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To Analyze the Sensitivity of RT-PCR Assays Employing S Gene Target Failure with Whole Genome Sequencing Data during Third Wave by SARS-CoV-2 Omicron Variant

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Abstract

Introduction: Omicron is a highly divergent variant of concern (VOCs) of a severe acute respiratory syndrome SARS-CoV-2. It carries a high number of mutations in its spike protein hence; it is more transmissible in the community by immune evasion mechanisms. Due to mutation within S gene, most Omicron variants have reported S gene target failure (SGTF) with some commercially available PCR kits. Such diagnostic features can be used as markers to screen Omicron. However, Whole Genome Sequencing (WGS) is the only gold standard approach to confirm novel microorganisms at genetically level as similar mutations can also be found in other variants that are circulating at low frequencies worldwide. This Retrospective study is aimed to assess RT-PCR sensitivity in the detection of S gene target failure in comparison with whole genome sequencing to detect variants of Omicron. **Methods:** We have analysed retrospective data of SARS-CoV-2 positive RT-PCR samples for S gene target failure (SGTF) with TaqPath COVID-19 RT-PCR Combo Kit (ThermoFisher) and combined with sequencing technologies to study the emerged pattern of SARS-CoV-2 variants during third wave at the tertiary care centre, Surat. **Results:** From the first day of December 2021 till the end of February 2022, a total of 321,803 diagnostic RT-PCR tests for SARS-CoV-2 were performed, of which 20,566 positive cases were reported at our tertiary care centre with an average cumulative positivity of 6.39% over a period of three months. In the month of December 21 samples characterized by the SGTF (70/129) were suggestive of being infected by the Omicron variant and identified as Omicron (B.1.1.529 lineage) when sequence. In the month of January, we analysed a subset of samples (n = 618) with SGTF (24%) and

without SGTF (76%) with Ct values < 25 for N, ORF 1ab and S gene with sequencing. We found other sublineages namely BA.1, BA.1.1, and BA.2 as a result over a period of time. Finally in the month of February-22 BA.2 lineage emerged and BA.2 was the most predominant and found to be increasing each week. It was clearly observed that Omicron has spread in the community and within two months the proportion of pre-existing Delta variants became zero. **Conclusions:** During the COVID-19 pandemic, it took almost more than 15 days to diagnose infection and identify pathogen by sequencing technology. In contrast to that molecular assay provided quick identification with the help of SGTF phenomenon within 5 hours of duration. This strategy helps scientists and health policymakers for the quick isolation and identification of clusters. That ultimately results in a decreased transmission of pathogen among the community.

Keywords

SARS-CoV-2, S Gene Target Failure, Whole Genome Sequencing, Omicron

1. Introduction

A highly mutated and infectious Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV) has become the global pandemic of the 21st century. Since the emergence of SARS-CoV, from December 2019 to till date, India has experienced three waves of the Coronavirus disease (COVID-19) caused by different variants. During this, the World Health Organization (WHO) has designated a total of five variants of SARS-CoV-2 as variants of concern (VOC) *i.e.* Alpha, Beta, Gamma, Delta, and Omicron [1]. At the beginning of the pandemic, detection of the virus using a PCR-based technique and clinical correlation was the only relevant and optimal method for diagnosing a coronavirus infection. Such diagnostic techniques can be cost and time-saving to monitor the emergence of new variants compared to sequencing technology [2]. However, their sensitivity is not always sufficient to detect viruses in low viral load biological samples. Whatever the reason may be, this pandemic has made known that due to deficient molecular surveillance, we were unable to identify and monitor the viral pathogen spread among the community [3].

