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SHORT COMMUNICATION

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Detection and isolation of SARS-CoV-2 Eta variant from the international travelers and local residents of India

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Abstract

International travel has been the major source for the rapid spread of new SARS-CoV-2 variants across the globe. During SARS-CoV-2 genomic surveillance, a total of 212 SARS-CoV-2 positive clinical specimens were sequenced using next-generation sequencing. A complete SARS-CoV-2 genome could be retrieved from 90 clinical specimens. Of them, 14 sequences belonged to the Eta variant from clinical specimens of international travelers ($n = 12$) and local residents ($n = 2$) of India, and 76 belonged to other SARS-CoV-2 variants. Of all the Eta-positive specimens, the virus isolates were obtained from the clinical specimens of six international travelers. Many variants of interest have been found to cause substantial community transmission or cluster infections. The detection of this variant with lethal E484K mutation across the globe and India necessitates persistent genomic surveillance of the SARS-CoV-2 variants, which would aid in taking preventive action.

KEYWORDS

Eta, India, SARS-CoV-2, variant under monitoring

1 | INTRODUCTION

With ongoing genomic surveillance of SARS-CoV-2 across the globe, more than 6,694,501 complete genome sequences have been generated till January 3, 2022.[1] Such extensive surveillance has helped in the timely identification of various SARS-CoV-2 variants. The first variant with D614G mutation in spike protein was identified in early 2020 which later became the dominant strain worldwide.[2] During December 2020, a new variant was identified in the United Kingdom which was initially labeled as variant of interest (VOI)—202012/01 and subsequently designated as variant of concern (VOC)—202012/01 or alpha (B.1.1.7). Since then, VOCs such as beta (B.1.351) gamma (B.1.1.28.1), delta (B.1.617.2), omicron (B.1.1.529) have been identified in South Africa, Brazil, and India. Globally, many severe public health events have been reported related to these variants.[2–4] Some of these variants were reported to be capable of higher transmission while the others could reduce the neutralization capabilities of the currently available vaccines.[2–4]

The World Health Organization (WHO) has classified the new variants other than VOC under the VOI category which includes the variants of which few are likely to pose a public health threat. Further, variants under monitoring (VUM) have been classified as a variant with genetic mutations which are suspected to affect virus characteristics that could pose a future risk. However, the phenotypic or epidemiological impact of these variants is unknown and hence requires continuous surveillance and repeat assessment based on new evidence. There are many formerly monitored variants that have been identified and reported across the globe such as AV.1, AT.1, P.2,

P.3, R.1, B.1.466.2, B.1.1.519, C.36.3, B.1.214.2, B.1.427, B.1.429, B.1.1.523, B.1.619, B.1.620, B.1.526, B.1.630, B.1.617.1 (Kappa), and B.1.525 (Eta).[3] Many of them are found to cause substantial community transmission or cluster infections.

Of these, the Eta variant has grabbed special attention within the predominance over other variants of concern. As per WHO, the Eta variant is different from all other variants because it carries both E484K and F888L mutations. The E484K has also been found in the gamma, zeta, and beta variants which are variants of concern.[3,4] The variant was initially reported for the first time from the UK and Nigeria during December 2020. Since then, the Eta variant has marked its presence in 85 countries with around 9176 sequences have been reported around the world, with a cumulative prevalence of <0.5% till November 3, 2021.[1,5] Eta variant has amino acid variation in the positions ORF1ab (L4715F), S (Q52R, E484K, Q677H, and F888L), E (L21F and I82T) and deletions [11288:9 nucleotides (nt), 21765:6 (nt), 28278:3(nt)]. Some of these mutations are reported to help the virus to resist the neutralizing antibodies response (E484K), alter the virus transmissibility (Q677H) in the earlier SARS-CoV-2 strains.[6] Eta variant also has the deletions in the NTD (Δ69-70) region of the spike protein reported from Alpha and Omicron variants.[4]

India has severely affected during the pandemic with it being listed at second place among the highest cases reported across the globe. Looking at the dreadful situation of pandemic and emergence of new SARS-CoV-2 variants globally, the Government of India has established "The Indian SARS-CoV-2 Consortium on Genomics (INSACOG)" composed of 10 National Laboratories. Since November 23, 2020, clinical specimens of all the international travelers who arrived in India were tested with SARS-CoV-2 specific rRT-PCR. Subsequently, genome sequencing of the positive specimens was carried out at referral laboratories. After the genomic surveillance of samples from international travelers, alpha, beta, and zeta variant has been identified among travelers who arrived in different States of the country.[6]

