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Coronavirus Pandemic

Surveillance of omicron variant in Kangra District of Himachal Pradesh, India during the 3rd disease wave of COVID-19

Suresh Kumar[1,2], Sahdev Choudhary[1,2], Sharad Thakur[1], Arbind Kumar[1], Sanjay Kumar[3]

[1] COVID-19 Testing Facility, D & NT Division, CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, India
[2] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India
[3] CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, India

Abstract

Introduction: The coronavirus disease 2019 (COVID-19) wave has fluctuated erratically around the globe over the past three years of the pandemic, sometimes declining and at other times surging. The cases of infection in India have remained low, despite the continued surge of Omicron sub-lineages reported in a few countries. In this study, we determined the presence of the circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strains in the population of Kangra District, Himachal Pradesh, India.

Methodology: In vitro diagnostic real-time reverse transcriptase polymerase chain reaction (RT-qPCR) was performed using Tata MD CHECK RT-PCR Omisure kit (Tata Medical and Diagnostics Limited, Maharashtra, India), to detect the presence of Omicron in target samples. A total of 400 samples were analyzed in this study; 200 each for the second and third waves, respectively. The S gene target failure (SG-TF) and S gene mutation amplification (SG-MA) primer-probe sets were used.

Results: Our results corroborated that during the third wave, SG-MA amplification was noted, while amplification of SG-TF was not, and vice versa in the case of the second wave, indicating that all the tested patients were infected with the Omicron variant during the third wave, while Omicron was absent during the second wave.

Conclusions: This study added more information about the prevalence of Omicron variants during the third wave in the chosen area, and it projected a use of in vitro RT-qPCR method for rapid prospective determination of the prevalence of the variant of concern (VOC) in developing countries with limited sequencing facility.

Key words: COVID-19; SARS-CoV-2; Omicron; real RT-PCR; third wave.

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Introduction

The first SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) infection case was detected in Wuhan, China in late 2019. Within two months, the viral infection had spread over the world, and in March 2020 the World Health Organization (WHO) classified the illness as a pandemic. A variety of therapy options arose over time and were implemented globally to combat the novel coronavirus [1-3]. Despite vaccination drives worldwide, instances are still being recorded globally, and this is because new "Omicron sub-lineage" variants are emerging [4].

The Omicron (B.1.1.529) variant was first reported in South Africa in November 2021 [5]. Within a month of its emergence, this variant was the most prevalent strain around the world. The third wave of the COVID-19 pandemic across all states in India is being driven by the fast-spreading Omicron variant [6]. Genome sequencing of SARS-CoV-2 demonstrated that point mutations, insertions, and deletions resulted in the emergence of variants. Several SARS-CoV-2 variants emerged during community transmission, with a few exhibiting high transmissibility, disease severity, immune invasion, breakthrough infections, and were highly lethal. These were classified as variants of concern (VOC) by the WHO. To date, a total of five VOC, Alpha, Beta, Gamma, Delta, and Omicron, have been reported [4]. All VOC caused rapid surge of infection worldwide at the time of onset, resulting in disease waves. In India, the first wave was primarily driven by the Alpha variant, second wave by the Delta variant, and third wave by the Omicron variant [7]. Omicron contains many variations in the SARS-CoV-2 genome. More than 60 variations (substitutions/deletions/insertions) have been reported and a few of them are alarming [8]. Different sub-

lineages of Omicron variants (BA.1, BA.2, BA.3, BA.4, BA.5) have evolved over time, but apart from a few specific changes, all share the same changes in spike proteins. The spike proteins in BA.4 and BA.5 are identical to those in BA.2, apart from L452R, F486V, R493Q, and 69-70 deletion [4,9]. Omicron has a much higher transmission rate than Delta, and the capability to neutralize antibodies gained through vaccination or prior infection, resulting in community transmission much more quickly than the Delta variant [10].

Omicron outperformed Delta during the third wave and became the most prevalent strain globally, including in India [7,10,11]. A timely detection of the prevalent VOC in a region is required in order to determine the extent of transmission as well as to strengthen public health and social measures, such as infection prevention and control (IPC) measures, to reduce the risk of disease transmission to other regions and for better patient care management [12]. The presence of variations in a population can only be identified through genomic sequencing [13]. However, it is expensive, time-consuming, and requires trained personnel. The sequencing of the SARS-CoV-2 genome has been delayed during the pandemic; all isolates cannot be sent for sequencing or sequenced during disease waves. However, due to Omicron's high transmissibility and based on a few variant-specific sequencing data from different locations, it can be assumed that Omicron is most likely to be the reason for a sudden surge of infection in those states during the third wave.

