Original Article

Genomic epidemiology of severe acute respiratory syndrome coronavirus 2 from Theni, Tamil Nadu

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Abstract

Introduction: The coronavirus disease 2019 (COVID-19) is a viral infection characterized by respiratory and gastrointestinal symptoms. The causative agent of this infection is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The genomic study helps in understanding the pathogenesis, epidemiology, and the development of therapeutic and preventive strategies in the combat against COVID-19. **Materials and Methods:** Nasopharyngeal and oropharyngeal swab samples were collected from asymptomatic and symptomatic patients during the time period of 2021–2022 for the detection of SARS-CoV-2 by employing real-time reverse transcriptase, cDNA synthesis, whole-genome sequencing by next-genome sequencing, analysis of SARS-CoV-2 sequence data and lineage and variant of concern assignment along with phylogenetic analysis. **Results:** Lineages BA.2.10 and BA.4.1.1 clustered with genomes from Senegal suggested the spread of infections. Similarly, high clustering among delta samples during the second wave showed possible importation and subsequent spread via local transmission. **Conclusions:** Studies like these are important to understand the characteristics and origins of locally circulating SARS-CoV-2 diversity in order to prevent further spread.

Keywords: cDNA synthesis, coronavirus, COVID-19, RT-PCR, SARS-CoV-2, whole-genome sequencing

Introduction

The coronavirus disease 2019 (COVID-19) is an infection with viral etiology and is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or nCoV). The SARS-CoV-2 is an RNA virus comprising positive-sense single-stranded RNA whose genome is approximately 27–32 kb in size. A wide range of hosts, such as humans, other mammals, and birds are susceptible to this infection. The infections are often displayed

in various clinical courses that are anywhere in the range of asymptomatic to severe.[1,2] The emergence of this virus was first recorded in Wuhan, China, in December 2019 and since then has spread throughout the world with an urgent need to explore the impact of the virus and its genome. This type of study can help in understanding the pathogenesis, epidemiology, and the development of therapeutic and preventive strategies in the combat against COVID-19. The SARS-CoV-2 has so far undergone rapid mutation and recombination with the other existing coronavirus in the body and possesses the ability to induce alterations in the tissue tropism, crossing the species barrier and can also now adapt to various epidemiological situations. Due to the high prevalence and wide distribution of coronaviruses among the reservoirs, there is an extensive

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and frequent recombination of their genomes contributed by increased human–animal interface activities and frequent cross-species infections. This paved for the way emergence of novel coronaviruses.[3]

Pathogenesis is the established next step in the infection which consists of the cell's protease TMPRSS2 cleaving and exposing the spike protein of the virus by exhibiting a fusion peptide.[4] The virus releases the RNA into the cell and this forces the cell to undergo the production of copies of the virus, thereby establishing a successful infection.[5]

Minimal numbers of the SARS-CoV-2 genome sequences sufficient for constructing of commonly used molecular tests were developed.[6] However, understanding the transmission patterns of the virus is highly essential, so the need for whole-genome studies arose. The changes in the nucleotide sequence of the virus impact the epidemiology of the outbreak and the clinical picture and severity of COVID-19.[7] On this account, the WHO has established the variant under monitoring (VUM), variant of interest (VOI), and variant of concern (VOC) for further surveillance and analysis. Both the VOI and VOC are characterized as risks to global public health, with the VOC additionally having either increased transmission or due to the causing of significant impact in the COVID-19 epidemiology.[8] The genetic alterations undergone by the virus affect the results of the routine molecular tests that are performed to the detection of the presence of SARS-CoV-2 RNA in the clinical samples. Even after the initial genetic pathogen identification, the importance of ongoing whole-genome sequencing (WGS)-based epidemiologic surveillance over the course of pandemics is critical for predicting the associated risks (pneumonia, breathlessness, bronchitis, etc.) and impact. The emergence in the cases infected by SARS-CoV-2 in Theni, Tamil Nadu, was reported in late February through early March 2020; multiple migrations of SARS-CoV-2 cases into Theni, Tamil Nadu, from countries worldwide initiated a rapid outbreak across the region. In this study, we have sequenced 48 SARS-CoV-2 complete genomes from imported and early circulating samples. The epidemiological data, including the country of importation, district of residence, and additional Theni-based sequences from the same time frame, were uploaded in a global initiative on sharing all influenza data (GISAID) and the National Center for Biotechnology Information. Thorough investigations on the mutation patterns to characterize the origins of viral evolution and spread patterns of SARS-CoV-2 in Theni, Tamil Nadu, were carried out in the study.

