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# Research Article
# Dynamic Evolution of SARS-CoV-2 in West Sumatra: Analyzing S Gene Mutations Across Variants and Their Impact on Public Health and Vaccine Strategies

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## Abstract

**Background and Objective:** The global SARS-CoV-2 pandemic highlights the importance of tracking virus evolution through genomic surveillance, especially concerning mutations in the SARS-CoV-2 spike protein, crucial for vaccine development. Despite global concern over variants, regions like West Sumatra, Indonesia, lack thorough genomic analysis, prompting this study to analyze S gene mutations across three pandemic waves in West Sumatra. **Materials and Methods:** Next-generation sequencing was conducted through the Illumina MiSeq instrument to leverage a dataset of 352 anonymized samples collected between March, 2020 and November, 2022 and rigorous analysis of S gene mutation using CLC Genomics Workbench® 21 version 21.0.3 were employed. Statistical analyses assessed mutation prevalence over time, exploring associations with clinical outcomes. **Results:** The findings revealed significant variability in mutation profiles across different variants. Notably, the Omicron variant (21K) exhibited a high mutation rate, suggesting enhanced immune evasion capabilities. Comparative analysis highlighted evolutionary trends, from early variants with fewer mutations to highly adapted forms like Delta (21I) and Omicron. The dynamic nature of SARS-CoV-2 evolution underscores the importance of continuous surveillance, rapid public health response and vaccine adaptation. **Conclusion:** This study contributes valuable insights into the virus's evolving landscape, emphasizing the need for ongoing research, global collaboration and adaptable vaccine strategies to manage the evolving threat of COVID-19 effectively.

Key words: SARS-CoV-2, spike gene mutations, genomic surveillance, West Sumatra, viral evolution

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The global pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has underscored the critical importance of genomic surveillance in understanding virus evolution and managing public health responses[1]. The spike (S) protein of SARS-CoV-2, a primary target for vaccine development, is prone to mutations, some of which may alter virus transmissibility, pathogenicity and immune escape capabilities[2]. The emergence of variants harboring mutations in the S gene has prompted worldwide concern, driving efforts to monitor these changes and assess their impact on the effectiveness of current vaccines and therapeutic interventions[3].

In this context, the geographic and temporal distribution of SARS-CoV-2 mutations, particularly in regions with limited genomic data representation, presents a critical gap in the global surveillance mosaic[1,4]. West Sumatra, Indonesia, exemplifies such a region where detailed analyses of viral genomic evolution are scarce yet imperative. Despite Indonesia's vast archipelago presenting a unique epidemiological landscape, studies focusing on the mutation dynamics of the SARS-CoV-2 S gene in West Sumatra during the pandemic's multiple waves remain under represented. Integrating data from such areas is essential for constructing a more comprehensive and nuanced picture of the pandemic's evolution, facilitating the development of targeted and effective interventions worldwide[4,5].

This study aims to bridge this knowledge gap by comprehensively analyzing the S gene mutations across three distinct pandemic waves in West Sumatra. By illuminating the mutation landscape within this locale, we contribute to a more nuanced understanding of SARS-CoV-2's evolutionary trajectories and offer insights that may inform vaccine development strategies and public health policies locally and globally. Additionally, current work underscores the importance of including diverse geographical regions in genomic surveillance efforts to ensure a comprehensive global response to the pandemic.

## MATERIALS AND METHODS

This research design is an analytical observational design with a cross-sectional approach. The foundation of this research is built upon a comprehensive dataset comprising isolates from patients in West Sumatra between March, 2020 and November, 2022. These isolates were obtained in collaboration with local health authorities and The Center for Infectious Disease Diagnostic and Research (PDRPI), Faculty of Medicine, Universitas Andalas, ensuring a representative sample of the viral population within the region. All isolates were anonymized to protect patient privacy by ethical guidelines for research. This research involved 352 samples with a qubit concentration greater than 20 ng and a Quantitative Polymerase Chain Reaction (qPCR) threshold value lower than 30, as determined by repeated qPCR before sequencing.