Mutation-specific polymerase chain reaction (PCR) - based techniques can be employed for detection of the SARS-CoV-2 strain for experimental confirmation in this case. These techniques are affordable and enable rapid high-throughput screening of SARS-CoV-2-positive samples [14]. Therefore, in the present study, SARS-CoV-2 positive samples from the Kangra District of Himachal Pradesh, India, were subjected to an in-vitro real time reverse-transcriptase polymerase chain reaction (RT-qPCR) analysis during the third wave to add more information about the prevalence of Omicron without the use of the genome sequencing technique.

Methodology

Study population and ethical clearance

Swab samples of patients were collected for routine COVID-19 testing at Palampur, Kangra district, Himachal Pradesh, India. Swab samples were collected in Viral Transport Media (VTM) (TRIVITRON Healthcare System, Chennai, India) at collection centers and transported to the COVID-19 Testing Facility, CSIR-Institute of Himalayan Bioresource Technology. A total of 400 swabs that were positive for SARS-CoV-2 (200 each from second and third waves) were included in the study. This included infected patients' swab samples from the second wave (n = 200) that were tested for SARS-CoV-2 infection between 1 June 2021 and 31 August 2021, and samples from the third wave (n = 200) that were collected between 1 July 2022 and 31 August 2022.

The collection of human swab samples was approved by the institute ethical committee of the CSIR-Institute of Himalayan Bioresource and Technology, Palampur, India. The Indian Council of Medical Research and the Indian Government's Ethical Guidelines for Biomedical Research on Human Subjects were followed in all the experiments. All required safety protocols were followed and all experiments were performed in B2 cabinet Esco Labculture® Class II Type B2 biosafety cabinet (ESCO Life Sciences, Singapore) in BSL-2+ facility.

Isolation of RNA

RNA was isolated from the samples using a column-based nucleic acid isolation kit (TRIVITRON Healthcare System, Chennai, India) according to the manufacturer's protocol. Briefly, the VTM-swabs were vortexed for a moment, and 200 µL of sample were added to 500 µL of lysis buffer (provided with the kit) and gently vortexed again and incubated for 15 minutes at room temperature. Then, 600 µL of absolute ethanol was added to the lysis-sample mixture and vortexed for 30 seconds. A total of 650 µL of solution was loaded into the spin column and centrifuged at 12000 rpm for 1 minute. The spin column capturing nucleic acid was washed with Wash 1 and Wash 2 buffers (supplied with the kit), and then dried. Elution buffer was added to the spin column and RNA was collected in Eppendorf tubes (Genaxy Scientific Pvt Ltd, Solan, India).

Reverse transcriptase-real time-polymerase chain reaction (RT-qPCR)

Reactions were carried out using a commercial MD CHECK RT-PCR Omisure (Tata Medical and Diagnostics Limited, Maharashtra, India), which contains both reverse transcription and DNA-polymerase enzyme activities. All reactions were carried out in 96-well plates in a CFX-96 Real-Time System (BioRad, Hercules, USA) and each plate included RNA samples as positive controls, and a non-template (negative control). Each 17 µL reaction consisted of 12.5 µL of 2X Master mix, 1.25 µL of

Table 1. Amplification signal for the three targets SARS-CoV-2 genes, demonstrating the presence of omicron in clinically diagnosed samples.

S-gene TF	S-gene MA	RdRp	RNase P	Interpretation of results
(-)	(+)	(+/-)	(+/-)	Omicron present; SARS-CoV-2 detected
(+)	(-)	(+/-)	(+/-)	Omicron absent; SARS-CoV-2 detected
(+)	(+)	(+)	(+/-)	Omicron present; SARS-CoV-2 detected

primer probe mix [spike gene-target failure (S-Gene TF) 5′-FAM, spike gene-mutation amplification (S-Gene MA) 5′- HEX, *RdRp*- CY5 (positive control), and internal control (*RNase P*)-Texas Red] and 3.25 µL of water as needed; 8 µL of isolated RNA was added in the final reaction mix. The cycling profile PCR was performed on an automated system that involved reverse transcription at 50 °C for 15 min, initial activation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 sec and 58 °C for 30 sec. AT combined annealing-extension steps fluorescence was measured, and the data were analyzed using the Bio-Rad CFX Maestro program (supplied with the thermocycler) [BiORAD, Hercules, USA]. The results were analyzed and interpreted in accordance with the manufacturer's guidelines (Table 1)