Subjects and Methods

Study design
This was a retrospective study.

Study population
A total of 48 samples were collected from COVID-19 patients in the period from 2021 to 2022 admitted to the isolation ward, Government Theni Medical College, Theni, Tamil Nadu.

Inclusion criteria
- Patients reported to Government Theni Medical College.
- Laboratory-confirmed cases of COVID-19.
- Cases of COVID-19 in ICU.

Exclusion criteria
- Severe cases of viral coinfection were admitted to ICU.

Clinical specimens
Nasopharyngeal and oropharyngeal swab samples were collected from asymptomatic and symptomatic patients to detect SARS-CoV-2 using real-time reverse transcriptase (RT-PCR). After confirmation, VTM (viral transport medium) was stored at −80°C until use of vaccination. All samples were collected after obtaining written informed consent from the patients. This study was approved by the Institutional Human Ethics Committee (Ref. No. 1515/MEII/21 dated 02.28.2022).

RNA extraction
RNA was extracted from clinical samples with a QIAamp viral RNA mini kit (QIAGEN) and HiPurA™ Viral RNA Purification Kit (HiMedia Laboratories) in according to the manufacturer's instructions. All specimens were handled in a biosafety cabinet in accordance with the laboratory biosafety guidelines of ICMR and WHO.[9]

Real-time RT-PCR
The assay was performed according to the manufacturer's instructions.[10] The nucleic acid was eluted into 50 μL of elution. Master Mix was prepared containing TaqPath 1-Step Multiplex Master Mix (No ROX™), COVID-19 real-time PCR assay multiplex, and nuclease-free water. About 20 μL of Master Mix was dispensed into wells in a 96-well plate followed by adding 5 μL of eluted specimen to the appropriate well. Each run also included a SARS-CoV-2 positive control and a negative control. Amplification was performed on the Applied Biosystems® 7500 Real-Time PCR Instrument (ThermoFisher Scientific, Waltham, MA). Testing was performed in batches of 94 specimens plus one negative and positive control. The results were interpreted using the Applied Biosystems™ COVID-19 Interpretive Software version 1.3. According to the manufacturer's instructions, a specimen was considered SARS-CoV-2 positive when two or more SARS-CoV-2 gene targets were called positive with cycle threshold values of ≤37.

cDNA synthesis
Reverse transcription and first-strand cDNA synthesis were performed using the Superscript III 1st Strand Synthesis Kit (Invitrogen, Cat No. 18080-051) following the manufacturer's protocol. Then, the extracted cDNA was stored at −80°C.

WGS by next-genome sequencing
The complete SARS-CoV-2 virus genome was analyzed with PCR amplicon sequencing using the Ion Torrent platform at the State Public Health Laboratory (SPHL), Chennai.

Analysis of SARS-CoV-2 sequence data

Data analysis was performed using the Ion Torrent platform Sequencing Technology (Torrent Suite-5.16.0.13 v).

Lineage and VOC assignment

Additional quality control, clade assignment, and mutation profiles were obtained using the NextClade tool v1.13.2[11] using a SARS-CoV-2 reference genome (accession NC_045512). All consensus sequences with a genome coverage >70 were classified using the PANGO lineage assignment tool (Pangolin v3.1.20 and PangoLearn v02.02.2022). Less genomic coverage is cost-effective and assists data analysis in population scaling.[12]

Phylogeny analysis

Phylogenetic trees were constructed using the neighbor-joining algorithm as a statistical method and maximum composite likelihood as model in MEGA X software. FIGTREE (version 1.4.4) was used for the graphical visualization of phylogenetic analysis.

