Original Article

COVID-19 pandemic – Cocktail of variants, a study from Northern India

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Abstract

Context: The aim of the study was to identify and monitor the circulating strains of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the samples received at our center and update the existing national and international genomic surveillance data. **Introduction:** SARS-CoV-2 is no exception to the basic nature of the viruses ability to change and evolve. Since its first report in December 2019 from Wuhan, China, multiple variants of the virus have emerged and been reported. Five variants of concern have been recognized and reported by the Centers for Disease Control and Prevention, which are associated with variable degrees of transmissibility and mortality. **Materials and Methods:** Nasopharyngeal and oropharyngeal swabs received in viral transport medium at the Viral Research Diagnostic Laboratory were processed for reverse transcription-polymerase chain reaction for SARS-CoV-2. Whole genome sequencing (WGS) was performed for selective positive samples using Oxford Nanopore sequencing technology, using MinKNOW software for data acquisition. **Statistical Analysis:** The clades were assigned using Nextclade v2.4.1 software. The statistical analysis was calculated using OpenEpi version 3, an open-source calculator, and two by two. **Results:** Variants reported over the study period included Alpha, Kappa, Delta, and Omicron. Delta dominated in the year 2021, while Omicron was the dominant variant in 2022. In both the dominant variants, asymptomatics contributed to around 30–40% of positives. Intensive care unit admissions and mortality were higher in the Delta variant, while vaccination history and travel history were higher in the patients with Omicron variant. **Conclusion:** The trend tracking of these variants has been important in view of public health, enabling early interventions to control the spread of the disease and foresight in preparation for the situation.

Keywords: RT-PCR, SARS-CoV-2, variants

Introduction

Genomic surveillance has been a crucial epidemiological tool all through the coronavirus disease 2019 (COVID-19) pandemic, monitoring the evolving nature of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. The character of this virus to continuously evolve has given multiple variants to humanity.[1] By October 2022, a total of 12 SARS-CoV-2 variants had been identified, and the Centers for Disease Control and Prevention (CDC) had classified them into four classes of variants.[1] Variant being monitored (VBM) – Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2 and AY lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), Mu (B.1.621, B.1.621.1), and Zeta (P. 2); variant of interest (VOI) – presently no variant in this class; variant of concern (VOC) – Omicron and its lineages (B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4, and BA.5); and variant of high consequence (VOHC) – no variant has been identified in this class till now.[1]

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Received: 06-07-2023 **Revised:** 08-12-2023
Accepted: 02-02-2024 **Published:** 14-06-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_1107_23

How to cite this article: Chaudhary P, Gulati N, Gupta V, Garg R, Walia S, Dhawan I, *et al*. COVID-19 pandemic – Cocktail of variants, a study from Northern India. J Family Med Prim Care 2024;13:2449-54.

These variants differ in their characteristics, including transmissibility, infectivity, and severity. The neutralization effect of the available vaccines and their detection by available diagnostic molecular essays also differ.[2] Knowledge of these emerging variants helped the authorities understand national and the local trends of the circulating variants. Further, the awareness of the circulating variant became a guide for the authorities and scientists to understand the changes that are often of concern to tackle and improve public health interventions. Genomics is one of the world's most advanced genetic technologies, which accurately sequences the new variant, and the availability of testing on a global scale has enabled the tracking of the variants on a nearly real-time basis. These genetic lineages were being monitored and shared on a common platform globally on Global Initiative on Sharing All Influenza Data (GISAID). India also took an initiative under the Ministry of Health and Family Welfare (MoHFW) by forming the Indian SARS-CoV-2 Genomics Consortium (INSACOG) in December 2020.[3] Its main aim was to co-ordinate and monitor the genome sequencing of the SARS-CoV-2 virus across the various designated laboratories nationwide to determine the viral dynamics and epidemiological trends in view of public health implications. This study aimed to recognize and report the circulating variants of SARS-CoV-2 at our center. The tracking record of these variants is not only important at the international and national level, but also the local data is equally contributing to monitoring the changes and the emerging variants, especially for the primary care physicians and the family physicians, as they are the frontline workforce in pandemic situations. Guisado-Clavero *et al.*[4] have described the importance of primary care during the COVID-19 pandemic. They should have appropriate knowledge regarding the circulating strain in the specific community, to take adequate measures on time, so that the transmission is decreased and the pandemic is controlled.