2 | MATERIALS AND METHODS

With the continued surveillance for SARS-CoV-2 variant in India, clinical specimens of 212 SARS-CoV-2 rRT-PCR positive cases with a history of international travel ($n = 146$) arrived at Delhi or Mumbai international airport and clustered cases ($n = 66$) with local transmission of SARS-CoV-2 at Mumbai were collected during January-March 2021. Next-generation sequencing was carried out on all 212 clinical specimens of SARS-CoV-2 confirmed cases (*Ct*-value < 30) using Illumina sequencing platform at Maximum Containment Facility of ICMR-National Institute of Virology, Pune. Briefly, the RNA extracted from the clinical specimens was quantified by Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies) using a Qubit RNA high sensitivity (HS) kit. NebnextrRNA depletion kit (Human/mouse/rat) was used for the ribosomal RNA depletion after which cDNA synthesis was performed using the first strand and second synthesis kit. The RNA libraries were prepared using TruSeq Stranded Total RNA library preparation kit. The amplified RNA library was quantified and loaded on the Illumina sequencing platform after normalization.[7] Reference-based mapping was performed to retrieve the complete genome sequence of the clinical specimens using the reference SARS-CoV-2 sequence (Accession Number: NC_045512.2) in CLC genome workbench V20.0.4 and submitted to the public repository GISAID. The complete genomes were typed using the Phylogenetic Assignment of Named Global Outbreak LINeages "Pangolin" tool. A set of lineages of interest was downloaded and selected for comprehensive analysis based on their predominant distribution worldwide. The sequences with >98% genome retrieval were included for further genomic analysis.

Virus isolation was attempted using throat/nasal swabs of 14 COVID-19 cases affected with Eta variant using Vero CCL-81 cells. Briefly, the clinical specimens were inoculated onto monolayers of Vero CCL-81 which was maintained in Eagle's minimum essential medium (MEM; Gibco, UK) supplemented with 10 percent fetal bovine serum (FBS) (HiMedia, Mumbai), penicillin (100 U/ml), and streptomycin (100 mg/ml). Likewise, 100 µl was inoculated onto 24-well cell culture monolayers of Vero CCL-81, before the growth medium was decanted. The cells were incubated for 1 h at 37°C to allow virus adsorption, with rocking every 10 min for uniform virus distribution. After the incubation, the inoculums specimen was

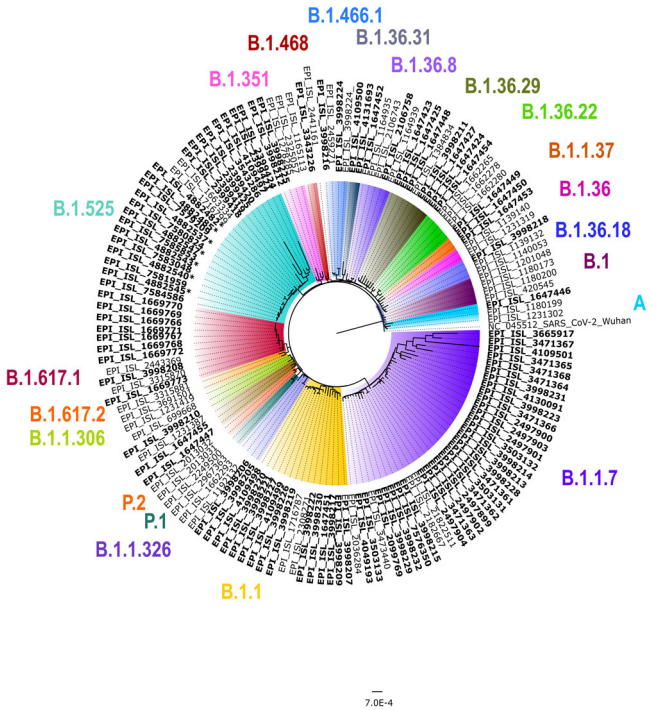

FIGURE 1 A neighbor-joining tree of the sequences retrieved from SARS-CoV-2 positive clinical samples and clinical isolates of the international travelers and local residents of India: A neighbor-joining tree was generated using a Tamura 3-parameter model with gamma distribution and a bootstrap replication of 1000 cycles. The sequences retrieved in this study are marked in black boldfaced. The alpha variant and Eta variant are marked with blue and orange color respectively

TABLE 1 Clinical details and sequencing data of the SARS-CoV-2 cases affected with Eta variant (clade-G)

