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Genomic sequencing of SARS-CoV-2 samples at the central public health laboratory of Alagoas (Brazil) in June 2023

Abstract

Genomic surveillance has played a crucial role in monitoring the evolution and spread of SARS-CoV-2, particularly in identifying emerging variants that may influence disease transmission, vaccine efficacy, and therapeutic strategies. In this report, we present the genomic sequencing results of 17 SARS-CoV-2-positive samples collected in Alagoas, Brazil, between May 17 and 29, 2023, and sequenced at the Central Public Health Laboratory of Alagoas (LACEN/AL) from June 5 to 13, 2023. The sequencing was performed using the Illumina MiSeq platform, and lineage classification was conducted using PangoLineages and Nextclade tools. Our analysis identified seven Omicron sublineages: FE.1.2, XBB.1.5, FE.1, FL.4, XBB.1.18.1, XBB.1.5.26, and XBB.1.9.1. The predominance of FE and XBB lineages highlights the ongoing viral evolution and the replacement dynamics of circulating variants. Phylogenetic analysis revealed clustering patterns consistent with global trends, demonstrating the importance of continuous genomic surveillance in tracking SARS-CoV-2 evolution. These findings reinforce the necessity of monitoring emerging variants to inform public health interventions and improve pandemic response strategies. The genomic data generated in this study contribute to a broader understanding of SARS-CoV-2 genetic diversity and its epidemiological impact.

Keywords: SARS-CoV-2, genomic surveillance, variant tracking, omicron lineages, phylogenetic analysis, Brazil

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Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has highlighted the importance of genomic surveillance in identifying and monitoring viral evolution.[1] Genomic sequencing has proven to be an essential tool for detecting emerging variants, contributing to the understanding of epidemiological dynamics and the development of disease control strategies.[2] In Brazil, genomic surveillance has been conducted by various reference laboratories, enabling the tracking of viral lineage circulation across different regions of the country.[3,4] In the state of Alagoas, the Central Public Health Laboratory (LACEN/AL) plays a crucial role in this process, performing sequencing of SARS-CoV-2-positive samples to monitor the spread and evolution of variants within the state.[4] This study presents the results of genomic sequencing conducted by LACEN/AL from June 5 to June 13, 2023, covering 17 samples collected from symptomatic patients between May 17 and May 29, 2023. The analyses include the characterization of the identified lineages, their geographical distribution, and their epidemiological implications, emphasizing the importance of continuous monitoring in responding to the pandemic and in developing more effective public health policies.[5–7]

Methods

During the period from June 5 to June 13, 2023, the Genomic Surveillance Laboratory team, located at the Central Public Health Laboratory of Alagoas (LACEN/AL), conducted genomic sequencing. This procedure was applied to a set of 17 samples that tested positive for the SARS-CoV-2 virus using the RT-qPCR method. The samples were collected from patients presenting COVID-19 symptoms between May 17 and May 29, 2023, with Ct (Cycle threshold) values ranging from 15 to 27, averaging 19. The selection of samples for sequencing followed the criteria established in the documents "Vigilância genômica do vírus SARS-CoV-2 no âmbito da SVS/MS"[8] and "Ofício Circular Nº 2/2022/CGLAB/DAEVS/SVS/MS".[9]

Results and discussion

As shown in Figure 1, the samples were selected from individuals residing in different municipalities of the state of Alagoas: Arapiraca (5), Maceió (2), Coruripe (1), Delmiro Gouveia (1), Limoeiro de Anadia (1), Maragogi (1), Pilar (1), Rio Largo (1), Santa Luzia do Norte (1), and São Miguel dos Campos (1). These municipalities are distributed across six of the ten health regions that make up the state of Alagoas, as represented in Figure 1. Additionally, two sequenced samples belong to patients residing in Araçatuba (SP) and Aparecida de Goiânia (GO). For genomic sequencing, the MiSeq M07636 platform from Illumina Inc. was used,[10] ensuring data quality through the inclusion of a negative control (NC), following the guidelines of Technical Note No. 114/2022-CGLAB/DAEVS/SVS/MS.[11] No signs of contamination were detected in the negative control (NC), ensuring the integrity of the generated data. Genome assembly was performed using version 1.0 of the ViralFlow pipeline, available at https://viralflow.github.io/.[12] Lineage assignment was carried out using PangoLineages (data v1.20), available at https://github.com/hCoV-2019/pangolin, and Nextclade, available at https://clades.nextstrain.org/.[13] Nucleotide and amino acid identity was subsequently

analyzed using custom Python scripts. This sequencing effort yielded 16 complete genomes with coverage exceeding 90.48% of the total genome, with 14 of these genomes having coverage greater than 99.31% (Table 1).

