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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

# Reinfection by SARS-CoV-2 by divergent Omicron sublineages, 16 days apart

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**Abstract**
Since the beginning of the SARS-CoV-2 pandemic, studies on the variants and sublineages stand out, mainly in the cases of reinfection in a short period. In this study, we describe a case of infection by BA.1.1 sublineage in an individual from Southern Brazil. The same patient acquired reinfection with sublineage BA.2 within 16 days after the first detection. The viral extraction and RT-qPCR were performed on the samples LMM72045 (collected in May 2022) and LMM72044 (collected in June 2022). After the confirmation of SARS-CoV-2 infection, we conducted the sequencing and viral genome analysis. This case of reinfection affected a 52-year-old male patient, without comorbidities, with three doses of vaccines against COVID-19, showing symptoms on May 19. These symptoms lasted for approximately six days. The patient returned to work activities on May 30. However, on June 4, the patient felt a new round of clinical signs that lasted for approximately seven days. Analysis of the viral genomes recovered from patients' clinical samples revealed that the two COVID-19 episodes were related to two divergent VOC Omicron sublineages, namely, BA.1.1 for the first round of symptoms and BA.2 for the second infection. Based on our findings, we can say that the present case of reinfection is the shortest described so far.

**Keywords** BA.1.1 · BA.2 · COVID-19 · Reinfection · SARS-CoV-2

## Introduction

Since the beginning of the severe acute respiratory syndrome virus Coronavirus 2 (SAR-CoV-2), which causes Coronavirus disease 19 (COVID-19), several studies have been carried out to elucidate the mechanisms of action, mainly due to the emergence of new lineage and sublineage coronaviruses. Thus, the world has been facing the consequences of the emergence of the pandemic waves guided by the Alpha, Beta, Gamma, Delta, and Omicron variants [1].

In November 2021, B.1.1.529 lineage—later named Omicron—was first detected in Botswana followed by South Africa. Due to its high transmissibility and lower susceptibility to neutralization by antibodies produced by previous viral exposure or vaccine administration, this variant was classified as a Variant of Concern (VOC) [1–3]. The mutations identified in Omicron occur largely in the spike protein (S) (antibody binding site), which attributes high infectivity and transmissibility characteristics. There are six sublineages (BA.1, BA.2, BA.4, BA.5, BA.2.12.1, and XBB.1.5) of Omicron currently (April 2023) described, and three sublineages are under monitoring (BQ.1, BA.2.75, and XBB) [1–3].

In addition to the antibody escape ability, another concern is the higher number of reinfections from Omicron and sublineages in those who had a previous natural infection [4]. According to literature reports, when comparing all VOCs, the Omicron variant presents an increased risk of reinfection, suggesting that Omicron is associated with a particular ability to evade natural immunity from a previous infection [5].

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In this study, we describe a case of infection by sublineage BA.1.1 of VOC Omicron in an individual from Southern Brazil (State of Rio Grande do Sul), and the same patient acquired reinfection with another sublineage (BA.2) in 16 days.

## Material and methods

### RNA extraction and RT-qPCR for SARS-CoV-2 detection

The Municipal Health Department of Porto Alegre (State of Rio Grande do Sul, Brazil) sent two samples (Naso-Oropharyngeal Swab) from the same patient to the Laboratório de Microbiologia Molecular (LMM) Universidade Feevale (Laboratory of Molecular Microbiology (LMM) Feevale University) for SARS-CoV-2 analysis. The samples were named LMM72045 and LMM72044, and viral RNA was extracted with the commercial MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA), using the automated equipment KingFisher™ Duo Prime (Thermo Fisher Scientific™). Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) for SARS-CoV-2 selective for the envelope (E) gene was performed according to the Charite Institute (Berlin, Germany) protocols [6] and using AgPathID One-Step RT-PCR Reagents (Thermo Fisher Scientific™).

### Whole-genome viral sequencing and phylogenetic analysis

Samples were prepared for sequencing using the COVIDSeq RUO kit (Illumina Inc., City of Foster, CA, USA), and for enrichment, ARTIC v4 Primer Pools (Illumina Inc.) were used according to the instructions provided by the manufacturer. Sequencing was implemented on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600 cycles) from Illumina Inc. All procedures were performed in a laminar flow to minimize the risk of contamination. The FASTQ reads were imported into Geneious Prime, trimmed to remove low-quality sequences and primers used for library generation (BBDuk 37.25), and mapped against the hCoV-19/Wuhan/WIV04/2019 reference sequence (EPI_ISL_402124) available in the EpiCoV database from GISAID. PANGO and Nextstrain lineage assignments were applied to characterize the consensus sequences. The phylogenetic tree was constructed through the Nextclade tool on the Nextstrain server [7]. Mutational sequence profiles and signatures were also analyzed to elucidate differences among the paired samples.