Results

Demographic details of the subjects

To improve our understanding on the infection and transmission of SARS-CoV-2 in Theni, oropharyngeal swab samples were collected from 48 confirmed COVID-19 cases from 2021 to 2022 by the Government Theni Medical College, Theni. Based on the epidemiological investigation, one-third of these cases had contact history in this population. In general, the gender and age distributions of the cases examined were similar to those of early cases in Theni, Tamil Nadu. About 44% of the sample population was female and 56% was male. There were five children in the 0–14 years age group, seven young individuals in the 15–24 years age group, 29 adults in the 25–64 years age group, and seven geriatric people above 65 years [Figures 1 and 2]. The timeline shows the influx of samples and the predominant strain circulating in our district. The April month shows the higher influx of samples indicating a higher spread and the predominant strain was B.1.617.2 [Figure 3].

Clinical characteristics of the COVID-19 patients

The clinical classification of patients was categorized into mild, moderate, severe, and ICU. Out of 48 COVID-19 cases, 33 cases were mild, 8 were moderate, 5 were severe, and 2 were in ICU. One case had travel history, while the others did not have. Forty-four people were vaccinated and four were nonvaccinated. Among the vaccinated, 17 were Covaxin the rest were Covishield. Thirty-seven cases had taken their second dose, seven cases had taken their preventive dose. Nine cases contracted the infection after the first dose of vaccine and three cases had after the second dose. Analysis of the comorbidities revealed two cases of cardiovascular and cerebrovascular diseases, two cases with lactate dehydrogenase, 26 cases with highly sensitive C-reactive protein, one case with chronic kidney disease (CKD), and one case with chronic obstructive pulmonary disease (COPD). Based on the signs and
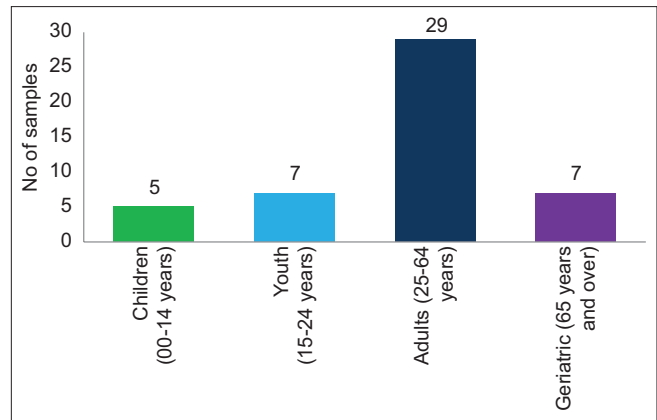

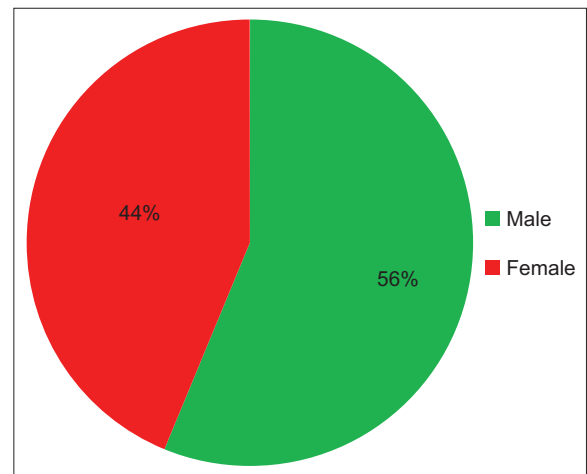**Figure 1:** Age-wise distribution of SARS-CoV-2 patients

