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BRIEF REPORT

SARS-CoV-2 BA.1 and BA.2 coinfection detected by genomic surveillance in Brazil, January 2022

Cristina Mendes de Oliveira[1] · Camila Malta Romano[2,3] · Luciane Sussuchi[1] · Bianca Della Croce Vieira Cota[1] · José Eduardo Levi[1,3]

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Abstract

In January 2022, our genomic surveillance network identified a SARS-CoV-2 BA.1 and BA.2 coinfection in a sample from a patient residing in Brazil. Our results suggest that the true number of SARS-CoV-2 coinfections remains largely underestimated.

On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. Since then, unprecedented genomic surveillance efforts have been made to track the diversity of circulating SARS-CoV-2 lineages and, in particular, to monitor the spread of variants of concern (VOCs) [1, 2]. In November 2021, the Omicron VOC was identified in South Africa, and it rapidly spread all over the world [2]. Since then, the Omicron VOC evolved into several sublineages, including BA.1, BA.2, BA.3, BA.4, and BA.5 [3]. Like some other VOCs, SARS-CoV-2 BA.1 and BA.2 share amino acid substitutions in the spike protein (G339D, S477N, T478K, and N501Y) that enhance binding to the human receptor angiotensin-converting enzyme 2 (ACE2) [4]. Some receptor-binding domain mutations (G496S, A67V, T95I, Del 69-70, Del 143-145, and the insertion EPE between amino acids 214 and 215) that are specific to the SARS-CoV-2 BA.1 lineage have been predicted to substantially reduce the protection provided by SARS-CoV-2 vaccines [5]. Humoral immunity induced by vaccines, to a certain extent, fails to protect against BA.1 and BA.2 [6]. Moreover, the reproduction number of BA.1 is 1.4-fold lower than that of BA.2, which is characterized by higher viral loads in human nasal epithelial cells [6].

On November 30, 2021, SARS-CoV-2 BA.1 was identified in Brazil by our DASA network (https://dasa.com.br/en/genov/). We began screening for BA.1 by targeted sequencing of S-gene target failures (SGTFs) when the presence of the spike protein del69/70 signature was found in our routine use of the COVID-19 TaqPath assay from Thermo Fisher [7]. By January 10, 2022, the Omicron BA.1 lineage represented 99% of all circulating SARS-CoV-2 variants in Brazil (https://portal.fiocruz.br/observatorio-covid-19). However, to investigate the presence of the BA.2 variant, which does not possess the del69/70 mutation, we conducted targeted sequencing of samples without the SGTF signature.

From January 17 to 25, 2022, we identified 47 samples that were S-gene target positive (SGTP). SGTP samples were subjected to next-generation sequencing (NGS) using the Illumina COVIDSeq Test on a NovaSeq 6000 instrument (Illumina, CA, USA) (local ethical approval, CAAE 45540421.0.0000.5455).

One of these samples, collected on January 19, 2022, from a 34-year-old male resident of the city of Volta Redonda, Rio de Janeiro state, was identified as lineage BA.1 using the Dragen™ Covid Lineage App, with S:Del 69-70. Intriguingly, TaqPath-based diagnostic PCR testing did not show the SGTF signature (C_t values: N, 19.64; S, 23.13; ORF1ab, 19.35), so we decided to investigate this sample in more detail.

A maximum-likelihood phylogenetic tree including this and other Brazilian sequences sampled in the same period

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Cristina Mendes de Oliveira and Camila Malta Romano contributed equally to this article.

✉ Cristina Mendes de Oliveira
cristina.mendes@dasa.com.br

[1] Research and Development, Dasa, Av. Juruá, 434, Barueri, São Paulo 06455-01, Brazil

[2] Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

[3] Universidade de São Paulo, Instituto de Medicina Tropical de São Paulo, São Paulo, Brazil

was built using IQTREE2 [8]. Our background reference dataset also included complete genome sequences of Omicron BA.1, BA.2, and BA.3, Delta, and the recombinant XD as references. As expected for recombinants or consensus sequences resulting from coinfection, the sample clustered with a long branch and low bootstrap support within BA.1 clade (Fig. 1A). This sequence, deposited in the GISAID database under ID no. EPI_ISL_11271349, had 99.59% non-N bases with a mean coverage of 2,833. However, NextClade pointed to several individual mutations that differed between the query sequence and the nearest neighbor sequence that are typically associated with the BA.2 clade, although the PANGO designation assigned it as lineage BA.1. We then analyzed the mutation profile of the sequencing reads in detail and observed the presence of BA.1 characteristic mutations in the spike protein (A67V, T95I, Y145D, G496S, T547K, N856K, L981F, del 69-70, and del 142-144), as well as BA.2-specific amino acid mutations (T19I, V213G, S371F, T376A, D405N, and R408S) in different proportions, strongly suggesting that the sequence obtained was not due to a BA.1/BA.2 recombinant but instead to a coinfection with both of these lineages (Table 1).