## Results

### Case description

A 52-year-old male patient, without comorbidities, with two doses of the vaccine (AstraZeneca) and a booster dose in January 2022 (Pfizer) started to show respiratory symptoms on May 19, 2022. The symptoms reported by the patient were sneezing, mild headache, runny nose with mucus, and a fever of 37.5 °C (the usual temperature is between 35 and 36 °C). These symptoms were more intense in the first two days (May 19 and 20) and lasted for approximately six days. Thus, the patient worked at home until May 29 and returned to work on May 30, as he no longer had any symptoms. However, on June 4, the patient started to present symptoms again: myalgia, intense headache, fever, runny nose with mucus, sneezing, coughing, and sore throat when coughing. These symptoms remained for seven days. Samples were collected on May 23 (LMM72045—first infection) and on June 8 (LMM72044—second infection) and were sent by Porto Alegre Municipal Health Department to LMM for analysis. A brief timeline of the case description is shown in Fig. 1.

### RT-qPCR, genome sequencing, and phylogeny analysis

Both samples evaluated by RT-qPCR were positive and had a Ct value of 37.70 (LMM72045) and 14.68 (LMM72044) (Fig. 1). After sequencing, it was identified that the first infection was by the sublineage BA.1.1, while the second was by BA.2, LMM72045 and LMM72044, respectively (Fig. 1), which proves that it was a case of reinfection and not a positive test even after the illness. The two sequences have been registered in the GISAID database (EPI_ISL_13696794 and EPI_ISL_13696793, respectively).

When we analyzed the sequences of the samples, we observed that LMM72045 contains long stretches of NNNs (27.67% of the total sequence), insertion of six nucleotides, and a gap of 30 nucleotides when compared to the reference sequences. Moreover, amino acid exchanges were observed in the following regions of the spike protein: E484A, G142D, G339D, G496S, ins214EPE, L212I, L981F, N211del, N501Y, N969K, Q493R, Q498R, Q954H, R346K, S371L, S373P, S375F, S477N, T478K, T547K, V143del, Y144del, Y145del, and Y505H. Other amino acid changes were observed in the E T9I, M A63T, N E31del, N G204R, N P13L, N R32del, N R203K, N S33del, NSP3 K38R, NSP4 T492I, NSP5

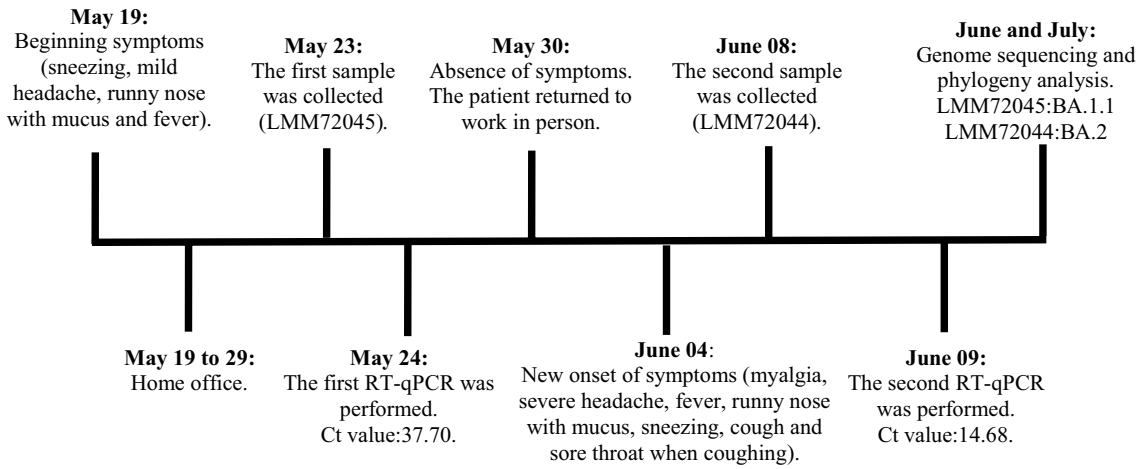


**Fig. 1** Timeline with case description (symptoms and date of samples collection) of a 52-year-old male patient who acquired infection and reinfection with two distinct Omicron sublineages. The results (Ct value and sequencing) are briefly described

