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MORTALITY-ASSOCIATED SARS-COV-2 GENOMIC VARIANTS FROM PATIENTS HOSPITALIZED FOR SEVERE PNEUMONIA IN AGUASCALIENTES, MEXICO FROM 2020 TO 2023

Running title: PNEUMONIA COVID-19 VARIANTS FROM MEXICO

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ABSTRACT (204 words)

Background

The SARS-CoV-2 virus has caused a global health crisis, resulting in a significant loss of human lives. It is essential to report disease and mutation associations to provide ideas for public health interventions and preventive measures.

Methods

In this study, to determine the association between genomic variants and the severity of pneumonia caused by SARS-CoV-2, a sequencing analysis of 150 patient samples with confirmed COVID-19 was conducted. These samples were collected between 2021 and 2023 and isolated in Aguascalientes, Mexico.

Results

The patient cohort had males and females ranging from 0 to 91 years old. Males accounted for 66% of the population analyzed. The Delta variant was the most prevalent lineage associated with deaths in 2021-2022, while the B.1.1.529 lineages emerged in mid-2022. Currently, the XBB lineage is the most commonly identified in Mexico. New mutations L95M and L46M in ORF 8 and ORF 9 were discovered in 30% and 20% of the sequences and are uniquely present in the studied population. These mutations are positively associated with patient death.

Conclusions

This study provides valuable data to aid in understanding the evolution of SARS-CoV-2 in specific populations and explores the severity of the disease and mutation correlations.

KEYWORDS

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molecular epidemiology, pandemic, regional evolution, virus adaptation, LatAm

INTRODUCTION

At the end of 2019, a new coronavirus emerged in Wuhan, China. Initially, it was identified as WH-Human 1 coronavirus (WCHV). It exhibited genomic similarity of 79.8% related to SARS-CoV and 59.1% associated with MERS-CoV, as well as showing 100% amino acid similarity in the E and nsp7 with bat SL-CoVZC45 proteins, all possibly derived from recombination events [Wu et al., 2020]. SARS-CoV was determined to have high homologous similarity to CoV bat virus RmYN02 in most of its genome but shows weak homology in the receptor binding domain (RBD) that makes up the S1 subunit of the spicule (S) protein, which represents a critical factor in host receptor recognition and the process of cell membrane fusion with angiotensin-converting enzyme 2 (ACE2). The tissue ubiquity of the latter explains the multiple manifestations and affectations of the disease [Ren et al., 2020].

Coronaviruses, members of the Orthocoronoviridae family within the Coronoviridae domain, are enveloped viruses with a single-stranded positive RNA genome (approximately 26 to 32 kb in length). They are classified into four genera: Alphacoranaviruses (□), Betacoronaviruses (□), Gammacoronaviruses (□), and Deltacoronaviruses (□). The viral genome typically encodes four structural proteins: Spike (s), envelope (E), membrane (M), and nucleocapsid (N), along with numerous non-structural proteins and multiple accessory proteins [Ren et al., 2020].

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Now recognized as Severe acute respiratory coronavirus 2 (SARS-CoV-2), it is a highly contagious virus that caused the COVID-19 pandemic disease that brought the world to a standstill, with a primary reproduction number (Ro), which depends on several factors, including the circulating variant, therefore, for Delta the Ro was 3.2 and for Omicron it was 9.5, which also depends on the population density, the mobility patterns, social interactions, and vaccination policies (Roy S, et al, 2023). It is a single-stranded positive RNA virus with a length of 29,891 nucleotides, encoding 9,860 amino acids. Its genome comprises 14 open reading frames (ORFs) and 27 proteins, of which the structural proteins are spike (S), envelope protein (E), membrane protein (M), and nucleocapsid (N) (Chan et al., 2020). As of December 2023, there have been 772 million confirmed cases of SARS-CoV-2 worldwide and 6.9 million deaths attributed to the disease. In Mexico, the first case of COVID-19 was detected on February 27, 2020, and as of today, there are 7.7 million confirmed cases and 334,917 deaths [WHO, 2023; Garcés-Ayala et al., 2020]. The pandemic propelled advancements in molecular biology techniques that allowed us to answer crucial questions, particularly the Delta and Omicron variants. It clarified everything related to the development of the disease, such as the relationship between the characteristics presented and the risk of death or infection susceptibility in prolonged-stay settings [Anderegg et al., 2023; Iqbal et al., 2023].

Epidemiological surveillance has proven indispensable in identifying mutations that affect the virus´s behavior, enhancing its infectious capacity, or evading the immune system.

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It has been reported that Latin American countries have experienced an increase in the frequency of virus mutation, with a particular prevalence in these countries compared to other regions. This geographical area could be the origin of new and potentially dangerous virus variants that could later spread globally. This report only covers the period of 2021-2022 [Dhruv Yadav et al. 2022].