Materials and Methods

Viral Research Diagnostic Laboratory (VRDL) at our center is collecting and testing both nasopharyngeal and oropharyngeal swabs for COVID-19 using reverse transcription-polymerase chain reaction (RT-PCR). Both diagnostic and surveillance samples were included from our tertiary care center and various other hospitals in Chandigarh, that is, sector 22, sector 45, and Mani Majra. This is retrospective data collected from April 2021 until July 2022. The samples were received in viral transport medium (VTM) and transported in triple packing, maintaining the cold chain. All the samples were tested for SARS-CoV-2 by RT-PCR on a Bio-Rad CFX96 Real-Time PCR machine using RT-PCR kits and extraction kits approved by the Indian Council of Medical Research (ICMR), Delhi. Target genes were two, among the S gene, E gene, N gene, ORF 1ab gene, or RdRp gene (depending on the kit used), along with the positive and negative controls. Interpretation was done as per the kit literature.

Only selective samples, as per the guidelines of the INSACOG, were sent for genomic sequencing at the epigenetics lab, Council of Scientific and Industrial Research – Institute of Microbial Technology (CSIR-IMTech), Chandigarh. INSACOG is a joint collaboration between MOHFW, the Department of Biotechnology (DBT), CSIR, and ICMR to assess SARS-CoV-2 variants with a network of multiple laboratories across India. The consortium targets establishing the co-relation between genome sequencing data with clinical and epidemiological data of SARS-CoV-2. Target samples for Whole genome sequencing (WGS) as per INSACOG have been international travelers, their primary contacts, community samples for sentinel surveillance, randomly chosen (15%) as population representatives, samples from healthcare facilities of those with severe illness, vaccine breakthrough infections, atypical clinical presentations, and surge surveillance with a sudden increase in positivity in a particular community. The aim has been to guide the health care authorities to identify the super spreader events, community outbreaks, and local trends to break the chain of transmission with appropriate interventions, targeting public health.[3] Samples with a lower cycle threshold value (Ct-value) with a high viral load were preferred for sequencing, which was sent on a weekly basis after ensuring triple packing.

At CSIR-IMTech, the samples were processed for next-generation sequencing by Oxford Nanopore sequencing technology. Ribonucleic acid (RNA) was extracted from the positive samples sent to VTM using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). The eluted RNA was purified using RNA-clean XP beads (Beckman Coulter, USA). The concentration of RNA was measured using the NanoDrop Spectrophotometer (Thermo Scientific, NanoDrop) and Qubit Fluorometer (Thermo Scientific, USA). The WGS was performed using the Midnight RT PCR Expansion Kit (EXP-MRT001) and following the Oxford Nanopore Technologies (ONT) Midnight protocol (midnight2_rev2 version, ONT).

Reverse Transcription: The RNA was reverse transcribed using LunaScript RT SuperMix in a 96-well RT-PCR plate.

PCR: For the amplification of generated viral cDNA, two separate PCR mixes (PCR pool A and PCR pool B) were prepared using midnight primer pool A and midnight primer pool B, respectively, using Q5 HS Master Mix, and the PCR program was carried out as per the manufacturer's instructions.

Addition of rapid barcodes: The contents of wells in PCR pool B were transferred to the corresponding wells in PCR pool A and mixed by pipetting. Unique rapid barcodes were added to the wells containing a mix of PCR pool A and PCR pool B. The PCR plate was sealed, centrifuged, and incubated at 30°C for 2 minutes and then at 80°C for 2 minutes.