Results

During the third wave of COVID-19 in India, there was a sudden surge of SARS-CoV-2 infections. We analyzed the presence of Omicron in the region through in vitro diagnostic real-time RT-PCR. A total of 200 clinically diagnosed COVID-19 samples from the third wave were included in this study. The sociodemographic data of the patients included in this study are given in Table 2. A total 121 males and 79 females, across all age groups, were included. In vitro diagnostic RT-qPCR results showed that no signal of SG-TF (S gene drop out or target failure) was detected in the third wave samples. The SG-MA (S gene mutation amplification) signal, on the other hand, was detected in all cases, with mean CT values of 25.3, indicating the presence of Omicron in the target samples. The signals of *RNase P* and *RdRp* (RNA-dependent RNA polymerase) genes were detected in all tested samples, which showed that the clinical samples were valid and positive for SARS-CoV-2 agonist. The mean CT value for *RdRp* and *RNase P* were 24.5 and 28.0, respectively (Figure 1A, B).

Overall, our finding confirmed that Omicron was detected in all 200 samples, which showed the prevalence of the dominant Omicron variant in India during the third wave (Figure 1C). Omicron mutations, which indicate the presence of the Omicron variant in a specific tested sample, can be detected using S-Gene mutation amplification (SG-MA). *RdRp* signal allowed the detection of SARS-CoV-2 virus in a sample regardless of variants. The *RNase P* signal indicated the presence of human swabs in clinical specimen.

During the second wave, the Delta and Delta+ variants were the dominant strains in India. A total of 200 clinically diagnosed COVID-19 samples from the second wave were included for a comparative study. A total of 121 males and 79 females, across all age groups, were included in this study. The results showed that the SG-TF signal was detected in all the second wave samples. The mean CT value for SG-TF was 25.0. In contrast, no SG-MA signal was reported, indicating that the Omicron was not present during the second wave. The signals of *RNase P* and *RdRp* genes were detected in all tested samples with mean CT values of 24.0 and 29.0, which showed that the clinical samples were valid and positive for SARS-CoV-2 agonist of variants (Figure 2).

Table 2. Sociodemographic characteristics of the patients included from the period of 3rd and 2nd disease waves.

Characteristics	Percentage of SARS-CoV-2 infected patients (COVID-19)	
	3rd wave	2nd wave
Number of patients included (n)	200	200
Gender		
Male	121 (60.50%)	121 (60.50%)
Female	79 (39.50%)	79.0 (39. 5%)
Age		
0 month to 17 years	36 (18.00%)	29 (14.50%)
18 to 35 years	65 (32.50%)	61 (30.50%)
36 to 53 years	56 (28.00%)	58 (29.00%)
54 to 71 years	34 (17.3%)	41 (20.50%)
72- 89 years	9.0 (4.50%)	11 (5. 50%)
Occupation		
Business/Farming/Professional	149 (74.5%)	157 (78.5) %
Others	51 (25. 5) %	43 (21.5) %

