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An imported human case with the SARS-CoV-2 Omicron subvariant BA.2.75 in Yunnan Province, China

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ABSTRACT

The Omicron variants spread rapidly worldwide after being initially detected in South Africa in November 2021. It showed increased transmissibility and immune evasion with far more amino acid mutations in the spike (S) protein than the previously circulating variants of concern (VOCs). Notably, on 15 July 2022, we monitored the first VOC / Omicron subvariant BA.2.75 in China from an imported case. Moreover, nowadays, this subvariant still is predominant in India. It has nine additional mutations in the S protein compared to BA.2, three of which (W152R, G446S, and R493Q reversion) might contribute to higher transmissibility and immune escape. This subvariant could cause wider spread and pose a threat to the global situation. Our timely reporting and continuous genomic analysis are essential to fully elucidate the characteristics of the subvariant BA.2.75 in the future.

1. Introduction

After the Delta variant created a severe coronavirus disease 2019 (COVID-19) pandemic in the second half of 2021, another highly transmissible variant of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged on 9 November 2021, which was named Omicron and soon listed by the World Health Organization (WHO) as the fifth variant of concern (VOC) on 26 November 2021 [1,2]. Omicron developed many mutations, including 15 mutations in the S protein's receptor-binding domain (RBD). It is well-known that the RBD is responsible for interacting with the angiotensin-converting enzyme 2 (ACE2) receptor [3]. Probably due to the contribution of these mutations, the Omicron variant exhibited increased transmissibility and immune escape, evolving into a globally dominant strain [4,5]. Omicron has evolved into about 220 genetic subvariants [6]. The BA.2.75 was classified by WHO as a subvariant under monitoring (VUM) on 7 July 2022 [2]. It has been detected across 15 countries as of 19 July 2022 [7].

2. Case presentation and results

On 5 July 2022, an international flight (H9784) from Katmandu, Nepal, arrived at Changshui International Airport, Kunming City. Passengers were transferred to the quarantine hotel for a routine 7-day medical observation with a standard nucleic acid assay of SARS-CoV-2. One of these passengers, a 33-year-old male international student, was reported as a positive case for SARS-CoV-2 nucleic acid testing with the cycle threshold (Ct) value (ORF1ab = 21.50, N = 18.50) on 6 July 2022. Then, he was transferred to Yunnan Provincial Infectious Disease Hospital for treatment by negative pressure ambulance. After admission, the patient was diagnosed as an asymptomatic case with a few nodules and cable foci at the apex of both lungs based on the chest computed tomography (CT) examination on 7 July 2022. During hospitalization, the patient had no particular discomfort. On July 20 and 21, 2022, the patient showed the single target positive results in two consecutive SARS-CoV-2 nucleic acid testing (Ct value: ORF1ab=-/N = 38.9, ORF1ab = 38.8/N=-, respectively). He was discharged on 22 July 2022 and transferred to medical isolation for observation. Later, he was released from medical isolation on 28 July 2022, after two consecutive negative results of SARS-CoV-2 nucleic acid testing from 26 July to 27 2022. The patient has been inoculated with two doses of SARS-CoV-2 vaccines and did not have exposure to other COVID-19 cases in the past 14 days.

A nasopharyngeal swab specimen from the patient was then transferred to the Yunnan Center for Disease Control and Prevention for

