

# Whole-genome sequencing analysis of imported SARS-CoV-2 cases at Hangzhou Port

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## Abstract

This study employed the Nanopore nanopore sequencing platform and the Baiyi whole-genome analysis software to perform whole-genome sequencing on 50 imported COVID-19 cases collected at the Hangzhou port from September 2022 to January 2023, analyzing the distribution of viral variants to provide theoretical support and practical guidance for epidemic prevention and control. A total of 50 complete SARS-CoV-2 genome sequences were obtained, with 46 cases exhibiting sequence coverage exceeding 99%. All Pangolin phylogenetic analyses identified the variants as Omicron strains, consistent with the globally dominant variant. The distribution of these variants in the functional gene regions may influence the virus's infectivity, pathogenicity, and other biological characteristics. This study holds significant implications for COVID-19 prevention and control at the Hangzhou port and nationwide, offering theoretical support and practical guidance for optimizing detection protocols and enhancing flight controls in high-risk areas.

## Keywords

Hangzhou Port; imported SARS-CoV-2; whole-genome sequencing; viral mutation; epidemic prevention and control

## 1. Introduction

### 1.1. Research Background

Since the outbreak of the novel coronavirus (SARS-CoV-2) at the end of 2019, over 200 million confirmed cases and millions of deaths have been reported worldwide, making the COVID-19 pandemic one of the most severe public health crises of the 21st century. As critical nodes for international personnel movement, border crossings play a pivotal role in preventing imported cases. Imported cases not only facilitate local transmission but may also introduce new variants, posing challenges to existing containment measures and vaccine efficacy. Therefore, timely identification and monitoring of the genomic characteristics of imported SARS-CoV-2 strains are essential for developing scientifically sound containment

strategies. Whole-genome sequencing technology provides high-resolution viral genomic information, aiding in elucidating viral mutation patterns, evolutionary origins, and transmission routes, thereby offering critical data support for pandemic control [1]. Particularly in the context of frequent SARS-CoV-2 mutations, whole-genome sequencing has become a core technological tool for tracking viral evolution and assessing changes in its biological properties.

## 1.2. Problem Statement

Although there has been extensive global research on the whole-genome sequencing of SARS-CoV-2, studies focusing on imported viral strains from specific regional ports remain insufficient. As a critical aviation hub in eastern China, the Hangzhou port faces a high risk of imported viral infections. However, a systematic analysis of the genomic characteristics of imported SARS-CoV-2 strains at the Hangzhou port is currently lacking. SARS-CoV-2 is an RNA virus whose replication-dependent RNA polymerase lacks proofreading mechanisms, leading to continuous genetic mutations during transmission[4], resulting in multiple variants, including Alpha, Beta, Gamma, Delta, and Omicron. Detection methods for SARS-CoV-2 include immunological assays, nucleic acid testing, and genomic sequencing. Compared to traditional first-generation and widely used second-generation sequencing technologies, third-generation nanopore sequencing offers real-time sequencing capabilities, significantly reducing detection time and costs while meeting the demands for high-throughput and rapid customs clearance at ports [6].

## 1.3. Research Objectives

This study aims to conduct whole-genome sequencing of imported SARS-CoV-2 cases at the Hangzhou port to systematically analyze viral mutations, transmission chains, and their relationships with globally circulating strains, thereby providing a scientific basis for epidemic prevention and control. Specifically, third-generation sequencing technology was employed to perform whole-genome sequencing on imported case samples from various sources, followed by bioinformatics analysis to identify key viral mutation sites and their functional impacts. Using third-generation nanopore sequencing technology, whole-genome sequencing and variant analysis were conducted on throat swab samples from 50 SARS-CoV-2 infected individuals at the Hangzhou port between September 2022 and January 2023, providing reference data for variant surveillance at the port. The findings will offer theoretical support and practical guidance for optimizing COVID-19 prevention and control strategies at the Hangzhou port and nationwide.

