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Genetic profile of Omicron subvariants in Vietnam by May 2024: The emergence of JN.1

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Abstract

SARS-CoV-2 Omicron and its subvariants continue to significantly impact global infection and mortality rates. Omicron is characterized by its rapid transmissibility and its ability to evade immune defenses and neutralizing antibodies. The widespread prevalence of Omicron and its subvariants is attributed to mutations within the spike (S) gene. This study update the status of the nucleotide changes and genetic diversity of Omicron in Vietnam. Our analysis recorded new Omicron variants, including BA.2.86 and its descendant: BA.2.86.1; JN.1 and its descendants: JN.1.20, JN.1.4, JN.1.4.5, JN.1.11, and JN.1.16 since December of 2023. These newly found variants formed a separated group in the haplotype network. Our analysis identified five Omicron subvariants isolated in Vietnam: BA.1*, BA.2*, BA.4*, BA.5*, and XBB*. Among these, BA.2* exhibited the highest frequency and haplotype diversity index. We observed that mutations in the S gene predominantly occur in the S1 region, while the S2 region remains relatively conserved across Omicron subvariants.

This study provides a comprehensive analysis of the nucleotide alterations and genetic diversity of Omicron in Vietnam, confirming the critical role of the S gene in the evolution and control of SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2; Spike gene; Genetic diversity; Omicron.

1. Introduction

By mid-2024, the global community continues to report COVID-19 cases and deaths caused by the SARS-CoV-2 virus, which belongs to the Coronaviridae family. This family of viruses is known for its capability of human-to-human transmission, as seen with SARS-CoV and MERS-CoV [1]. The Coronaviridae family comprises four structural proteins: M (membrane), E (envelope), S (spike), and N (nucleocapsid) [2]. The spike (S) protein, in particular, is crucial for viral entry into host cells. The S protein consists of two main subunits: S1 and S2. The S1 subunit contains the N-terminal domain (NTD) and the receptor binding domain (RBD), which interacts with the ACE2 receptor, while the S2 subunit includes the fusion peptide (FP), heptapeptide repeat sequence 1/2 (HR1/2), transmembrane domain (TM), and cytoplasm domain, facilitating membrane fusion and cell entry [3]. Upon entry, the virus synthesizes proteins and assembles new particles using its ss+RNA genome. Due to the lack of a proofreading mechanism during transcription, SARS-CoV-2 exhibits a high mutation rate, leading to the emergence of thousands of variants [4]. Variants that spread rapidly or exhibit high virulence are classified by the WHO as Variants of Concern (VOC). Previously identified VOCs included Alpha, Beta, Gamma, Delta, and Omicron [5] [6]. However, by mid-2024, only Omicron subvariants remain in circulation, with the rapid transmission rate of Omicron variants leading to the decline of other VOCs.

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The success of the Omicron variant has been attributed to the large number of mutations present in S protein. Omicron contains over 30 amino acid mutations compared to the Wildtype [7]. Some of these mutations have been shown to increase Omicron's transmissibility and decrease its sensitivity to neutralizing antibodies [8] [9] [10]. Consequently, Omicron has rapidly generated hundreds of subvariants with enhanced viral characteristics compared to its pedigree. The WHO and CDC have classified Omicron subvariants based on mutations in the S protein. Currently, Omicron subvariants are classified into six main groups: BA.1*, BA.2*, BA.3*, BA.4*, BA.5*, and recombinant Omicron subvariants (notably the XBB* variants) [11]. This classification highlights the role of the S protein not only in facilitating SARS-CoV-2 transmission but also in distinguishing between different variants. Therefore, continuous monitoring of nucleotide changes in the S gene, which encodes the S protein, is crucial for global COVID-19 control efforts.