MCL No	Details of the SARS-CoV-2 positive cases/ specimen type	Age	Sex	Symptomatic/ asymptomatic	Co-morbid conditions	Clinical outcome	District/ State	Date of collection	International Travel History	Date of arrival in India/ Arrival Airport	SARS CoV-2 result with *Ct* value (E gene)	% retrieved	GISAID accession id for clinical specimen	GISAID accession id isolates
MCL-21-H-542	International traveler/ Clinical specimen	28.1	Male	Asymptomatic	No	Recovered	Bihar	13.02.2021	Sharjah	13.02.2021/ Delhi	17.16	99.33	EPI_ISL_2399421	NA
MCL-21-H-529	International traveler/ Clinical specimen	40	Male	Asymptomatic	No	Recovered	Mumbai/ Maharashtra	08.02.2021	South Africa	08.02.2021/ Mumbai	21.74	98.87	EPI_ISL_2399420	NA
MCL-21-H-594	International traveler/ Clinical specimen	39	Male	Asymptomatic	No	Recovered	Mumbai/ Maharashtra	16.02.2021	Qatar	09.02.2021/ Mumbai	16	99.66	EPI_ISL_2399418	NA
MCL-21-H-609	Community Cluster/ Clinical specimen	53	Male	Asymptomatic	No	Recovered	Mumbai/ Maharashtra	17.02.2021	No	NA	14	99.73	EPI_ISL_2399419	NA
MCL-21-H-824	Community Cluster/ Clinical specimen	46	Male	Asymptomatic	No	Recovered	Mumbai/ Maharashtra	20.01.2021	No	NA	19	99.20	EPI_ISL_3543286	NA
MCL-21-H-892	International traveler/ Clinical specimen	42	Male	Asymptomatic	No	Recovered	Bilaspur/ Chattisgarh	25.02.2021	Nigeria	25.02.2021/ Delhi	21.31	99.65	EPI_ISL_2399425	NA
MCL-21-H-1075	International traveler/ Clinical specimen	30	Male	Asymptomatic	No	Recovered	Rupnagar/ Punjab	02.03.2021	Ghana	02.03.2021/ Delhi	17.08	99.56	EPI_ISL_4109497	NA
MCL-21-H-1098	International traveler/ Clinical specimen	63	Male	Asymptomatic	No	Recovered	New Delhi	03.03.2021	Sudan	03.03.2021/ Delhi	28.66	99.12	EPI_ISL_2399424	NA
MCL-21-H-1090	International traveler/ Virus isolate	26	Male			Recovered	New Delhi	03.03.2021	UNITED ARAB	03.03.2021/ Delhi	15.4	99.98	EPI_ISL_7579209	EPI_ISL_48824-85

TABLE 1 (Continued)

MCL No	Details of the SARS-CoV-2 positive cases/ specimen type	Age	Sex	Symptomatic/ asymptomatic	Co-morbid conditions	Clinical outcome	District/ State	Date of collection	International Travel History	Date of arrival in India/ Arrival Airport	SARS CoV-2 result with *Ct* value (E gene)	% retrieved	GISAID accession id for clinical specimen	GISAID accession id isolates
									EMI-RATES					
MCL-21-H-1426	International traveler/ Virus isolate	39	Male			Recovered	New Delhi	11.03.2021	UNITED ARAB EMI-RATES	11.03.2021/ Delhi	16	99.6	EPI_ISL_7580824	EPI_ISL_4882537
MCL-21-H-1397	International traveler/ Virus isolate	25	Male			Recovered	New Delhi	16.03.2021	UNITED ARAB EMI-RATES	16.03.2021/ Delhi	22	99.51	EPI_ISL_7581959	EPI_ISL_4882540
MCL-21-H-1398	International traveler/ Virus isolate	41	Male			Recovered	New Delhi	16.03.2021	UNITED ARAB EMI-RATES	16.03.2021/ Delhi	18	99.59	EPI_ISL_7583048	EPI_ISL_4882543
MCL-21-H-1411	International traveler/ Virus isolate	30	Male			Recovered	New Delhi	17.03.2021	UNITED ARAB EMI-RATES	17.03.2021/ Delhi	19	99.55	EPI_ISL_7584586	EPI_ISL_4882545
MCL-21-H-1186	International traveler/ Virus isolate	30	Male			Recovered	New Delhi	09.03.2021	UNITED ARAB EMI-RATES	09.03.2021/ Delhi	22	99.65	EPI_ISL_7585853	EPI_ISL_4882547

Abbreviations: Ct, cyclic threshold; NA, not applicable.

removed and the cells were washed with 1X phosphate-buffered saline (PBS). The MEM supplemented with two percent FBS was added to each well. The cultures were incubated further in a 5% CO_2 incubator at 37°C and observed daily for CPEs under an inverted microscope (Nikon). Cellular morphological changes were recorded using a camera (Nikon).[8]

3 | RESULTS AND DISCUSSION

Of the total 212 SARS-CoV-2 positive cases, 90 SARS-CoV-2 sequences (>98%) were retrieved from 52 samples of international travel history and 38 samples with local transmission of SARS-CoV-2. A signature of Alpha variant was identified in 33 specimens followed by Eta (14), kappa (9), B.1.1 (8), B.1.36.29 (5), beta (2), B.1.36.22 (2), B.1.1.306 (2), B.1.1.326 (2), B.1.1.216 (2), B.1.466.1 (2), A (1), B.1 (1), B.1.1.174 (1), B.1.1.198 (1), B.1.1.37 (1), B.1.1.372 (1), B.1.1.419 (1), B.1.36 (1), and B.1.36.18 (1).

