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SARS-CoV-2 reinfection in a healthcare professional in inner Sao Paulo during the first wave of COVID-19 in Brazil

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ABSTRACT

Coronavirus Disease 2019 pandemic remains a threat to public health. We report 2 cases of Coronavirus Disease 2019 infection in the same healthcare professional in Brazil. Genomic analysis identified that primoinfection was caused by the endemic lineage B.1.1.33 while reinfection by the lineage B.1.1.44, a lineage with an additional V1176F mutation in S protein.

1. Case report

Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) emerged in China and rapidly evolved to a Public Health Emergency of International Concern (Harapan et al., 2020). Until end of 2020, >75 millions of cases and >1,5 millions of deaths were reported worldwide (Dong et al., 2020).

Phylogenetic studies report different lineages of SARS-CoV-2 simultaneously circulating in a given region with particular specificities (Mercatelli and Giorgi, 2020), mainly associated with neutral mutations not associated with enhanced transmissibility (van Dorp et al., 2020). According to Global Initiative on Sharing All Influenza Data (GISAID) database, in Brazil, the most common concurrently circulating lineages are B.1.1.28, B.1.1.33 and P.1 and P.2 lineages, variants of concern derivatives of B.1.1.28.

Although cases of reinfection are still rare (Larson et al., 2020, Mulder et al., 2020, Prado-Vivar et al., 2020, Tillett et al., 2020, To et al., 2020), weak or absent immunological response to SARS-CoV-2 after natural infection can occur (Wu et al., 2020), which may contribute to reinfections as also observed with other seasonal coronavirus (Edridge et al., 2020).

The epidemiologic definition of reinfection considers the presence of distinct phylogenetic lineages in an interval of >90 days, and low CT in RT-PCR assays (Yahav et al., 2020). Here, we report a confirmed episode of SARS-CoV-2 reinfection in a patient from Brazil.

All biological samples suspected to be from cases of reinfection are sent to Central Instituto Adolfo Lutz Laboratory for further analysis and confirmation. For those samples, the RNA was reextracted using the automatic Extracta 32 (Loccus, Brazil) and the presence of *E, N, RdRP* and *RNAseP* genes were confirmed using the Allplex 2019-nCoV Assay (Korea, Seegene Inc.) on the QuantStudio 5 Real-Time PCR Systems (Applied Biosystems, USA).

For genome sequencing, AmpliSeq™ SARS-CoV-2 (Thermo Fisher Scientific Inc., USA) was employed. Next, analysis were performed after quality control checking, assembly by IRMA (https://wonder.cdc.gov/amd/flu/irma/) and phylogenetics analysis in the GISAID database and BioNumerics 8.0 software (Applied Maths, Sint-Martens-Latem, Belgium). Sequences were deposited in the GISAID database under the accession IDs EPI_ISL_708529 and EPI_ISL_708530 and compared with reference WIV04-sequence (EPI_ISL_402124).

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Table 1
Clinical and genomic characteristics of SARS-Cov-2 infection and reinfection cases in Brazil.

Parameter	First episode – primo infection	Second episode – reinfection
Collection date	June 29, 2020	November 21, 2020
Symptoms	headache, myalgia, nonproductive cough, shortness of breath, ageusia and anosmia	headache, myalgia, nonproductive cough, fever, diarrhea, appetite loss, dizziness, and chest pain
SARS-CoV-2 lineage	B.1.1.33	B.1.1.44
GISAID Accession ID	EPI_ISL_708529	EPI_ISL_708530
Average coverage	15,677x	15,848x
Genetic differences[a]	Spike D614G	Spike D614G
	N R203K	Spike V1176F
	N G204R	N R203K
	N I292T	N G204R
	N P383L	NSP2 V577F
	NS6 I33T	NSP7 L71F
		NSP12 P323L
		NSP16 R216N

[a] In comparison with WIV04 sequence (EPI_ISL_402124).

Also, GISAID database was analyzed to compare the mutations present in S protein in all reinfection cases available (as of January 22, 2021).

On December 10, 2020, the Central Laboratory received 2 biological samples from a female nursing assistant, aged 41 years old, living in Fernandopolis, Sao Paulo, Southeast Brazil. Patient did not report comorbidities except for a previous gastroplasty. The first sample was positive for SARS-CoV-2 on June 29, 2020 with CT values of 18 and 32 for *E* and *RNAseP* genes on qPCR, respectively. In her first episode, the patient reported headache, disseminated body pain, nonproductive cough, shortness of breath, ageusia, and anosmia. She did not require hospitalization or any other aggressive intervention.

On November 21, 2020, after the patient presented again with symptoms of headache, cough, tiredness and myalgia, a second sample nasopharyngeal swabs were collected and positive for SARS-CoV-2 with CT values of 22 and 30 for *E* and *RNAseP* genes. In this second episode, the patient also reported diarrhea, appetite loss, dizziness, and chest pain. Temperature of 36.6°C and O_2 saturation of 98% were measured, and chest x-ray did not show abnormalities. Persistent fatigue and shortness of breath were reported 30 days after the diagnostic of the second SARS-CoV-2 infection, but with normal O_2 saturation.

Both samples were retested in Central Laboratory with CT values of 19, 19, 22, and 26 for *E, N, RdRP* and *RNAseP* genes on the first sample and 17, 17, 19, and 25 for the same genes on the second sample, respectively.

Complete aligned sequences of SARS-CoV-2 of 29,852bp and 29,862bp genomes with a coverage of 15,677x and 15,848x were obtained. GISAID classified these 2 genomes into 2 different lineages (as classified on January 22, 2021): B.1.1.33 in the primoinfection and B.1.1.44 in reinfection. Both lineages belong to Nexstrain clade 20B, but in different subbranches (Supplementary Figure).