Figure 1B illustrates the mix present in the regions 21765-21770 and 21633-21641, where SARS-CoV-2 BA.1 and BA.2, respectively, are expected to have deletions. To confirm this observation, we performed an RT-PCR using specific probes to detect del69-70 and wild type, and both variants were amplified. Like most of the samples sent to

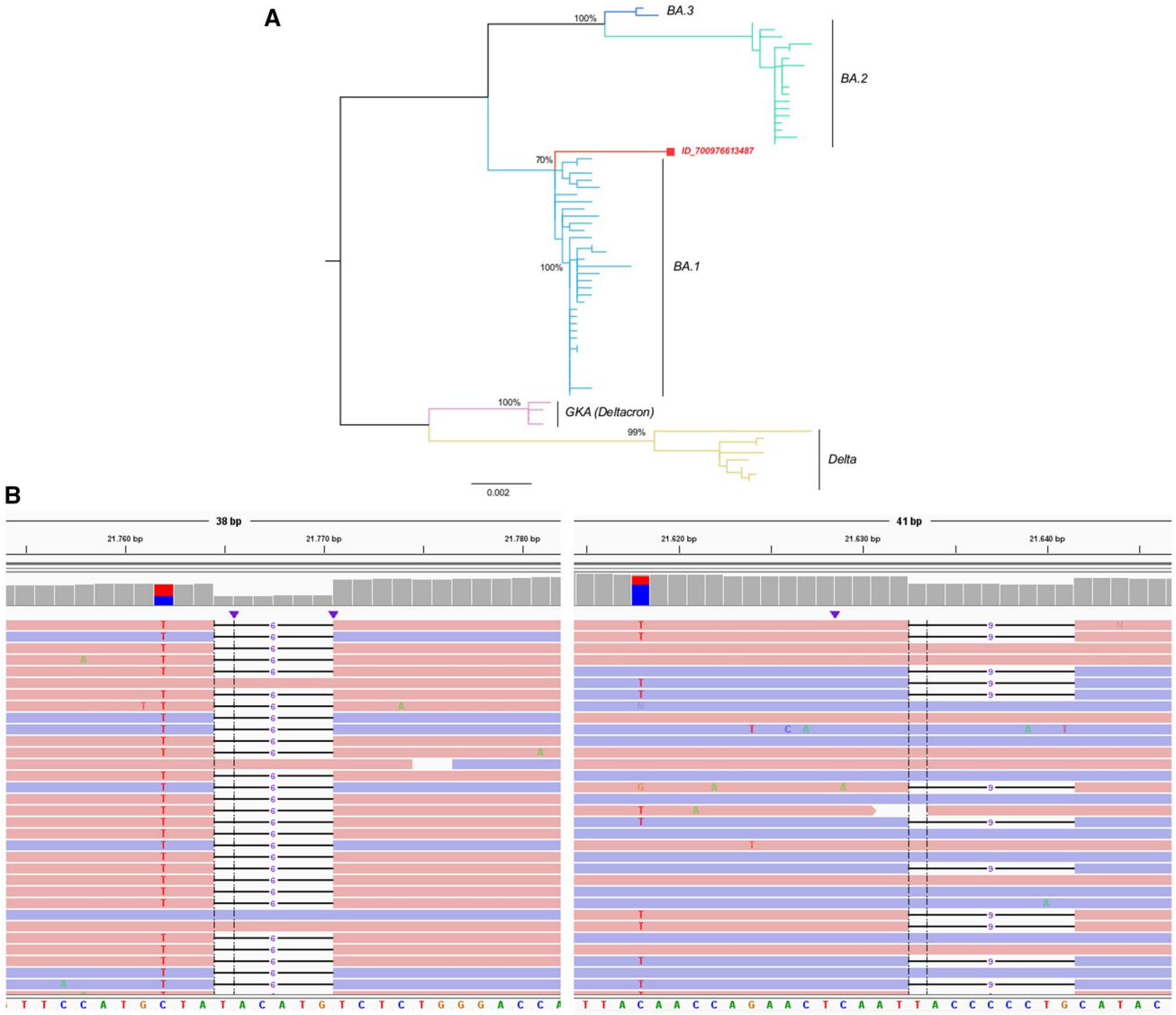

Fig. 1 (A) Maximum-likelihood phylogenetic tree including the study sequence and other Brazilian sequences sampled in the same period, constructed using IQTREE2. (B) Assembly of the reads of the study sequence showing lower coverage in the regions 21765-21770del and 21633-21641del, where SARS-CoV-2 BA.1 and BA.2, respectively, were expected to have deletions.