In the first year of the pandemic in Mexico, numerous lineages were detected, some transient. In contrast, others, such as B.1, B.1.1.1, and B.1.1.519, among others, represented up to 76% of the circulating lineages in Mexico during this first year, in addition to exhibiting the mutations P314L in protein nsp12 and D614G in spike protein with a prevalence of 99% and 98 % respectively [Taboada et al., 2021]. The spike protein has been a focal point of numeral studies, given its high mutation rate, which gives it the ability to have immune escape. This capability allows the virus to infect even vaccinated or previously infected individuals [Harvey et al., 2021].

After the first year of the pandemic, the Delta variant, with AY20 and AY.26 lineages, replaced other variants and marked the third wave. Finally, the Omicron variant and its respective lineages entered in December 2021, initiating the fourth wave becoming the variant with the highest daily case count. This variant and the recombinant XBB sublineage have spread even among vaccinated populations [García-López et al., 2022]. It is unclear whether the reported regional mutations are related to the alarming case-fatality rates in Mexico. Before vaccination, the rate was 10%, but it has dropped to 4,5% in 2024. These rates are higher than the worldwide average (John Hopkins University, 2024).

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Therefore, this project aims to determine the genomic sequence of SARS-Cov2 in samples detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR) in central Mexico from patients with severe respiratory infection to establish a possible association between the severity of pneumonia, death incidence, and genomics variants.

METHODS

Clinical data were collected from 300 biosamples from deaths in the state of Aguascalientes who tested positive for COVID-19, confirmed by RT-qPCR, spanning from August 2021 to May 2023. The clinical information includes demographic data (age, gender, and vaccination status), comorbidity details, symptoms, and their duration until death (Table 1). Samples with CT values of 28 or less were selected for sequencing using the Illumina CovidSeq protocol following the manufacturer's instructions and utilizing the Illumina MiniSeq Sequencing System. In total, 173 samples were subjected to sequencing.

The obtained FASTQs from the sequencing process were assembled using CLC Genomics Workbench software, with Wuhan-Hu 1 (NC_045512.2) as the reference genome. Subsequently, the Genome Annotation of the BV-BRC platform (https://www.bv-brc.org/app/Annotation) was used to visualize the general genome structure. Based on the quality of each genome, nucleotide contents were analyzed, and those containing 10% or more Ns quantity were excluded from the study. Finally, 150 good-quality sequences were selected for analysis and

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uploaded to the GISAID platform (https://gisaid.org/) for consultation. Sequence identifiers are stated in Table 2.

Following the PANGO designation (https://cov-lineages.org/lineage_list.html), the lineage data were interpreted as delta and omicron variants for further analysis. Frequency and percentages were calculated for all the subject characteristics. The mean (±SD) was calculated for the age and duration of the symptoms until death. The normality of data was assessed using the Shapiro-Wilk test. The p-value was calculated through the chi-square test for categorical variables, comparing the presence and absence of each comorbidity and symptom and the male or female gender. Mann - Whitney test was used for non-parametric data comparing the ages of the subjects infected with the delta or omicron variant and the duration of the symptoms until death between the two variants.

Multiple sequence alignments were generated using the online program Multiple Sequence Alignment and SNP / Variation Analysis at the BV-BRC platform (https://www.bv-brc.org/app/MSA) for the different structural and non-structural proteins in different groups and as a whole. The representative genomes of the B, B.1.1.529, BA.1, B.1.351, B.1.617, B.1.1.7, B.1.429, and XBB lineages were used for alignments, using Wuhan-Hu 1 as the reference genome. In the same way, a control group of 443 genomes of the circulating lineages in Aguascalientes between 2021 to 2023 was generated. The genomes selected for the control group are of the period mentioned and at least one sequence of each state of Mexico was introduced.

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The alignments were carefully inspected to identify mutations with at least 45% prevalence among the total analyzed sequences. These selected mutations provided an overview of circulating mutations in the center of México between 2021 and 2023. A matrix was made considering the absence and presence of the mutation in each sequence. Using R programming language, a heat map was generated to represent the presence of mutations in each sequence visually. Similarly, the mutations not found in the genome references used in the alignments were considered New Mutations until they were not referenced in official media or previous works. Subsequently, another matrix was used to create a heat map with clustering showing the prevalence of each mutation against lineages from 0 to 100%.

DATA AVAILABILITY: Sequence data is available on the GISAID platform. All other data is presented in the included tables and figures.

RESULTS

All patients that were included in this study lost their lives due to the COVID-19 infection or associated conditions. Out of 173 samples analyzed in the genetic analysis, 150 were found to have sufficient quality for further examination and were identified with SARS-CoV2 variants. Of these 150 results, 63 (42%) were identified as delta variants and 87 (58%) as omicron variants. The characteristics, symptoms, and comorbidities of the delta and omicron-infected patients are presented in Table 1. The average age of the patients was 64 years, and there was only a 3-year age difference between averages for the age of the omicron and

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delta-infected patients. Male patients passed away more frequently than female patients, with 66% and 34%, respectively, and this trend was observed in both groups of variants.