During the initial phase of the COVID-19 pandemic, Vietnam stood out as one of the few nations successfully managing domestic outbreaks [12]. The situation escalated with the advent of the Delta variant and further intensified when the Omicron variant made its way into the country, leading to a surge in infection rates and mortality. Our previous study [13] documented the persistent presence of multiple Omicron subvariants in Vietnam by the end of 2023. Continuing until mid-2024, the recent detection of new variants such as BA.2.86, JN.1, and KP.2 has notably increased COVID-19 cases across Europe and the USA, sparking concerns about the potential impact if these variants spread to Vietnam. In light of this, our current study aims to evaluate the genetic diversity within the S gene region of Omicron subvariants circulating in Vietnam.

2. Materials and Methods

2.1. Data collection

Whole genome sequences of Omicron subvariants isolated in Vietnam were collected from GISAID. The S gene sequences were extracted and aligned using the MAFFT server [14], based on the reference sequence from GenBank (accession number: NC_045512.2) [15].

2.2. Haplotype numbering and mutation scanning

An in-house Python script was employed to investigate mutations in the S gene. The S gene sequences were grouped and assigned haplotype numbers using DnaSP [16] for downstream analysis.

2.3. Estimating genetic diversity

Nucleotide diversity, haplotype diversity, and molecular distance were determined using Arlequin software [17].

2.4. Haplotype network analysis

The haplotype network was built using PopART [18] software with a Minimum Spanning Network approach. Gephi software was used to visualize and determine the centrality of each node in the network.

3. Results and Discussion

3.1. Haplotype diversity

We obtained 6562 S gene sequences from Omicron subvariants isolated in Vietnam. Compared to the last collection in December 2023, 214 more sequences were reported. Among these, 232 Omicron subvariants were classified into five groups: BA.1*, BA.2*, BA.4*, BA.5*, and XBB*. BA.2* and BA.5* were still predominant subvariants circulating in Vietnam, constituting 51.5% and 28.9% of the distribution, respectively (Table 1). Conversely, BA.4* exhibited a lower prevalence in Vietnam, accounting for 0.5%. The recombinant variant XBB* accounted for a higher proportion at 13.0% compared to BA.1*. Analysis of genetic diversity revealed a high haplotype diversity index of 0.9690 ± 0.0010 for the Omicron in Vietnam. Similarly, Omicron subvariants also demonstrated substantial diversity, indicating extensive genetic diversity and robust circulation of these subvariants in Vietnam. Especially, by this time, the analysis revealed the existence of BA.2.86.2, JN.1 and its descendant such as JN.1.20, JN.1.4, JN.1.4.5, JN.1.11 and JN.1.16 in the community.

Table 1 Haplotype diversity of Omicron variants in Vietnam

Variants	Frequency	Haplotype diversity
BA.1*	6.1%	0.9409 ± 0.0060
BA.2*	51.5%	0.9260 ± 0.0032
BA.4*	0.5%	0.9617 ± 0.0203
BA.5*	28.9%	0.8794 ± 0.0058
XBB*	13.0%	0.9554 ± 0.0046
Omicron	100%	0.9690 ± 0.0010

3.2. Nucleotide diversity

The genetic diversity of the S gene of Omicron subvariants in Vietnam is evident through number of mutations, polymorphic sites, and nucleotide diversity. The apprearance of new JN.1 variants increased the average number of mutation of each Omicron subvariant to 43.4 ± 8.7 mutations (Table 2) from 42.9 ± 7.6 as in the last study. The S1 region recorded an average of 35.0 ± 8.4 mutations, slightly higher than that in December 2023, while the S2 region remained unchanged at 8.4 ± 0.8 (Table 2), confirming the higher mutation density in the S1 region. Within the S1, the NTD harbored more mutations than the RBD, with averages of 17.0 ± 5.3 and 16.8 ± 4.9 mutations, respectively (Table 2). Because of the recent apprearance, these variants only affected some statistic values and could not change the overall mutation landscape of Omicron. Mutation counts and distributions across the S gene were analyzed among various Omicron subvariants, revealing distinct mutation profiles. BA.1* and XBB* exhibited the highest mutation counts, with 54.7 ± 5.9 and 54.6 ± 3.1 mutations, respectively. In contrast, BA.2*, BA.4*, and BA.5* displayed 40.2 ± 8.2, 42.4 ± 3.9, and 41.7 ± 5.0 mutations, respectively. Additionally, the distribution of mutations across the S gene regions varied among the Omicron subvariants. BA.1*, BA.4*, and BA.5* showed a higher mutation frequency in the NTD compared to the RBD. Conversely, BA.2* and XBB* exhibited a higher mutation count in the RBD relative to the NTD (Table 2).

