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Microbiology Section

# Molecular Epidemiology of Emerging Subvariants of the SARS-CoV-2 Omicron Lineage: A Cross-sectional Study from Himachal Pradesh, India

PRIYANKA RAO[1], SUNITE A GANJU[2], LATA R CHANDEL[3]



## ABSTRACT

**Introduction:** Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) shows continuous genetic variation, resulting in the emergence of multiple viral lineages worldwide. These mutations can influence viral transmissibility, pathogenic potential, and immune escape, resulting in changed disease pattern. Genomic surveillance allows systematic tracking of viral evolution and circulating variants; thus, monitoring is essential for informed public health planning and clinical preparedness.

**Aim:** To detect the emergence of variants of Omicron lineage in the hilly state of Himachal Pradesh, India.

**Materials and Methods:** This cross-sectional study was conducted at the Shri Lal Bahadur Shastri Medical College, Mandi, Himachal Pradesh, India from January 2024 to June 2024. All the SARS-CoV-2 positive samples with Ct value ≤30 received across the state from the four Virus Research and Diagnostic Laboratories (VRDLs) of the state were included. Ribonucleic acid (RNA) extraction was performed using the Q-Line Molecular Viral RNA Extraction Kit, and Whole Genome Sequencing (WGS) was carried out on the Oxford Nanopore MinION platform. Consensus sequences were generated using Medaka and Nanopolish for variant identification and phylogenetic analysis. All demographic and clinical data, like age, sex, clinical presentation, and vaccination status, were recorded and tabulated in Microsoft Excel 2021. The study focused on genomic sequences generated, and the data were presented as numbers and percentages.

**Results:** A total of 30 SARS-CoV-2 positive samples were received. Among SARS-CoV-2 positive cases, adult females constituted the highest proportion, 17 (56.7%), with most patients aged 19-60 years, 17 (56.7%), and the majority of samples originating from Shimla, 11 (36.7%). The study identified JN.1 sublineages in 19 (63.3%) as the predominant cause of infection. Most cases, 20 (66.7%) reported to the outpatient department. Mild symptoms such as fever and cough were seen in 7 (35%) cases. Even though 23 (76.7%) of cases were vaccinated with the COVID-19 vaccine, 10 (33.3%) required admissions. However, no mortality was reported.

**Conclusion:** Tracking SARS-CoV-2 variants revealed the predominance of the JN.1 sublineage of Omicron with generally mild symptoms. Continuous genetic evolution of SARS CoV-2 highlights the need for robust, ongoing genomic surveillance integrated with epidemiological data to enable early detection of emerging variants and strengthen control strategies.

**Keywords:** Epidemiology, Genomic surveillance, Respiratory tract infections, Severe acute respiratory syndrome coronavirus 2

## INTRODUCTION

The SARS-CoV-2 was initially detected in Wuhan City, Hubei Province, China, in December 2019 and has undergone continuous evolution due to genetic mutations or recombination occurring during genome replication [1]. This has led to the emergence of variants distinct from the original virus, each with distinct characteristics related to transmissibility and resistance [2,3].

The five major variants of concern, Alpha, Beta, Gamma, Delta, and Omicron, each show increased transmissibility, infectivity, and immune evasion. Besides, the subvariants of Omicron like BA.2, BA.5, and XBB have gained attention for their rapid spread and resistance to neutralising antibodies and monoclonal therapies [4]. In August 2023, a new COVID-19 Omicron variant called BA.2.86 appeared. It is a sublineage of the older BA.2 variant [5]. At the same time, XBB variants were most commonly circulating globally [6]. BA.2.86 had over 30 changes in its spike protein. It was first found in Israel on August 13, 2023 and recognised by the World Health Organisation (WHO) in August 2023, as a Variant Under Monitoring. Later, it was upgraded to a Variant of Interest (VOI) as it started to spread, as confirmed by genomic surveillance data [5].

Further lineage BA.2.86 evolved to give rise to the JN.1 (BA.2.86.1.1) lineage, which was first detected in the United States in September 2023 and simultaneously identified in 12 other countries, with the highest proportions reported in Canada, France, Singapore, Sweden, the United Kingdom, and the United States. The rapid and widespread transmission of JN.1 across multiple nations led WHO to recognise it as a VOI on December 20, 2023, with distinct characteristics compared with the parent lineage, BA.2.86 [7]. According to WHO reports during January 2024, JN.1 was the most frequently reported VOI globally. It was detected in 71 countries, accounting for 65.5% of all sequences in the final week of December 2023 [8]. By January 2024, the JN.1 variant had become the dominant strain in India, emphasising its significant global impact and transmission dynamics [9].

