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Sequence analysis of Indian SARS-CoV-2 isolates shows a stronger interaction of mutant receptor-binding domain with ACE2

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\textbf{Abstract}

\textbf{Objective:} Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected the whole world, including Odisha, a state in eastern India. Many people have migrated to the state from different countries as well as other states during this SARS-CoV-2 pandemic. The aim of this study was to analyse the receptor-binding domain (RBD) sequence of the spike protein from isolates collected from throat swab samples of COVID-19-positive patients and further to assess the RBD affinity for angiotensin-converting enzyme 2 (ACE2) of different species, including humans.

\textbf{Methods:} Whole-genome sequencing for 35 clinical SARS-CoV-2 isolates from COVID-19-positive patients was performed by ARTIC amplicon-based sequencing. Sequence analysis and phylogenetic analysis were performed for the spike region and the RBD region of all isolates. The interaction between the RBD and ACE2 of five different species was also analysed.

\textbf{Results:} The spike region of 32 isolates showed one or multiple alterations in nucleotide bases in comparison with the Wuhan reference strain. One of the identified mutations, at position 1204 (Ref A, RMRC 22 C), in the RBD coding region of the spike protein showed stronger binding affinity for human ACE2. Furthermore, RBDs of all the Indian isolates showed binding affinity for ACE2 of different species.

\textbf{Conclusion:} As mutant RBD showed stronger interaction with human ACE2, it could potentially result in higher infectivity. The binding affinity of the RBDs for ACE2 of all five species studied suggests that the virus can infect a wide variety of animals, which could also act as natural reservoir for SARS-CoV-2.

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\section*{Introduction}

Coronaviruses have been studied for more than 50 years and are known to infect multiple animal species, including humans. Although the pathogenesis of the diseases caused by these viruses and their mechanism of replication have already been well described because of previous outbreaks (severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2003 and Middle East respiratory syndrome coronavirus in Saudi Arabia in 2012), the current COVID-19 pandemic has propelled the whole world to study and investigate more deeply the pathogenesis of diseases caused by these viruses. Coronaviruses belong to a diverse virus family which consists of four genera: \textit{Alphacoronavirus}, \textit{Betacoronavirus}, \textit{Gammacoronavirus} and \textit{Deltacoronavirus} (Fehr and Perlman, 2015). According to the nucleic acid sequence similarity, SARS-CoV-2, the organism that causes COVID-19, is a betacoronavirus. The spike glycoprotein of coronaviruses facilitates their entry into the host cell and also gives the virus a crown-like structure on its surface. Binding of pathogenic particles of SARS-CoV-2 with host cell receptors remains the crucial step for
initiation of infection. Besides, the ability of the virus to bind to the specific receptor of other host species is an essential requirement for transmission across different species (Lu et al., 2015). SARS-CoV interacts with human angiotensin-converting enzyme 2 (ACE2) for entry, and after the COVID-19 outbreak, researchers found that SARS-CoV-2 also interacts with ACE2 for entry into host cells (Li et al., 2003; Zhou et al., 2020). The SARS-CoV-2 spike protein is cleaved by host proteases into an S1 domain and an S2 domain, which mediate receptor recognition and membrane fusion, respectively (Lai et al., 2007; Wang et al., 2020) reported that the S1 domain of the spike protein of SARS-CoV-2 contains a receptor-binding domain (RBD), which forms a complex with human ACE2 and facilitates viral entry. However, emerging mutations in the SARS-CoV-2 genome might alter the processes of infection transmission and replication and the potential for viral attachment to ACE2. According to epidemiological data, SARS-CoV-2 was first identified in bats in Wuhan, China, and then spread to other parts after its zoonotic transmission via Malayan pangolins (Chinazzi et al., 2020; Zhang et al., 2020). In a country such as India with a diversified geographical distribution, it is important to understand the origin of different strains of SARS-CoV-2 isolated from different parts of the country. As interaction with ACE2 is the main pathway of entry of this virus into its host, knowledge of the RBD binding affinity of different Indian SARS-CoV-2 isolates for ACE2 of the natural reservoir, including humans, is very important. However, this has remained a grey area with little information available. Our study aimed to analyse the spike sequences of Indian SARS-CoV-2 isolates collected from COVID-19 patients in Odisha (an eastern state in India) and further understand the interaction between their RBDs and ACE2 of different probable natural hosts of coronavirus: bats, pangolins, hamsters and humans. The mutant RBD of one isolate shows stronger binding affinity for human ACE2 than the wild-type RBD, providing important information regarding its virulence as well as drug targeting.