Figure 2: Gender-wise distribution of SARS-CoV-2 patients
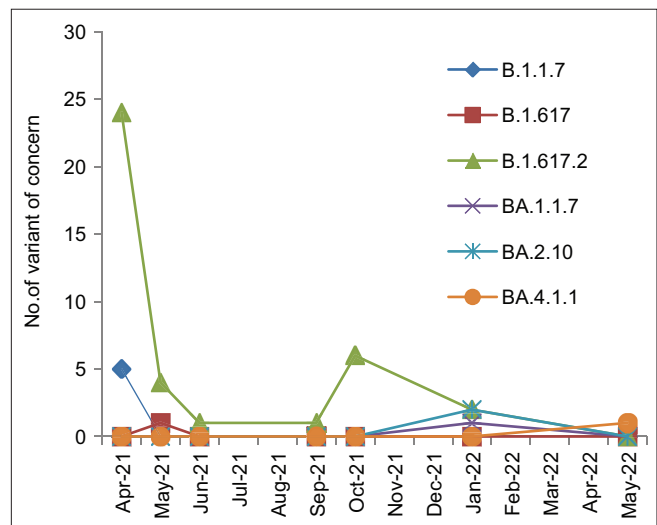

Figure 3: Timeline of variant of concern circulated in Theni district

symptoms at admission, 47 cases had fever, 43 had cough, 36 had cold, 38 had myalgia, 12 have diabetes, six had breathing difficulty, 41 cases had headache, 26 had loss of smell and taste sensation, 12 cases had sore throat, three cases had nausea and vomiting,

two cases had hypertension, 48 had no abnormal density shadow in the chest CT, and 48 were under antibiotic treatment [Table 1].

Genome sequences and variant analysis

For all 48 samples, we examined the *Ct* value of the quantitative RT-PCR targeting SARS-CoV-2 and found it ranged from 16 to below the detection limit, with a median of 24. Nucleic acids of SARS-CoV-2 were enriched by the probe hybridization method and sequencing was performed on Ion Torrent platforms (Torrent Suite-5.16.0.13 v). On average, 12 million paired-end reads were obtained for each sample, and a median number of 228,310 (range: 5,163–358,458) reads per million (RPM) could be mapped to the SARS-CoV-2 reference genome. From the analysis, it was revealed that 38 had infection by delta variant from the B.1.617.2 lineage, one case had infection by Kappa variant from the B.1.617.1 lineage, four had infection by Omicron subvariants from the BA.2.10 and BA.4.1.1 lineage, five had infection by alpha variant from the B.1.1.7 lineage. The highest cases of infection were caused by the delta variant [Figure 4 and Table 2].

Clustering and phylogenetic analysis

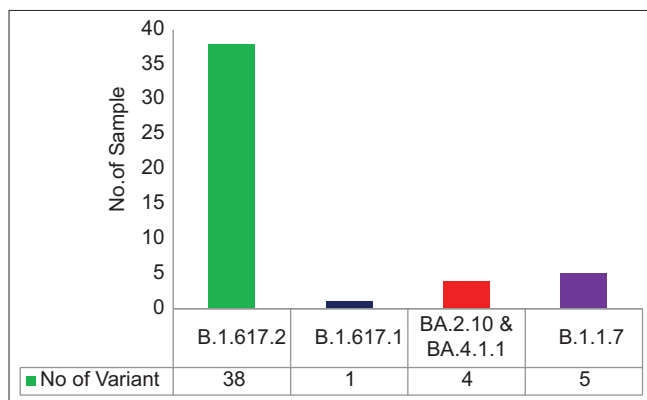Using phylogenetic analysis, 48 unique and strongly supported

Figure 4: SARS-CoV-2 genomic epidemiology of NGS variant

clades were identified based on lineage types in the SARS-CoV-2 data [Figure 5]. High local transmission events during the second and third waves were shown by phylogenetic clustering utilizing the nearest global sequences. Given that the bulk of the cases belonged to lineages BA.2.10 and BA.4.1.1, their clustering with genomes from Senegal suggests the spread of infections. Similar to this, there was evidence of potential importation and subsequent local transmission through strong clustering among delta samples during the second wave. Overall, cluster analysis suggested that the virus may have been imported primarily from India.