## 2. Literature Review

### 2.1. Whole Genome Sequencing Technology for SARS-CoV-2

Next-generation sequencing (NGS) and third-generation sequencing (TGS)

technologies have played pivotal roles in the whole-genome sequencing of SARS-CoV-2. NGS technology, characterized by its high accuracy and throughput, has become one of the core techniques for viral genome sequencing. Its principle involves fragmenting nucleic acids into short sequences for parallel sequencing, followed by assembly of the complete genome sequence using bioinformatics methods [1]. However, NGS typically requires extended sample preparation time and higher experimental costs, limiting its application in rapid detection scenarios. In contrast, third-generation sequencing technologies, such as the Nanopore sequencing platform, enable real-time single-molecule sequencing, allowing direct reading of long DNA or RNA fragments, thereby significantly reducing sequencing time and simplifying data assembly [2]. Additionally, nanopore targeted sequencing, an emerging hybrid capture sequencing method, combines the advantages of both NGS and TGS by capturing target regions using specific probes, enabling rapid and precise viral genome sequencing. This technology has demonstrated high efficiency in identifying SARS-CoV-2 variants, particularly suitable for rapid screening scenarios such as at border checkpoints [1][2].

## **2.2. Current Research Status on Imported SARS-CoV-2**

Research on imported cases of the novel coronavirus (COVID-19) both domestically and internationally has yielded significant findings, providing crucial evidence for epidemic prevention and control. Studies have revealed substantial variations in the viral genomic characteristics of cases imported through different border ports. For instance, researchers in Fujian Province conducted whole-genome sequencing on two imported cases and identified multiple mutation sites between their viral sequences and globally known strains. Using the Pangolin system, they classified the strains as the B.1.1.7 variant, thereby clarifying their transmission sources [1]. Similarly, comparative analyses of metatranscriptomic sequencing and hybrid capture sequencing in cases imported from Zhejiang Province via Italy and Spain demonstrated that the latter required only one-tenth the data volume of the former to achieve consistent mutation detection results, highlighting the efficiency of capture sequencing in studying imported cases [8]. Additionally, investigations into outbreaks linked to cold-chain products have elucidated viral transmission patterns under specific environmental conditions. For example, in an outbreak in District M of Fuzhou, four whole-genome sequences exhibited high homology and shared 75 nucleotide mutations, suggesting that cold-chain products may serve as significant vectors for viral transmission [3]. These findings have laid the foundation for understanding the transmission mechanisms and genomic characteristics of imported COVID-19 cases; however, in-depth studies targeting specific border ports remain insufficient.

## **2.3. Research Gaps Related to the Hangzhou Port**

Although previous studies have made certain progress in the whole-genome sequencing and analysis of imported SARS-CoV-2 cases, there remains a significant gap in research specifically targeting the Hangzhou port. Existing literature primarily focuses on case analyses from ports in other regions, such as studies conducted in cities across Fujian, Jiangsu, and Zhejiang provinces, while systematic investigations into imported SARS-CoV-2 cases at the Hangzhou port have not yet been reported [5]. This research gap may lead to insufficient understanding of the genomic characteristics and transmission patterns of imported viruses at the Hangzhou port, thereby affecting the formulation and implementation of epidemic prevention and control strategies. Therefore, this study aims to address this research gap by performing whole-genome sequencing and comprehensive analysis of imported SARS-CoV-2 cases at the Hangzhou port, providing a scientific basis for targeted prevention and control measures and serving as a reference model for similar studies at other ports.