Epidemiological data was also collected on the ability of SARS-CoV-2 variants to spread among hosts, as assessed by the speed at which the peak phase of cases is reached, by the variant experiencing an outbreak during that period, with the category being fast if the peak phase of positive cases is reached within $\leq$30 days and slow if the peak phase of positive cases is reached within >30 days.

**Sequence analysis:** Upon collection, next-generation sequencing was performed using the Illumina MiSeq platform (Illumina, California, United States of America). The MiSeq system automatically trims the primary and adapter sequences during the demultiplexing process, generating a FASTQ file. Subsequently, all files were analyzed using CLC Genomics Workbench® 21 version 21.0.3 (Qiagen, Hilden, Germany). To ensure data quality reads with poor quality (quality score <Q30, length <26 bp, or containing more than two consecutive ambiguous bases) were filtered out. The final consensus sequences of the genome were obtained by aligning them with the SARS-CoV-2 reference genome (GenBank accession number MN908947, Wuhan-Hu-1 isolate) using the CLC Genomics Workbench app, providing a consensus sequence for the SARS-CoV-2 virus in West Sumatra. Variant classification of the genome sequences was performed using Phylogenetic Assignment of Named Global Outbreak LINeages (Pangolin) and Nextclade. The genomic sequences underwent rigorous analysis to identify mutations within the S gene. Particular attention was given to mutations that affect the spike protein's structure and function, such as those impacting receptor binding or antibody neutralization.

**Statistical analysis:** The statistical analysis were conducted using Chi-square tests to determine the significance of the relationship between mutations in the S gene of the SARS-CoV-2 virus in West Sumatra and transmission capability. The level of significance was set at $p<0.05$.

**Ethical considerations:** This study was conducted strictly with ethical principles, including respect for persons, beneficence and justice, as outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethical Committee of the Faculty of Medicine, Universitas Andalas (Approval Number: 664/UN.16.2/KEP-FK/2022). This study followed the relevant guidelines and regulations outlined in the granted approval.

## RESULTS

The variant categorization of the West Sumatra genome sequences according to Phylogenetic Assignment of Named Global Outbreak LINeages (Pangolin) and Nextclade can be seen in Fig. 1.

**Comparative mutation prevalence:** Our analysis revealed significant variability in the mutation profiles across different SARS-CoV-2 variants identified in West Sumatra during the study period. Here, we highlight key findings related to the prevalence of specific mutations within these variants and offer a comparative analysis to illustrate their evolutionary implications (Fig. 2).

**Variant 19A:** Characterized by a low mutation rate, with mutation L5F being the only significant mutation observed at 33.333%. This suggests that early variants circulating in the region had fewer mutations, potentially indicating a limited evolutionary adaptation at the initial pandemic stages.

**Variant 20A:** A diversification in mutation patterns, with mutations Q14-, C15-, V16- and L18F observed at around 0.813 to 4.878%. These mutations suggest an evolutionary response to host immune pressures or environmental factors.

**Variant 20B:** This variant did not exhibit a significant prevalence of the early identified mutations, suggesting a different evolutionary lineage or selective pressures affecting its mutation spectrum.

**Variant 21J:** Notably, this variant showed a 100% prevalence of T19R and D950N mutation, indicating a potential shift towards mutations that might confer advantages in transmissibility or immune escape.