The vaccinated population represented 27%, and further data is needed to determine the effect on disease severity. Hypertension was the most common comorbidity, present in 51% of the subjects, followed by diabetes mellitus at 38%. Asthma was the pre-existing medical condition with the smallest number of cases, occurring only in 3 subjects (2%).

An analysis was conducted to determine the duration between the onset of COVID-19 symptoms and death among infected patients. The mean duration was found to be 16±10 days. However, patients infected with the omicron variant had a shorter mean duration of 14 days, while patients infected with the delta variant had a statistically significant more extended duration of hospitalization by an additional four days ($p<0.05$). All infected patients exhibited at least two symptoms. Dyspnoea was the most frequent symptom reported by 90.6% of patients. Cough was reported by 115 (76.6%) patients, with 54 and 61 patients infected by delta and omicron variants, respectively, and had statistically significant ($p<0.05$). Fever was the most frequent complication reported (72.6%), followed by headaches and diarrhea, with diarrhea being the least frequent (12.6%).

Table 1. Demographic and clinical characteristics of study subjects.

n (%)	Total (n=150)	Delta (n=63)	Omicron (n=87)	p-value
Demographics				
Age, years, mean (±SD)	64 (19.95)	62 (19.84)	65(20.00)	0.068*

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Male Female	99 (66) 51 (34)	40 (63.4) 23 (36.5)	59 (67.8) 28 (32.1)	0.581** ***
COVID Vacinated	41 (27.3)	14 (22.2)	27 (31.0)	0.231**
Comorbidities	116 (77.33)	47 (74.6)	69 (79.31)	0.496**
Diabetes	57 (38)	21 (33.3)	36 (41.4)	0.316**
Hypertension	77 (51.3)	32 (50.8)	45 (51.7)	0.910**
Chronic kidney disease	18 (12)	5 (7.9)	13 (14.9)	0.192**
Smoking	15 (10)	5 (7.9)	10 (11.5)	0.473**
Heart disease	7 (4.6)	2 (3.2)	5 (5.7)	0.460**
Asthma	3 (2)	1 (1.6)	2 (2.3)	0.758**
COPD	8 (5.3)	4 (6.3)	4 (4.6)	0.641**
Immunosuppression	10 (6.6)	0 (0.0)	10 (11.5)	**0.005****
Obesity	15 (10)	5 (7.9)	10 (11.5)	0.473**
Presenting complaints				
The duration between the appearance of symptoms and death, days mean (±SD)	16 (9.9)	18 (11.1)	14 (8.4)	**0.008***
Fever	109 (72.6)	46 (73.0)	63 (72.4)	0.934**
Cough	115 (76.6)	54 (85.7)	61 (70.1)	**0.025****
Odynophagia	63 (42.0)	27 (42.9)	36 (41.4)	0.856**
Dyspnoea	135 (90.6)	58 (92.1)	77 (88.5)	0.473**
Diarrhea	19 (12.6)	5 (7.9)	14 (16.1)	0.138**
Chest pain	33 (22.0)	17 (27.0)	16 (18.4)	0.209**
Headaches	96 (64)	44 (69.8)	52 (59.8)	0.204**

* The p-value is calculated from the Mann - Whitney test, and the ** p-value from the chi-square test.*** p-value is shown in Male cell, Gender was used as a categorical variable.

Over the three years of study, the evolution of the SARS-CoV2 virus was monitored for epidemiological surveillance in Aguascalientes. Genomic surveillance began in June 2021 and continued until May 2023 (Fig.1a). At the start of the study, the most prevalent lineages in the samples belonged to the delta variant (Table 2), with the AY.20 lineage having the highest number of deaths in the state. However, in early 2022, the prevalence of omicron lineages began to increase, with the B.1.1.529 lineages being the most prevalent. By early 2023, the XBB lineages occupied at least 50% of the COVID-19 cases and rapidly increased to 90% of the sequenced instances over the next few months. Despite this, the BA.1 (belonging to the omicron variant) and AY.3 (belonging to the o delta variant)

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lineages were responsible for most of the recorded deaths, with 40 and 30 cases, respectively (Fig. 1b).

Figure 1. Genomic data in Aguascalientes from June 2021 to May 2023. **a** Lineage prevalence of SARS-CoV2. **b** Number of genomes sequenced from each SARSCoV2 lineage. Lineages for less than one genome are collated into “Other Lineage.”.

The analysis of alignments revealed mutations in all sequences compared to the reference. Mutations present in at least 45% of the sequences were selected for inspection. As expected, sequences identified in 2021 have fewer mutations than those found in 2022 and 2023 (Figure 2). Nevertheless, some sequences belonging to delta variants have more mutations than other sequences of the same lineage (AY.3). Among all the mutations found, the spike protein has the most mutations compared to other structural or non-structural viral proteins. However, the most common mutation in most samples, except for five genomes, is T492I in the nsp4 protein sequence. The mutations P323L for RNA-dependent RNA polymerase (RdRP) and T485K for the spike protein are also present in most sequenced genomes.