Table 2 A mount of mutations in the S gene of Omicron and its subvariants.

	S gene	S1	NTD	RBD	S2
BA.1*	54.7 ± 5.9	44.6 ± 5.8	26.2 ± 5.4	16.3 ± 1.5	10.1 ± 0.5
BA.2*	40.2 ± 8.2	31.9 ± 7.9	14.3 ± 5.0	16.5 ± 3.6	8.3 ± 0.7
BA.4*	42.4 ± 3.9	33.3 ± 3.9	18.8 ± 1.1	13.5 ± 4.0	9.1 ± 0.8
BA.5*	41.7 ± 5.0	33.5 ± 5.0	18.5 ± 2.2	13.9 ± 4.3	8.2 ± 0.6
XBB*	54.6 ± 3.1	46.1 ± 2.9	20.0 ± 2.6	25.0 ± 1.0	8.5 ± 0.8
Omicron	43.4 ± 8.7	35.0 ± 8.4	17.0 ± 5.3	16.8 ± 4.9	8.4 ± 0.8

Nucleotide substitution analysis revealed that the majority of mutations were transition substitutions, including C to T (C>T), T to C (T>C), A to G (A>G), and G to A (G>A). Among transversions, only G to T (G>T) was noted with high frequency (Table 3). This distribution pattern was consistent across Omicron subvariants. In 1141 identified polymorphic sites, transitions were predominant with 752 sites, while the remaining were 368 transversions and 196 indel sites (Table 4). BA.2* exhibited not only the highest number of polymorphic sites but also the highest nucleotide diversity index (Table 5). Additionally, descendants of BA.2* displayed significant divergence, with the pairwise nucleotide difference index being the highest among the Omicron subvariants.

Table 3 Nucleotide changes in the S gene of Omicron.

Number	Nucleotide changes	Frequency	Number	Nucleotide changes	Frequency
1	A>T	3.1%	7	G>A	8.4%
2	A>C	2.8%	8	G>T	8.0%
3	A>G	9.0%	9	G>C	3.1%
4	T>A	2.5%	10	C>T	18.1%
5	T>C	12.5%	11	C>A	2.9%
6	T>G	1.5%	12	C>G	0.8%

Table 4 Number of polymorphic sites and nucleotide changes of Omicron subvariants.

	BA.1*	BA.2*	BA.4*	BA.5*	XBB*
Polymorphic sites	182	772	69	435	386
Transition sites	72	492	31	279	236
Transversion sites	37	234	20	117	123
Indels	76	137	18	77	62

Table 5 Nucleotide diversity of Omicron subvariants appeared in Vietnam.

Variants	Nucleotide diversity	Nucleotide pairwise difference
BA.1*	0.003057 ± 0.001535	11.712870 ± 5.315446
BA.2*	0.003130 ± 0.001564	12.131180 ± 5.484564
BA.4*	0.002295 ± 0.001210	8.737903 ± 4.140754
BA.5*	0.001776 ± 0.000925	6.811582 ± 3.209758
XBB*	0.001358 ± 0.000728	5.182005 ± 2.511525
General	0.005145 ± 0.002515	19.966048 ± 8.828662

With the rising of JN.1 descendants, BA.2* continually emerged as the predominant circulating variant in Vietnam, exhibiting the highest genetic diversity at both haplotype and nucleotide levels. This suggests that BA.2* harbors mutations that enhance its transmissibility. Our additional molecular docking analysis indicates that BA.2* has a higher number of mutations in the RBD compared to BA.1*, BA.4*, and BA.5*, resulting in the accumulation of novel mutations in the RBD. Previous studies have highlighted that mutations in the RBD of BA.2* increase its affinity for ACE2 receptors [19] [20]. Compared to BA.1*, BA.4*, and BA.5*, BA.2* demonstrates the highest ACE2 binding affinity, underscoring its potent transmissibility [19] [20]. Therefore, BA.2* emerges as the primary circulating variant in Vietnam.