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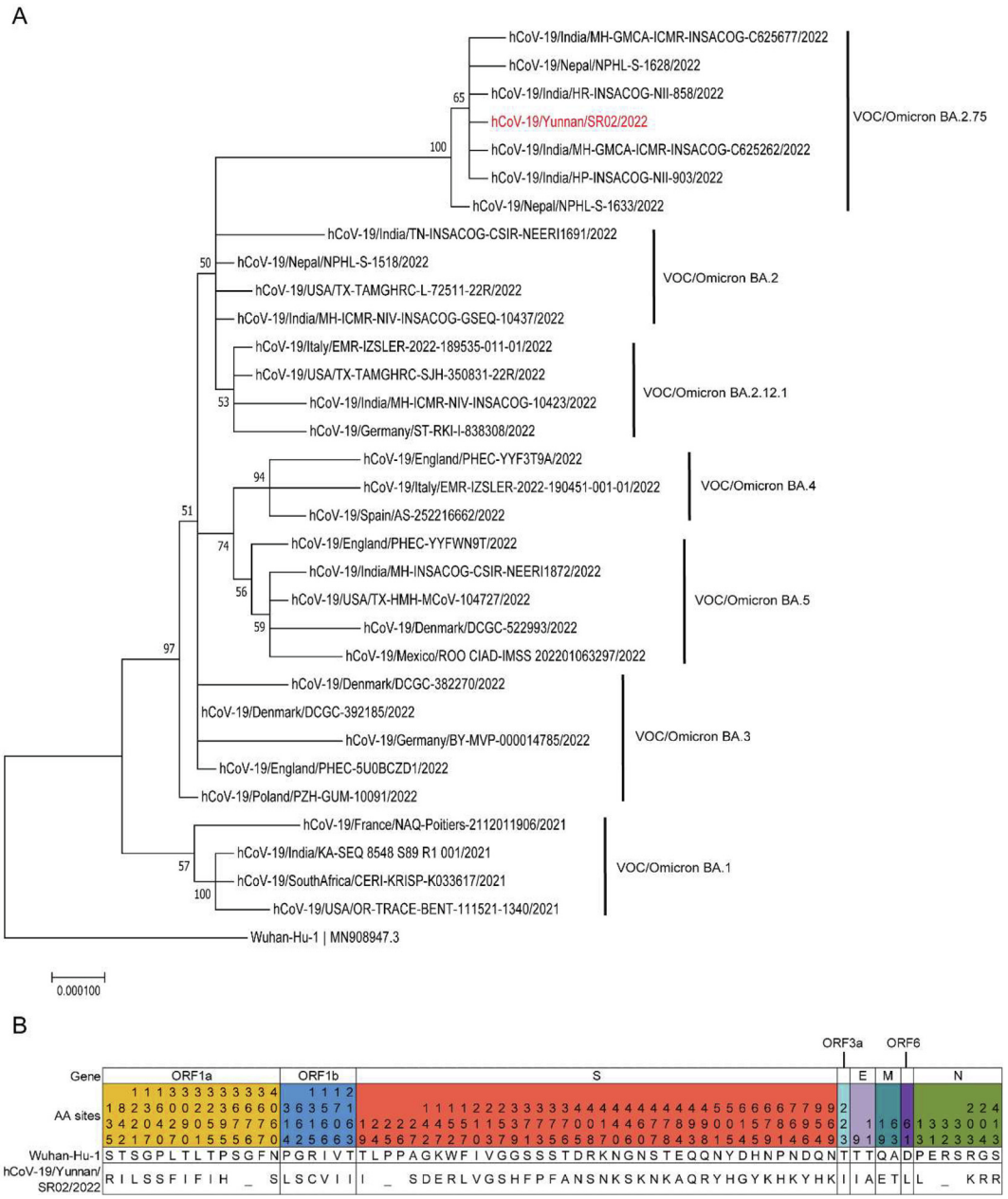

Fig. 1. Genomic analysis of the first imported case of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron subvariant BA.2.75 in Yunnan Province, China. A) A maximum likelihood tree analysis of SARS-CoV-2 genome sequence of the subvariant BA.2.75. The Yunnan Province imported Omicron subvariant BA.2.75 is indicated with red color. B) The analysis of amino acid mutations of the imported BA.2.75 strain, compared to the reference strain Wuhan-Hu-1. "_" represents deletions of amino acids. Abbreviations: VOC = variant of concern.

genome sequencing. First, the viral nucleic acids were extracted by the automatic nucleic acid extraction instrument (BioPerfectus, China), and the products were applied for cDNA synthesis and PCR amplification by ULSEN® Ultra-Sensitive SARS-CoV-2 whole-genome Capture Kit (MicroFuture, China). Then, the amplification products were purified and quantified by MinElute PCR Purification Kit and Qubit™ dsDNA HS Assay Kit (Qiagen, Germany). Next, the sequencing libraries were prepared with Nextera XT DNA Library Prep kit (Illumina, USA) and AMPure XP beads (Beckman Coulter, USA) referring to the kit instructions and were further sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) [8,9]. The raw sequencing reads were trimmed by CLC Genomics Workbench v22 software with parameters: quality scores >95 %, ambiguous nucleotides <2, and automatic read-through adapter trimming. The high-quality reads were mapped to the Wuhan-Hu-1 reference (GenBank accession code: MN908947.3) using the improved mapping options [match score = 1, mismatch cost = 2 (affine gap cost), length fraction = 0.5, similarity fraction = 0.8, auto-detect paired distances, non-specific match handling: map randomly] [8,10]. Eventually, the consensus sequence was obtained for the downstream analysis on 15 July 2022.

