Genomic evidence of a SARS-CoV-2 reinfection case with E484K spike mutation in Brazil

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Abstract

To date, uncertainty remains about how long the protective immune responses against SARS-CoV-2 persists and reports of suspected reinfection began to be described in recovered patients months after the first episode.[1] Viral evolution may favor reinfections, and the recently described spike mutations, particularly in the receptor binding domain (RBD) in SARS-CoV-2 lineages circulating in the UK, South Africa, and most recently in Brazil, have raised concern on their potential impact in infectivity, immune escape and reinfection.[2,3,4] We report a case of reinfection from distinct SARS-CoV-2 lineages presenting the E484K mutation in Brazil, a variant associated with escape from neutralizing antibodies. [5,6,7]

Text

A 45-year-old female healthcare executive, resident in Salvador, Bahia state, Northeast Brazil, with no comorbidities, presenting symptoms of viral infection on two occasions (May 26, 2020 and October 26, 2020). In the first episode, the patient presented diarrhea, myalgia, asthenia and odynophagia for approximately 7 days. She used 40 mg prednisone for 5 days and returned to activities 21 days later without sequelae or complaints.

In the second episode, symptomatically more severe, the patient presented headache, malaise, diarrhea, cough and sore throat that evolved to myalgia and respiratory distress, ageusia, muscle fatigue, insomnia, mild dyspnea on exertion and shortness of breath.

Viral RNA was extracted from nasopharyngeal swabs and tested for SARS-CoV-2 by multiplex real-time PCR Allplex™ SARS-CoV-2 assay (Seegene Inc, Seoul, Korea). On both occasions, results of RT-PCR tests targeting 3 genes (N, E and RdR) were positive for SARS-CoV-2 (**Figure 1A**). Cycle threshold values (Cts) of N, E and RdRp targets were 25, 26, and 27 in the first episode and 21, 12 and 17 in the second episode. In the second episode the patient presented a high viral load, presumed by the low Cts detected. Four weeks after testing positive by RT-PCR in the second episode, an IgG test against S1 protein by chemiluminescence (VITROS®, Ortho Clinical Diagnostic, New Jersey, United States), was performed and showed a positive result (index value: 2.15 on 11/23/2020).

Sequencing was then conducted by PGM Ion Torrent (Life Technologies, USA), according to the manufacturer's instructions. A total of 1.405.009 mapped reads for sample A and 2.570.182 reads for sample B were obtained, resulting in a sequencing mean depth >1,000X for both samples and a coverage >99%.

We further assessed the distinct viral origin of the two infections by phylogenetic inference. To do so, we combine our two new isolates (EPI_ISL_756293 and EPI_ISL_756294) with all Brazilian SARS-CoV-2 genomes available on GISAID (https://www.gisaid.org/) up to January 14th, 2021. Only genomes >29,000bp and <1% of ambiguities were retrieved (n=1164). Sequences were aligned using MAFFT[8] and submitted to IQ-TREE for maximum likelihood (ML) phylogenetic analysis.[9] We inferred time-scaled trees by using TreeTime.[10]

Phylogenetic analysis, of the two newly whole genome sequences compared with contemporaneous sequences from Brazil (**Supplementary Table 1**), clearly demonstrated that the two COVID-19 episodes, separated by a 147-day interval, were indeed caused by different SARS-CoV-2 lineages, confirming reinfection (**Figure 1B**). In the first episode, the lineage B.1.1.33 was detected, whereas lineage P.2 (an alias for B.1.1.28.2) was detected in the second infection (**Figure 1 panel B**), according to the Pangolin lineage classification (pangoLEARN version 2021-01-11). Further, we identified several mutations distinguishing the two genomes (**Figure 1C**), three of them in the SARS-CoV-2 spike glycoprotein. In the first infection, the retrieved genome presented the S:G1219C, while in the second infection, S:E484K and S:V1176F were observed.

The reinfection case here described, aligns with another reinfection case recently describe in Brazil that also documented a first infection with the B.1.1.33 lineage followed by a second one with the P.2 lineage (https://virological.org/t/spike-e484k-mutation-in-the-first-sars-cov-2-reinfection-case-confirmed-in-brazil-2020/584). The E484K mutation, located in the viral RBD, has been emerging independently in several SARS-CoV-2 variants and its monitoring is of a pivotal importance in the current stage of the pandemic. At least, three main lineages harbor E484K: a) B.1.351, first identified in South Africa and widespread worldwide[3] (https://virological.org/t/tracking-the-international-spread-of-sars-cov-2-lineages-b-1-1-7-and-b-1-351-501y-v2/592); b) P.1, recently described in Manaus, Brazil, and harboring a constellation of new mutations (including the N501Y) (https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586); c) P.2, also described in Brazil[4] and already detected in UK, USA, Canada and Argentina (https://cov-lineages.org/lineages.html). This case reporting a SARS-CoV-2 reinfection with a E484K variant corroborates *in vitro* and *in silico* studies that estimated the potential of lineages carrying this mutation to escape from neutralizing antibodies[6,7], and also highlights the importance of genomic surveillance to detect and monitor the emergence of new viral linages with possible implications in public health policies and immunization strategies.

Ethical Statement

This research was approved by the São Rafael Hospital Ethics Review Committee (CEP/CAAE: approval number 41528620.1.0000.0048).

Data Sharing

Newly generated SARS-CoV-2 sequences have been deposited in GISAID under accession numbers EPI_ISL_756293 and EPI_ISL_756294.

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Declaration of interests

The authors declare no competing interests.

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Figure legend

Figure 1. Molecular characterization of a COVID-19 reinfection case in Salvador, Bahia state, Northeast Brazil. A) Timeline of symptom onset, molecular and serological diagnosis. B) Time-scaled ML tree including the newly SARS-CoV-2 genomes (EPI_ISL_756293 and EPI_ISL_756294) recovered from a 45-year-old female resident in Salvador, Bahia state, Northeast Brazil, plus Brazilian full-length viral genomes available on GISAID (https://www.gisaid.org/) up to January 14th (**Supplementary table 1**). New genomes are highlighted with red circles. Branch support (SH-aLTR >0.8) is shown at key nodes. C) Mutational pattern of the two isolates obtained from the same patient with a 147-day interval. Only unique mutations and lineage defining mutations for B.1.1.33 and P.2 are shown.