**Table 1** Sequencing results (reads, coverage, and length) and signature mutation at spike protein for both samples evaluated

| | | Samples | |
|---|---|---|---|
| | | LMM72045[1] | LMM72044[2] |
| Reads | Total reads | 242,155 | 326,802 |
| | Coverage | 85.3% | 99.5% |
| | Length (mean) | 130 bp | 133 bp |
| Signature mutation at spike protein[3] | Total | 36 | 31 |
| | Shared mutations[4] | 13 | 21 |
| | Specific mutations | 15 | 10 |
| | Mutations detected | 19 | 31 |

*bp* base pairs

[1]First infection, classified as BA.1.1 sublineage (clade 21 K).

[2]Second infection, classified as BA.2 sublineage (clade 21L).

[3]According to the website https://covariants.org/shared-mutations

[4]Total number of signature mutations shared between BA.1.1 and BA.2 that was detected.

P132H, NSP6 G107del, NSP6 L105del, NSP6 L146del, NSP6 S106del, and NSP12 P323L. On the other hand, the sample LMM72044 presented 1.47% of stretches of NNNs in the general sequence and a gap of 53 nucleotides when compared to the reference sequences.

When comparing with the reference and analyzing the sequencing results, the number of reads performed for the sample LMM72045 (classified as BA.1.1—clade 21 K), was 242,155 and for LMM72044 (classified as BA.2—clade 21L) was 326,802 readings. Coverage was 85.3% and 99.5%, respectively, for LMM72045 and LMM72044 (Table 1). Due to the high Ct values and the percentage of gaps detected in LMM72045, analysis was performed on the mutations present in both samples. Thus, based on the list of shared and specific mutations for each sublineage (https://covariants.org/shared-mutations), we evaluated the signature mutations in the spike protein (S) in both samples to verify that they were different sublineages and exclude the possibility of coinfection. It should be noted that BA.1.1 and BA.2 share 21 signature mutations and have a total of 36 and 31 mutations at S, respectively. Therefore, we observed that in the sample LMM72045, 24 mutations were detected, of which 19 are signature (19/24—79.1%) and 13 are in common with LMM72044. We also evaluated the specific mutations of BA.2 (LMM72044) and observed that it has all the characteristic and shared signature mutations, but does not have the specific mutations of the BA.1.1, and LMM72045 does not have the mutations that are specific to BA.2. Thus, after analyzing the data, we conclude that the findings are consistent with a reinfection case.

Figure 2 shows the phylogenetic tree containing the sequence samples of the present study, the reference sequences, and sequences of the sublineages BA.1.1 and BA.2 from different countries. Two groups of Omicron sublineages are observed: 21 K, which belongs to sample LMM72045 (BA.1.1), and 21L, which belongs to sample LMM72044 (BA.2), reaffirming that the evaluated samples belong to different Omicron sublineages (Fig. 2).

## Discussion and conclusions

The findings of VOC Omicron and its sublineages have led to the development of several studies to understand the viral mechanisms, especially, due to the high transmissibility capacity and the cases of reinfection. Hirotsu and Omata [8] evaluated 12 cases of reinfection and observed that in 5 cases both the first and second infections were

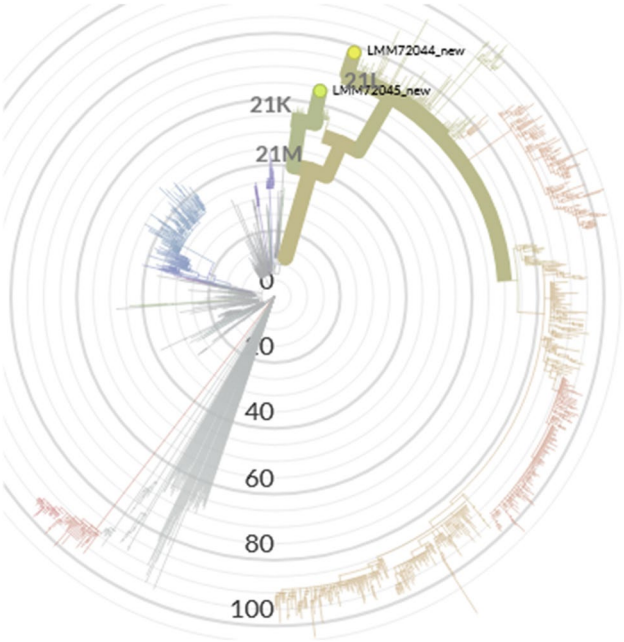


**Fig. 2** Phylogenetic tree SARS-CoV-2 variant analysis in a reinfection case from Rio Grande do Sul State, Southern Brazil, developed through the Nextstrain server (Nextclade tool), using GISAID data. LMM72045 (BA.1.1) sample (first infection) belongs to 21 K clade and LMM72044 (BA.2) sample (second infection) belongs to 21L clade

