



Correspondence

Imported SARS-CoV-2 V501Y.V2 variant (B.1.351) detected in travelers from South Africa and Tanzania to India



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Dear Editor,

The SARS-CoV-2 has been continuously mutating, leading to the emergence of new variant strains since the emergence of the pandemic (2020–21). The first SARS-CoV-2 variant, 20I/501Y.V1 (B.1.1.7 Pangolin lineage) was reported from the United Kingdom (UK) which had 14 mutations and three amino acid deletions that influence the transmissibility of the virus in humans [1]. Subsequently, the emergence of new variants V501Y.V2 and 20J/501Y.V3 was also reported from South Africa [2] and Brazil [3] respectively. Although the 50% increased transmissibility has been observed with V501Y.V2, the clinical severity associated with the variant is not known. The variant strains of SARS-CoV-2 have raised serious concerns related to their increased transmissibility and also their ability to evade the immune response elicited by available S gene-based vaccines [3]. The World Health Organization (WHO) has also reported a resurgence of SARS-CoV-2 infection in few countries due to the emergence of the variant strains.

Extensive research is being done across the globe to monitor the spread of new variants of SARS-CoV-2 along with their impact on the virus transmission, severity of the disease, diagnostics, therapeutics and vaccine efficacy. Recognizing the importance of genomic surveillance, the Public health system in India has also tracked the spread of the SARS-CoV-2 variant across the country. With this initiative, VUI-202012/01 variant (lineage B.1.1.7) have been recently identified and reported from India [4]. An increase in the reports of these SARS-CoV-2 Variants of Concern (VOC) among the Indian nationals returning from different countries to India has raised a serious concern. These travelers might have transmitted the infection to close contacts before being diagnosed, leading to the spread of new variants in India. With ongoing surveillance activities, the Indian Council of Medical Research (ICMR)-National Institute of Virology (NIV), Pune has carried out the screening of SARS-CoV-2 among the international travelers with special emphasis on the Indian national returning from South Africa (Johannesburg) and Tanzania. The presence of SARS-CoV-2 was detected from clinical samples (nasal/throat swabs) of four returnees from South

Africa using the real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) method.

Two cases (age: 44 and 39 years; male) had a low-grade fever and cold from 5 days and cough with breathlessness from 8 days respectively; while the other two cases were asymptomatic (age: 19 and 56 years). All the cases were followed for 14 days. No new symptoms developed in any of the cases and all cases recovered completely.

The positive samples were used for next-generation sequencing to retrieve the genome of SARS-CoV-2 using Hybrid capture-based approach for the identification of the variants. Briefly, viral RNA was extracted from the clinical samples using MagMAX™ viral pathogen nucleic acid isolation kit (Thermo Fisher Scientific, USA) as per manufacturer's instructions and quantified using Qubit RNA High Sensitivity kit (Invitrogen). Up to 100 ng of RNA was taken for preparation of libraries using Illumina RNA Prep Enrichment (L) Tagmentation kit. Purified cDNA was tagmented using Enrichment Bead-Linked Transposomes to tagment double-stranded cDNA. After tagmentation, the fragments are purified and amplified to add P7 and P5 adapters for dual indexing. Amplified samples were enriched as single-plex reactions using the Respiratory Virus Oligos Panel v2 (Illumina, Catalog no.20044311) [5]. Prepared libraries were quantified, normalized, diluted to a final loading concentration of 1.25 pmol as per the Miniseq system and loaded on the Illumina machine for sequencing.

The reads generated from the Illumina machine were mapped to the reference SARS-CoV-2 sequence (Accession No. NC_045512.2) on the CLC Genomics workbench version 20 (CLC, Qiagen) using reference-based assembly. The percent genome retrieved (with respect to reference sequences) was ranged between 98.93 and 99.96%. It was observed that the retrieved SARS-CoV-2 sequences had the nucleotide and amino acid mutation characteristics of the South Africa V501Y.V2 (Fig. 1). The common amino acid mutation observed in all the four samples was at the ORF1ab (T265I, K1655 N and K3353R), spike (L18F, D80A, E484K, N501Y, D614, and A701), ORF3a (S17L) and N (P17L and T208I) proteins. The variation in the nucleotide along with the phylogenetic tree was generated using the highlighter plot (<https://www.hiv.lanl.gov/cgi->

