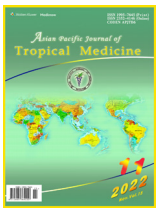

Case Report

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.359789

Impact Factor: 3.041

Detection of SARS-CoV-2 Eta VOI among international travelers using COVIDSeq-NGS

Mati Ur Rehman[1,2], Rooh Ullah[1], Narmeen Arshad[1], Muhammad Ammad[1], Qurat Ul Ain[1], Anam Razzak[1], Muhammad Yousaf[4], Shabana Perween[1], Syed Sajjad Naqvi[1], Tarique N. Hasan[1,3]✉

[1]*Pure Health Laboratory, Mafraq Hospital, Abu Dhabi, United Arab Emirates*

[2]*Ascencia Business School, College de Paris, France*

[3]*School of Life Science, Manipal Academy of Higher Education, Dubai, United Arab Emirates*

ABSTRACT

Rationale: SARS-CoV-2 has been identified as a highly infective and contagious viral infection. The SARS-CoV-2 pandemic has been spread worldwide and affected more than 210 countries. Globally, the fast spread of novel SARS-CoV-2 variants has been mostly attributed to international travel.

Patient concerns: We are reporting the genomic evidence of SARS-CoV-2 Eta VOI among two international travelers. Both travelers were males from Nigeria aged 24 and 34 years and both were asymptomatic.

Diagnosis: The nasopharyngeal swab samples were in both travelers positive by real-time RT-PCR followed by COVIDSeq-NGS.

Interventions: Paracetamol 3 times daily for 5 days.

Outcomes: Patient recovered completely within 10 days and discharged after 14 days of quarantine duration.

Lessons: This report highlights genomic variation of SARS-CoV-2 among the travelers. For managing the present health crisis, molecular identification of viral variants present in different geographical locations will be very helpful.

KEYWORDS: COVID-19; SARS-CoV-2; Eta; VOC; VOI

1. Introduction

SARS-CoV-2 genomic surveillance is ongoing worldwide. A total of 13 081 448 whole genome sequences have been reported till September 13, 2022[1]. This huge SARS-CoV-2 genomic surveillance has helped out in the timely detection of many SARS-CoV-2 variants. Early in 2020, the first variant of SARS-CoV-2 was identified with D614G-mutation in spike (S) region, which later emerged as the dominant strain globally[2]. A new-found variant was detected in UK during December 2020, which was first described as VOI-202012/01 and later described as alpha (B.1.1.7) a (VOC-202012/01). Since then, Brazil, India, and South Africa have reported the presence of VOCs such as beta B.1.351 (beta), B.1.1.28.1 (gamma), B.1.617.2 (delta), and B.1.1.529 (omicron). Worldwide, many serious public health incidents have been reported that was associated to these variants[2-4]. Some of these SARS-CoV-2 variants are able to have greater transmission while some variants can decrease the neutralizing capability of the available vaccines[2-4].

Besides these VOCs, the Eta VOI has taken more attention than other VOCs. As per the World Health Organization (WHO) guidelines, Eta VOI is distinct from all others because it has both F888L and E484K mutations. E484K mutation has also been found in the beta, gamma, and zeta VOCs[3,4].

Eta variant, also called as B.1.525 and VUI-21FEB-03, was first found in Nigeria and UK in December 2020[5]. The Eta variant is categorized as VOI due to the existence of certain genetic markers that are also present in other VOCs, which includes 1) E484K mutation, existing in the different VOCs like beta, gamma, and zeta; 2) Q677H mutation, related with a higher transmission; 3) 144 mutation linked with escape from immune system; 4) 106-108 mutation, previously noticed in the other variants like alpha, beta, and gamma; 5) N439K mutations found in B.1.258, Y453F, B.1.141 variants; and finally 6) 2 deletions ΔH69 and ΔV70 found

✉To whom correspondence may be addressed. E-mail: tariquenh@gmail.com

How to cite this article: Rehman MU, Ullah R, Arshad N, Ammad M, Ain QU, Razzak A, et al. Detection of SARS-CoV-2 Eta VOI among international travelers using COVIDSeq-NGS. Asian Pac J Trop Med 2022; 15(11): 518-521.

Article history: Received 15 September 2022 Revision 12 October 2022
Accepted 9 November 2022 Available online 30 November 2022

in B.1.1.7.3 variant. Eta VOI does not have the mutation N501Y, but inversely from all the other variants it holds F888L and E484K mutations[6]. Over the time with rising number of new cases, Eta VOI is increasing the risk to the public health globally[5].

2. Case history

Here, we report the genomic evidence of the SARS-CoV-2-Eta-VOI among two international travelers. Both travelers were males with age 24 and 34 years from Nigeria. Both were asymptomatic and were detected positive during screening process for international travelers. The positive COVID-19 travelers were quarantined for 14 days. Both were receiving paracetamol medicine for 5 days. Though, the disease was mild, both were cured at quarantine center, not in need of hospitalization.