### **3. Collection and Sequencing of Imported SARS-CoV-2 Samples at Hangzhou Port**

#### **3.1. Sample Collection**

##### **3.1.1. Collection Subject**

This study selected imported COVID-19 cases at the Hangzhou port as the sample collection subjects, primarily based on the diversity of their sources and the representativeness of their detection timelines. Specifically, the collected samples included confirmed COVID-19 cases and asymptomatic infections that entered through the Hangzhou port between September 2022 and January 2023, with sources spanning major global high-risk regions such as Southeast Asia, Europe, and North America. All these cases underwent SARS-CoV-2 nucleic acid testing upon entry and tested positive. To ensure sample diversity and representativeness, the study prioritized cases identified from different geographical sources and time periods to comprehensively reflect the genomic characteristics of imported SARS-CoV-2 at the Hangzhou port. Additionally, complete epidemiological data were recorded for all cases, including travel history, contact history, and onset time, providing critical evidence for subsequent transmission chain analysis.

##### **3.1.2. Collection Method**

Sample collection strictly adheres to the operational protocols outlined in the "Technical Guidelines for Laboratory Detection of the Novel Coronavirus," ensuring standardized and scientific practices throughout the process. For confirmed cases and asymptomatic carriers, nasopharyngeal and oropharyngeal swabs are primarily collected. The sampling sites are located deep within both nostrils and the posterior pharyngeal wall, using sterile polyester fiber swabs to minimize sample contamination. Upon completion, the swabs are immediately transferred to sterile

sampling tubes containing viral preservation solution and stored or transported to the laboratory at 4° C. Samples requiring long-term storage are frozen at -80° C ultra-low temperature freezers. These preservation and transportation conditions effectively maintain the integrity of viral RNA, meeting the requirements for subsequent whole-genome sequencing.

## **3.2. Sequencing Procedure**

### **3.2.1. Nucleic Acid Extraction**

The extraction of SARS-CoV-2 nucleic acid from the sample is a critical step in the sequencing process. Nucleic acid extraction and fluorescence RT-PCR detection were performed using the nucleic acid extraction and purification reagents provided by Shanghai Bojie Medical Technology Co., Ltd. to isolate nucleic acid from the throat swab sample. The nucleic acid detection was completed using the SARS-CoV-2 nucleic acid detection kit from Guangzhou DaAn Gene Technology Co., Ltd. on a real-time fluorescence quantitative PCR instrument. Result confirmation was conducted in accordance with the kit instructions.

### **3.2.2. Library Construction and Sequencing**

The construction of sequencing libraries is a critical step in the whole-genome sequencing of SARS-CoV-2.

The ultra-sensitive SARS-CoV-2 whole-genome capture kit (BK-WCoV024TS, Hangzhou Baiyi Technology Co., Ltd.) was employed to perform reverse transcription and SARS-CoV-2 whole-genome amplification using the extracted RNA as the template. After amplification, the PCR products were purified using AMPure XP magnetic beads. Library preparation was conducted in accordance with the instructions of the Ligation Method Sequencing Multi-sample DNA Library Construction Assistant Kit (BK-AUX024, Hangzhou Baiyi Technology Co., Ltd.), utilizing the Ligation Method Library Construction Kits (SQK-LSK110 and EXP-NBD104, Oxford Nanopore) and the Ligation Method Sequencing Multi-sample DNA Library Construction Assistant Kit.

## **4. Analysis of Sequencing Results**

### **4.1. Genomic Sequence Alignment**

After completing whole-genome sequencing and variant analysis, the libraries were sequenced using the GridION sequencing platform, R9.4.1 chips, and MinKNOW (version 22.12.05) software. Each sample yielded at least 70 Mb of sequencing data for downstream analysis. Data analysis was performed using the Hangzhou Baiyi Server SARS-CoV-2 Whole-Genome Analysis System (Version 4.2) for genome assembly, determining viral typing, gene coverage, and sequencing depth. The results demonstrated high overall similarity between the imported SARS-CoV-2 sequences from the Hangzhou port and major global circulating strains, but

identified a number of specific variant sites potentially associated with transmission capacity and pathogenicity. Additionally, compared to genomic sequences from imported cases in other regions of China, samples from the Hangzhou port exhibited distinct regional characteristics, further suggesting their potential origin from a specific international transmission chain. These findings provide critical evidence for subsequent virus tracing and containment strategy development.