**Variant 21K, 21L, 22A, 22B, 22D, 22E and 22F (Omicron):** Marked by a high mutation rate in the S gene, Omicron

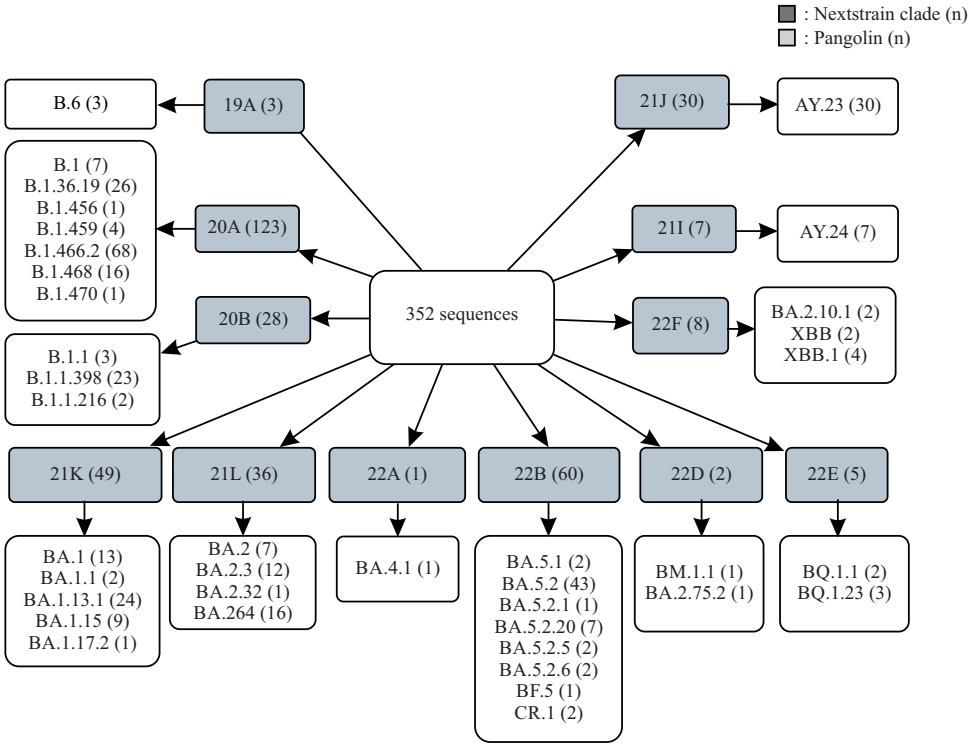


Fig. 1: West Sumatra SARS-CoV-2 variant during the pandemic

Results of next-generation sequencing from 352 isolates collected over three waves of the pandemic are classified according to Nextstrain and Pangolin

Table 1: Statistical analysis of the relationship between mutations in the S gene of the SARS-CoV-2 virus in West Sumatra and transmission capability