In India first two cases of the Omicron (B1.1.1.529) were reported on 3rd December 2021 among international travelers [1]. This variant is highly contagious and infectious therefore, it had been found in more than 29 countries across the globe before being detected in India [4]. The characteristic feature of Omicron is mutation in the S-spike protein; few out of them overlap with the Alpha, Beta, and Delta VOCs. Since the discovery of Omicron or the Pango lineage B.1.1.529, this VOC has been split into four sub-lineages namely BA.1, BA.1.1, BA.2, and BA.3. As per the literature survey Omicron BA.2 isolates does not contain the 69 - 70 del mutation in the S protein; in contradictory to that BA.1 and BA.3 iso-

lates, contain the del 69/70 mutation. The presence or absence of del 69/70 affects the final outcome of S gene amplification during the Polymerase Chain Reaction (PCR). The commercially available TaqPath COVID-19 diagnostic tests by Applied Biosystems are capable of differentiating Omicron variants [3]. This kit helps to detect SARS-CoV-2 infections by identifying the presence of screening and/or confirmatory genes *i.e.* S, N and ORF a/b of the virus. Omicron variants having BA.2 lineages are showing positive amplification for the N and ORF 1a/b genes while negative for the S gene target. Therefore, S Gene Target Failure (SGTF) can be used as a proxy marker to identify the Omicron variants. On the other hand, Omicron BA.1 and BA.3 variants without spike protein mutation, are able to amplify all three target genes of the virus [5]. However, whole genome sequencing is the only gold standard approach to finding point mutation as; such mutation can be found in other circulating VOCs.

Sequencing technology remains the ideal tool for surveillance, detection and confirmation of novel SARS-CoV-2 VOCs, although many laboratories in India are unable to effectively implement this tool due to lack of resources, facilities, or expertise. Due to this, it took a lot of time to generate and release sequencing data that ultimately affected the quality of surveillance all over India during the pandemic. In this study, we have analyzed the data combining RT-PCR and sequencing technologies for the rapid identification of infectious COVID-19 VOCs, during the third wave in India. As a result, it highlights the effectiveness and significance of rapid molecular assay, especially in settings where sequencing facilities may not be readily available.

2. Material and Method

2.1. Study

The study cohort includes people of all age groups for whom upper respiratory tract samples were received for SARS-CoV-2 diagnosis, at the New Civil Hospital, Surat from December 2021 to February 2022. This includes samples collected from indoor patients, outdoor patients, and community surveillance.

2.2. Study Samples

The majority of respiratory samples received included nasopharyngeal and/or oropharyngeal swabs in commercially available Viral Transport Medium (VTM) along with specimen referral forms.

2.3. Total Nucleic Acid Extractions

Total nucleic acids extraction was extracted from 200 µl of respiratory specimens using the Thermo Scientific KingFisher Flex automated extraction coupled with MagMAX™ Viral/Pathogen II (MVP II) nucleic acid isolation kit. Total nucleic acids extraction was also performed on the Himedia Insta NX® Mag96 automated extraction system using the HiPurA® Viral RNA automated extraction combi kit, as per manufacturer's instructions.

2.4. SARS-CoV-2 Diagnostics

Multiplex real-time reverse transcription-based PCR assays were performed on extracted nucleic acids, according to the manufacturers' instructions. The assays performed included: 1) All the samples were subjected to RT-PCR analysis performed using the meril COVID-19 one-step RT-PCR kit (Meril Diagnosis) which targets the conserved sequence encoding the ORF 1ab gene and the the nucleocapsid (N) gene, was run on the Insta Q96® Plus real-time platform (Himedia) according to the manufacturers' instructions. 2) Random positive samples with Cycle Threshold (Ct) value of <25 from test a) RT-PCR were subjected to detect spike (S) gene target failure using the TaqPath COVID-19 combo assay kit (Thermo Fisher Scientific). Targets N, S, and open-reading frame 1ab (ORF 1ab) gene were detected using the QuantStudio™ 5 real-time platform (Thermo Fisher Scientific). Samples with a Cycle Threshold (Ct) value of <25 in RT-PCR were alone selected and sent for sequencing studies.