Additionally, we found two mutations not present in the reference sequences used for different genomes or the control group, shown as New Mutations in Figure 2. The first is L95M for the ORF 8 sequence, representing 31.33%, was found for the first time in the sequence obtained in December 2021, is located in the C-terminal end of the protein (Fig. 5b). The second is L46M for the ORF 9 sequence,

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representing 22.66%, and appeared in a sample of January 2022, this sample presented both mutations as most of the identified sequences located in the region of interaction with human TOM70 (Fig 5c). Dyspnoea, cough, and hypertension were the clinical data most common in patients whose specimens presented at least one mutation.

Figure 2. Heat map of the presence and absence of mutations found in each genome sequence of the 150 used. The lineage, year of detection, and mutation sequence have different color codes for better identification. Likewise, the New Mutations show a different color than the previously Reported Mutations.

The figure 3 shows the absence of these mutations in the control group of 443 sequences from all the states of Mexico, this behavior allows us to assume the discovery of mutations associated with a population. They seem exclusively present in the Aguascalientes state, making this a significant finding for the region. However, further investigations are crucial to determine if these mutations exist in other parts of Mexico. This discovery could have implications for our understanding of genetic variations and their potential impact on human health.

Figure 3. Comparison of LOGO obtained from Multiple sequence alignment (MSA) for ORF 8 and ORF9 sequences between the control and study groups. **a** Schematic LOGO of MSA of the study group highlighting the L95M mutation site for ORF 8 sequence. **b** Schematic LOGO of MSA of the control group highlighting the absence of the L46M mutation for ORF 8 sequence. **c** Schematic LOGO of MSA of the study group highlighting the L46M mutation site for ORF 9 sequence.

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d Schematic LOGO of MSA of the control group highlighting the absence of the L95M mutation for ORF 9 sequence.

As shown in Figure 4, the BA.5 lineage has all the mutations found, showing a prevalence of at least 75% in the corresponding sequences of this lineage. The new mutations have the highest prevalence in the lineages BA.1, BA.2, and BA.5, reaching up to 75% in the corresponding sequences. On the other hand, lineages AY.26, AY.43, and AY.20 have fewer mutations, with only four mutations in all three cases. The mutation T492I for nsp4 is present in all lineages with 100% frequency except for the AY.26 lineage, where it is present in only three genomes, representing 37.5% of the sequences of this lineage.

Figure 4. Heat map of mutation prevalence (0 to 100%) in the genomes belonging to lineages found in deaths reported in México between 2021 and 2023. The mutation sequence and the New and Reported Mutations have different color codes for better identification.

The mutation distribution (Figure 5) shows that most mutations are present in the structural and accessory regions of SARS-CoV2. The Spike sequence, in particular, has nine representative mutations out of the 150 genomes analyzed in this study. The graphical genome is a general representation of the SARS-CoV2 genome structure that circulated in the center of México between 2021 and 2023.

Figure 5. Graphical representation of the SARS-CoV2 and overview of accessory proteins with the new mutations. **a** Genome organization of SARS-CoV-2, the nonstructural proteins are shown in green, the structural proteins are in red, and

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the accessory proteins are in blue. The mutations are highlighted in their location, with the new ones colored red. **b** Schematic representation of ORF 8 protein with the L95M mutation site highlighted by the amino acid methionine. **c** Schematic representation of ORF 9 protein with the L46M mutation site highlighted by the amino acid methionine.

Table 2. Accession numbers and metadata of viral genome sequenced.

GISAID Identifier	Gender	Intubated	Age	Comorbidity	Vaccinated	Vaccine type
EPI_ISL_7697229	Male	Yes	50	DM, HTN	Not Applied	
EPI_ISL_3460083	Male	No	36	SMK	Not Applied	
EPI_ISL_3265656	Female	Yes	32	DM, HTN, CKD	Not Applied	
EPI_ISL_3265404	Male	Yes	50	DM	Not Applied	
EPI_ISL_4199549	Male	Yes	82	DM, HTN, CKD	Not Applied	
EPI_ISL_4199283	Male	Yes	83		Not Applied	
EPI_ISL_4199489	Female	Yes	71	DM, HTN	Complete	Pfizer BioNTech
EPI_ISL_4199270	Male	No	60	DM, HTN	Not Applied	
EPI_ISL_4199250	Male	Yes	46		Not Applied	
EPI_ISL_4199501	Male	Yes	45		Not Applied	
EPI_ISL_4199548	Female	Yes	85	HTN	Incomplete	Sinovac
EPI_ISL_4199452	Female	Yes	26		Not Applied	
EPI_ISL_4199274	Male	No	60	DM, HTN	Not Applied	
EPI_ISL_4199536	Female	No	60	DM, HTN, Obes.	Not Applied	
EPI_ISL_4199523	Male	No	48		Not Applied	
EPI_ISL_4199540	Male	Yes	76	HDS	Complete	Sinovac
EPI_ISL_4298937	Female	Yes	54		Not Applied	
EPI_ISL_4298949	Male	Yes	62	SMK	Not Applied	
EPI_ISL_4298944	Male	No	50		Not Applied	
EPI_ISL_5428098	Male	Yes	84	HTN	Not Applied	
EPI_ISL_4602916	Male	No	41		Not Applied	
EPI_ISL_18082278	Male	Yes	72	HTN	Not Applied	
EPI_ISL_4602989	Female	No	55	ATA	Not Applied	
EPI_ISL_5686679	Female	Yes	40	Obes.	Not Applied	
EPI_ISL_5686678	Female	Yes	23	DM, HTN, CKD, Obes.	Not Applied	
EPI_ISL_6571042	Male	Yes	59	HTN	Complete	CanSino
EPI_ISL_6571144	Male	No	74	HTN	Not Applied	
EPI_ISL_6571037	Female	Yes	82		Not Applied	
EPI_ISL_18090397	Female	No	82	HTN	Complete	Pfizer BioNTech
EPI_ISL_7235730	Female	Yes	85	DM, HTN	Not Applied	
EPI_ISL_7717027	Male	No	67	HTN	Complete	Sinovac
EPI_ISL_7716894	Female	No	62	HTN	Not Applied	