An attempt of virus isolation with six clinical specimens of international travelers showed a typical rounding and detachment of the infected cells on 3rd PID which further progressed to fusion of the infected cells and the generation of a large mass of cells on 5th PID. The characterization of six clinical isolates with the next generation sequencing confirmed them as Eta variants (Figure 1).

All the sequences of clinical specimens and virus isolates of Eta affected cases showed nucleotide changes in the spike regions (21717 A > G, 21991_3del, 23012 G > A, 23593 G > C, 24224 T > C), similar to the Eta variant. A couple of additional mutations were observed at positions 3037 C > T and 16138 T > C in the reported sequences. Figure 1 depicts the neighbor-joining tree for the retrieved SARS-CoV-2 sequences (>98%) and the reference sequences. Out of the 14 Eta-affected cases; 12 cases were linked with international travel to multiple countries that is, United Arab Emirates, South Africa, Qatar, Nigeria, Ghana, and Sudan. The remaining two cases were identified from the community in Mumbai with no travel history. All the cases were male with the age group ranging from 28 to 63 years. All 14 cases were asymptomatic during infection and subsequently recovered completely (Table 1).

Many of the formerly monitored variants such as Kappa, Iota, and Eta has proved their existence along with the other VOCs in different countries. The first case of the Eta variant was identified in India on February 12, 2021. Since its first detection in December 2020, a total of 9006 sequences of Eta have been reported globally till November 3, 2021. With the dominance of Alpha and Delta variants in the country, the cumulative prevalence of Eta was reported to be less than 0.5%. Still, the presence of the Eta variant is worrisome as it carries the E484K mutation[4] which helps the virus to escape the body's immune response hence called "Escape mutation." A recent study on the genomic analysis of breakthrough infections in partially vaccinated individuals with BNT162b2 identified an immunocompromised patient with an escape mutation (E484K; Eta variant) who developed COVID-19 four days post-vaccination.[9] In addition, Liu et al., reported effective neutralization of the Eta variant with the sera of BNT162b2 two-dose vaccinated individuals. However, a mild reduction in the neutralizing antibody (NAb) titer was observed against the Eta variant (NAb-320) compared to wild type USA-WA1/2020 strain (NAb-502).[10] This suggests the continuous monitoring of VUI and VUM with lethal mutations specifically related to the vaccine efficacy. Hence, this study focused on the Eta variant, a variant under monitoring.

In conclusion, our study confirms the detection of Eta variants among travelers from the countries such as the United Arab Emirates, South Africa, Qatar, Nigeria, Ghana, and Sudan. A heavy amount of international travel could have been the important factor for the importation of this variant. Although the VOCs have been reported to enhance the transmissibility of the virus, there is little information available on the VUIs and VUMs. The researchers are working on the VUIs and VUMs to determine the transmissibility, pathogenicity, and neutralization efficacy in naturally infected individuals or vaccinated individuals. Genomic sequencing and epidemiological studies are continuing to further analyze the genomic variants of SARS-CoV-2 and associated public health events.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study was approved by the Institutional Biosafety Committee and Institutional Human Ethics Committee of ICMR-NIV, Pune, India under project "Molecular epidemiological analysis of SARS-COV-2 circulating in different regions of India" (20-3-18 N).

AUTHOR CONTRIBUTIONS

Pragya D. Yadav contributed to study design, data collection, data analysis, interpretation and writing, and critical review. Dimpal A. Nyayanit and Abhinendra Kumar contributed to data analysis and interpretation, writing, and critical review. Nivedita Gupta, Rima R. Sahay, Deepak Y. Patil, Anita M. Shete, and Triparna Majumdar, Savita Patil and Manisha Dudhmal contributed to data collection, data interpretation, writing, and critical review. Jayanthi Shastri, Harmanmeet Kaur, Sachee Agrawal, Alpana Razdan, and Neeraj Aggarwal contributed to data collection, writing, and critical review.

DATA AVAILABILITY STATEMENT

All data from the study were made available in the study.

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