Material and methods

Sequencing of different Indian isolates of SARS-CoV-2 from throat swab samples

The current study was part of a whole-genome sequencing study performed by the Odisha Study Group, which consists of different government organizations: the Regional Medical Research Centre (RMRC), Bhubaneswar, and the Institute of Life Sciences, Bhubaneswar. As Indian Council of Medical Research RMRC, Bhubaneswar, is a government-authorized testing laboratory for COVID-19 testing, we received throat swab samples of suspected cases from different hospitals in Odisha. For COVID-19 diagnosis, viral RNA isolation from all samples was performed with a QIAamp Viral RNA Mini Kit followed by quantitative PCR (Taqpath™ 1-step master mix, ThermoFisher Scientific). The libraries were prepared for whole-genome sequencing with use of ACTIC amplicon-based sequencing kits from Qiangen as per the manufacturer’s recommended protocol. Among all positive samples, whole-genome sequencing of 35 isolates from different

Table 1

| Serial no. | Sample ID | RMRC spike nucleotide sequence identity with the reference (%) | RMRC RBD nucleotide sequence identity with the reference (%) |
|-----------|-----------|---------------------------------------------------------------|---------------------------------------------------------------|
| 1         | RMRC 2    | 99                                                            | 100                                                           |
| 2         | RMRC 5    | 99                                                            | 100                                                           |
| 3         | RMRC 6    | 99                                                            | 100                                                           |
| 4         | RMRC 7    | 99                                                            | 100                                                           |
| 5         | RMRC 22   | 99                                                            | 99 (Mutation)                                                 |
| 6         | RMRC 23   | 99                                                            | 100                                                           |
| 7         | RMRC 24   | 99                                                            | 100                                                           |
| 8         | RMRC 27   | 99                                                            | 100                                                           |
| 9         | RMRC 28   | 99                                                            | 100                                                           |
| 10        | RMRC 30   | 99                                                            | 100                                                           |
| 11        | RMRC 38   | 99                                                            | 100                                                           |
| 12        | RMRC 46   | 99                                                            | 100                                                           |
| 13        | RMRC 90   | 99                                                            | 100                                                           |
| 14        | RMRC 103  | 99                                                            | 100                                                           |
| 15        | RMRC 104  | 100                                                           | 100                                                           |
| 16        | RMRC 106  | 99                                                            | 100                                                           |
| 17        | RMRC 108  | 99                                                            | 100                                                           |
| 18        | RMRC 110  | 99                                                            | 100                                                           |
| 19        | RMRC 112  | 99                                                            | 100                                                           |
| 20        | RMRC 154  | 99                                                            | 100                                                           |
| 21        | RMRC 155  | 99                                                            | 100                                                           |
| 22        | RMRC 156  | 99                                                            | 100                                                           |
| 23        | RMRC 157  | 100                                                           | 100                                                           |
| 24        | RMRC 158  | 100                                                           | 100                                                           |
| 25        | RMRC 159  | 99                                                            | 100                                                           |
| 26        | RMRC 160  | 99                                                            | 100                                                           |
| 27        | RMRC 162  | 99                                                            | 100                                                           |
| 28        | RMRC 163  | 99                                                            | 100                                                           |
| 29        | RMRC 164  | 99                                                            | 100                                                           |
| 30        | RMRC 165  | 99                                                            | 100                                                           |
| 31        | RMRC 166  | 99                                                            | 100                                                           |
| 32        | RMRC 167  | 99                                                            | 100                                                           |
| 33        | RMRC 168  | 99                                                            | 100                                                           |
| 34        | RMRC 170  | 99                                                            | 100                                                           |
| 35        | RMRC 171  | 99                                                            | 100                                                           |
COVID-19 patients with different travel histories was performed with the Illumina platform. Detailed information and a description of the method can be found in Raghav et al. (2020).