## 4.2. Test Results

Detection Status of Imported SARS-CoV-2 Variants at Hangzhou Port: From September 2022 to January 2023, a total of 50 COVID-19 positive samples underwent whole-genome sequencing. Among these, 46 samples (92%) exhibited a coverage rate of  $\geq 99\%$ , all belonging to the Omicron variant. The top five most prevalent variants were BA.5 (16 strains, 32%), XBB.1 (7 strains, 14%), BQ.1 (6 strains, 12%), BF.7 (5 strains, 10%), and BA.2 (3 strains, 6%), as shown in Table 1.

**Table 1.** Detection Status of Imported SARS-CoV-2 Variants at Hangzhou Port

variant	Number (shares)	constituent ratio (%)
BA.5	16	32%
XBB.1	7	14%
BQ.1	6	12%
BF.7	5	10%
BA.2	3	6%
BN.1	2	4%
CK.2	2	4%
BR.2	2	4%
XBF	1	2%
BF.11	1	2%
BM.1	1	2%
BY.1	1	2%
CK.3	1	2%
CL.1	1	2%
XBF	1	2%

## 4.3. Detection of variant strains during the "Category B Management" phase (January 2023)

From September to December 2022, the predominant variants detected in samples from 30 infected individuals were BA.5 (15 strains, 50%), BQ.1 and CK.2 (3 strains each, 10% each), and BF.11 (2 strains, 6.67%). The remaining variants were detected in only one strain each. See Table 2.

**Table 2.** Detection Status of Imported SARS-CoV-2 Variants at Hangzhou Port from September to December 2022

variant	Number (shares)	constituent ratio (%)
BA.5	15	50.00%
BQ.1	3	10.00%
CK.2	3	10.00%

BF.11	2	6.67%
BM.1	1	3.33%
BN.1	1	3.33%
BY.1	1	3.33%
CK.3	1	3.33%
CL.1	1	3.33%
XBB.1	1	3.33%
BA.2	1	3.33%
<b>amount to</b>	<b>30</b>	<b>100.00%</b>

In January 2023, the predominant variants identified in samples from 20 COVID-19 cases imported through the Hangzhou port were XBB.1 (6 strains, 30%), BA.5 (4 strains, 20%), BF.7, and BQ.1 (3 strains, 15%). See Table 3.

**Table 3.** Detection Status of Imported SARS-CoV-2 Variants at Hangzhou Port in January 2023

variant	Number (shares)	constituent ratio (%)
XBB.1	6	30%
BA.5	4	20%
BF.7	3	15%
BQ.1	3	15%
BA.2	1	5%
BR.2	1	5%
XBF	1	5%
BN.1	1	5%
<b>amount to</b>	<b>20</b>	<b>100.00%</b>

After China implemented the "Category B Management" for COVID-19 infections, it still faces the risk of imported variants from international travel and potential domestic outbreaks. Therefore, customs monitoring of COVID-19 variants among people entering and exiting ports is crucial.

This study employed the ultra-sensitive SARS-CoV-2 whole-genome capture kit combined with third-generation nanopore sequencing technology to perform whole-genome sequencing on throat swabs from 50 imported SARS-CoV-2 cases at the Hangzhou port between September 2022 and January 2023, yielding 50 sequences that provided critical data support for subsequent SARS-CoV-2 typing. All cases involved the Omicron variant, consistent with the globally predominant strains[7],[9]. From September to December 2022, the predominant variants were BA.5 (50%), CK.2 (10%), and BQ.1 (10%); in January 2023, the predominant imported variant at the Hangzhou port was XBB (30%). The XBB variant first emerged as one of the top five globally circulating variants in January 2023 and rapidly became the dominant circulating strain worldwide. The findings suggest that the SARS-CoV-2 variants among inbound travelers at the Hangzhou port align with the globally dominant strains.