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EPI_ISL_18090385	Male	No	72		Not Applied	
EPI_ISL_18082258	Male	Yes	80	DM, HTN	Complete	Pfizer BioNTech
EPI_ISL_18090404	Male	Unknown	68	DM	Not Applied	
EPI_ISL_18090399	Male	Yes	68	HTN	Not Applied	
EPI_ISL_18082259	Male	Yes	83	SMK	Not Applied	
EPI_ISL_12658033	Male	Yes	63	DM, HTN	Complete	Sinovac
EPI_ISL_18090395	Male	Unknown	69	DM, HTN, Obes.	Incomplete	CanSino
EPI_ISL_18090405	Male	Yes	74	HTN, CKD	Complete	Sinovac
EPI_ISL_12643878	Male	No	66		Complete	Sinovac
EPI_ISL_18082250	Female	No	74	DM, HTN	Incomplete	Sinovac
EPI_ISL_18090400	Male	Yes	55	DM, HTN	Not Applied	
EPI_ISL_18082251	Female	No	70	DM, HTN	Not Applied	
EPI_ISL_18090401	Male	Yes	79	HTN, HDS	Not Applied	
EPI_ISL_18090403	Male	No	65		Not Applied	
EPI_ISL_18082252	Male	Yes	84	HTN	Complete	Sinovac
EPI_ISL_18090398	Male	Yes	55		Not Applied	
EPI_ISL_18082279	Male	Yes	67		Not Applied	
EPI_ISL_9140156	Female	No	85	HTN	Complete	Pfizer BioNTech
EPI_ISL_8814194	Male	No	39		Not Applied	
EPI_ISL_18082253	Female	Yes	59	COPD, HTN	Not Applied	
EPI_ISL_18082281	Male	Yes	45	Obes.	Not Applied	
EPI_ISL_9030990	Male	No	58	DM, HTN	Complete	AstraZeneca
EPI_ISL_18090402	Female	No	45	DM	Not Applied	
EPI_ISL_18082254	Female	Yes	80	COPD, HTN	Complete	Pfizer BioNTech
EPI_ISL_18082294	Male	No	45	DM	Not Applied	
EPI_ISL_18082249	Male	Unknown	51	DM, SMK, Obes.	Not Applied	
EPI_ISL_9031700	Female	Unknown	59	HTN	Not Applied	
EPI_ISL_18082280	Male	Yes	64	HTN, CKD	Not Applied	
EPI_ISL_18082282	Male	No	32	HTN, SMK	Not Applied	
EPI_ISL_9707547	Male	Yes	62	DM, HTN, CKD	Complete	Sinovac
EPI_ISL_9086658	Male	No	33	DM, HTN, SMK	Not Applied	
EPI_ISL_18082261	Female	Yes	3		Not Applied	
EPI_ISL_9804095	Male	Unknown	92	COPD	Not Applied	
EPI_ISL_12658274	Male	No	75	Obes.	Not Applied	
EPI_ISL_9754766	Male	Yes	90	DM, HTN	Complete	Sinovac
EPI_ISL_18082283	Male	Yes	56		Not Applied	
EPI_ISL_12658263	Male	No	91	HTN	Complete	Sinovac
EPI_ISL_12658275	Female	Yes	86		Complete	Pfizer BioNTech
EPI_ISL_12658276	Male	No	86	HTN	Complete	Sinovac
EPI_ISL_18090391	Male	No	5	DM	Not Applied	
EPI_ISL_11533135	Male	Yes	5	DM	Not Applied	
EPI_ISL_9760051	Male	No	65	DM, HTN	Not Applied	
EPI_ISL_18090396	Female	Yes	1		Not Applied	
EPI_ISL_9761233	Male	No	87	HTN, SMK	Incomplete	Sinovac
EPI_ISL_9706609	Female	No	63	HTN	Not Applied	
EPI_ISL_9799167	Female	Yes	84	COPD	Not Applied	
EPI_ISL_9804226	Female	No	90		Not Applied	
EPI_ISL_10158345	Male	Unknown	69	Obes.	Not Applied	
EPI_ISL_18090392	Male	No	75	DM, CKD	Not Applied	