The viral RNA from both travelers were extracted from nasopharyngeal-swab sample and analyzed for the presence of SARS-CoV-2 by rt-PCR using LabGun™ COVID-19 ExoFast RT-PCR assay (LabGenomics Co., Ltd. Korea) and then were submitted to the COVIDSeq-NGS (NovaSeq 6000 System, Illumina) following the manufacturer instructions.

To precisely determine the evolutionary links between the newly produced sequence from our case study and other variants of SARS-CoV-2, we then subjected a combined dataset to phylogenetic inference (including SARS-CoV-2 viral genomes available on GISAID: https://www.gisaid.org). The low quality genomes such as >10% of ambiguous positions were eliminated; only the genomes <1% of ambiguities and >29 000 bp were retrieved. Phylogenetic-analysis was conducted using the NextClade available at https://clades.nextstrain.org (Figure 1A and 1B).

Furthermore, in our case study, we explored the mutational-

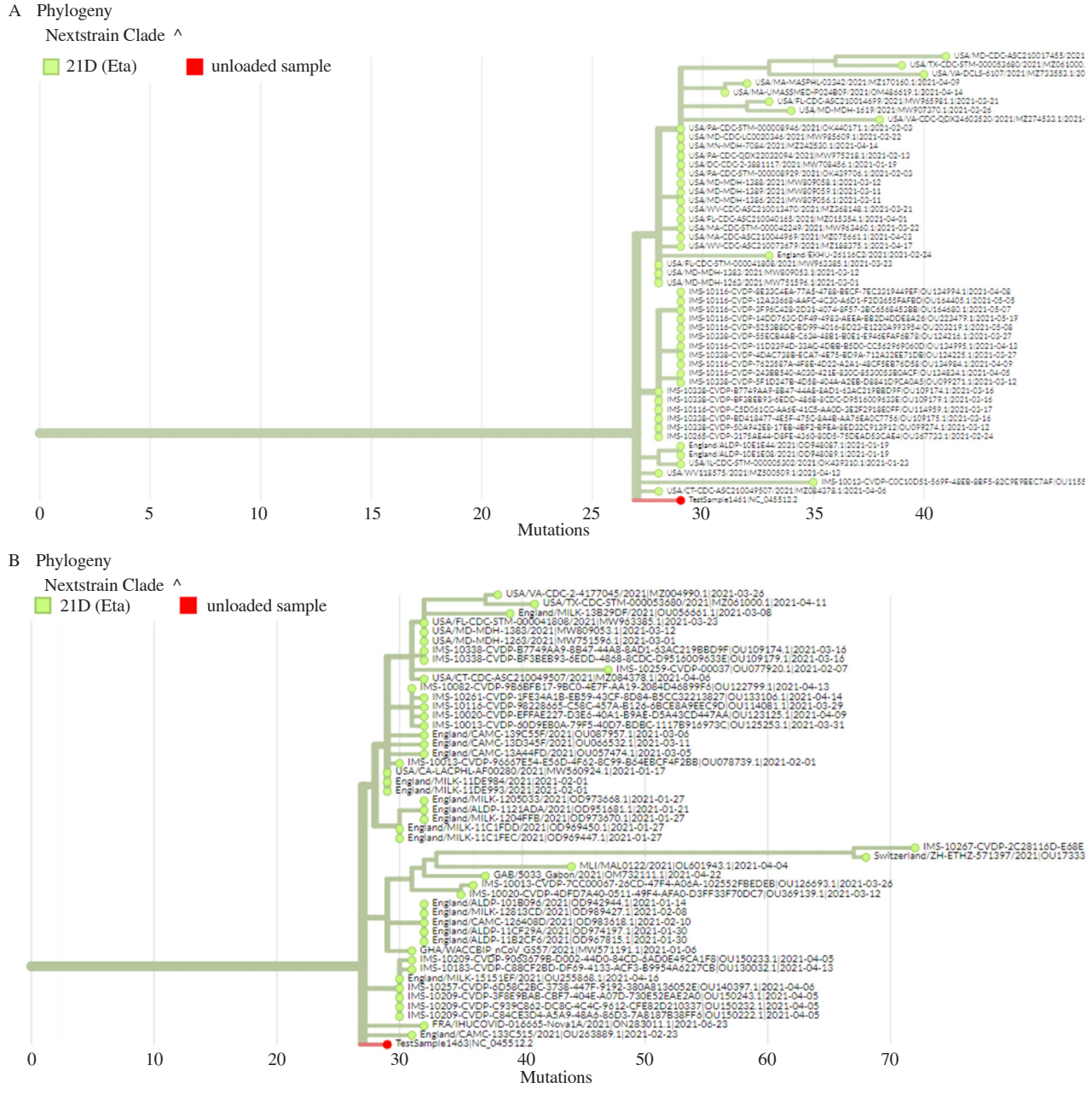

Figure 1. Phylogenetic tree generated by NextClade, showing (A) Eta VOI for traveler sample ID: 1461; (B) Eta VOI for traveler sample ID: 1463.