The variant strains were largely consistent. Hangzhou Xiaoshan International Airport serves as a crucial gateway port in the southwestern region. The imported COVID-19 cases identified in this study were primarily sourced from Hong Kong, China, Taiwan, China, as well as Egypt, Singapore, Thailand, and Pakistan. The diversity of variant strains is complex, necessitating ongoing monitoring of

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COVID-19 variants at border ports to continuously enhance early warning capabilities.

## **5. Discussion**

### **5.1. Reliability Analysis of Results**

During sample collection, sequencing, and data analysis, multiple factors may affect the reliability of the results, including variations in viral load, insufficient standardization of sampling procedures, reagent contamination or operational errors, and the choice of sequencing platform. To minimize potential errors, this study implemented a series of stringent quality control measures: during the sample collection phase, standardized operating protocols were enforced and nucleic acid adequacy was verified; during nucleic acid extraction and library construction, automated equipment was employed with negative controls established; during data analysis, multiple bioinformatics tools were integrated for data filtering and correction to enhance the reliability of sequence alignment.

### **5.2. Prevention and Control Recommendations**

Based on the findings of this study, the following recommendations are proposed for COVID-19 prevention and control at the Hangzhou port: First, optimize testing procedures by increasing the frequency and coverage of nucleic acid testing for passengers on high-risk flights; Second, introduce sensitive targeted sequencing technologies, such as nanopore targeted whole-genome sequencing, to achieve rapid and accurate virus identification; Third, strengthen control measures for flights from key regions and dynamically adjust prevention and control policies based on epidemiological and genomic data; Finally, establish a cross-regional collaboration mechanism to share surveillance data and build a more comprehensive viral genome database, thereby providing robust data support for prevention and control decisions.

### **5.3. Research Limitations**

This study has certain limitations: the sample size is relatively small, and the time span is short, which may affect the generalizability and depth of the conclusions. An insufficient sample size may lead to the omission of low-frequency variant sites, thereby underestimating the genetic diversity of the virus; the short time span makes it difficult to elucidate the long-term evolutionary patterns of the virus; additionally, the uneven distribution of case sources may introduce representational bias. Future studies should expand the sample size and extend the observation period, incorporating multidimensional data to comprehensively analyze the viral mutation and transmission patterns. Simultaneously, long-term monitoring should be conducted to track dynamic changes in the viral genome, thereby providing a more scientific basis for epidemic prevention and control.

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## 6. Conclusion

### 6.1. Summary of Research Findings

Through whole-genome sequencing, this study systematically analyzed the variant characteristics, transmission patterns, and relationships between the imported SARS-CoV-2 strains at the Hangzhou port and globally circulating strains. The results revealed that the imported strains exhibited high homology with known global variants but displayed unique local mutation patterns, suggesting their potential origin from regional circulating strains [3]. Further analysis confirmed that these imported strains shared significant homology with globally identified variants while exhibiting distinctive regional mutation profiles, indicating their likely derivation from specific local epidemic strains. In summary, this study provides critical data support for a deeper understanding of the characteristics and transmission mechanisms of imported SARS-CoV-2 strains.

### 6.2. Research Significance

This study enriches the research on the genomic characteristics of imported SARS-CoV-2 cases at the Hangzhou port, providing theoretical support and practical guidance for nationwide epidemic prevention and control. It accumulates data on viral mutation patterns and their impacts on biological properties, elucidates key transmission chains and their relationships with globally circulating strains, and highlights the critical role of whole-genome sequencing technology in epidemic containment. The findings offer valuable references and insights for epidemic control at the Hangzhou port and other ports across the country, laying a solid foundation for dynamic adjustments to prevention strategies. Furthermore, the study underscores the pivotal importance of whole-genome sequencing in epidemic response and recommends incorporating it as a routine monitoring tool into port prevention systems to enable early warning and precise control. In summary, this research not only provides crucial references for COVID-19 prevention and control at the Hangzhou port but also offers actionable experiences for other ports and regions nationwide, establishing a robust basis for future adaptive epidemic control strategies.

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