Table 1 List of samples sequenced at LACEN/AL in June 2023

ID sample	CT	Municipality	Collection date	Age	Sex	Reads	Coverage	Identity NT
270249868	23.45	Araçatuba (SP)	19/05/2023	2 months	Female	569643	99.31	99.67
270249869	15.20	Limoeiro de Anadia	19/05/2023	80	Female	364949	99.52	99.66
270249870	16.03	Arapiraca	17/05/2023	52	Female	453896	99.52	99.67
270249872	20.83	Arapiraca	17/05/2023	57	Male	577149	99.33	99.68
270249874	27.19	Maceió	21/05/2023	79	Male	182949	90.48	99.72
270249875	18.73	São Miguel dos Campos	23/05/2023	56	Female	422218	99.59	99.66
270249876	19.41	Maragogi	24/05/2023	44	Female	498565	99.59	99.67
270249879	15.28	Coruripe	24/05/2023	29	Female	572251	99.59	99.67
270249881	18.72	Delmiro Gouveia	24/05/2023	45	Female	426225	99.39	99.66
270249882	23.94	Rio Largo	23/05/2023	78	Male	375116	98.38	99.68
270249884	17.99	Pilar	22/05/2023	27	Female	662972	99.67	99.67
270249885	17.59	Aparecida de Goiânia (GO)	26/05/2023	26	Female	800685	99.70	99.67
270249886	21.45	Arapiraca	25/05/2023	40	Female	809981	99.67	99.68
270249901	21.06	Arapiraca	25/05/2023	28	Male	814717	99.68	99.67
270249904	16.08	Santa Luzia do Norte	28/05/2023	86	Male	557118	99.74	99.66
270249906	18.61	Arapiraca	29/05/2023	12	Male	508440	99.59	99.68

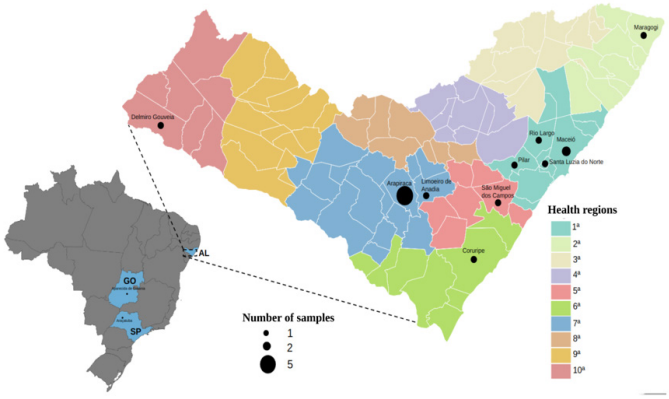

Figure 1 Geographic distribution of the sequenced samples in June 2023 in Alagoas (Brazil).

The genomic sequencing analysis conducted in May 2023 in Alagoas revealed the circulation of seven distinct lineages of the Omicron variant of SARS-CoV-2, with a predominance of the sublineages FE.1.2 (5 genomes) and XBB.1.5 (4 genomes).[14] These findings reinforce the virus's evolutionary dynamics and high genetic diversity, reflecting global trends in the dissemination of recombinant variants.[15] The XBB.1.5 lineage, detected in four samples, has been widely reported in several countries due to its high transmissibility and immune escape capability.[14] Previous studies have shown that this subvariant possesses mutations in the spike protein that enhance its affinity for the ACE2 receptor, contributing to its efficient spread.[16] The presence of other XBB-related lineages, such as XBB.1.18.1, XBB.1.5.26, and XBB.1.9.1, indicates that this lineage continues to evolve and acquire new genetic adaptations.[17–19] The predominance of the FE.1.2 lineage, derived from XBB, suggests a possible local adaptive advantage. This sublineage has been reported in various regions worldwide, particularly in the second half of 2023, and is associated with mutations that potentially enhance immune evasion.[20] The detection of FE.1 in a lower proportion (3 genomes) also highlights the diversity within this subfamily and its ability to co-circulate with other emerging variants. The identification of FL.4 (1 genome), another subvariant of XBB, is relevant for monitoring viral evolution, as these lineages frequently present additional mutations that may impact transmissibility and immune response. Although its presence was detected at a lower frequency, it is necessary to track its spread and epidemiological impact.[21,22] The co-circulation of these lineages underscores the need for continuous genomic surveillance to monitor the introduction of new variants and assess their consequences on transmissibility, immune escape, and response to public health interventions. These data are essential for guiding COVID-19 control strategies, including vaccine updates and preventive measures tailored to the circulating variants (Figure 2).[20]