**Sequence alignment of RMRC spike genes with the reference sequence**

For sequence alignment, the full-length genome sequence of SARS-CoV-2 isolate Wuhan-Hu-1 (accession no. NC_045512) was downloaded from the US National Center for Biotechnology Information (NCBI GenBank Database) database and used as the reference sequence for all further analyses. Alignment of all 35 RMRC spike nucleotide sequences with the reference genome was performed with BLASTN (align two/more sequences). From the data available for the reference strain, we retrieved information on the coding region of the spike RBD sequence, and all RMRC spike sequences were aligned with the reference sequence to identify the respective RBD coding region mutations by our using BLASTN. Multiple sequence alignment of all 35 RBDs (amino acid sequences) was performed with Clustal X. Mutations specific to RMRC isolates were identified by our comparing the RBD coding regions with the reference strain. A phylogenetic tree was generated with MEGA version 6 with 1000 bootstrap replications as in the instructions for MEGA.

**Identification and phylogenetic analysis of the RBD of RMRC SARS-CoV-2 isolates**

![Figure 1](image_url)

*Figure 1.* (A) Multiple sequence alignment of receptor-binding domain (RBD) protein sequences of Regional Medical Research Centre (RMRC) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolates. All the sequences were aligned with the reference strain, and the arrow indicates the alteration found in the RBD of the RMRC 22 isolate. (B) Phylogenetic analysis of RBD protein sequences of RMRC isolates using the neighbour-joining method. The bootstrap consensus tree inferred from 1000 replicates has been taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed by the Poisson correction method and are in the unit of the number of amino acid substitutions per site.
isolated sequences of the were from C7ECV1, human CoV-2. The percentage interaction for ACE2 receptor-binding domain (RBD): (A) predicted 3D structure of ACE2; (B) Ramachandran plot and Z score; (C) ACE2 interaction with wild-type RBD; (D) ACE2 interaction with mutant RBD.

Sequence and 3D structure analysis of ACE2

From the phylogenetic tree analysis, four RMRC RBD sequences were selected from four random clusters for further investigation of their interactions with ACE2 of probable natural hosts of SARS-CoV-2. Hamsters were reported to be the most suitable animal model to perform SARS-CoV-2-related experiments; therefore, it was essential to understand the interaction of hamster ACE2 with isolated Indian SARS-CoV-2 RBDs (Imai et al., 2020). For the interaction study, hamster (Mesocricetus auratus) ACE2 (UniProtKB C7ECV1, 785 amino acids), pangolin (Manis javanica) ACE2 (NCBI XP_017505752, 805 amino acids), Chinese bat (Rhinolophus sinicus) ACE2 (UniProtKB E2DH7, 805 amino acids), Indian bat (Cynopterus sphinx) ACE2 (UniProtKB A0A6M3WQG9, 807 amino acids) and human (Homo sapiens) ACE2 (UniProtKB Q9BYF1, 805 amino acids) sequences were included. Before inception of structure prediction, the pangolin, hamster, Chinese bat and Indian bat ACE2 sequences were aligned with the human counterpart to identify the percentage of similarity and dissimilarity between these sequences.

The experimental 3D structure of human ACE2 was retrieved from the RCSB Protein Data Bank (PDB) (ID 6M0J) with a resolution of 2.45 Å positioning from 19 to 615 amino acids. The PDB did not provide any experimental structures of pangolin, hamster, Chinese bat or Indian bat ACE2, which prompted us to predict their 3D structure through homology modelling using Modeller version 9.19 followed by structure validation. Suitable templates were identified for 3D model building of ACE2 by a BLASTp search against the PDB (Altschul et al., 1990). The templates with PDB IDs 1R42, 6CS2, 6LZG, 3SCI and 2AJF were found to be good homologues for target–template alignment and modelled 3D structure prediction. On the basis of the optimized target–template alignment, Modeller 9.19 tool was used to predict the three dimensional structures of the proteins with unknown structures through homology modelling, the models with the lowest discrete optimized protein energy score were retained for further structural refinement (Webb and Sali, 2017). Side chain optimization was performed with WHATIF and GalaxyRefine (Hekkelman et al., 2010; Heo et al., 2013). The optimized models of ACE2 were finalized on the basis of the overall quality and stereochemical geometry and energy. The geometry of the predicted model was evaluated with PROCHECK and Ramachandran plot analysis (Laskowski et al., 1993; Pontius et al., 1996). The program ERRAT was used to calculate the accuracy of the non-
bonded atoms for the predicted model (Colovos and Yeates, 1993). Verify3D was used to evaluate the compatibility of the 3D model with its own amino acid sequence by our assigning a structural class based on its location and environment and comparing the results with good-quality structures (Bowie et al., 1991). The structure was uploaded to the Qualitative Model Energy Analysis (QMEAN) server to resolve the model quality. The energy potential of the predicted model was calculated with the ProSA-web server (Wiederstein and Sippl, 2007).