Molecular distance analysis was conducted to determine the genetic distances between Omicron subvariants. BA.2* exhibits a close genetic distance (F_{ST}) with BA.4* at 0.37831 and BA.5* at 0.39929, while it is relatively distant from XBB* at 0.59614 when compared to BA.1*. BA.4* and BA.5* share a closely related relationship, with F_{ST} value of 0.09983. Previous studies have indicated that BA.4* and BA.5* are descendants of BA.2* through recombination mechanisms [21]. Additionally, the proximity of BA.2* to XBB* has been identified in several prior studies [22-24]. This underscores BA.2*'s central role, as it maintains close genetic relationships with other Omicron subvariants.

3.3. Haplotype network

To comprehensively assess the relationships between Omicron subvariants, we constructed a haplotype network and examined the central roles of each haplotype within the network. The network analysis revealed distinct clustering of Omicron variants into separate groups, namely BA.1*, BA.5*, and XBB*. In contrast, BA.2* exhibited a diverse distribution with numerous smaller clusters within the network. Especially, the network showed the appearance of a small group of JN.1 variants (Figure 1), marking the appearance of this variant in Vietnam (on January 26th, 2024 - as in the GISAID's metadata). We observed that haplotypes of BA.5* formed from intermediate nodes of BA.2*, indicating a direct relationship between BA.2* and BA.5*. Similarly, the XBB* cluster showed direct connections with BA.2*. This underscores the central role of BA.2* in the haplotype network, highlighting its significance in the evolutionary dynamics of SARS-CoV-2.