The symptoms of the JN.1 variant appeared to be very similar to those of previous variants, but generally less severe. The patients reported sore throat as their initial symptom, often followed by congestion of the nose. Symptoms such as dry cough and loss of smell or taste were observed less frequently than with earlier variants. However, in severe cases, symptoms like difficulty in breathing and chest discomfort were reported. Overall, severity was less compared to the early stages of the pandemic [10].

Although WHO declared the end of COVID-19 as a public health emergency on May 5, 2023, SARS-CoV-2 continues to evolve with the emergence of new variants. This highlights the need for a robust

ongoing vigilance through continuous monitoring. Surveillance of viral genomes is vital for the rapid detection of variants, guiding global public health strategies and targeted interventions essential for developing effective treatments and vaccines, ensuring a strong response to future threats [11].

The study aimed to detect the emerging subvariants of the Omicron variant of SARS-CoV-2 by WGS in COVID-19 positive samples received from various districts within Himachal Pradesh, India.

## MATERIALS AND METHODS

This was a cross-sectional study, conducted at the Shri Lal Bahadur Shastri Medical College, Mandi, Himachal Pradesh, India from January to June 2024, wherein all COVID-19 positive samples received from the four VRDLs of the state of Himachal Pradesh were subjected to WGS at the SARS-CoV-2 WGS laboratory recognised by the Indian SARS-CoV-2 Genomics Consortium. Ethical approval was obtained from the Institutional Ethics Committee vide letter no. HFW(H)/SLBSGMC/IEC/2018-90, Protocol No. 20/2022.

This was a time-bound study, and all subjects available during the study period were included; hence, no formal statistical sample size calculation was performed.

**Inclusion criteria:** All SARS-CoV-2 RT-PCR positive samples that met the predefined eligibility conditions were included for WGS. These comprised samples with a cycle threshold (Ct) value ≤30, collected in viral transport medium, and transported under appropriate cold chain conditions [12].

**Exclusion criteria:** Samples with Ct value >30, an insufficient sample volume, or compromised sample quality that could affect sequencing reliability were excluded from the study.

### Study Procedure

All the demographic and clinical data, including age, sex, district of sample collection, clinical symptoms, disease severity, and vaccination status, were compiled, collated and analysed. Clinical severity was categorised based on presenting symptoms and need for hospital care. Mild illness was defined as symptomatic disease without hypoxia ($SpO_2$ ≥94% on room air) or radiological pneumonia, while minimal hospitalisation referred to brief admission for observation or supportive care without oxygen or Intensive Care Unit (ICU) requirement [13]. All samples were processed in a Biosafety Level (BSL)-2 laboratory within a Class II Microbiological Safety Cabinet while following BSL-3 precautions for handling potentially infectious material. The RNA extraction was done according to the manufacturer's instructions using the Q-Line Molecular Viral RNA Extraction Kit from Q-Line Biotech, by a silica membrane-based spin column method [14]. All samples with Ct values ≤30 were subjected to next-generation WGS using Oxford Nanopore MinION technology. The MinION is a compact and portable sequencing device managed by MinKNOW software version 24.11.8, which handles essential operations during sequencing runs. These tasks range from sample tracking and data acquisition to real-time analysis, base calling, and data streaming using MinKNOW software. The library was prepared and sequenced using the Oxford Nanopore MinION Mk1B device for 18 hours. All eligible samples received during the study period were included for testing, irrespective of disease severity, and no additional randomisation was performed. In the current study, among 30 SARS-CoV-2 samples analysed, the genome coverage greater than 70% was achieved in 20 samples.

The bioinformatics data analysis was done by interARTIC software version 0.4.4 incorporating the ARTIC bioinformatics pipeline (version 1.2.1) which uses two inbuilt tools, namely 'Medaka' and 'Nanopolish.' These were employed to create consensus sequences (single nucleotide variation, insertion, deletions, coverage) from nanopore sequencing data and visualisation was done by using UShER (Ultrafast Sample placement on Existing tRee) and Nextclade. UShER enables the comparison of the genetic similarity of the test samples to the sequences available in the public repository, including GISAID (Global Initiative on Sharing All Influenza Data) and shows the relationship between genetically similar sequences.