In silico translation of RBD sequences and their interaction with ACE2 of different natural reservoirs

Four nucleotide sequences of RMRC RBD coding regions were selected and translated to the protein sequence with use of the EMBOSS Transeq tool of the European Bioinformatics Institute. The 3D structure of the reference human RBD (UniProtKB P0DT2, amino acids 333–526 of the spike protein, PDB ID 6M0J) was considered as the wild type. The experimental structure of the wild-type RBD was altered at the position 402 (I402L) with use of Discovery Studio Visualizer (version 4.1) to obtain a mutant RBD (RMRC 22) as required for further computational analysis. Finally, protein–protein interaction between wild-type/mutant RBDs and ACE2 from different organisms was studied with the online HawkDock server, which is a powerful tool to predict the binding structures and identify the key residues of protein–protein interactions (Weng et al., 2019).

Results

Sequence information and analysis of the spike gene of Indian SARS-CoV-2 isolates

All SARS Co-V-2 isolates included in the study were from patients with a travel history from either outside the country or from other states in India. Among the 35 isolates, one was from a patient with a foreign travel history, 11 were from patients who had returned from Nizamuddin (cluster detected in New Delhi, India, during April 2020) and the rest (23) were from patients who had migrated from Surat, Gujarat, India. The detail of the demographic and clinical status of the patients included in the study has been described in one of our unpublished reports (Turuk
et al., unpublished). We did not record any deaths among the patients whose samples were included in the current study.

In the current study, the spike region was identified at the position 21,563 to position 25,384 of the whole genome and consists of 3822 nucleotides. The BLAST alignment analysis of RMRC spike nucleotide sequences showed that three spike sequences (RMRC 104, 157 and 158) were 100% identical to the Wuhan reference spike sequence, whereas all other RMRC spike sequences shared 99% identity, with one or multiple altered bases at different positions (Table 1).

**Sequence identification, alignment and phylogenetic analysis of RBD**

The alignment of the spike sequences of RMRC isolates with the reference strain RBD revealed the RBD region of Indian SARS-CoV-2 isolates is located in the 996–1578 bp region of the spike gene, consisting of a total of 582 bases. The protein sequence of the RBD spans from amino acid 333 (threonine) to amino acid 526 (glycine) of the spike protein (Lan et al., 2020). RBD sequence alignment results showed that RMRC 22 has 99% sequence identity with the reference RBD and harbours a mutation at position 1204 (nucleotide: Ref A, RMRC 22 C; protein: Ref I, RMRC 22L) (Figure 1A). Another three isolates shared 100% identity with the reference RBD (UniProtKB PO0T2C2, amino acids 333–526 of the spike protein). As only RMRC 22 had a mutation, it was named a mutant isolate, whereas all other isolates were considered to be the wild type.

The phylogenetic analysis was performed with RBD sequences of all 35 RMRC isolates and showed that they formed four different clusters. RMRC 22, having a mutation, belonged to the first cluster, RMRC 171 RBD formed the second cluster, four RBDs (RMRC 2, 5, 6 and 7) formed the third cluster and all other RBDs were in the fourth cluster, which describes their phylogenetic distribution (Figure 1B).