Pooling of samples: The contents of all barcoded wells were transferred to a single microcentrifuge tube and purified using AMPure XP Beads. The beads and the sample were mixed on a hula mixer for 10 minutes and the DNA was eluted from the beads in nuclease-free water. The DNA was quantified using

Qubit dsDNA BR Assay (Thermo Scientific, USA). Furthermore, 1 µl of Rapid Adapter F was added to the elute and incubated at room temperature for 10 minutes.

Priming and loading the SpotON Flow Cell: The Sequencing Buffer II, Loading Beads II, Flush Tether, and Flush Buffer were thawed and mixed at room temperature. The priming mix was prepared by mixing 30 µl of flush tether and 1.17 ml of flush buffer through pipetting. The Flow Cell was placed on the GridION and the number of pores was checked. The Flow Cell has two ports: a priming port and a SpotON sample port. The priming port cover was removed, and about 20 µl of buffer from the SpotON Flow Cell was pipetted out to remove bubbles, if any, which can hinder the sequencing process. Then, 800 µl of priming mix was added slowly through the priming port and left for five minutes. The SpotON sample port cover was opened, and an additional 200 µl of priming mix was added to the flow cell via the priming port. The Sequencing Buffer II and Loading Beads II were added into the DNA to get the final library. Up to 1 µg of library was loaded to the flow cell via the SpotON sample port in a dropwise manner. The SpotON sample port cover and the priming port cover were closed and the sequencing process was started.

Data acquisition, basecalling, and clade assignment: MinKNOW software was used for the data acquisition using the PCR DNA sequencing protocol for 48 h. The post processing was done in MinKNOW software using the Nextflow pipeline. The clades were assigned using the Nextclade v2.4.1 software.

A total of 316 samples were sent to CSIR-IMTech Chandigarh for WGS. Among them, 38 samples were rejected because of degraded RNA. Sequencing was done for 278 samples, and mutation results were available for 251 samples, sequencing failed in the other 27 samples. The significance of the gender and symptomatology comparisons was calculated using OpenEpi, version 3, an open-source calculator – two by two.

Results

VRDL at our center had tested 2,39,816 samples for SARS-CoV-2 by RT-PCR, till July 31, 2022. The average positivity rate reported was 6.57%, ranging from 0.2% to 31.9%. Variants were reported according to the Pango Lineage and the World Health Organization (WHO) nomenclature (based on Greek alphabets). Reported variants were Alpha (B.1.1.7), Kappa (B.1.617.1), Delta (B.1.617.2), and Omicron (B.1.1.529, BA.2, and B.1.1) including Delta and Omicron sub-lineages. Eleven samples were reported with the Q677H mutation. Table 1.

The prevalence of these variants varied over the pandemic. During May 2021 to December 2021, Delta and its sub-lineages were the dominant variant, while after that, until July 2022, Omicron and its sub-lineages dominated the reported variants. The pattern of the variants reported on a monthly basis over the pandemic is shown in Figure 1.

The reported variants were further analyzed for all the possible parameters to add to the epidemiological surveillance data. Male predominance was observed in both the Delta (52.5%) and the Omicron (51.8%) variants including their sub-lineages, though the gender difference was not significant (*P* value = 0.99). In our study, most of the positive cases (40.2%) were observed in the age group of 20–40 years of age. Symptomatics were more positive in each group (Delta – 55% and Omicron – 63%), but asymptomatics still contributed to around 30–40% of the positives. In our study, 46.6% of the people infected with Delta and its sub-lineages were either partially or fully vaccinated, while Omicron positives were 84.3% vaccinated, and higher than 90% of them were fully vaccinated. History of travel was also more in the Omicron variant, 15.7%, as compared to only 2.5% in the Delta variant. Severe disease with intensive care unit (ICU) admission and mortality was reported more in the Delta variant. Both ICU admissions and mortality were reported only in non-vaccinated individuals. Table 2.