The genotyping results showed that the patient was infected with a variant of concern (VOC)/Omicron subvariant BA.2.75 using the pangolin tool [6,11]. Further phylogenetic analysis by the maximum likelihood method based on the Hasegawa-Kishino-Yano (HKY) model and Gamma Distributed (G + 5) (Bootstraps = 1000) confirmed that the virus strain belonged to this lineage (Fig. 1A) [12,13]. We also used the MrBayes v3.2.1 software under the HKY + I + G nucleotide substitution model to verify it (Supplementary Fig. 1) [14]. The chain

length was set to 10,000,000, with the first 1,000 samples burnin and other parameters regarded as defaults. Compared with the Wuhan-Hu-1 reference (MN908947.3) [15], the strain had 61 amino acid missense mutations. It also involved S, envelope (E), membrane (M), nucleocapsid (N), and nonstructural proteins (Fig. 1B). Among them, a total of 34 amino acid mutations (T19I, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K), three deletions (L24del, P25del, P26del) and one reversion mutation (R493Q) were detected on the S protein, two of which (K147E and W152R) were key sites defining the signature of BA.2.75 [16]. The sequence has been submitted to the GISAID database (under the Accession ID: EPI_ISL_13902032).

The whole genome sequence of BA.2.75 was first uploaded to GISAID on 15 June 2022 from a patient's viral transport media (VTM) swab collected on 7 June 2022 in India [17]. As of 18 July 2022, 263 BA.2.75 sequences screened by completed collection dates and high-quality sequences had been submitted to the GISAID database, of which 199 sequences (75.95%) came from India and the rest were from 18 other countries (e.g., the United Kingdom, United States, Australia and Japan) [18]. This indicated that the BA.2.75 could be predominant in India.

3. Discussion and conclusion

After the first detection of BA.2.75 in a sample collected at the end of May 2022, the genomic surveillance indicated that the proportion of BA.2.75 amongst reported SARS-CoV-2 sequences had increased to 20 % in some regions of India by mid-July 2022 [19]. Furthermore, BA.2.75 infections have been reported in more than 25 countries worldwide [20]. Studies revealed that BA.2.75 had more neutralization effect than BA.2.12.1 against the plasma from post-vaccination BA.2 infection, but lower than BA.4 and BA.5 [21]. However, BA.2.75 seemed more immune-evasive than BA.4 and BA.5 against immune background due to Delta-infection [21], which may explain the apparent increase of BA.2.75 in India, which reminds us that the regions with Delta-infection background in China need to pay special attention to the possible risk of associated infection caused by imported cases infected with BA.2.75.

Concerning mutations, the BA.2.75 has the representative triple mutations (K417N, E484A, and N501Y) of Omicron, contributing to higher infectivity and immune escape [22,23]. Furthermore, it has nine additional mutations than BA.2 on the *S* protein, with five (K147E, W152R, F157L, I210V, and G257S) on the *N*-terminal domain (NTD) and four (G339H, G446S, N460K, and R493Q) on the RBD. Furthermore, four (W152R, G339H, G446S, and N460K) have been shown to enhance the ability of the virus to evade recognition by neutralizing antibodies [3,24]. In addition, the R493Q reversion mutation, similar to that of BA.4/BA.5, has been found to restore receptor affinity [25]. Moreover, the number of mutation sites in the S protein of the subvariant BA.2.75 was also more than that of BA.4/BA.5. Preliminary research has revealed that this difference may increase the likelihood that the BA.2.75 significantly reduced susceptibility to therapeutic monoclonal antibodies compared to the BA.2, BA.4 and BA.5 [26]. These latest studies all implied that the BA.2.75 might have higher transmissibility and more robust immune escape. Consequently, on 20 July 2022, the WHO closely monitored Omicron BA.2.75 subvariant.

Whether it will become the dominant variant in India or other countries remains uncertain due to limited data and duration. However, timely reporting and risk assessment, such as routine 7-day medical observation and regular SARS-CoV-2 nucleic acid testing, should be well carried out to control the possible spread of this subvariant. In addition, ongoing genomic monitoring and analysis will be necessary to provide a solid scientific assessing basis for this.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Meiling Zhang: Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing. **Zhixiao Chen:** Data Curation. **Jienan Zhou:** Data Curation. **Xiaonan Zhao:** Data Curation. **Yaoyao Chen:** Formal Analysis. **Yanhong Sun:** Data Curation. **Zhaosheng Liu:** Data Curation. **Wenpeng Gu:** Formal Analysis. **Chunrui Luo:** Formal Analysis. **Xiaoqing Fu:** Conceptualization, Resources. **Xiang Zhao:** Conceptualization, Writing – Review & Editing.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2022.10.003.

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