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EPI_ISL_10029425	Male	Yes	61	DM, HTN	Not Applied	
EPI_ISL_9804200	Male	No	72	HTN	Not Applied	
EPI_ISL_10030127	Male	No	64	DM	Not Applied	
EPI_ISL_18082262	Female	No	65	DM, HTN	Not Applied	
EPI_ISL_18082284	Female	Unknown	73		Not Applied	
EPI_ISL_18090386	Male	Yes	62	DM, HTN, HDS	Not Applied	
EPI_ISL_12658120	Male	Unknown	72	DM, HTN,, SMK	Not Applied	
EPI_ISL_10027505	Male	Unknown	61	DM, HTN, CKS, SMK	Not Applied	
EPI_ISL_18090393	Male	No	48	HTN	Not Applied	
EPI_ISL_10158771	Female	No	72		Complete	Moderna
EPI_ISL_18090387	Female	No	47	DM	Not Applied	
EPI_ISL_10157697	Female	No	39	DM, HTN, CKD	Complete	CanSino
EPI_ISL_18082285	Male	No	85	HDS	Not Applied	
EPI_ISL_18082265	Male	Yes	71	SMK	Not Applied	
EPI_ISL_18082263	Female	Unknown	47	HTN, CKD	Complete	Pfizer BioNTech
EPI_ISL_18082264	Male	No	39	HTN, CKD	Not Applied	
EPI_ISL_11013529	Male	No	4		Not Applied	
EPI_ISL_18090394	Female	Yes	58		Not Applied	
EPI_ISL_11999110	Female	Yes	36	DM, IS, HTN, CKD, SMK	Not Applied	
EPI_ISL_18090388	Female	No	69	HTN	Not Applied	
EPI_ISL_18082296	Female	Yes	88	DM, HTN, CKD	Complete	AstraZeneca
EPI_ISL_18082266	Female	Yes	63	DM, HTN	Not Applied	
EPI_ISL_12000207	Female	No	82	HTN	Not Applied	
EPI_ISL_12657413	Male	No	74	HTN	Incomplete	Sinovac
EPI_ISL_18082267	Male	No	70	SMK	Complete	Unknown
EPI_ISL_16818752	Female	No	61	DM, HTN, CKD	Complete	Sinovac
EPI_ISL_18082260	Male	No	79		Not Applied	
EPI_ISL_9084929	Female	No	85	HTN	Complete	Pfizer BioNTech
EPI_ISL_14378963	Male	Unknown	75	HTN, SMK	Incomplete	Pfizer BioNTech
EPI_ISL_18082268	Male	No	81	HTN	Complete	Pfizer BioNTech
EPI_ISL_14333734	Male	No	76	COPD, HTN, SMK	Complete	Unknown
EPI_ISL_14528539	Female	No	67	DM, HTN, CKD	Not Applied	
EPI_ISL_18082286	Male	No	82	COPD, IS, SMK	Not Applied	
EPI_ISL_18082270	Male	No	51	DM, HTN, CKD	Not Applied	
EPI_ISL_18082269	Female	Unknown	64	DM, HTN, Obes.	Not Applied	
EPI_ISL_18082255	Female	Unknown	89	HTN	Complete	Sinovac
EPI_ISL_14817514	Male	Yes	61		Not Applied	
EPI_ISL_18082300	Male	No	70	DM, HTN	Not Applied	
EPI_ISL_16474247	Male	No	81	DM	Incomplete	AstraZeneca
EPI_ISL_16474239	Male	No	69	HTN	Complete	Pfizer BioNTech
EPI_ISL_16486634	Male	Yes	79		Not Applied	
EPI_ISL_16486632	Male	No	67		Not Applied	
EPI_ISL_18082271	Male	Unknown	77	DM	Not Applied	
EPI_ISL_16515071	Male	No	87		Not Applied	
EPI_ISL_16515070	Male	Yes	41	DM, CKD	Not Applied	
EPI_ISL_16515087	Male	No	72	DM, HTN, Obes.	Not Applied	
EPI_ISL_12658184	Male	Yes	49	DM	Not Applied	
EPI_ISL_18082287	Male	Unknown	73		Not Applied	