Discussion

Variants are a group of viruses that share distinctive mutations, and if these multiple mutations accumulate over a period of time in a lineage, these viruses may evolve and result in newer strains.[5] The present pandemic also witnessed multiple variants of SARS-CoV-2 over a period of almost 3 years. The major variants reported from our institute, as per the genomic surveillance results, are Alpha (B.1.1.7),

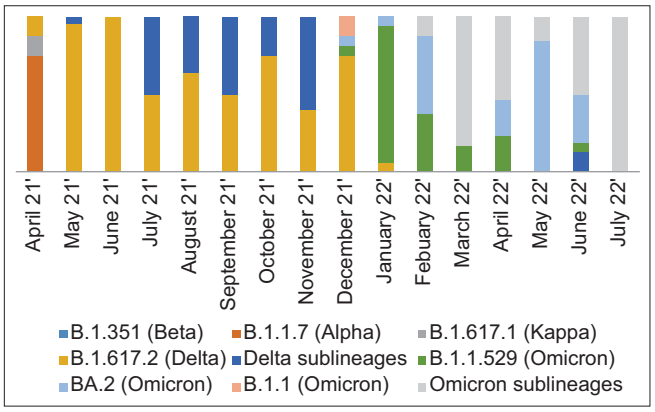

Figure 1: Pattern of the variants reported in each month

Table 1: Number of isolates reported in each variant

B.1.1.7 Alpha	B.1.617.1 Kappa	B.1.617.2 Delta	Delta sub-lineages	B.1.1.529 Omicron	BA.2 Omicron	B.1.1 Omicron	Omicron sub-lineages
12 (4.8)	2 (0.8)	87 (34.7)	31 (12.4)	42 (16.7)	30 (11.9)	2 (0.8)	34 (13.5)

Table 2: Various parameters of the reported variants in detail

	B.1.1.7 Alpha (12) (*n*, %)	B.1.617.1 Kappa (2) (*n*, %)	B.1.617.2 Delta (87) (*n*, %)	Delta sub- lineages (31) (*n*, %)	B.1.1.529, BA.2, B.1.1 Omicron (74) (*n*, %)	Omicron sub-lineages (34) (*n*, %)
Gender						
Males	5 (41.6)	1 (50)	41 (47.1)	21 (67.8)	38 (51.4)	18 (52.9)
Females	7 (58.3)	1 (50)	46 (52.9)	10 (32.2)	36 (48.6)	16 (47.1)
Age						
<10 years	0	0	2 (2.2)	1 (3.2)	3 (4.05)	1 (2.9)
11–20 years	0	0	8 (9.1)	5 (16.1)	3 (4.05)	4 (11.8)
21–30 years	5 (41.6)	0	10 (11.5)	6 (19.4)	19 (25.7)	8 (23.5)
31–40 years	2 (16.6)	1 (50)	22 (25.3)	5 (16.1)	17 (22.9)	6 (17.6)
41–50 years	2 (16.6)	0	16 (18.3)	5 (16.1)	8 (10.8)	5 (14.7)
51–60 years	2 (16.6)	1 (50)	9 (10.3)	4 (12.9)	10 (13.5)	4 (11.7)
>60 years	1 (8.3)	0	20 (22.9)	4 (12.9)	15 (20.3)	6 (17.6)
Asymptomatic	5 (41.6)	0	39 (44.8)	14 (45.2)	29 (39.2)	11 (32.4)
Symptomatic	7 (58.3)	2 (100)	48 (55.2)	17 (54.8)	45 (60.8)	23 (67.6)
Clinical features						
Fever	5	2	25	11	33	18
Cough	5	2	18	8	16	9
Sore throat	3	0	7	2	15	6
Body aches	3	0	2	6	5	5
Breathlessness	1	1	9	4	9	4
Loss of smell	0	0	5	4	1	0
Loss of taste	0	0	3	4	1	0
GI symptoms	0	0	3	3	0	1
Travel	NA	NA	2	1	16	1
Vaccination						
1st	NA NA	NA NA	39 (44.8) 10 (11.4)	16 7 (22.5)	60 (83.3) 2 (2.7)	31 (91.2) 3 (8.8)
2nd	NA	NA	29 (33.3)	9 (29.0)	50 (67.5)	27 (79.4)
>2	NA	NA	0	0	8 (10.8)	1 (2.9)
Severity						
ICU admission	0	0	4 (4.6)	1 (3.2)	0	0
Mortality	0	0	2 (2.3)	1 (3.2)	0	0