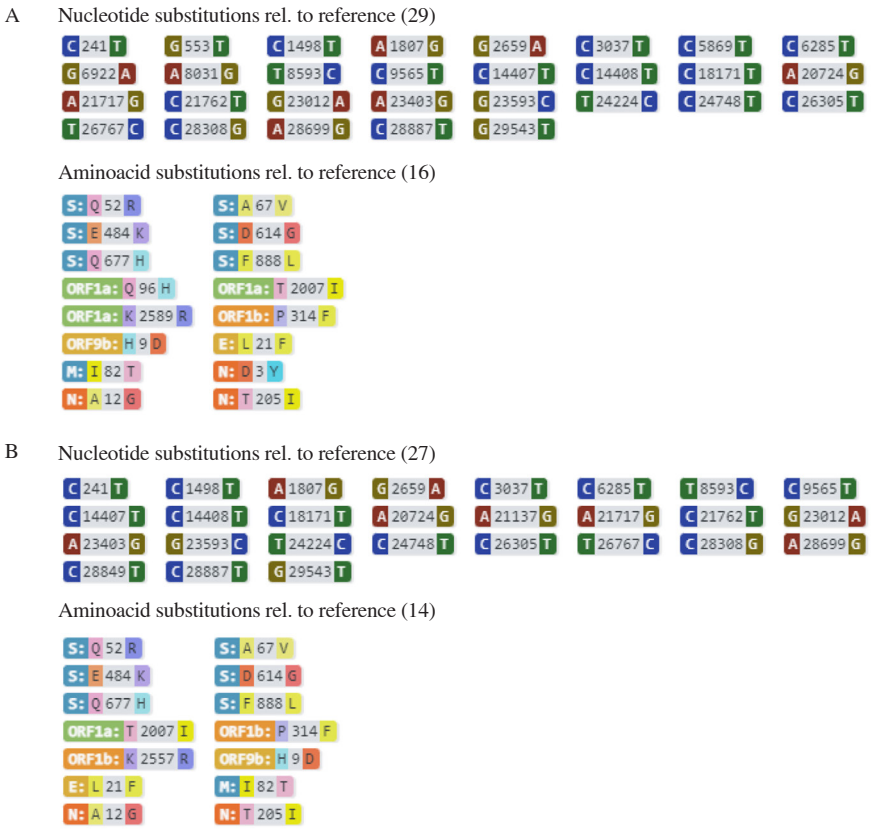

Figure 2. Mutational-profile of the newly produced Eta VOI generated by NextClade, showing nucleotide and amino acid substitution (A) in traveler sample ID: 1461; (B) in traveler sample ID: 1463.

profile of the newly produced strain to define its lineage defining mutations using NextClade. As likely, the found lineage carried all the mutations specific to lineage B.1.525 (Figure 2A and 2B).

3. Discussion

To the best of our knowledge, it is the first report describing the presence of Eta VOI in travelers from Nigeria. Yadav *et al*. also detected 14 Eta VOI among peoples having international travel history[7].

The COVIDSeq-NGS confirmed the two cases from travelers as Eta VOIs, showed in Figure 1A and 1B. The Eta VOIs effected cases showed some nucleotide changes in spike protein highlighted in Figure 2A and 2B. Study conducted by Yadav *et al*. also showed the similar pattern as: 24224 T>C, 23593 G>C, 23012 G>A, 21991_3del, and 21717 A>G. A couple of some other mutations were detected at positions 16138 T>C and 3037 C>T[7]. According to our case study, both travelers were male with age of 24 and 34 years. In relation to our case, all the studied travelers were males with age groups from 28 to 63 years in the study conducted by Yadav *et al*[7]. Both the Eta VOI travelers showed no symptoms during illness and subsequently fully recovered. The same pattern was observed in the study conducted by Yadav *et al*[7].

Most of the earlier observed variants such as Iota, Eta and Kappa have confirmed their presence along with other VOCs globally. The accumulative occurrence of Eta VOIs was reported to be less than 0.5%. Still, the existence of Eta VOI is terrible as it carries the E484K mutation (escape mutation) which helps the variant to evade from the immune response of the body[4]. A recent study on vaccinated individuals with BNT162b2 showed the development of COVID-19 infection after four days post-vaccination, molecular studies revealed the presence of E484K mutation-Eta variant[8,9].

In conclusion, our case report supports the finding of Eta variants among Nigerian travelers. Many international travelers could have been one of the factors for importing of Eta variant. Even though the VOCs have been described to increase the viral transmission, few evidence available on the VOIs and VUMs. The scientists are still working on VUMs and VOIs to find the neutralization, pathogenicity, transmissibility in un-vaccinated and vaccinated infected individuals. Epidemiological studies and genomic sequencing are ongoing to investigate more related to SARS-CoV-2 genomic variants and linked public health issues.

Ethical approval

The Study was ethically approved and informed consent were taken from both the patients.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to acknowledge the support we received from the medical experts working at Pure Health Laboratory, Mafraq Hospital, Abu Dhabi, UAE and School of Life Sciences, Manipal Academy of Higher Education, Dubai, United Arab Emirates.

Funding

The authors received no extramural funding for the study.

Authors' contributions

TNH, MR, SSN and RU conceived and designed the study. RU, NA, SP and MA analyzed the data. RU, QUA, AR and MY wrote the manuscript. All authors read and approved the final version of the manuscript.

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