Comparative analysis with the SARS-CoV-2 Wuhan-Hu-1 reference genome (NC_045512.2) revealed the presence of six distinct Omicron sublineages among the sequenced samples. The six Omicron sublineages were classified based on Pango lineage assignment from WGS data using lineage-defining mutation profiles. The pango lineage of the sequenced SARS-CoV-2 samples was determined using UShER, which enables rapid and precise phylogenetic placement. Utilising a maximum parsimony-based approach, UShER ensures robust and reliable lineage classification grounded in the global diversity of SARS-CoV-2 genomes. The Nextclade phylogenetic placement shows a clearer picture of the viral evolution, placing the samples onto the same background tree. The phylogenetic tree was constructed using the Nextclade online tool (version 3.13.2) and evaluated [15].

## STATISTICAL ANALYSIS

All demographic and clinical data were recorded and tabulated in Microsoft® Excel version 2021 and presented as numbers and percentages.

## RESULTS

A total of 30 samples were received in the laboratory, and in all, the Ct value was ≤30. Among all SARS-CoV-2 positive cases, a higher proportion of infection was noted, 17 (56.7%) in adult females. The majority of cases, 17 (56.7%) were in the age range of 19-60 years. A total of 11 (36.7%) samples were received from district Shimla, followed by 9 (30%) from district Hamirpur and district Nahan [Table/Fig-1].

| Demographic characteristics | n (%) |
|---|---|
| **Gender wise distribution** | |
| Male | 13 (43.3) |
| Female | 17 (56.7) |
| **Age-wise distribution (in years)** | |
| 0-18 | 07 (23.3) |
| 19–60 | 17 (56.7) |
| ≥60 | 06 (20) |
| **Area-wise distribution** | |
| Bilaspur | 01 (3.3) |
| Hamirpur | 09 (30) |
| Shimla | 11 (36.7) |
| Nahan | 09 (30) |
| **Rural and Urban distribution** | |
| Rural | 19 (63.3) |
| Urban | 11 (36.7) |

**[Table/Fig-1]:** Demographic characteristics of SARS-CoV-2 patients.

Out of all the 30 SARS-CoV-2 positive cases, 10 (33.3%) were asymptomatic, while 20 (66.7%) were symptomatic. However, 10 patients (33.3%) required inpatient admission, compared to 20 (66.7%) who attended the outpatient department. Vaccination for COVID-19 was recorded in 23 (76.7%) of cases [Table/Fig-2].

Month-wise distribution of different lineages of the Omicron variant showed that the maximum number of isolates was detected in month of February 2024, followed by March 2024. No sample was received in the month of May, and also from July to December 2024 [Table/Fig-3].

The most common sublineage detected was JN.1.1 belonging to the JN.1 lineage. The further phylogenetic analysis revealed the presence of seven distinct Nextstrain clades among the 30 sequenced samples: 21L, 22F, 23B, 23I, 24A, 24B, and 24C. The majority of the viruses 20 (66.7%) were clustered in clade 23I

indicating a dominant clade. Clade 23I, 24B and 24C, comprised of the KP strains. Clades 21L and 24C each contained three clustered viruses. Each clade 22F, 23B, 24A, and 24B contained a single virus showing lower representation of these clades [Table/Fig-4].

| Symptoms | n (%) |
|---|---|
| Asymptomatic | 10 (33.3) |
| Symptomatic | 20 (66.7) |
| **IPD and OPD attendees** | |
| IPD | 10 (33.3) |
| OPD | 20 (66.7) |
| **Clinical features** | |
| Cough | 01 (5) |
| Fever | 05 (25) |
| Fever with cough | 07 (35) |
| Fever with SOB | 03 (15) |
| Fever with pneumonia | 02 (10) |
| Respiratory distress | 02 (10) |
| **Vaccination** | |
| Not eligible for vaccination | 3 (10) |
| Non vaccinated | 4 (13.3) |
| Vaccinated | 23 (76.7) |

**[Table/Fig-2]:** Clinical characteristics of SARS-CoV-2 patients.
IPD: Inpatient department, OPD: Outpatient department; SOB: Shortness of breath