Delta (B.1.617.2), and Omicron (B.1.1.529). A few strains of Beta (B.1.351) and Kappa (B.1.617.1) were also reported. The time of isolation of these major variants coincides with the variants reported from other parts of India as well.[6] The first wave subsided in September 2020, with Alpha as the dominant variant. The second wave in India started from May 2021; the dominant variant was Delta (B.1.617.2) and its sub-lineages, our study also had similar results.[7] The third wave started in December 2021 with Omicron as the dominant variant. Omicron and its sub-lineages were reported from our institute also, from January 2022 until July 2022 (until data were reported). Presently, Omicron is the dominant variant reported throughout India, with new sub-lineages, BQ.1 and XBB being the most recent.[8] In our study, male dominance was observed in both Delta and Omicron variants. Kahn *et al.*[9] have reported female dominance in both the variants while, on the other hand Zirpe *et al.*[10] and Domingo *et al.*[11] has reported more cases among males and also higher mortality among males. No possible explanation has been given in the literature for the gender difference. The maximum number of cases were reported in the age group of 20–40 years in both variants. Higher mortality in the elderly group is reported in the literature.[9,10,12]

The Delta variant has three sub-lineages (B.1.617.1, B.1.617.2, and B.1.617.3) and was the major variant reported in the second wave in India, with variations among the sub-lineages.[1] From our institute, 98% of the strains were B.1.617.2, and B.1.617.1 was reported in only 1.6% of strains, while B.1.617.3 was not reported at all. Delta and its sub-lineages had approximately 45% of asymptomatics and 55% of symptomatics; though the percentage of symptomatics was higher, the difference was not significant (*P*- value = 0.23). The reporting of asymptomatics is equally important, as their role in transmitting the infection and fueling the pandemic is already established in the literature.[13,14] The Delta variant was first reported in October 2020 from Maharashtra (India) and was known to harbor two major mutations, E484Q and L452R, in the receptor binding domain (RBD) region of spike protein.[5] These mutations increase the affinity for angiotensin converting enzyme 2 (ACE2) receptors, increase the transmission capacity of the virus, and decrease the binding capability to selective monoclonal antibodies aiding immune escape and further increasing the virulence. India started its vaccination drive in January 2021. By the time, second wave started in April 2021, only partial vaccination (only one dose) was done and that too in the high-risk groups.[5] This was one of the contributing factors to the severity of the second

wave in India. Delta and its sub-linages were both reported in the vaccinated population, though the mortality was not reported in this group in our study. Literature on the dynamics of vaccination in India has also reported decreased hospital admissions and mortality among vaccinated individuals.[7,15]

Omicron, is presently the only VOC as per CDC.[1] First reported in November 2021, it subsequently spread rapidly across the globe. Omicron was known for its unusual constellations of mutations, and these mutations resulted in its increased affinity for ACE2 receptors, increased its capability for immune invasion and its transmissibility also.[16] This variant particularly had the capacity to escape from vaccine-induced protection as well as, from post-infection-induced immunity though the disease was reported to be less severe.[6,9,17] We also reported Omicron and its sub-lineages in the vaccinated population, approximately 90%, reflecting the vaccine escape. The history of travel was reported more in the Omicron variant; this might be attributed to the fact that the Delta variant originated from India while the Omicron was from South Africa.