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EPI_ISL_18082272	Male	No	78	IS	Not Applied	
EPI_ISL_18082297	Male	Unknown	70	Obes.	Not Applied	
EPI_ISL_18082256	Female	Unknown	76	DM, ATA, IS, HTN, HDS, Obes.	Complete	Pfizer BioNTech
EPI_ISL_18082273	Female	Unknown	68	COPD, HTN	Not Applied	
EPI_ISL_18090389	Male	Unknown	70	IS, HTN, Obes.	Not Applied	
EPI_ISL_18082288	Female	Unknown	83		Not Applied	
EPI_ISL_18082290	Male	Unknown	79	COPD, HDS, CKD	Not Applied	
EPI_ISL_18090384	Male	Unknown	65		Incomplete	Pfizer BioNTech
EPI_ISL_18082289	Male	Unknown	67	DM, HTN, Obes	Not Applied	
EPI_ISL_18082298	Male	Unknown	31	DM	Not Applied	
EPI_ISL_18090390	Female	Unknown	54	IS, HTN	Not Applied	
EPI_ISL_18082291	Male	Unknown	88		Not Applied	
EPI_ISL_18082274	Female	Unknown	89	DM, ATA, IS, HDS	Not Applied	
EPI_ISL18082292	Female	Unknown	74	HTN	Not Applied	
EPI_ISL_18082275	Male	Unknown	91	DM, IS, HTN, Obes.	Incomplete	AstraZeneca
EPI_ISL_18082276	Female	Unknown	0	IS	Not Applied	
EPI_ISL_18082293	Male	Unknown	81	DM, CKD	Not Applied	
EPI_ISL_18082295	Male	Unknown	68		Complete	Sinovac
EPI_ISL_18082257	Female	Unknown	83	DM, HTN	Complete	Pfizer BioNTech
EPI_ISL_18082277	Male	Unknown	71	IS	Not Applied	
EPI_ISL_18082299	Male	Unknown	84	DM, HTN, CKD	Not Applied	

Abbreviations: DM: Diabetes Mellitus, HTN: Hypertension, CKD: Chronic kidney disease, SMK: Smoking, HDS: Heart disease, ATA: Asthma, COPD: Chronic obstructive pulmonary disease, IS: Immunosuppression, Obes: Obesity

DISCUSSION

Mexico established a comprehensive epidemiological follow-up of the SARS-CoV-2 pandemic, carried out by various entities such as public health organizations, academic institutions, and private entities. The collaborative efforts of these entities underscore the significance of a multi-sectoral approach to tackling pandemics of this nature [Hernández-Huerta et al. 2021, Taboada et al. 2021, Taboada et al. 2023]. The World Health Organization (WHO) has confirmed the presence of all Variants of Concern (VOCs) in Mexico as of the latest reporting period. The timely identification of VOCs is paramount for successfully implementing public health measures and mitigating future outbreaks.

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297 According to data from the INEGI (National Institute of Statistics, Geography, and
298 Information), the State of Aguascalientes has an area of 5,680 km^2, a population of
299 1.4 million inhabitants, and a population density of 265.2 habs/km^2, the
300 metropolitan area of the Aguascalientes City concentrates 81% of the total
301 population of the State, people over 60 years of age represent 10.3%. Which
302 undoubtedly represents a risk for the spread of the virus.

303 According to information from ENSANUT 2018 (National Public Health and
304 Nutrition Survey), abdominal obesity in Aguascalientes reaches up to 72.8% in the
305 adult population, with a prevalence of obesity of 32.6%, high blood pressure of
306 31.3%, and diabetes of 10.9%. % respectively. In addition, it has an incidence rate
307 of chronic kidney disease of 134.5 per 100,000 inhabitants. (Arreola-Guerra, et al,
308 2019) being factors that favor the severity of the disease.

309 The relationship between a host and a virus can be described as co-evolutive,
310 meaning that both entities evolve together over time. This relationship can
311 significantly impact the development of new virus variants, especially in
312 hospitalized patients. This is because when a virus infects a host, it adapts and
313 mutates to survive better and replicate within the host's body. The host's immune
314 system also evolves to combat the virus.

315 However, this co-evolutionary process can become problematic when a virus
316 mutates in a way that allows it to evade the host's immune system. This can result
317 in the development of new VOCs that are more infectious or virulent than the

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original virus. This can be particularly dangerous in hospitalized patients, where the immune system may weaken.

Therefore, it is essential to closely monitor the co-evolutionary relationship between hosts and viruses, especially in healthcare settings. By doing so, we can better understand the development of VOCs and take steps to prevent their spread and minimize their impact on public health.

In Mexico, from 2020 to March 2022, 10% of COVID-19 patients required hospitalization, and the lethality rate was less than 1% [García-López et al. 2022]. Hospitalized patients had a higher lethality rate of around 40%. Among these hospitalized patients, around 1% required mechanical ventilation and had an even higher lethality rate of around 80%. Analyzing patient demographics, age, and patient type are crucial factors in COVID-19 outcomes in Mexico [Torres-Ibarra et al., 2022]. Patients under the age of 65 who require hospitalization have a mortality rate of less than 50%, which increases significantly with advancing age. Patients aged 85 years or older have the highest mortality rate, reaching up to 60% [García-López et al., 2022]. Connecting specific virus mutations to particular cases is challenging due to their rarity.