Discussion

WGS is an important tool for the determination of the geographical prevalence, the evolution of viruses over time, prediction of the trends of disease transmission, and understanding the most effective designs and platforms for the development of vaccines and therapeutics.[13] It additionally assists in tracing the transmission chains of the virus. The number of cases of COVID-19 is continuously rising all across the world.[14] In India, the first SARS-CoV-2 wave paved the way for maximum number of cases in the period between September and October 2020, and subsequently declined until February 2021. The next exponential upsurge (second wave) of the COVID-19 cases in India was observed from April 2021, with more than 0.2 million new cases being reported as of 17 April 2021 (https://www.worldometers.info/coronavirus/country/india/(accessed on March 22, 2022). Then the extracted RNA will be stored at −80°C. However, owing to the recent diversity of the new SARS-CoV-2 variants, the development of a dynamic nomenclature based on the phylogenetic framework was used to identify the lineages with an active spread.[15] Among the 48 virus isolates sequenced in this study, 38 (38/48) belong to lineage B.1.617.2.

In the study done by Banu *et al.*,[16] on the phylogenetic cluster of SARS-CoV-2 in India, members of the Clade I/A3i formed the predominant class of isolates from the states of Delhi,
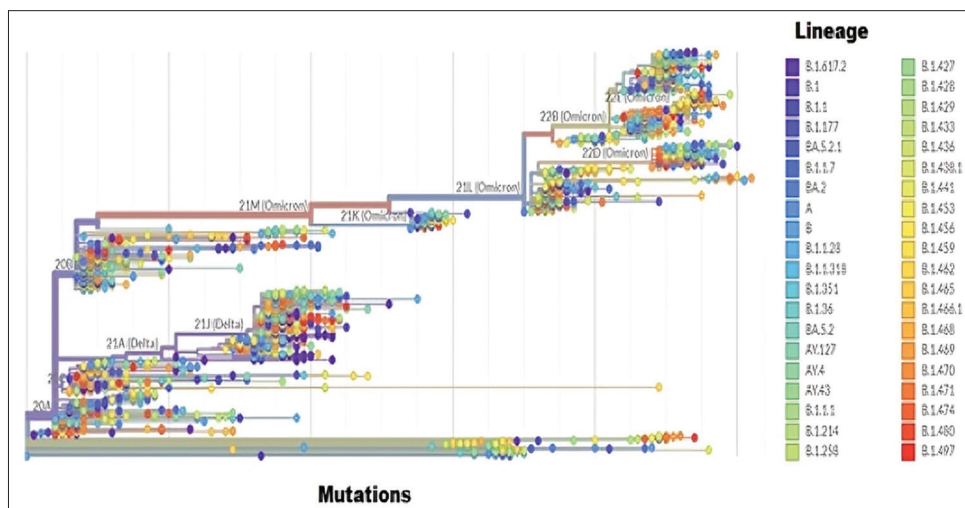

Figure 5: Phylogenetic analysis of SARS-CoV-2 sequences

Table 1: Demographic, baseline, and clinical characteristics of 48 patients with COVID-19 in Theni, Tamil Nadu

Variables	Patients (*n*=48)
Age group (%)	
00–14 years	5 (10%)
15–24 years	7 (14%)
25–64 years	29 (61%)
65 years and over	7 (15%)
Sex, n (%)	
Male	27 (56%)
Female	21 (44%)
Clinical classification (%)	
Mild type	33 (69%)
Moderate type	8 (17%)
Severe type	5 (10%)
ICU	2 (4%)
Exposure to confirmed cases (%)	
Yes	32 (67%)
No	16 (33%)
Signs and symptoms at admission	
Fever	47
Cough	43
Cold	36
Myalgia	38
Diabetes	12
Breathing difficulty	6
Headache	41
Loss of smell and taste sensation	26
Sore throat	12
Nausea and vomiting	3
Hypertension	2
Comorbidities	
Cardiovascular and cerebrovascular diseases, cerebrovascular diseases	2
Chronic lung diseases	0
Lactate dehydrogenase	2
High-sensitive C-reactive protein	26
Chronic kidney disease (CKD)	1
Coronary artery disease (CAD)	0
Chronic obstructive pulmonary disease (COPD)	1
Endocrine system diseases	0
Respiratory system diseases	0
Malignant tumor	0
Wave of infection	
Second wave	38
Third wave	10
Travel history	
Yes	1
No	47
Chest radiograph and CT findings	
Bilateral pneumonia	0
Unilateral pneumonia	0
No abnormal density shadow	48 (100%)
Vaccine details	
Vaccinated	44
Nonvaccinated	4
Treatment (%)	

Contd...