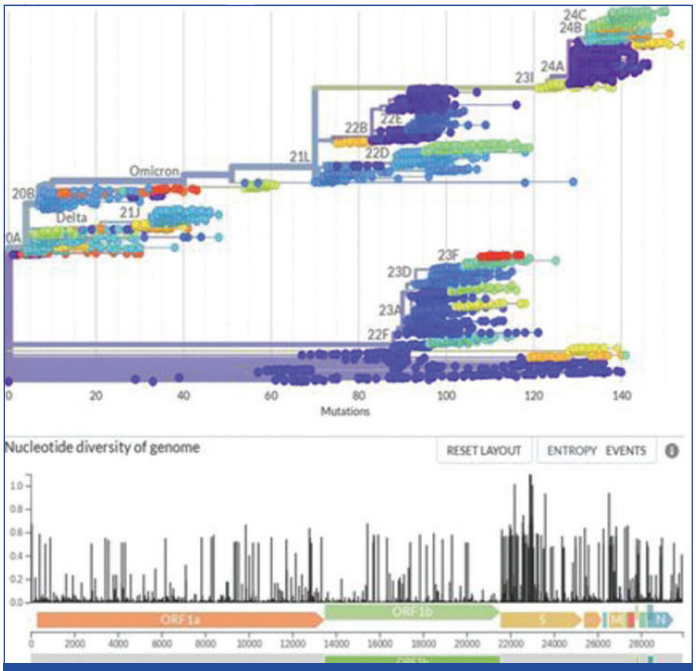
| Months (2024) | XBB.1 | XBB.1.16 | BA.2 | BA.2.86/ SL | JN.1/ SL | KP.1/ SL | Total |
|---|---|---|---|---|---|---|---|
| January | - | 1 | - | 01 | 05 | - | 07 |
| February | - | - | 02 | - | 08 | - | 10 |
| March | 01 | - | 01 | - | 04 | 02 | 08 |
| April | - | - | | - | 02 | 02 | 04 |
| May | - | - | - | - | - | - | 00 |
| June | - | - | - | - | - | 01 | 01 |
| Total | 01 | 01 | 03 | 01 | 19 | 05 | 30 |

**[Table/Fig-3]:** Month-wise distribution of different lineages of the Omicron variant.
Among the remaining 10 samples, nine (90%) achieved ≥50% genome coverage (including 6 with >60%); one sample had <50% coverage; SL: Spike Lineage

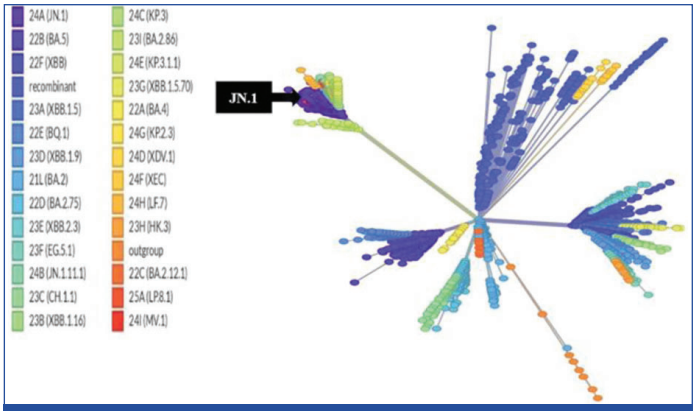
| *Clade | †Lineages (UShER) | Neighbouring sample in tree (UShER) | n (%) |
|---|---|---|---|
| 22F | XBB.1 | CHN(C_AA042590.1) | 1 (3.33) |
| 23B | XBB.1.16 | USA(OQ893733.1) | 1 (3.33 |
| 21L | BA.2 | USA(PP065061.1) BHR(PP029740.1) USA(PP214269.1) | 3 (10) |
| 23I | BA.2.86.1 | - | 1 (3.3) |
| 23I | JN.1 | USA(PP219068.1) USA(OR985353.1) USA(PP316924.1) USA(PP319924.1) | 4 (13.3) |
| 23I | JN.1.1 | USA(PP064083.1) England (OZ010414.1) | 8 (26.7) |
| 23I | JN.1.11 | USA(PP189003.1) USA(PP159170.1) | 6 (20) |
| 24A | JN.1.30 | England (OY990737.1) | 1 (3.3) |
| 23I | KP.1 | USA(PP280861.1) | 1 (3.4) |
| 24B | KP.1.1 | USA(PP808528.1) | 1 (3.4) |
| 24C | KP.3.1 | England (OZ082168.1) | 3 (10) |