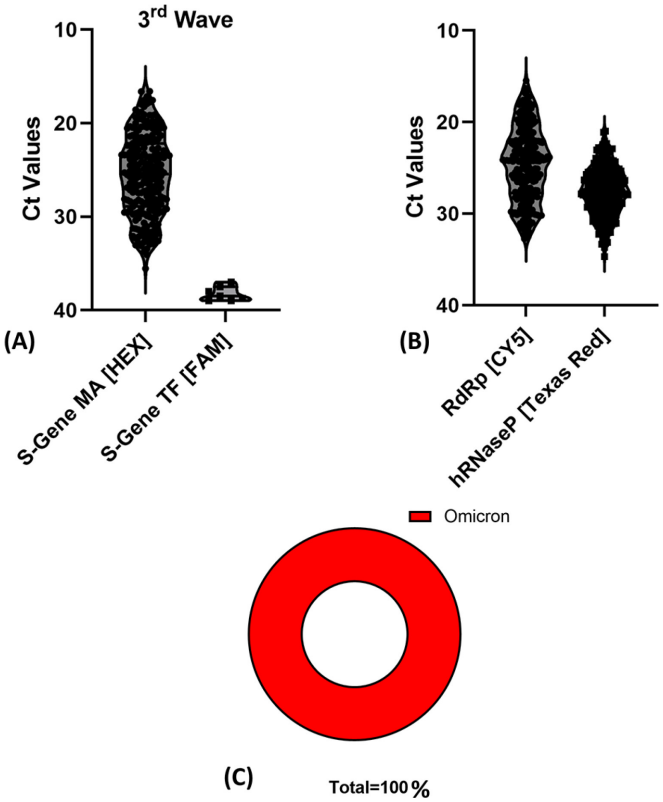
The comparative sociodemographic data of the patients included in the second and third waves clearly indicated that the age group 18-53 years was most affected, accounting for 60.94% of the samples during the third wave and 59.33% of the samples during the second wave. When analysing the incidence rate for the age group 0-17 years, an interesting observation was made during the third wave. The 0 to 17-year-old age groups were comparatively more affected, accounting for 17.36% of the total samples for the third wave, compared to 14.52 % for the second wave (Table 2).

Discussion

The WHO categorizes the VOC of SARS-CoV-2 based on transmission, hospitalisation, and mortality. Among all the reported VOC, the Omicron (B.1.1.529) variant was the most transmissible, contributing to new COVID-19 waves in many countries [15]. Sub-lineages of Omicron have evolved and spread rapidly over the last six months. The infections caused by the Omicron sub-lineage is still causing widespread panic due to breakthrough infections and COVID-19 waves [16,17]. The most effective way of keeping track of SARS-CoV-2 variations during a pandemic is whole genome sequencing (WGS). However, limited sequencing facilities make it challenging to use WGS to track every infection during a pandemic. In vitro RT-qPCR offers a high throughput method for rapid surveillance of VOC, allowing for better management of infection control and prompt treatment. In this study, we describe the use of in vitro diagnostic RT-qPCR for the rapid identification of Omicron, a highly infectious VOC of SARS-CoV-2.

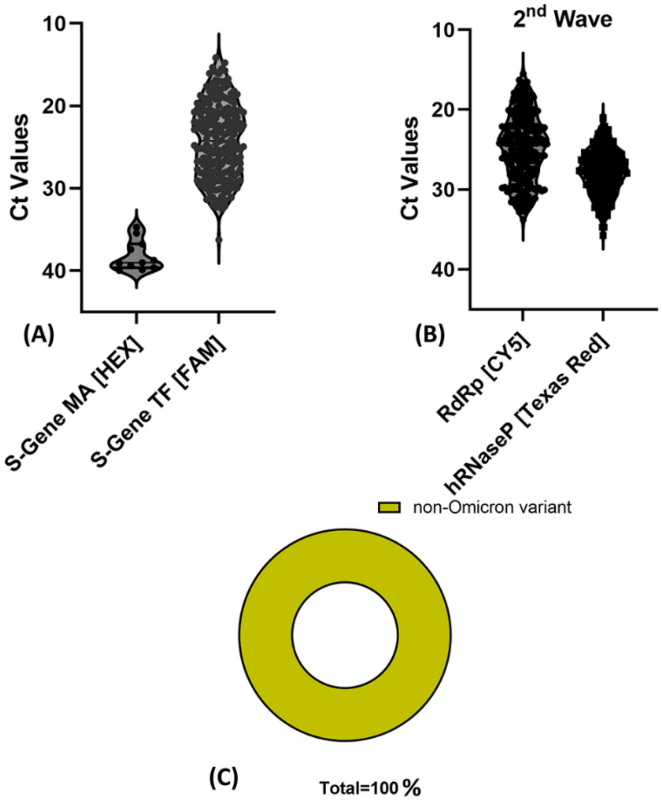
Genome sequencing of the Omicron variant shows numerous mutations, including deletions and

Figure 1. The SG-TF (S gene drop out or target failure), SG-MA (S gene mutation amplification), RdRp (RNA-dependent RNA polymerase), and RNaseP (Ribonuclease P) amplification signals from the samples of the third wave.