| | | Speed of Transmission | | |
|---|---|---|---|---|
| S gene | Mutation | Fast n (%) | Slow n (%) | p-value |
| A67V | Positive | 47 (97.9) | 1 (2.1) | 0.000 |
| | Negative | 105 (34.5) | 199 (65.5) | |
| D796Y | Positive | 99 (61.1) | 63 (38.9) | 0.000 |
| | Negative | 53 (27.9) | 137 (72.1) | |
| D950N | Positive | 35 (94.6) | 2 (5.4) | 0.000 |
| | Negative | 117 (37.1) | 198 (62.9) | |
| E484A | Positive | 97 (61) | 62 (39) | 0.000 |
| | Negative | 55 (28.5) | 138 (71.5) | |
| G142D | Positive | 75 (54.3) | 63 (45.7) | 0.001 |
| | Negative | 77 (36.0) | 137 (64.0) | |
| H655Y | Positive | 100 (61.7) | 62 (38.3) | 0.000 |
| | Negative | 52 (27.4) | 138 (72.6) | |
| K417N | Positive | 87 (59.2) | 60 (40.8) | 0.000 |
| | Negative | 65 (31.7) | 140 (68.3) | |
| N440K | Positive | 90 (60.8) | 58 (39.2) | 0.000 |
| | Negative | 62 (30.4) | 142 (69.6) | |
| N501Y | Positive | 98 (61.3) | 62 (38.8) | 0.000 |
| | Negative | 54 (28.1) | 138 (71.9) | |
| N679K | Positive | 99 (61.5) | 62 (38.5) | 0.000 |
| | Negative | 53 (27.7) | 138 (72.3) | |
| N764K | Positive | 99 (61.9) | 61 (38.1) | 0.000 |
| | Negative | 53 (27.6) | 139 (72.4) | |
| N969K | Positive | 99 (61.9) | 62 (38.5) | 0.000 |
| | Negative | 53 (27.6) | 138 (72.3) | |
| P681H | Positive | 99 (61.9) | 71 (41.8) | 0.000 |
| | Negative | 53 (27.6) | 129 (70.9) | |
| P681R | Positive | 51 (49) | 53 (51) | 0.151 |
| | Negative | 101 (40.7) | 147 (59.3) | |
| Q498R | Positive | 98 (61.3) | 62 (38.8) | 0.000 |
| | Negative | 54 (28.1) | 138 (71.9) | |
| Q954H | Positive | 99 (61.9) | 62 (38.8) | 0.000 |
| | Negative | 53 (27.6) | 138 (71.9) | |
| R158G | Positive | 35 (94.6) | 2 (5.4) | 0.000 |
| | Negative | 117 (37.1) | 198 (62.9) | |
| S371F | Positive | 51 (46.8) | 58 (53.2) | 0.36 |
| | Negative | 101 (41.6) | 142 (58.4) | |
| S373P | Positive | 99 (61.5) | 62 (38.5) | 0.000 |
| | Negative | 53 (27.7) | 138 (72.3) | |
| S375F | Positive | 99(61.5) | 62 (38.5) | 0.000 |
| | Negative | 53(27.7) | 138 (72.3) | |
| S477N | Positive | 98 (61.3) | 62 (38.8) | 0.000 |
| | Negative | 54 (28.1) | 138 (71.9) | |
| T19R | Positive | 35 (94.6) | 2 (5.4) | 0.000 |
| | Negative | 117 (37.1) | 198 (62.9) | |
| T478K | Positive | 132 (67.3) | 64 (32.7) | 0.000 |
| | Negative | 20 (12.8) | 136 (87.2) | |
| T95I | Positive | 47 (97.9) | 1 (2.1) | 0.000 |
| | Negative | 105 (34.5) | 199 (65.5) | |
| Y505H | Positive | 98 (61.3) | 62 (38.8) | 0.000 |
| | Negative | 54 (28.1) | 138 (71.9) | |
| L24- | Positive | 50 (45) | 61 (55) | 0.632 |
| | Negative | 102 (42.3) | 139 (57.7) | |
| P25- | Positive | 50 (45) | 61 (55) | 0.632 |
| | Negative | 102 (42.3) | 139 (57.7) | |
| P26- | Positive | 50 (45) | 61 (55) | 0.632 |
| | Negative | 102 (42.3) | 139 (57.7) | |
| E156- | Positive | 35 (94.6) | 2 (5.4) | 0.000 |
| | Negative | 117 (37.1) | 198 (62.9) | |
| F157- | Positive | 35 (94.6) | 2 (5.4) | 0.000 |
| | Negative | 117 (37.1) | 198 (62.9) | |
| N439K | Positive | 15 (23.4) | 49 (76.6) | 0.000 |
| | Negative | 137 (47.6) | 151 (52.4) | |