2.5. SARS-CoV-2 Whole Genome Sequencing (WGS)

During the time of the COVID pandemic, Surat a city in Gujarat did not have a whole genome sequencing (WGS) facility in place. Therefore, only a fraction of Samples with Ct < 25 were randomly selected on a weekly basis for sequencing on an ad-hoc basis at the Gujarat Biotechnology Research Centre (GBRC), as a part of a national genomic surveillance program.

2.6. Data Analysis

Data was analyzed using Microsoft excel.

2.7. Ethical Statement

Ethical approval is obtained for this study from Institute Ethics Committee.

3. Result

From the first day of December 2021 through the end of February 2022, a total of 321,803 diagnostic tests for SARS-CoV-2 were performed, of which 20,566 positive cases were reported at our tertiary care centre with an average cumulative positivity of 6.39% over a period of three months (Table 1). Among them, 59.72% were females and the highest positivity 23.76% was seen among the age group of 20 - 30 years. Our study found that the majority of COVID positive patients were asymptomatic 92.18% during the third wave of the COVID-19 pandemic (Table 1).

During the study period, the third wave was found at its highest peak from 05th January 2022 until 20th January 2022 and started to decline from 25th February onwards. The number of samples received and tested declined during peak of the study was indicative of weekends and public holidays (Figure 1). The detection rate of SARS-CoV-2 was below 1% during December 2021, and by the first 10 days of January, the detection rate increased steadily up to 22.3%. At the

Table 1. Clinical characteristics of SARS-CoV-2 variant during third wave at our tertiary care centre.

Sr. No	Parameters		Percentage %
1	Time Period Of COVID-19 Pandemic	Dec-21 to Feb-22	
2	Total number Of tests done	321,803	
3	Total Positive	20,566	6.39%
	Total Negative	301,227	93.60%
	Gender (n = 20,566)		
4	Male	8284	40.28%
	Female	12,282	59.72%
	Age (n = 20,566)		
5	0 - 10	674	3.28%
	10 - 20	3105	15.10%
	20 - 30	4886	23.76%
	30 - 40	3563	17.32%
	40 - 50	2659	12.935
	50 - 60	2088	10.15%
	>60	1684	8.19%
	Symptoms (n = 20,566)		
6	Symptomatic	1609	7.82%
	Asymptomatic	18,957	92.18%