Table 1: Contd...

Variables	Patients (*n*=48)
Antibiotic treatment	48 (100%)
Antiviral treatment	0
Hormone therapy	0
Intravenous immunoglobulin therapy	0
Mechanical ventilation	5 (10%)
Clinical outcome	
Negative and discharged	48 (100%)
Died	0

Telangana, Maharashtra, Karnataka, and Tamil Nadu and the second largest in membership in Haryana, Madhya Pradesh, West Bengal, Odisha, Uttar Pradesh, and Bihar. This is in disagreement to our study as in our district, predominant cases belonged to clade A and clade J. In the study done by Raju *et al.*,[17] on the epidemiological, clinical characteristics, and outcomes of 1,175 SGTF COVID-19 patients (suspected Omicron) in Tamil Nadu, about one-third of the suspected Omicron patients did not report any symptoms, and majority had received either one or two doses of COVID-19 vaccination. The findings indicate a milder course of illness in majority of the suspected Omicron patients. Clinical presentation and vaccination status were similar in both suspected Omicron and non-Omicron patients.[18] This was found to be in agreement with our study. In the study by Raghav *et al.*,[19] molecular modeling and docking analysis identified that D614G mutation resulted in the enhanced affinity of Spike S1–S2 hinge region with TMPRSS2 protease, possibly the reason for the increased shedding of S1 domain in G614 as compared to D614. This type of study assisted in providing knowledge about the need for analysis of the genome and research on the mutation. In the study by Potdar *et al.*,[20] the temporal data of the Indian SARS-CoV-2 genomes revealed that except for Uttarakhand, West Bengal, and Haryana that showed the circulation of GISAID clade O even after July 2020, the rest of the states showed a complete switch to GR/GH clades. The Pangolin lineages B.1.1.8 and B.1.113 were identified within GR and GH clades, respectively, were noted to be indigenous evolutions. This is in disagreement with our study, as the predominant circulating strain was B.1.617.2. The motive of the studies done was similar to ours in assisting the surveillance of the circulating strains. These data obtained in Theni, Tamil Nadu, can serve as a tool for monitoring and providing real-time information on the spread of emerging SARS-CoV-2 variants in the population with important implications for public health and immunization strategies. Studies on genomic and epidemiology surveillance would also be useful in investigating vaccine effectiveness against circulating variants that appear to have a high turnover.

Conclusion

NGS is a powerful means of viral surveillance. The data obtained from the study reinforces the importance of genomic surveillance in Theni, Tamil Nadu. The optimal timing of the introduction of public health interventions is very important as it is often