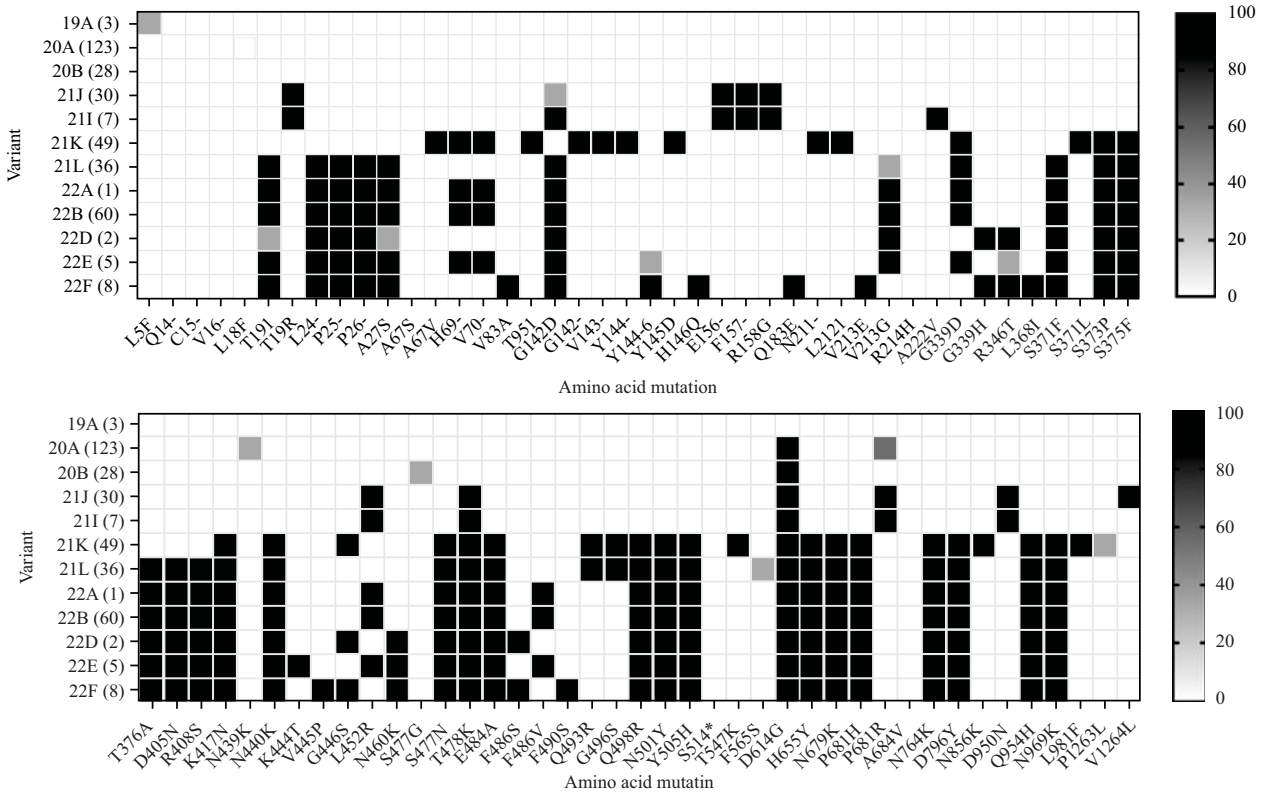


Fig. 2: Amino acid mutation in the SAR-CoV-2 variant S gene

Percentage of amino acid mutations found in each variant is depicted with varying levels of black color gradient

displayed a multitude of mutations, including N501Y, E484A and K417N, some of which have been associated with significant reductions in neutralizing antibody recognition. This suggested a notable advancement in the virus's ability to evade immune responses, leading to higher rates of breakthrough infections despite vaccination but generally causing less severe disease, necessitating updates in vaccine strategies.

Table 1 shows the mutation data found in the S gene of the SARS-CoV-2 virus, which is associated with the transmission speed spread in hosts. The results of the statistical tests indicate that there was a significant relationship between mutations in the S gene of the SARS-CoV-2 virus in West Sumatra, namely A67V, D796Y, D950N, E484A, G142D, H655Y, K417N, N440K, N501Y, N679K, N764K, N969K, P681H, Q498R, Q954H, R158G, S373P, S375F, S477N, T19R, T478K, T95I, Y505H, E156-, F157- and N439K, with the transmission ($p<0.05$).

## DISCUSSION

Rapidly identifying and characterizing novel SARS-CoV-2 variants through genomic surveillance has been paramount in the global response to the COVID-19 pandemic. Techniques and platforms for sequencing and data sharing, such as GISAID and Nextstrain, have facilitated near-real-time monitoring of the virus's evolution. These efforts have allowed for the early detection of variants of concern (VOCs), including those identified in our study, enabling swift public health interventions[6].