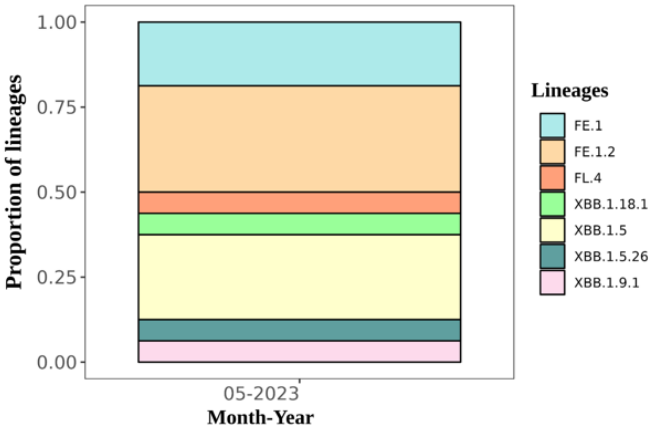

Figure 2 Proportion and distribution of SARS-CoV-2 sublineages by epidemiological week.

According to the obtained results, among the 16 sequenced samples, the FE lineages represent the majority of the collected

samples. FE.1.2 was detected in samples from Arapiraca, São Miguel dos Campos, Delmiro Gouveia, Pilar, and Santa Luzia do Norte, while FE.1 was observed in samples from Araçatuba (SP), Limoeiro de Anadia, and Coruripe. The XBB.1.5 lineage was detected in samples from Arapiraca, Maceió, and Maragogi. The XBB.1.9.1, FL.4, XBB.1.18.1, and XBB.1.5.26 lineages were detected in Arapiraca, Aparecida de Goiânia (GO), Rio Largo, and Arapiraca, respectively. Since January 2023, a significant increase in the XBB.1.5 lineage has been observed in Alagoas. In addition to the findings presented in this study, XBB.1.5 accounted for 36.4% (60 out of 165) of all identified lineages between January and May, followed by FE.1 at 17.62% (29 out of 165). These data, obtained from January to June, suggest a gradual replacement of XBB.1.5 by FE sublineages, following the global trend.[23–25] According to the study by Qu et al.,[26] the predominance of the XBB.1.5 lineage was attributed to its increased infectivity and pathogenicity, driven by additional mutations in the Spike protein. As observed by Wang et al.,[27] the G252V mutation present in XBB.1.5 is considered the main factor responsible for its rapid spread.[28] With the emergence of additional mutations in viral genomes, evolutionary differences become more pronounced, particularly in the S gene, which encodes the Spike protein, playing a key role in viral entry into human cells.[1,24,25,29] Figure 3 presents a phylogenetic analysis using Pangolin classification of sequenced genomes, grouping them into different clades that demonstrate their evolutionary differences, as described by Zhao et al.[30] The XBB.1.5 and XBB.1.5.26 lineages belong to clade 23A, while FE.1.2, FE.1, FL.4, XBB.1.18.1, and XBB.1.9.1 belong to clade 22F.[21,31,32] The predominant lineages in Alagoas, classified as XBB lineages, share the S:N460K mutation, along with other mutations that may confer evolutionary advantages, allowing them to evade specific antibodies targeting the Spike protein. This enhances their spread, as observed in previous studies.[33,34] It is important to emphasize that Omicron variants are in constant evolution and diversification, acquiring mutations that may facilitate their rapid dissemination and increase the pathogenicity of certain lineages.[35] Moreover, the evolution of SARS-CoV-2 is an ongoing process, and our understanding of viral lineage differences continues to evolve as new insights are gained through genetic sequencing.[36,37]