A: Amplification signals (Ct values) of SG-TF and SG-MA, which showed that the signal of SG-TF was not detected, while amplification of SG-MA was observed. **B:** RdRp amplification signal demonstrating the infection agonist of VOCs. RNase P amplification as an internal control, which shows valid sampling. (C) Prevalence of Omicron in tested samples; all samples tested positive for Omicron variant during the third wave. Ct values ≤ 32 was taken for demonstrations, signal considered negative for the value ≥ 36.

Figure 2. Amplification signals of samples of the 2nd wave.



A: Amplification signals (Ct values) of SG-TF (S gene drop out or target failure) and SG-MA (S gene mutation amplification), which showed that the signal of SG-TF was recorded in each tested samples, while amplification of SG-MA was not detected. **B:** RdRp (RNA-dependent RNA polymerase) amplification signal demonstrating the infection agonist of VOCs. RNase P amplification as an internal control, which shows valid sampling. **C:** Prevalence of non-Omicron in tested samples; all samples tested negative for the Omicron variant during 2nd wave. Ct values ≤ 32 was taken for demonstrations, signal considered negative for the value ≥ 36.

substitutions. These variants can be recognised by the proxy marker of S-gene target failure due to the deletion in spike, and a specific mutation that enables the identification of the definite signal for a particular VOC [18-22]. Biomarker S gene target failure in SARS-CoV-2 testing was used in several studies to distinguish the VOC from non-VOC [18,19]. The two targets method offer a better sensitivity for the detection of VOC in a population. A specific region of the S gene drop-out or target failure (SG-TF) is absent in Omicron (including its sub-lineages BA.1 and BA.2) and our results showed a negative signal in the FAM channel, indicating Omicron. In contrast, this region is present in all other variants, such as Alpha, Beta, Gamma, or Delta, and resulted in a positive signal in the FAM channel with the 2nd wave human swab samples. Our findings are consistent with the study of Bal *et al*. They used the TaqPath RT-PCR COVID-19 kit (Thermo Fisher, Massachusetts, US) which has three target probes, including SG-TF, to determine the transmissibility of a deletion mutant (H69/V70) [18]. In order to explore the trend of VOC frequency over time, Volz *et al*. employed SG-TF data to distinguish between VOC and non-VOC [19]. Similarly, a specific spike gene mutation was also included in various studies to determine the presence of VOC in a target population [20-22]. Our study corroborated that the Omicron was the prevalent strain during the third wave, as shown by the signal unique to an S-gene mutation during the third wave and an undetectable signal during the second wave. Overall, this study clearly showed that the Omicron variant was the cause of the third wave, and all tested cases were found to be infected with Omicron. Omicron cases were not detected during the 2nd wave. The comparative second and third wave study clearly demonstrate that the current strategy can be employed to rapidly determine the prevalence of Omicron in the target community.

However, the current study also has some limitations. This strategy cannot differentiate the different sub-lineages of Omicron that have evolved over time globally.

Sociodemographic data collected during the period of study clearly indicated that the younger age groups were comparatively highly affected during the third wave. The data, however, can be validated further by employing a larger sample size and considering different age groups.

Conclusions

It is difficult to sequence all infected samples during COVID-19 waves. According to the findings of this study, in vitro diagnostic RT-qPCR can quickly determine the prevalence of VOC. The SG-TF and SG-MF signals were used to distinguish Omicron from other VOC using in vitro diagnostic RT-qPCR. Our results clearly demonstrated that the Omicron strain was the prevalent strain during the third wave. This diagnostic method enables rapid retrospective and prospective assessments of Omicron prevalence, even in nations with subpar sequencing infrastructure. This study demonstrated that in vitro real time PCR detection of VOC offers rapid results at or near the time of diagnosis assisting in better patient care. This study also highlights the use or development of in vitro diagnostic RT-qPCR methods to detect the presence of prevalent VOC for countries having limited sequence facility.

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Authors' Contributions

SuK, SH and ST: performed the experiments, Ark: designed and performed the experiment, and wrote the manuscript, SK: devised the project, scientific suggestions, and conceived this study.

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Corresponding author

Arbind Kumar, PhD.
COVID-19 Testing facility, CSIR-Institute of Himalayan Bioresource Technology (IHBT),
Palampur, India.
Tel: +91 9417235692
Email: arbind.tripathi01@gmail.com

Sanjay Kumar, PhD.
CSIR-Institute of Himalayan Bioresource Technology (IHBT),
Palampur, India.
Tel: +91-1894-230411
Fax: +91-1894-230433
Email: sanjaykumar@ihbt.res.in

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