**[Table/Fig-4]:** Distribution of various variants, lineages, sub-lineages of SARS-CoV-2.
*Next Strain Clade, †Pango Lineages

JN.1, a descendant of the BA.2.86 lineage, exhibits specific mutations in the spike protein Receptor-Binding Domain (RBD), enhancing ACE2 binding and immune evasion. In the current study, JN.1 circulation declined over time, with KP.1, KP.3, and other JN.1-derived variants becoming more prevalent. Phylogenetic analysis showed that JN.1 forms a distinct, long branch, reflecting its evolutionary divergence from other variants. Rooted trees represent genetic changes over time, while unrooted trees depict sequence relatedness without implying ancestry. The phylogenetic tree in rooted and unrooted layouts is shown in [Table/Fig-5,6].




**[Table/Fig-5]:** Phylogenetic tree (Rooted): Evolutionary relationship of clades.




**[Table/Fig-6]:** Phylogenetic tree (Unrooted layout).

JN.1 shows 24 spike protein mutations compared to the original Wuhan-Hu-1 strain. Similar mutations like T19I, D614G, N764K, D796Y, Q954H, and N969K were found when compared to Wuhan-Hu-1 strain, but these were not detected in BA.2 and XBB sequences. In contrast, the Q146H mutation appeared when compared to the XBB variant, but was not found in the comparison with Wuhan-Hu-1. The list of mutations detected is illustrated in [Table/Fig-7].

Comparison of JN.1 with Wuhan-Hu-1, BA.2, XBB, and BA.2.86 revealed universal, unique, and random spike protein mutations. Unique mutations included L50S, V83A, F127V, G123E, E554K, S939F, and V1104L. Universal mutations like T547K, A570V, and D614G were also present. Most mutations were random, with A27S found in the N-Terminal Domain (NTD) being the most common. The mutations detected are shown in [Table/Fig-8].

## DISCUSSION

SARS-CoV-2 being an RNA virus, continuously mutates during transmission and leads to emergence of SARS-CoV-2 variants. This not only can reduce the sensitivity of SARS-CoV-2 detection techniques but also poses challenges for epidemic control [16,17].

| S. No. | Comparison of JN.1 variant with other variants (n=30) | Shared spike mutations | Unique spike mutations |
|---|---|---|---|
| 1 | Wuhan-Hu-1 | R21T, A27S, F157S, R158G, Q183E, L212I, V213G, L216F, H245N, G252V, E554K, T547K, A570V, D614G, P621S, N764K, D796Y, S939F, Q954H, N969K, K1086R, V1104L, P1143L | T19I, D614G, N764K D796Y, Q954H, N969K |
| 2 | BA.2 | R21T, A27S, F157S, R158G, Q183E, L212I, V213G, L216F, H245N, G252V, E554K, T547K, A570V, P621S, S939F, K1086R, V1104L, P1143L | V83A |
| 3 | XBB | R21T, A27S, F157S, R158G, Q183E, L212I, V213G, L216F, H245N, G252V, E554K, T547K, A570V, P621S, S939F, K1086R, V1104L, P1143L | Q146H |

**[Table/Fig-7]:** Mutational pattern in spike protein among different lineages.

| Mutation Type | NTD | RBD | SD | HR1/HR2 | FP |
|---|---|---|---|---|---|
| Universal | — | — | T547K | — | — |
| — | — | — | A570V | — | — |
| — | — | — | D614G | — | — |
| — | — | — | | — | — |
| Random | T19I | — | P621S | Q954H | D796Y |
| — | R21T | — | N764K | N969K | |
| — | A27S | — | | P1143L | |
| | Q146H | | | | |
| — | F157S | — | — | — | — |
| — | R158G | — | — | — | — |
| | Q183E | | | | |
| — | L212I | — | — | — | — |
| — | V213G | — | — | — | — |
| — | L216F | — | — | — | — |
| — | H245N | — | — | — | — |
| — | K1086R | — | — | — | — |
| Unique | V83A | E554K | | S939F | |
| — | L50S | — | — | V1104L | — |
| | F127V | | | | |
| | G123E | | | | |

**[Table/Fig-8]:** Unique, universal, and random mutations in JN.1 spike proteins (n=30).
FP: Fusion peptide, HR1/2: Heptad repeat, NTD: N-terminal domain, RBD: Receptor binding domain, SD: Spike subdomain

Nanopore sequencing effectively monitors SARS-CoV-2 mutations and the spread of the COVID-19 pandemic in different regions and is extensively used for diagnostic sequencing of SARS-CoV-2, and genome sequencing [18,19].