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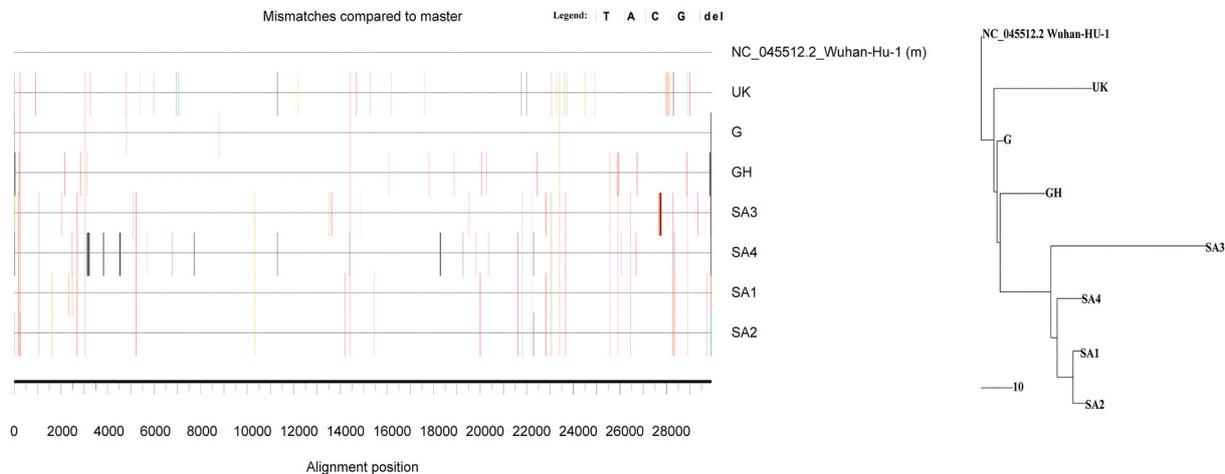


Fig. 1. Characterization of B.1.351 VOC from International travelers arrived in India: The aligned SARS-CoV-2 sequences retrieved from the clinical samples of COVID-19 positive cases having travel history to the South Africa and Tanzania with the reference isolate of Wuhan-HU-1 (Accession No.: NC_045512.2) and other representative sequences. A) The mismatches in the nucleotide position of the alignment B) the tree for the alignment were generated using the highlighter plot (<https://www.hiv.lanl.gov/cgi-bin/HIGHLIGHT/highlighter.cgi>). The nucleotide changes are marked indifferent colours. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bin/HIGHLIGHT/highlighter.cgi) (Fig. 1). All the four sequences belonged to the “GH” clade, according to GISAID nomenclature. Presently in India, multiple SARS-CoV-2 clades have been identified to be circulating [6]. The ‘G’ clade and its variants (GH and GR) were found to be predominant in the country [6]. The presence of this South Africa V501Y.V2 in travelers has alerted the country and dense search of these variants is in focus now during diagnosis and sequencing in those where cases are suddenly rising after a control situation in-country now.

Genetic mutations are part of the natural life cycle of RNA viruses hence, reducing the spread of new variant strains of SARS-CoV-2, using already established pandemic control measures should be the key approach to control further transmission. This will curtail the opportunity of the virus to mutate further. Genomic surveillance of SARS-CoV-2 should be continued to monitor the emergence and spread of new variant strains. This will enable the policy makers to make evidence-based decisions for curtailing the spread of the variant strains.

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Ethical approval

The study is approved by Institutional Biosafety Committee and Institutional Human Ethics Committee of ICMR-NIV, Pune, India.

Author contributions

PDY and NG contributed to study design, data collection, data analysis, interpretation and writing and critical review. DAN contributed to data analysis and interpretation, writing and critical review. RRS, AMS contributed to data collection, interpretation, writing and critical review. CN, JP, TM, SP, HK, NA, NV and JN contributed to data collection, writing and critical review. DYP contributed to data interpretation, writing and critical review.

Declaration of competing interest

Authors do not have conflict of interest.

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