30, AY.79, AY.85, B.1.617.2, Alpha: B.1.1.7, Beta: B.1.351.*

Discussion

The detection and characterization of SARS-CoV-2 variants in Thailand from 2020 to 2023 provide valuable insights into the country's viral evolution and transmission dynamics. The study utilized next-generation sequencing (NGS) platforms, including Ion Torrent, Oxford Nanopore Technologies, and Illumina, to identify and track various SARS-CoV-2 lineages. A total of 2,592 specimens were analyzed.

From January 2020 to May 2023, the Omicron variant emerged as the predominant strain, accounting for 93.44% of the sequenced samples (2,422/2,592). Within the Omicron lineage, the BA.2 subvariant was the most prevalent, constituting 54.86% (1,422/2,592) of cases, followed by BA.5 (20.22%, 524/2,592) and XBB (14.51%, 376/2,592). The 95% confidence interval (CI) for the proportion of Omicron cases ranged from 92.52% to 94.36%, reflecting a high confidence level in this variant's dominance. The Delta variant was identified in 2.66% (69/2,592) of the cases, with AY.30 and AY.85 as the most frequently detected sub-lineages, accounting for 1.20% (31/2,592) and 1.27% (33/2,592) of the total samples, respectively. The 95% CI for Delta cases ranged from 2.04% to 3.28%, highlighting its lower but notable presence compared to Omicron. The Alpha (B.1.1.7) and Beta (B.1.351) variants were present in much smaller proportions, with Alpha representing 1.27% (33/2,592) of cases and Beta a mere 0.04% (1/2,592). The 95% CI for Alpha ranged from 0.85% to 1.69%, and for Beta ranged from 0.00% to 0.12%, underscoring their limited detection during the study period.

This study indicated a shift in the SARS-CoV-2 variant landscape in Thailand, particularly with the emergence and subsequent dominance of the Omicron lineage after early 2022. This trend is consistent with global observations, where Omicron's high transmissibility and immune escape capabilities have led to its widespread prevalence despite vaccination efforts.[22,23] The use of NGS platforms in this study was instrumental in providing comprehensive genomic data and identifying SARS-CoV-2 variants. The selection of specimens with a nucleocapsid protein (N Gene) cycle threshold, Ct≤25.0, ensured high viral loads, enhancing the reliability of sequencing results. This methodological rigor allowed for the accurate detection of multiple variants and subvariants, offering a detailed overview of the circulating strains in Thailand.

Comparing this data with global trends, it is evident that Thailand's SARS-CoV-2 variant distribution aligns with broader patterns observed worldwide. The dominance of Omicron and the decline of earlier variants such as Alpha and Beta reflect similar dynamics seen in other regions, underscoring the interconnected nature of viral spread.[26,27] The implications of these findings are significant for public health strategies in Thailand and the broader Southeast Asian region. Continuous genomic surveillance is essential for early detection of new variants, understanding their epidemiological impact, and informing public health interventions. The high throughput and accuracy of NGS platforms are invaluable for tracking viral mutations and aiding in developing targeted response strategies.[28,29] In addition, from the study to track the changes of SARS-CoV-2 strains in Thailand by Puenpa J *et al*., it was found that in June 2021, both Alpha and Delta variants were reported, with a higher proportion of Alpha than Delta variants. In July-August 2021, the Alpha variant decreased, but the Delta variant increased. From September 2021 to

November 2021, the Delta variant was 100%. In December 2021, the Omicron BA.1 variant was detected as the main variant from January 2022 to March 2022. The Omicron BA.2 variant was also identified in January 2022.[30] The strain report's period and the proportion of variations discovered are compatible with this study. Except for June 2021, the small number of samples utilized in the study may have influenced the fraction of variations at that time. The Omicron variety has been found since December 2021, with reports of its proliferation in Thailand.[7]

Most infections in the community with omicron BA.2, BA.4/5, and XBB subvariants were moderate, but this did not change when omicron subvariants evolved in this highly vaccinated population. Because many sick people did not have fevers, more sensitive screening than fever alone may be required to prevent community transmission. The trend of a steadily decreasing correlation of vaccination and an increasing connection of previous infection with the symptom profile supported the idea of vaccine escape or immunological fading when additional SARS-CoV-2 omicron subvariants emerged.[31]

Due to the limited sample volume used in this study and the fact that most patients were from Nonthaburi, Nakhon Phanom, and Tak Province, the detected variants were not diverse. Although the detected variants tended to be in the same direction as the Department of Medical Sciences reports, the proportion of variants detected each month may change if the study is conducted with various sites.[6-8] Decoding the SARS-CoV-2 genome using NGS techniques is essential for monitoring the changes in variants. It allows us to know the origin and source of the virus that spreads in each period. It could be linked to molecular epidemiology, and the data could be used for disease prevention and control measures, including selecting appropriate strains for vaccine development in Thailand.

Conclusion

This study provides the SARS-CoV-2 variant in Thailand from 2020 to 2023, highlighting shifts in variant prevalence. The early dominance of the original lineage was succeeded by the rapid emergence of more transmissible and immune-evasive variants such as Alpha, Delta, and Omicron subvariants. These findings underscore continuous genomic surveillance's importance in informing public health strategies and vaccine development. The emergence and dominance of variants like Delta and Omicron subvariants illustrate the virus's adaptive capabilities, impacting the efficacy of public health measures and vaccines. Ongoing genomic monitoring, combined with data sharing, is crucial for early detection of new variants and for guiding effective public health responses and control measures against COVID-19.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was supported by the Division of Global Health Protection, Thailand-U.S. CDC Collaboration.

Acknowledgements

We extend our gratitude to Dr. Beth Skaggs, Dr. Pongpun Sawatwong, and Dr. Saithip Bhengsri from the Division of Global Health Protection, Thailand MOPH-U.S.CDC. Collaboration (TUC), for their contributions. We would like to thank Dr. Navin Horthongkham, Dr. Piroon Jenjaroenpun, and Dr. Thidathip Wongsurawat from the Faculty of Medicine, Siriraj Hospital, Mahidol University, for their technical support.

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