due to Omicron sublineages, and in all cases, the second infection was also due to Omicron sublineages. In addition, the authors showed that in the 5 cases of Omicron reinfection, viral loads increased in 3 samples between the first and second infections, as was also observed in the present study. Burkholz et al.[9] using the Next-Generation Sequencing method evaluated the strains related to cases of reinfection and found an increased frequency of reinfection related to Omicron. In addition, they suggested that reinfections with Omicron are occurring more frequently and in a shorter time interval than that observed for other strains during the pandemic's course.

Research indicates that the Omicron sublineages present substantial genomic differences between them, especially BA.1 and BA.2. In the present study, we describe the case of infection by the sublineage BA.1.1 in an individual who acquired reinfection with another sublineage, BA.2, in 16 days. In Denmark, Stegger et al.[10] carried out a study to elucidate the question that BA.2 could escape natural immunity acquired soon after a BA.1 infection. For this, they selected more than 1.8 million cases of infections from November 22, 2021, to February 11, 2022. Therefore, individuals with two positive samples—more than 20 and less than 60 days apart—were selected. From 187 reinfection cases, we identified 47 cases of BA.2 reinfection soon after a BA.1 infection. The authors concluded that Omicron BA.2 reinfections occur soon after BA.1 infections but are rare.

We emphasize that the case reported here is an individual who had had three vaccine doses already, the last one in January 2022. In a survey also carried out in Denmark, Lyngse et al. [4] studied cases of individuals infected and reinfected with Omicron sublineages for both vaccinated and unvaccinated. The conclusions pointed out by the study were that the BA.2 strain is more transmissible than the BA.1 and, in addition, an increase in the transmissibility of BA.2 was observed among unvaccinated individuals. This same study also states that both booster-vaccinated and fully vaccinated individuals had reduced susceptibility and transmissibility compared to unvaccinated individuals for both sublineages, suggesting that the effectiveness of the vaccines remains significant.

In Brazil, few studies involving cases of reinfection with Omicron sublineages have been carried out. In the northeast region (State of Rio Grande do Norte), extensive research was carried out. The authors evaluated data from March 2020 to mid-February 2022. In this period, in a total of 172,965 individuals with symptoms, 58,097 tested positive for SARS-CoV-2 and 444 cases were of reinfection. Among these reinfection cases, nine were sequenced, and the genomic analyses revealed that the virus strains diverged between the primary infection and the reinfection, the latter being caused by the Omicron variant (BA.1) among individuals fully vaccinated against SARS-CoV-2. Since all individuals whose samples were sequenced had previous SARS-CoV-2 infection and were also fully vaccinated, the authors stated that the Omicron variant avoids both natural and vaccine-induced immunities, confirming the continued need to decrease transmission and develop effective blocking vaccines. Studies on the escape ability of the Omicron variant from the immune system have also been carried out [11].

Planas et al. [12] evaluated the resistance of Omicron to neutralization by monoclonal antibodies. Sera from recipients of Pfizer or AstraZeneca vaccines, sampled five months after complete vaccination, did little to inhibit the variant. Sera from convalescent COVID-19 patients collected 6 or 12 months after symptoms showed low or no neutralizing activity against Omicron. Thus, the authors concluded that Omicron eludes most therapeutic monoclonal antibodies and, largely, vaccine-induced antibodies. However, antibodies generated by a booster dose of vaccine neutralize Omicron. Hence, as demonstrated by other studies, vaccination does not lose its importance, as it avoids aggravation in cases of infection and reinfection by SARS-CoV-2 and its variants.

Based on this study and under the literature review, we can say that the present case of reinfection is the shortest described so far. Moreover, these points to the current situation of evolution and spread of SARS-CoV-2, in which reinfections may be an important consequence of

the potential escape from the immune response generated by previous infections and/or vaccination.

**Data availability** The sequences from this study have been registered in the GISAID database. More information are available upon request.

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