Cataloging mutations within the S gene across multiple SARS-CoV-2 variants provides profound insights into the virus's evolutionary mechanisms. The variability observed among different variants, from the early stages of the pandemic with variant 19A to the emergence of highly mutated forms such as the Omicron variant, underscores the virus's capacity for rapid genetic adaptation. This adaptation is driven by selective pressures to enhance transmissibility, evade immune responses and possibly increase virulence[7,8].

Variant 20A demonstrated a diverse array of mutations in the S gene, including L5F, Q14-, C15-, V16- and L18F, with varying prevalences. These mutations suggested an early adaptations of the virus, potentially influencing its binding affinity, antigenicity and immune evasion capabilities to a certain extent. Difference studies have shown that

specific spike protein mutations can enhance SARS-CoV-2 transmissibility and reduce neutralization by antibodies. For instance, the L5F mutation, observed in other contexts, has been associated with changes in the spike protein's structure that could affect the virus's binding affinity or antigenicity, potentially impacting virus-host interactions[9].

The mutations within the 20A variant underscore the necessity for ongoing assessment of vaccine efficacy against emerging variants. While current vaccines are designed to be effective against a broad range of SARS-CoV-2 variants, the accumulation of mutations, especially in the spike protein, warrants continuous monitoring to ensure sustained vaccine protection[10].

The diversity of mutations within the 20A variant highlights the importance of genomic surveillance in identifying and tracking the spread of variants. Enhanced surveillance and rapid public health responses are crucial for mitigating the impact of variants with potential public health implications[11].

The absence of significant mutations in the tracked regions of the S gene for the 20B variant suggests it might retain a closer genetic similarity to the original strain or have mutations outside the studied S gene regions. This could imply different evolutionary pressures or a distinct adaptation pathway in the population it circulated within. Without specific mutations that increase transmissibility or pathogenicity (like those seen in variants such as Alpha, Delta, or Omicron), the 20B variant might not have the same level of concern regarding spread or severity. However, the absence of these mutations does not necessarily mean the variant is less capable of causing outbreaks, as transmissibility can also be influenced by human behavior and immunity. If the 20B variant lacks mutations that significantly alter the spike protein's structure, vaccines designed around the original strain's spike protein might remain effective against it. This, however, requires empirical validation through neutralization studies and real-world vaccine effectiveness assessments.

The Delta variant (21I, 21J) mutations like L452R, T478K, P681R and D614G significantly enhance transmissibility and partially evade immune responses. These mutations facilitated the variant's rapid global spread, becoming dominant in many regions due to its increased infectivity and potential for immune escape. It Increased vaccine breakthrough cases and reduced antibody neutralization, necessitating booster doses for enhanced protection. Variants such as 21J, with high prevalence rates of specific mutations, may represent an adaptive response to the host environment, potentially impacting virus spread and vaccine efficacy. In contrast, the relatively stable mutation profile of early variants like 19A points to the evolutionary infancy of the virus in the region[12-14].

The distinct mutation patterns observed across variants suggest multiple evolutionary pathways within the SARS-CoV-2 virus as it spreads through populations. For instance, the evolution from variant 19A to 20A and subsequently to 21J highlights a trend towards increasing mutation diversity, possibly reflecting the virus's adaptation to evade host immune responses or increase its transmissibility. Both 20A and Delta (21I) show the virus's evolutionary trajectory towards increased fitness in human hosts, with Delta exhibiting a significant leap in transmissibility and potential immune escape. While 20A introduced mutations that suggested early adaptations, Delta's mutations were directly linked to enhanced transmissibility and partial immune escape, which were observed to impact public health measures and vaccine effectiveness considerably. The transition from 20A to Delta underscored the need for robust genomic surveillance, rapid public health response and vaccine adaptation to address the evolving threat of SARS-CoV-2.