Table 2: Description of SARS-CoV-2 lineages observed in Theni, Tamil Nadu					
Sequence ID	Clade	Lineage	Unaliased	Mutation	Coverage (%)
IONCODE—0349	21I (Delta)	B.1.617.2	B.1.617.2	33	99.7
IONCODE—0350	21I (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0351	21A (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0459	21J (Delta)	B.1.617.2	B.1.617.2	38	99.6
IONCODE—0460	21A (Delta)	B.1.617.2	B.1.617.2	32	99.7
IONCODE—0352	21J (Delta)	B.1.617.2	B.1.617.2	41	99.5
IONCODE—0353	21J (Delta)	B.1.617.2	B.1.617.2	49	99.7
IONCODE—0433	21A (Delta)	B.1.617.2	B.1.617.2	32	99.7
IONCODE—0354	21J (Delta)	B.1.617.2	B.1.617.2	43	99.7
IONCODE—0355	21J (Delta)	B.1.617.2	B.1.617.2	42	99.7
IONCODE—0356	21J (Delta)	B.1.617.2	B.1.617.2	41	99.7
IONCODE—0357	21J (Delta)	B.1.617.2	B.1.617.2	41	99.7
IONCODE—0358	21A (Delta)	B.1.617.2	B.1.617.2	33	99.7
IONCODE—0359	21B (Kappa)	B.1.617.1	B.1.617.1	32	99.7
IONCODE—0360	21I (Delta)	B.1.617.2	B.1.617.2	34	99.7
IONCODE—0361	21J (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0362	21J (Delta)	B.1.617.2	B.1.617.2	39	99.7
IONCODE—0363	21J (Delta)	B.1.617.2	B.1.617.2	39	99.8
IONCODE—0366	21K (Omicron)	BA.1.1.7	BA.1.1.7	58	99.6
IONCODE—0367	21L (Omicron)	BA.2.10	BA.2.10	67	99.5
IONCODE—0368	21A (Delta)	B.1.617.2	B.1.617.2	37	99.7
IONCODE—0461	21A (Delta)	B.1.617.2	B.1.617.2	35	99.7
IONCODE—0369	21A (Delta)	B.1.617.2	B.1.617.2	37	99.7
IONCODE—0370	21A (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0371	21A (Delta)	B.1.617.2	B.1.617.2	36	99.7
IONCODE—0372	21A (Delta)	B.1.617.2	B.1.617.2	36	99.7
IONCODE—0373	20I (Alpha, V1)	B.1.1.7	B.1.1.7	39	99.7
IONCODE—0374	20I (Alpha, V1)	B.1.1.7	B.1.1.7	37	99.7
IONCODE—0375	21J (Delta)	B.1.617.2	B.1.617.2	37	99.7
IONCODE—0376	21A (Delta)	B.1.617.2	B.1.617.2	31	99.7
IONCODE—0377	20I (Alpha, V1)	B.1.1.7	B.1.1.7	40	99.7
IONCODE—0378	21J (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0379	20I (Alpha, V1)	B.1.1.7	B.1.1.7	32	99.7
IONCODE—0380	21J (Delta)	B.1.617.2	B.1.617.2	37	99.7
IONCODE—0381	21J (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0382	21A (Delta)	B.1.617.2	B.1.617.2	33	99.7
IONCODE—0383	21A (Delta)	B.1.617.2	B.1.617.2	32	99.7
IONCODE—0384	21A (Delta)	B.1.617.2	B.1.617.2	43	99.8
IONCODE—0385	21J (Delta)	B.1.617.2	B.1.617.2	38	99.7
IONCODE—0386	20I (Alpha, V1)	B.1.1.7	B.1.1.7	40	99.7
IONCODE—0387	21A (Delta)	B.1.617.2	B.1.617.2	31	99.7
IONCODE—0388	22A (Omicron)	BA.4.1.1	BA.4.1.1	78	99.5
IONCODE—0389	21J (Delta)	B.1.617.2	B.1.617.2	39	99.6
IONCODE—0462	21J (Delta)	B.1.617.2	B.1.617.2	37	99.7
IONCODE—0434	21A (Delta)	B.1.617.2	B.1.617.2	34	99.7
IONCODE—0390	21A (Delta)	B.1.617.2	B.1.617.2	38	99.6
IONCODE—0391	21L (Omicron)	BA.2.10	BA.2.10	78	99.7
IONCODE—0392	21J (Delta)	B.1.617.2	B.1.617.2	39	99.7

too late for the control strategies to act on the curbing of local transmission. Studies like these are important to understand the characteristics and origins of locally circulating SARS-CoV-2 diversity in order to prevent further spread. Surveillance of SARS-CoV-2 genomes is crucial for understanding its evolution and spread patterns and may aid in decision-making concerning public health issues. Moreover, studies on genomic surveillance would also be useful in investigating vaccine effectiveness against circulating variants which appear to have a high turnover.

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