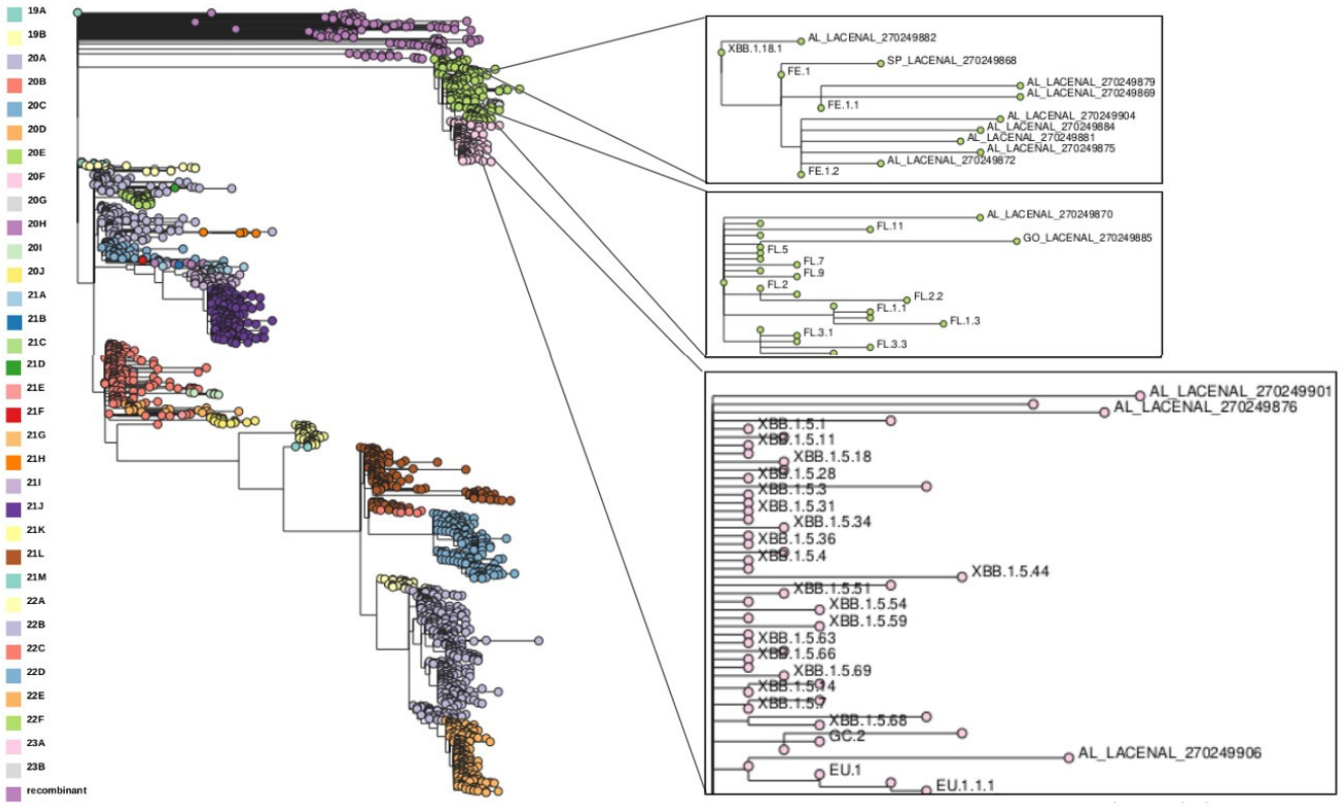

Figure 3 Phylogenetic tree of SARS-CoV-2 in the state of Alagoas, using genomic sequences isolated in Alagoas and the Nextclade database.

Conclusion

SARS-CoV-2 genomic sequencing plays a crucial role in combating the pandemic. This technique provides valuable insights into circulating viral lineages and variants, enabling continuous monitoring of viral evolution. This helps identify mutations, improve our understanding of transmissibility, virulence, and potential resistance to treatments and vaccines.[38] This study confirms the prevalence and evolution of the Omicron variant in Alagoas, highlighting the importance of epidemiological surveillance and regular genomic sequencing to detect new circulating variants and understand their phylogeny. Additionally, it underscores the central role of LACEN in this context, significantly contributing to monitoring viral evolution, early detection of concerning variants, and implementing effective strategies to control disease spread.

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Conflicts of interest

The authors declared that there are no conflicts of interest.

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