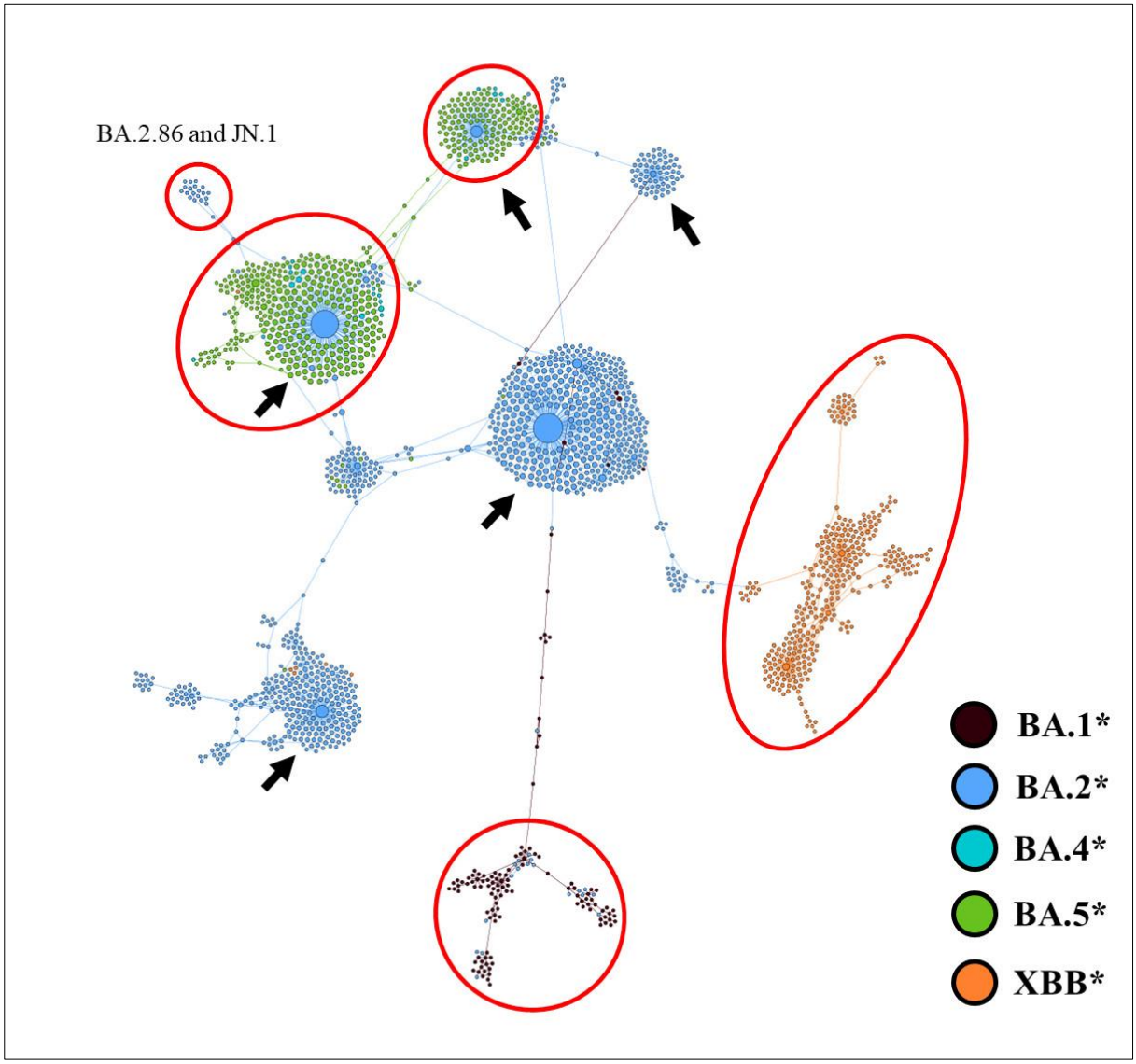

Figure 1 The haplotype network of Omicron subvariant in Vietnam.

(In this figure, each haplotype represented as a node. Each color represents each haplotype belonging to the Omicron subvariants and the size of each node reflects the eigenvector centrality value.)

4. Discussion

BA.2* continues to exhibit evolutionary dynamics by giving rise to numerous novel variants. Notably, BA.2.75 was classified as a VOI due to its significant virological properties. This variant not only showed the ability to evade neutralizing antibodies but also exhibited a stronger affinity compared to other Omicron subvariants such as BA.1* and

BA.5*. These characteristics were attributed to mutations like R346T, G446S, and N460K [25] [26]. By the end of 2023, BA.2.86 rapidly supplanted XBB* globally. The dominance of BA.2.86 was linked to mutations like R403K, which increased ACE2 affinity [27], along with other mutations such as N450D, K356T, L452W, A484K, V483del, and V445H, enhancing immune evasion [28]. Subsequently, JN.1 emerged as a descendant of BA.2.86, featuring the L455S mutation at the binding interface between human ACE2 and RBD, potentially increasing its ability to evade class 1 antibodies [29]. Consequently, the WHO classified JN.1 as a VOI, and it swiftly became the dominant lineage worldwide, replacing BA.2.86 [30]. By mid-2024, a descendant of JN.1, KP.2, emerged in over 30 countries. This variant exhibited a higher reproduction number than JN.1, indicating increased viral fitness and the potential to become the predominant lineage globally [31]. However, by this time, the KP.2 has not been introduced into Vietnam. The continuous accumulation of mutations in the S protein raises concerns that new BA.2* variants may more effectively evade immunity, thus increasing transmission rates. Therefore, ongoing surveillance and assessment of the impact of emerging mutations on the S gene are crucial for controlling COVID-19.

5. Conclusion

In conclusion, our updated study reveals significant genetic diversity among Omicron subvariants in Vietnam and records the appearance of JN.1 variants in the community. Mutations in the Omicron S gene were predominantly found in the S1 region, with the S2 region remaining relatively conserved. Notably, BA.2* has emerged as a key variant in the molecular evolution of the Omicron S gene. Consequently, ongoing surveillance of the evolutionary trends of Omicron subvariants is essential for a deeper understanding of the virus's dynamics and for the development of effective disease control strategies.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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