In this study, the majority of cases were in the age group 19-60 years, which could be due to more outdoor activities and more contact with persons in comparison to extremes of age. Similar findings were observed in a study by Kushwaha S et al., [20]. In a study conducted in Tamil Nadu, India, the proportion of male and female patients was equal, with 50% males and 50% females among JN.1 positive cases [4]. In contrast, a study from Maharashtra reported a higher percentage of male patients, with 55.45% males and 44.55% females [7]. These differences might also be due to biological variations like immunological and genetic differences. In the current study, the variants identified during this period caused only mild illness requiring minimal hospitalisation for observation or supportive care without oxygen or any intensive care requirement. Similar to the study done by Levy ME et al., where the hospitalisation due to JN.1 variants was much less than other variants [21]. In this study, symptomatic patients had only mild symptoms like cough, fever or both, similar to findings shown by Selvavinayagam ST et al., mild symptoms and fewer complications due to new variants despite their higher rate of transmission could be associated with a high rate of vaccination in the state [4]. The study depicted that most of the SARS-CoV-2 positivity was seen in the rural population, and these findings are quite comparable to a study done by Sahoo PK et al., which showed that returning migrants (mainly day labourers) brought the disease back to their home, which triggered significant spread of the virus to semiurban and rural areas. This highlighted serious concern in rural India, where access to sophisticated healthcare and mitigation strategies were lacking [22].

The highest number of positive samples was reported from Shimla district. This could be attributed to Shimla being the state capital and a focal point for inter-state and inter-district travel, resulting in increased movement of people, which likely contributed to a higher risk of virus transmission and, consequently, a greater number of positive cases followed by Hamirpur District. Nahan accounted for 30% of the samples which is a border area of the state.

The significant increase in cases of the JN.1 variant after vaccinations suggested breakthrough infections with a heightened ability to evade immunity [20]. While the previous XBB variant exhibited 82% immune evasiveness in vaccinated individuals, the JN.1 variant had acquired multiple mutations which resulted in structural changes to the spike protein, leading to an immune evasion rate of 95% [23]. Also, the reason for acquiring infection even after getting vaccinated could be attributed to COVID-19 inappropriate behaviour like not wearing masks and not taking care of transmission-based precautions [24]. Six major Omicron lineage/sublineage groups are reported using Pango classification, while seven clades are described using Nextstrain. The Pango lineage and Nextstrain systems represent two different classification frameworks. The Pango lineage system offers a fine-scale, hierarchical classification of SARS-CoV-2 sublineages, while the Nextstrain system categorises sequences into broader phylogenetic clades reflecting major evolutionary patterns and global spread.

The major variants detected in present study in 2024 were JN.1.1, JN.1.11, JN.1, JN.1.30 in 63.3% samples. A study done by Lu Y et al., also revealed that the global prevalence of JN.1 exceeded 60% in early 2024 [25]. The spike protein is responsible for binding to the human ACE2 receptor and is the main target of neutralising antibodies, so mutations here can affect transmissibility, immune evasion, and vaccine effectiveness. Mutations detected in current study such as A27S, L50S, V83A, F127V, S157F, and G123E are situated in the NTD, a region known to be involved in immune recognition. Changes here may alter the antigenic properties of the virus, potentially aiding in immune evasion. The A27S mutation in the S protein may present an evolutionary marker and should be tracked in future studies [26]. L216F lies near the RBD, which is directly involved in binding to the ACE2 receptor on host cells. Alterations in this area could influence binding affinity and, consequently, transmissibility. Mutations like T547K, K1086R, and V1104L are located in the S2 subunit, which facilitates membrane fusion during viral entry. Changes here might affect the fusion process, potentially impacting viral infectivity [27,28]. In the present study, the V1104L mutation was detected, which is shown to stabilise the spike conformation and decrease infectivity [29].