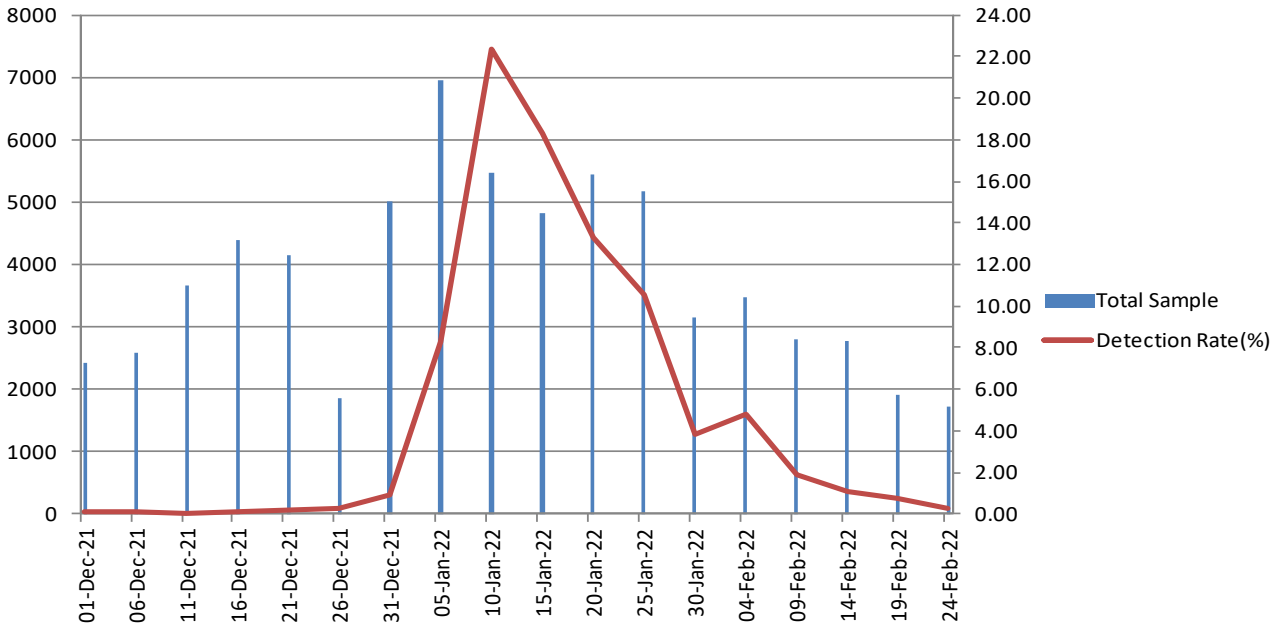

Figure 1. Detection rate of SARS-CoV-2 during the third wave at tertiary care centre, South Gujarat, India. The vertical line in graph represents the total number of samples received for SARS-CoV-2 diagnostics. The red line in graph represents the detection rate (number of positive tests/number of samples received per day).

beginning of February 2022 detection rate gradually started to decrease and it came around 0.25% at the end of February 2022 (Figure 1). As per the Guidelines by the Government of India, around 2% of all tested positive PCR samples were investigated to assess S gene target failure (SGTF) and the same was sent for Genome sequence at GRBC, Gandhinagar to have a complete picture of the circulation of variants in a particular territory. Priority was given to certain groups such as hospitalized patients and international travelers for sample selection criteria. Overall, we have ensured that viral load among the selected samples was correctly detected without loss of performance, by the correct amplification of N and ORF 1ab genes (ct < 25) by Meril COVID-19 one-step RT-PCR kit. In the month of December-21 samples characterized by the SGTF (70/129) were suggestive of being infected by the Omicron variant and identified as Omicron (B.1.1.529 lineage) when sequence (Table 2). On the other hand Delta variant (B.A.617.2) was also found in 12 samples out of 59 negative SGTF samples in the same month. In our study Omicron, a VOC has been found split into four sub lineages namely BA.1, BA.1.1, BA.2, and BA.3 over period. Omicron BA.1 and BA.3 isolates harbor the del69/70 mutation in the S protein as contradictory to BA.2. The del69/70 affects the amplification of the S gene target during polymerase chain reaction resulting in SGTF. Keeping this fact in mind we have analyzed a subset of samples (n = 618) with SGTF (24%) and without SGTF (76%) with Ct values < 25 for N, ORF 1ab and S gene, were evaluated with sequencing in month of January-22. We found initially the B.1.1.529 lineage among the Omicron cases but later In January-22, BA.1, BA.1.1, and BA.2 emerged and BA.2 was the most predominant and found to be increasing each week. It was clearly observed that Omicron has spread in the community and within two months the proportion of pre-existing Delta variants became zero.

4. Discussion

This retrospective study was conducted at the Viral Research and Diagnostic Laboratory of GMC Surat, a tertiary care institute. The study incorporated, the individuals that were tested at the microbiology department, during the third wave of the pandemic (December 2021 to February 2023) with an average positivity of

Table 2. Dynamics of SARS-CoV-2 variant at virology, GMC, Surat by month from December 2021 to February 2022 by S gene target failure and whole-genome sequencing.

Month	Number of Tests Done for SGTF & Genome Sequencing	Number of Test Positive for SGTF	Found Omicron Variant in Genome Sequencing*				Other Than Omicron
			B.1.1.529	BA.2	BA.1.1	BA.1	B.1.617.2
Dec-21	129	70	8	0	0	0	12
Jan-22	618	148	23	29	2	2	-
Feb-22	51	0	0	36	0	0	-

* Result subjected to sample screening criteria considered by GBRC.