Table 1 Percentage of bases in key positions of the spike gene that can be used to discriminate between SARS-CoV-2 BA.1 and SARS CoV-2 BA.2

SARS-CoV-2 lineage	Mutation	Nucleotide Position	Nucleotide (%)
BA.2	T19I	21,618	C (69), T (28)
BA.1	A67V	21,762	T (56), C (43)
BA.1	T95I	21,846	T (66), C (33)
BA.2	V213G	22,200	G (93), T (7)
BA.2	T376A	22,688	G (95), A (4)
BA.2	D405N	22,775	A (96), G (3)
BA.2	R408S	22,786	C (96), A (4)
BA.1	G496S	23,048	A (71), G (26)
BA.1	T547K	23,202	A (58), C (41)
BA.1	N856K	24,130	A (64), C (35)
BA.1	L981F	24,503	T (68), C (32)

us for routine SARS-CoV-2 surveillance, this sample did not have associated clinical data such as vaccination history.

Coinfections with SARS-CoV-2 sublineages have been reported previously [9–12]. However, to our knowledge, this is the first report of coinfection with sublineages BA.1 and BA.2. SARS-CoV-2 coinfection should be closely monitored, since it is a *sine qua non* precondition for the emergence of recombinant viruses. Unfortunately, SARS-CoV-2 genomic surveillance is suboptimal in many countries [13], and rare events like these are likely to go unnoticed. Moreover, standard bioinformatics protocols using the Dragen™ Covid Lineage App and the absence of detailed genetic analysis of NGS reads often obscure the detection of putative recombinants or mixed infections. Therefore, we conclude that the number of coinfections with different SARS-CoV-2 sublineages most likely remains underestimated.

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References

1. World Health Organization Tracking SARS-CoV-2 variants
2. Tegally H, Wilkinson E, Giovanetti M et al (2021) Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 592:438–443. https://doi.org/10.1038/s41586-021-03402-9
3. Viana R, Moyo S, Amoako DG et al (2022) Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature. https://doi.org/10.1038/s41586-022-04411-y
4. Starr TN, Greaney AJ, Hilton SK et al (2020) Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell 182:1295-1310.e20. https://doi.org/10.1016/j.cell.2020.08.012
5. Planas D, Saunders N, Maes P et al (2022) Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 602:671–675. https://doi.org/10.1038/s41586-021-04389-z
6. Yamasoba D, Kimura I, Nasser H, et al (2022) Virological characteristics of SARS-CoV-2 BA.2 variant. Microbiology
7. Scott L, Hsiao N, Moyo S et al (2021) Track Omicron's spread with molecular data. Science 374:1454–1455. https://doi.org/10.1126/science.abn4543
8. Minh BQ, Schmidt HA, Chernomor O et al (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534. https://doi.org/10.1093/molbev/msaa015
9. Hosch S, Mpina M, Nyakurungu E et al (2022) Genomic surveillance enables the identification of co-infections with multiple SARS-CoV-2 lineages in equatorial Guinea. Front Public Health 9:818401. https://doi.org/10.3389/fpubh.2021.818401
10. Li Y, Jiang Y, Li Z, et al (2021) Both Simulation and Sequencing Data Reveal Multiple SARS-CoV-2 Variants Coinfection in COVID-19 Pandemic. Bioinformatics
11. Pedro N, Silva CN, Magalhães AC et al (2021) Dynamics of a dual SARS-CoV-2 lineage co-infection on a prolonged viral shedding COVID-19 case: insights into clinical severity and disease duration. Microorganisms 9:300. https://doi.org/10.3390/microorganisms9020300
12. Rockett RJ, Draper J, Gall M, et al (2022) Co-infection with SARS-COV-2 Omicron and Delta Variants Revealed by Genomic Surveillance. Infectious Diseases (except HIV/AIDS)
13. Brito AF, Semenova E, Dudas G, et al (2021) Global disparities in SARS-CoV-2 genomic surveillance. Epidemiology