In comparison with the WIV04-sequence, mutations were identified in S, N and NSP proteins of both genomes, but reinfection case (lineage B.1.44) presented an additional V1176F mutation in S protein (Table 1). In addition, both sequences presented the R203K and G204R aminoacid change in the nucleocapsid (N) protein. On the other hand, only the EPI_ISL_708529 sequence presented the I292T and P383L in N protein, besides an I33T change in NS6 - Accessory protein 6 (NS6). Mutations found only in EPI_ISL_708530 included NSP2 V577F, NSP7 L71F, NSP12 P323L, and NSP16 R216N. Analyzing the publicly available sequences of infection and reinfection episodes, we found that the average value of mutations in S protein in

Table 2
Reported cases of SARS-Cov-2 infection and reinfection, and the mutations in Spike proteins, according to GISAID database (as of January 22, 2021).

Case	Patient gender/age	Country	Type	Collection date	Accession ID	Spike protein mutations[a]
1[b]	Female/41	Brazil	primo infection	2020/06/29	EPI_ISL_708529	D614G
			reinfection	2020/11/21	EPI_ISL_708530	D614G, V1176F
2	Female/29	Brazil	primo infection	2020/03/24	EPI_ISL_811148	D614G
			reinfection	2020/12/30	EPI_ISL_811149	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
3	Female/37	Brazil	primo infection	2020/06/23	EPI_ISL_792561	D614G
			reinfection	2020/10/13	EPI_ISL_792562	E484K, D614G, S929I, V1176F
4	Male/unknown	Ecuador	reinfection	2020/07/22	EPI_ISL_516650	None
			primo infection	2020/05/20	EPI_ISL_525430	D614G
5	Female/45	Brazil	primo infection	2020/06/01	EPI_ISL_756293	D614G, G1219C
			reinfection	2020/10/26	EPI_ISL_756294	E484K, D614G, V1176F
6	Female/57	Brazil	primo infection	2020/03/24	EPI_ISL_636834	D614G
			reinfection	2020/05/29	EPI_ISL_636835	D614G
7	Male/34	Brazil	primo infection	2020/03/24	EPI_ISL_636836	D614G
			reinfection	2020/05/29	EPI_ISL_636837	D614G
8	unknown	USA	primo infection	2020/04/18	EPI_ISL_514673	D614G
			reinfection	2020/06/05	EPI_ISL_514674	D614G
9	unknown	Netherlands	primo infection	2020/04/06	EPI_ISL_523507	None
			reinfection	2020/06/08	EPI_ISL_523510	T20N, T572I
10	Male/33	Hong Kong	primo infection	2020/03/26	EPI_ISL_516798	E780Q
			reinfection	2020/08/17	EPI_ISL_516799	L18F, A222V, D614G
11	Male/51	India	primo infection	2020/10/24	EPI_ISL_826274	M153I, D614G
			reinfection	2020/12/13	EPI_ISL_826275	L54F, D614G
			reinfection	2020/12/13	EPI_ISL_826276	L54F, D614G

[a] In comparison with WIV04-sequence (EPI_ISL_402124).
[b] Cases reported in this study.

reinfections episodes was numerically higher than those in the primoinfections (2.82 vs 1.09, respectively; $P = 0.0974$) (Table 2).

Detection of reinfection in a patient from Brazil underscores the continuous threat of COVID-19 in a country suffering from uncontrolled epidemic about to be exacerbated by a second disease wave. Although clinical significance of COVID-19 reinfection remains to be totally elucidated, apprehensions clearly arise from the possibility that, in at least some individuals, the immunological response may be not enough to prevent a second infection.

The reinfection case presented here is supported by the fact that clinical specimens were collected more than 90 days apart from the same symptomatic patient presenting positive results with low CT values for SARS-CoV-2. Moreover, the first episodes was caused by a common lineage circulating in Brazil, B.1.1.33, which is highly disseminated in the country, according to GISAID database (Rambaut et al., 2020). B.1.1.44 is reported exclusively in this case in Brazil but another 616 entries (mainly from Europe) are deposited in the GISAID repository. Lineage B.1.1.44 was previously characterized as B.1.1.248 and B.1.1.28, another recurrent lineage circulating in Brazil since April, which originated the variants of concern P.1 (Gamma) and P.2, in increasing prevalence (Sabino et al., 2021). A remarkable difference found in B.1.1.44 is an additional V1176F substitution besides the highly disseminated D614G amino acid substitution prevalent worldwide (Rahimi et al., 2020); increased number of mutations in S protein are also reported in other reinfection episodes (Table 2). Mutation in the spike S2 domain V1176F is recognized to be the second more frequent in SARS-CoV-2 from South America, but its frequency is <3% in available viral genomes. Besides our epidemiological and genomic data, absence of patient serologies against SARS-CoV-2 cannot be ignored as potential limitation in this study.

Finally, we report a reinfection case of COVID-19 in a patient from Brazil, demonstrated by genome sequencing, highlighting the potential of Brazilian lineages (and its derivatives) in causing reinfection. The impact of passive immunization on lineages circulation and effective protection against COVID-19 reinfection, however, deserves further evaluation.

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Authors' contributions

Conceptualization: AA, AB, CTS
Data collection: CRG, EVRGP, KRC, JOMM, MNPF, MMCSN, FMTB, PMF, ALFY, TRMPC
Data analysis: CHC, AA, AB, CTS
Writing – original draft: CHC, CTS
Writing Review and Editing: CHC, AA, AB, CTS

Data availability

Sequences were deposited in the GISAID database under the accession IDs EPI_ISL_708529 and EPI_ISL_708530.

Ethical approval

This study was approved by the Local Ethics Committee (CAAE 139 37513020.7.0000.0059).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2021.115516.

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