**Protein structure analysis and interaction of RBD with ACE2**

The sequence alignment analysis showed that ACE2 of pangolin, hamster, Chinese bat and Indian bat shared 85%, 84%, 80% and 78% identity with human ACE2, respectively (Supplementary Figure 1).
The quality of the modelled structures of ACE2 was validated by several computational methods (pangolin in Figure 2A; hamster in Figure 3A; Chinese bat in Figure 4A; Indian bat in Figure 5A; human in Figure 6A). In pangolin, of 805 amino acid residues, the Ramachandran plot analysis illustrated 637 residues (88.0%) in the most favoured regions, 66 (9.1%) in additional allowed regions, 14 (1.9%) in generously allowed regions and 7 (1.0%) in disallowed regions (Figure 2B). In hamster, of 785 amino acid residues, the Ramachandran plot analysis illustrated 613 residues (87.2%) in the most favoured regions, 70 (10.0%) in additional allowed regions, 12 (1.7%) in generously allowed regions and 8 (1.1%) in disallowed regions (Figure 3B). In Chinese bat, of 805 amino acid residues, the Ramachandran plot analysis illustrated 634 residues (88.8%) in the most favoured regions, 66 (9.2%) in additional allowed regions, 12 (1.7%) in generously allowed regions and 2 (0.3%) in disallowed regions (Figure 4B). In Indian bat, of 807 amino acid residues, the Ramachandran plot analysis illustrated 630 residues (86.8%) in most favoured regions, 73 (10.1%) in additional allowed regions, 15 (2.1%) generously allowed regions and 8 (1.1%) in disallowed regions (Figure 5B). We determined the model quality using the QMEAN server. The overall quality of the model was good as indicated by its QMEAN Z score and QMEAN4 global score. Low-quality models are expected to have a negative QMEAN Z score. QMEAN4 ranges from 0 to 1, with a higher value indicating a good-quality model (Benkert et al., 2008). Additionally, the overall quality of the model was evaluated with ProSA-web, which provides a quality score (Z score) as compared with all known protein structures from X-ray crystallography as well structural NMR spectroscopy. The Z scores obtained were −6.33 (pangolin), −4.76 (hamster), −6.24 (Chinese bat) and −6.73 (Indian bat), which indicates the high quality of the models compared with known protein structures (Figures 2B, 3B, 4B and 5B).

As the 3D structure of human RBD was already available in the database (PDB ID 6M0J), we inserted the observed mutation of the RMRC 22 isolate (protein: Ref 1, RMRC 22L) and predicted the structure of the mutant RBD (Figure 6B). Since the specific binding of the viral RBD with host ACE2 determines the establishment of infection, we analysed the interaction of Indian SARS-CoV-2 RBDs (both mutant and wild type) with human ACE2 as well as ACE2 of other species which are reported to be natural reservoirs for this virus. The interaction analysis showed that the mutant RBD of the RMRC 22 isolate has stronger interaction with human ACE2 (Figure 6D) as compared with the wild-type RBD (Figure 6C). The interaction between mutant RBD and human ACE2 has a binding
energy of −65.95 kcal/mol, with six hydrogen bonds, whereas wild-type RBD–human ACE2 interaction has a binding energy of −63.09 kcal/mol, with four hydrogen bonds. For ACE2 of all other species (pangolin in Figure 2C and D; hamster in Figure 3C and D; Chinese bat in Figure 4C and D; Indian bat in Figure 5C and D), the wild-type RBD seems to have stronger binding affinity, with no difference in the number of hydrogen bonds (except for hamster, for which wild-type RBD–ACE2 has two hydrogen bonds and mutant RBD–ACE2 has three hydrogen bonds). The details of the interaction analysis with hydrogen-bond-forming residues and the average distance of hydrogen bonds are given in Table 2. However, if we compare the interaction of the mutant and wild-type RBD with ACE2 of all species, the interaction between the mutant RBD and human ACE2 is the strongest, with the highest binding energy and highest number of hydrogen bonds.

Discussion

The complex trajectory of the recent COVID-19 pandemic in India poses great risk towards control and containment of the infection. It is high time to understand its mobility pattern in the country and the viral genetic properties favouring virulence. Although during the early phase of the pandemic Odisha had comparatively very few positive cases as well as a small number of deaths, the virus has gradually become rapidly infectious, making the clinical scenario worsen. Many people from Odisha were working outside the state, and during the pandemic they returned to their home state for many reasons. The samples included in our study were collected mainly from individuals with suspected COVID-19 who had a travel history from different states or a foreign travel record.