6.39%. In absolute numbers, more females are infected with COVID-19 compared to males, which is contradictory to the findings of the other studies from India where gender is associated with infection rates [4] [6]. The reason for infected female predominance in our study may be due to the role of care obligations where women are the main care providers. In this study, the highest positivity (23.76%) was found among the age group of 20 - 30 years old. The possible reason behind this could be their more active involvement in outdoor activities where they fail to maintain social and individual behavior as during the third wave lockdown was not imposed. In this study, it was observed that a major chunk of patients who were infected with the COVID variant during the third wave were commonly found Asymptomatic (92.18%). A similar observation was reported in a number of studies indicating that the clinical severity of infection is lower for omicron than for the delta variant [7]. The reason behind asymptomatic individuals might be that omicron replicates more in upper airway cells and less in the lungs hence leading to less severe disease [8]. Apart from that many other factors like age, geographic area, immunization coverage, etc can affect the severity of the disease. Since the majority of patients were asymptomatic, it was difficult to trace and track the positive persons which ultimately resulted in widely spread among the community.

A causative agent of the COVID-19 pandemic has been concerned about its emerging mutations. To date, multiple novel strains of SARS-CoV-2 have been found in circulation worldwide. Many out of them share the same deletion of amino acids H69 and V70 in the virus spike protein. Nowadays it is possible to detect such spike gene mutation through commercially available real-time PCR assays for more rapid detection compared to sequencing. Omicron was first observed among the samples received in our laboratory on 9th December, 2021. From December 2021 onwards the number of positive SARS-CoV-2 cases started to double on a daily basis, with the majority of cases being attributed to Omicron. The TaqPath™ COVID-19 assay targets the N, ORF 1ab, and S genes of the virus. Among these, S gene fails to amplify due to the deletion at amino acid position 69/70. These ultimately result in SGTF as described by the World Health Organization [9]. Therefore, such analysis can serve as a marker for early detection of infection caused by emerging variants.

As per our study inclusion criteria, we selected both COVID-19 RT-PCR positive samples identified as SGTF and non-SGTF for WGS. Our tertiary care centre doesn't have a WGS facility therefore, only a small fraction of all new SARS-CoV-2 cases were sequenced on an ad-hoc basis at GBRC, Gandhinagar. Only a few SARS-CoV-2 variants like Alpa and Omicron harbor the mutation and hence, demonstrate SGTF. Therefore, this phenomenon can be applicable to such variants only. In our study majority of the non-SGTF cases identified from December, 2021 to February, 2022 at our laboratories have been subsequently confirmed as omicron BA.2 by GBRC coordinated with WGS sequencing. Contradictory to Omicron BA.2, it was evident that Omicron BA.1 contains the 69/70 deletion which is associated with SGTF [10] [11]. Therefore, similarly majority

of COVID positive samples having SGTF were found omicron BA.1 in our study. There are a few limitations of the study first; the fraction of the sample is much less in comparison to the total positive cases identified during the study period. As a result, data distribution might be affected for which samples had been processed to identify SGTF. Second, among all omicron variants, only the BA.2 variant shows a negative result for SGTF. In contrast to those other variants like BA.1, BA.1.1, and BA.3 show positive. As a result, this study would not be able to differentiate between Omicron BA.1, BA.1.1, and BA.3 by TaqPath COVID-19 combo assay as they share similar mutations. However, this strategy was found useful for patients to be placed immediately in self-quarantine. In addition to that this phenomenon can help scientists and public health policymakers for the rapidly implement effective surveillance and preventive measures. These ultimately resulted in a decreased spread of community transmission.

Our results suggest that molecular assays can provide a quick, accurate, reliable and cost-effective approach to identify pathogen. Although WGS is the gold standard technique to identify newly emerging pathogen but, due to a lack of facility and time-consuming procedure it would not be possible during the pandemic to provide quick identification. We have demonstrated that the identification of SGTF not only provided a time and cost-effective approach but also helped to differentiate the infectious approach. Such an approach can be used to combat infectious communicable diseases in the future.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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