Of the 30 samples, 20 had high genome coverage (>70%). Among the remaining 10 samples, nine had coverage ≥50% (including 6 with >60% coverage), while only one sample had <50% coverage. While higher genome coverage provides greater confidence for detailed mutational analysis, however, partial genome coverage still permits reliable lineage assignment depending on the genomic regions sequenced, and

lineage determination was consistent across samples. All samples were included to preserve epidemiological representation.

In this study, KP sublineages became the predominant circulating strains following the decline of JN.1 sublineages. According to the WHO the current circulating VUMs are KP.3, KP.3.1.1, LB.1, XEC, LP.8.1 with genetic features of JN.1+S: F456L, S: Q493E, S: V1104L, KP.3+S: S31-, JN.1+ S: S31-, S: Q183H, S: R346T, S: F456L, JN.1+S: T22N, S: F59S, S: F456L, S: Q493E, S: V1104L and JN.1+S: S31-, S: F186L, S: R190S, S: R346T, S: V445R, S: F456L, S: Q493E, S: K1086R, S: V1104L, respectively [30].

In the study, the first confirmed case of the JN.1 variant was recorded in a sample collected in January 2024. The JN.1 lineage was first identified in the United States in September 2023, followed by its initial detection in India in December 2023 in the state of Kerala [31]. It was observed that the emergence and detection of new COVID-19 variants in our state typically lagged by one to two months when compared to states like Maharashtra and Kerala. This delay could be attributed to several factors. Maharashtra and Kerala have significantly higher population densities, which can accelerate the spread of infectious diseases due to closer human contact. Additionally, both states experience much higher levels of domestic and international mobility, particularly through major international airports in cities like Mumbai. These airports serve as key entry points for travelers from around the world, increasing the likelihood of earlier introduction and detection of new variants. In contrast, our state, being a hilly state with a relatively lower population density and limited international travel, may naturally experience a slower spread and later detection of emerging variants. Consistent with the findings of Lu Y et al., no deaths were reported in the present study, suggesting that the newly emerged Omicron variant may not be associated with an increase in mortality. Nonetheless, ongoing genomic surveillance and clinical monitoring are imperative to assess its evolving pathogenic potential [25].

The study highlights the predominance of the JN.1 sublineage in Himachal Pradesh, emphasising the importance of regional genomic surveillance to guide public health measures and vaccine strategies. Expanded genomic monitoring with larger sample sizes and integration of clinical and vaccination data will help assess variant impact, disease severity, and vaccine effectiveness, enabling timely public health responses.

### Limitation(s)

The limitations of the study were the smaller number of samples, as only 30 SARS-CoV-2 positive samples were received from different VRDLs during the study period. As this was a time-bound study and included all available subjects, no prior statistical sample size calculation was done, which may limit the generalisability of the findings. Besides, patient follow-up was not possible, which restricted the assessment of clinical outcomes or disease progression in relation to the specific variants detected. Despite these limitations, the study provides valuable insights into genomic surveillance data from the region.

## CONCLUSION(S)

This study uncovers the rapidly evolving SARS-CoV-2 landscape, with sublineages like JN.1 carrying distinct spike protein mutations. Such changes may alter viral infectivity and immune interactions, highlighting their real-world impact. Though the disease was less severe and led to a lower hospitalisation rate, the WGS approach by NGS using nanopore represents the gold standard, which helps in tracking the spread of the virus, local transmission trends and variant-specific evolutionary patterns to identify emerging strains. Early detection of these mutations through surveillance provides actionable insights for effective public health intervention, like targeted infection prevention, vaccine development and research.

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**PARTICULARS OF CONTRIBUTORS:**
1. Junior Resident, Department of Microbiology, Shri Lal Bahadur Shastri Government Medical College, Mandi, Himachal Pradesh, India.
2. Professor, Department of Microbiology, Shri Lal Bahadur Shastri Government Medical College, Mandi, Himachal Pradesh, India.
3. Assistant Professor, Department of Microbiology, Shri Lal Bahadur Shastri Government Medical College, Mandi, Himachal Pradesh, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Dr. Sunite A Ganju,
214-B, Sector-3, New Shimla, Himachal Pradesh, India.
E-mail: suniteaganju@gmail.com

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