As the spike protein of SARS-CoV-2 mediates viral entry into the host and houses the RBD, which binds to ACE2 of the host cell, understanding the spike RBD distribution in the genome of Indian isolates is crucial for therapeutic design. The SARS-CoV-2 spike protein is reported to have stronger binding affinity for ACE2 than the SARS-CoV spike protein, and higher affinity means a lower viral load is required to infect the cell, which may explain the high transmission of SARS-CoV-2 (Chen et al., 2020). In the current study, the spike region of 32 isolates showed altered nucleotide bases at multiple positions as compared with the Wuhan reference strain, suggesting mutations in these Indian isolates during the
spread. For SARS-CoV-2, specific RBD–ACE2 binding ensures infection as well as serves as a potential target for developing treatment strategies for this infection (Chen et al., 2020). According to Premkumar et al. (2020), the RBD of SARS-CoV-2 is an immunodominant and potential target of antibodies in COVID-19 patients. An earlier study confirmed the presence of 25 alterations in the spike protein of SARS-CoV-2 Indian isolates, and some of those were capable of altering secondary structure and dynamicity of the spike protein (Chand et al., 2020). Our earlier published work provided detailed information on the D614G alteration in the spike protein of these isolates, which falls parallel with the findings of other researchers (Chand et al., 2020; Raghav et al., 2020). However, the current study does not focus on the D614G alteration as here the RBD sequence (which spans amino acids 333–526 of the spike protein) analysis is of prime importance rather than analysis of the whole spike protein. The mutation found in the RMRC 22 isolate in our study might play a role in altering the antigenicity or binding affinity of the respective RBD. The beauty of the current study is that it describes the role of this mutation in increasing the binding affinity of the RBD of this isolate for ACE2, which could be a potential cause of aggressive infection. Because of rapid spread and evolution, the SARS-CoV-2 RBD is known to acquire several alterations leading to increased binding affinity for human ACE2 (Ortega et al., 2020). In France, multiple alterations were identified in the RBD of SARS-CoV-2 contributing to higher receptor binding capacity, which might be responsible for increased virus spread and infectivity (Ou et al., 2020). On the other hand, an alteration in the spike protein has been found to be associated with a decrease in receptor binding affinity (Jia et al., 2020; Saha et al., 2020). In the current study, mutation in the RBD region of Indian isolates did not seem to affect interaction of the RBD with ACE2 of other species prominently except that of humans. Surprisingly, the patient from whom the RMRC 22 isolate was obtained had a travel history of returning from Nizamuddin (cluster detected in New Delhi, India, during April 2020) before he tested positive for SARS-CoV-2. However, no mutation was observed in the isolates obtained from his other family members (his father and two brothers), who were also COVID-19 positive. A study including SARS-CoV-2 isolated from Indian patients showed that a few genes, such as ORF6 and ORF10, completely lack any mutation and the E gene contains a single mutation, suggesting therapeutic strategies against these genes could be beneficial (Hassan et al., 2020). It appears that the emergence and role of a mutation in any region of the SARS-CoV-2 genome depends on multiple factors, including the geographical distribution, rate of spreading, alteration in the virulence of the virus and immune response of the host. The interaction analysis of mutant and wild-type RBDs with ACE2 indicated that bats and pangolins could be suitable natural reservoirs for Indian isolates of this virus, and this finding agrees with earlier reports (Lam et al., 2020; Zhou et al., 2020). As hamster has been reported to be a suitable animal model to study SARS-CoV-2 related pathogenesis, the interaction of the RBD of the Indian isolates included in the current study makes the earlier report more relevant (Imami et al., 2020). Although the susceptibility to infection and the death rate could be affected by several factors, mutation in the virus genome and its ability to adapt to a new environment could be crucial.

Being an important determinant in SARS-CoV-2 infection, RBD–ACE2 interaction has already become a potential target for developing treatment against this deadly pathogen. The current study provides important information regarding the structural basis of the spike and RBD regions of a few Indian SARS-CoV-2 isolates and gives an idea about their evolution and spreading. The mutation observed in the RBD region of one of the isolates sheds light on drug targeted therapy for different strains of the virus. Further studies with a larger number of isolates of a wider origin would be helpful to understand this mutation pattern in the RBD region of Indian isolates.

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Conflicts of interest

None.

Ethical approval

Has been approved from state research and ethics committee. This work was done and was part of the Raghav et al. (2020) study.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.01.020.

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