In all the variants, fever was the predominant symptom followed by cough. No specific symptom predominance was reported in any particular variant, though the loss of smell and taste was reported more in the delta variant. Comparing the severity among both the major variants of the second and third wave, that is, Delta and Omicron, ICU admission and mortality at our institute were reported only in the Delta variant. Omicron was far more transmissible than any other SARS-CoV-2 variant, as more clustering of positive cases was observed by many authors. Though the mortality was lower as compared to the Delta, the variant is still of concern as people are suffering from severe disease.[17-20]

Knowledge regarding all the emerging and reported variants is not only of epidemiological importance but is also a guiding tool for physicians. The majority of healthcare providers in India are primary care physicians and family physicians; therefore, their responsibility increases in a pandemic situation. Apart from managing the patient, they have a major role in counseling the patients, advising appropriate precautions and basic measures, further facilitating early diagnosis, thereby decreasing transmission, and controlling pandemic situation. Measures taken appropriately at the primary level further reduce the burden on the tertiary centers, enabling them to focus on critical patients, thereby performing triage.

Inversely, during the pandemic, in view of serious health complications, patients did not choose primary physicians, adding to the tertiary burden.[21] Recognizing this issue, authorities took all the possible measures to equip the primary care health system for early diagnosis. RT-PCR machines were provided where trained technicians were available; others were provided with the TruNat system; and at many centers, the cartridge-based nucleic acid amplification test (CBNAAT) for tuberculosis was upgraded for the COVID-19 diagnosis. This pandemic has solely provided a different perspective to the primary care physician, enabling them to handle crisis like outbreaks, natural disasters, epidemics etc.

The limitations of the study are that the WGS was not performed on all the positive samples. Though the WGS results in our study cannot be generalized to the whole population in view of age, gender, and vaccination status, with limited resources the information is sufficient for the authorities to act in the public interest.

Throughout the pandemic, humanity has faced a number of COVID variants, starting with the original Wuhan strain in December 2019. In the next year, Alpha, Beta, and Gamma were detected and designated as variants of concern in December 2020. The next and deadliest variant was Delta, the first case detected in India in October 2020. Subsequently, it became the most common variant to be reported in April 2021 and was declared a VOC in June 2021.[1] In our study, it was reported to be the dominant variant from May 2021 until December 2021. The Delta variant was followed by the dominance of Omicron variant, which was first reported in November 2021. Omicron and its sub-variants were dominant from January 2022 until the date of our study. A major concern related to the emergence of new variants is the efficacy of the available vaccines against them. Breakthrough infections in fully vaccinated individuals are also a matter of concern. Foreseeing that the virus will still be going around the world, changing and evolving, we need to keep track of these evolving variants and, subsequently, their potential for infection. Genome sequencing is a genetic tool used to decipher the genetic code of the microorganism's emergence of new variants with their altered behavior. The capacity of the reference laboratories to perform WGS needs to be increased, thus enabling early interventions to prevent the spread of emerging new strains of SARS-CoV-2 viruses at the earliest in regard to control measures.

Acknowledgement

We are thankful to the medical ethical committee and the administration of Government Medical College and Hospital Sec-32, Chandigarh, for providing us with the facilities and support for the testing of samples.

Abbreviations

Abbreviation	Definition
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
CDC	Centers for Disease Control and Prevention
VRDL	Viral Research Diagnostic Laboratory
VTM	Viral transport medium
RT-PCR	Reverse transcription-polymerase chain reaction
ICU	Intensive care unit
COVID-19	Coronavirus disease 2019
VBM	Variant being monitored
VOC	Variant of concern
VOI	Variant of interest
VOHC	Variant of high consequence
GISAID	Global Initiative on Sharing All Influenza Data
MoHFW	Ministry of Health and Family Welfare

INSACOG	Indian SARS-CoV-2 Genomics Consortium
ICMR	Indian Council of Medical Research
CSIR-IMTech	Council of Scientific & Industrial Research – Institute of Microbial Technology
DBT	Department of Biotechnology
WGS	Whole genome sequencing
RNA	Ribonucleic acid
RBD	Receptor binding domain
ACE2	Angiotensin converting enzyme 2

Financial support and sponsorship

WGS testing was done at IMTech Chandigarh, funded by the CSIR core fund.

Conflicts of interest

There are no conflicts of interest.

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