The evolution of SARS-CoV-2, particularly from variant 20A to the Delta variant, underscores the dynamic nature of the virus as it adapts to overcome host defenses[15]. This evolution has significant implications for vaccine efficacy and public health strategies, as new variants may possess increased transmissibility, immune escape and diagnostic challenges[16]. The plasticity of SARS-CoV-2, driven by mutation and natural selection, further complicates the development of effective vaccines and immunotherapies[17]. The Delta variant, in particular, has been associated with superior transmissibility and reduced vaccine efficacy[18]. Continuous monitoring and research are crucial to understand the implications of emerging variants and mutations and to develop effective preventive and therapeutic strategies.

Our study's findings on the mutations within the S gene of SARS-CoV-2 variants in West Sumatra after variant 21I contribute valuable insights into the virus's evolving landscape. Identifying a broad spectrum of mutations, particularly in the Omicron and Delta variants, underscores the dynamic nature of the virus and its potential for rapid adaptation. These results align with global observations, indicating an ongoing evolutionary arms race between the virus and host immune defenses[7].

The emergence of these variants post-21I represents a critical juncture in the pandemic's evolution within West Sumatra. With its extensive mutation profile, variant 21K (Omicron) contrasts sharply with earlier variants, suggesting

a significant leap in the virus's evolutionary trajectory aimed at survival and spread in a partially immune population. The analysis indicates an evolutionary trend where subsequent variants, particularly Omicron, have accumulated mutations at a rate and in configurations that challenge current vaccine-induced immunity. This rapid evolution highlights the virus's capacity for adaptability and underscores the need for ongoing surveillance and vaccine adaptation[19-21].

The emergence of variants with significant mutations in the S gene has prompted a reevaluation of vaccine design and efficacy. The mRNA vaccine platforms have demonstrated flexibility in adapting to new variants, with manufacturers testing updated vaccines targeting mutations found in variants like Delta and Omicron[8,22]. This rapid adaptability underscores the potential of mRNA vaccines to keep pace with the evolving virus. This necessitates booster campaigns with updated vaccine formulations and a global strategy to ensure equitable vaccine distribution and administration.

The diversity of mutations across the variants studied suggests multiple evolutionary pathways, likely influenced by regional differences in population immunity, virus exposure and public health interventions. This variability reinforces the importance of localized genomic surveillance to guide public health responses tailored to the specific viral landscape. Moreover, our analysis emphasizes the critical role of international collaboration in sharing genomic data and resources to enhance the global response to emerging variants[4,23].

Further research is needed to elucidate the functional impacts of specific mutations and their combinations in the context of viral pathogenesis and immune escape. Longitudinal studies tracking the emergence and spread of variants in different geographic and immunologic landscapes will enhance our understanding of viral evolution. Moreover, research into pan-coronavirus vaccines targeting more conserved regions of the virus may offer a long-term solution to the challenge posed by emerging variants.

## CONCLUSION

The interpretation of mutation data across all SARS-CoV-2 variants identified in West Sumatra highlights the virus's capacity for rapid evolution and its implications for public health and vaccine strategy. Understanding these genetic changes is crucial for developing effective countermeasures and controlling the pandemic. As the virus evolves, a global, coordinated response that includes ongoing surveillance, vaccine adaptation and equitable public health measures will be essential in managing COVID-19.

## SIGNIFICANCE STATEMENT

The aim of this study was to address knowledge gaps by comprehensively analyzing S gene mutations across three distinct pandemic waves in West Sumatra, to gain a deeper understanding of the evolutionary trajectory of SARS-CoV-2. The key findings of the study revealed significant variability in S gene mutation profiles across different SARS-CoV-2 variants identified in West Sumatra. It provides insights into the evolutionary mechanisms of the virus, potentially influencing its transmissibility, pathogenicity and immune evasion capabilities. Understanding these mutations is crucial for developing effective countermeasures and controlling the pandemic, informing vaccine development strategies and public health policies. Additionally, the study emphasizes the importance of ongoing genomic surveillance to monitor variant emergence and spread, enabling swift public health interventions.

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