However, the State Public Health Microbiology Laboratory (LESP) in Aguascalientes has the advantage of having access to such valuable samples and the ability to monitor the corresponding mutations in this particular population continuously. This unique advantage allows for a comprehensive analysis of the

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339 genetic variations of the virus. This analysis can significantly contribute to
340 understanding its regional evolution and spread.

341 This work focuses on molecular epidemiology and COVID-19-infected patient
342 demographics for all pneumonia-associated deaths in the Aguascalientes state
343 from the pandemic's beginning until May 2023. Aguascalientes is a small central
344 state situated in the Mexican Bajio region, serving as a connecting hub between
345 various large states in the center of the country, including San Luis Potosi,
346 Queretaro, Guanajuato, Zacatecas, and Nayarit due to its leading económic sector
347 being the manufacturing industry. The state is located in the Mexican Plateau,
348 which connects Mexico's Central and North regions. Previous country-wide
349 molecular epidemiology efforts have included this region, but only a few sequences
350 were analyzed compared to other states, such as the capital.

351 The CDC has reported that severe COVID-19 infection is more likely to occur in
352 patients with underlying chronic diseases, with 90% of hospitalized COVID-19
353 patients having at least one chronic condition. Hispanics, who have a higher
354 burden of chronic diseases such as obesity, diabetes, and renal disease, are at
355 greater risk for severe COVID-19 outcomes [Gil et al. 2020]. Worldwide,
356 comorbidities such as obesity and diabetes may be associated with a younger age
357 of severe disease in the Mexican population [Herrera-Esposito et al. 2022]. In
358 accordance, in the study group, the most prevalent co-morbidities were diabetes
359 and hypertension.

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It is essential to consider that mortality rates are low in younger people, which we also see here. The average age of the studied group is 64 years, significantly higher than the publicly reported average age of 44 years in the Mexican population. This is because older age is associated with worse health outcomes and higher death rates for COVID-19 patients.

On average, in Mexico, it was previously reported that the delta variant infection led to the highest maximum viral load and shortest time from symptom onset to maximum viral load [Ribeiro et al., 2023]. There were similarities in the genomic sequences of SARS-CoV-2 found in Mexico, Belize, and Guatemala in 2021, which are associated with a highly infectious and virulent strain [Hernández-Huerta et al. 2021]. This demonstrates the rapid virus mutation rate during the pandemic's first months to one year. Our results corroborate these findings and show that the mutation rate has not slowed from 2021 to the end of 2023.

This study acknowledges that the aforementioned general aspects of the infection apply to our data. The patient population had an average age of 64 years. The mean age of patients infected with the Omicron variant was only three years different from those infected with the Delta variant. Male patients exhibited a higher mortality rate than female patients, with males accounting for two-thirds of the analyzed population as compared to females.

This study could also confirm that children who are immunocompromised and under the age of three who have contracted COVID-19 and have comorbidities,

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particularly diabetes or obesity, are at a greater risk of developing pneumonia, leading to death.

This study also confirms previous findings that BA.5 Omicron variant dominated the fifth epidemic wave (summer 2022) in Mexico, replacing BA.1 and BA.2 and that Aguascalientes was among the states with a transitory presence of BA.2 and a low presence of BA.4 [Taboada et al. 2023, Castelán-Sánchez et al. 2023, [García-López et al., 2023].

The most intriguing discovery involves the identification of two mutations that appear to be associated with the population of Aguascalientes. Both proteins are linked to immune system evasion [Zandi et al. 2022]. The first, L95M in ORF8, has demonstrated a high mutation rate at amino acids 119 and 120, conferring resistance to evasion. Nevertheless, this mutation is located in the region of highest diversity found in North America and Oceania, despite not being previously reported [Alkhansa et al. 2021]. On the other hand, ORF9 protein has shown interaction with TOM70 (mitochondrial import receptor), reducing active IFN. The L46M mutation is within the 11 amino acid residues that form the binding complex with TOM70 [Gao et al. 2021], making it necessary to investigate whether it has a more significant impact on this binding and, therefore, a more significant role in the immune response. These findings open the door to analyzing the effects of these mutations and their potential implications for viral infectivity, disease severity, or clinical characteristics.

CONCLUSIONS

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After evaluating patient demographics, virus evolution dynamics, and changes over time, we identified unique characteristics of SARS-CoV-2 infection in patients hospitalized for severe pneumonia in central Mexico during the pandemic. Our findings revealed two new mutations specific to the lineages in this geographical area. We also observed only two prevalent SARS-CoV-2 genomic lineages in 2023. Continuing genomic surveillance is crucial to detect emerging variants that could potentially threaten public health. This study emphasizes the significance of maintaining such vigilance to safeguard our communities. Understanding the virus's genetic makeup and evolution patterns is essential for developing effective vaccines and treatments.

AUTHOR STATEMENTS

CONTRIBUTIONS

BMG, MSLG, CDC, and APM developed the idea and wrote and edited the manuscript. BMG MSLG performed laboratory and bioinformatics analyses. BMG prepared the manuscript figures. We acknowledge using Grammarly AI to improve the manuscript's English writing [Grammarly 2024].

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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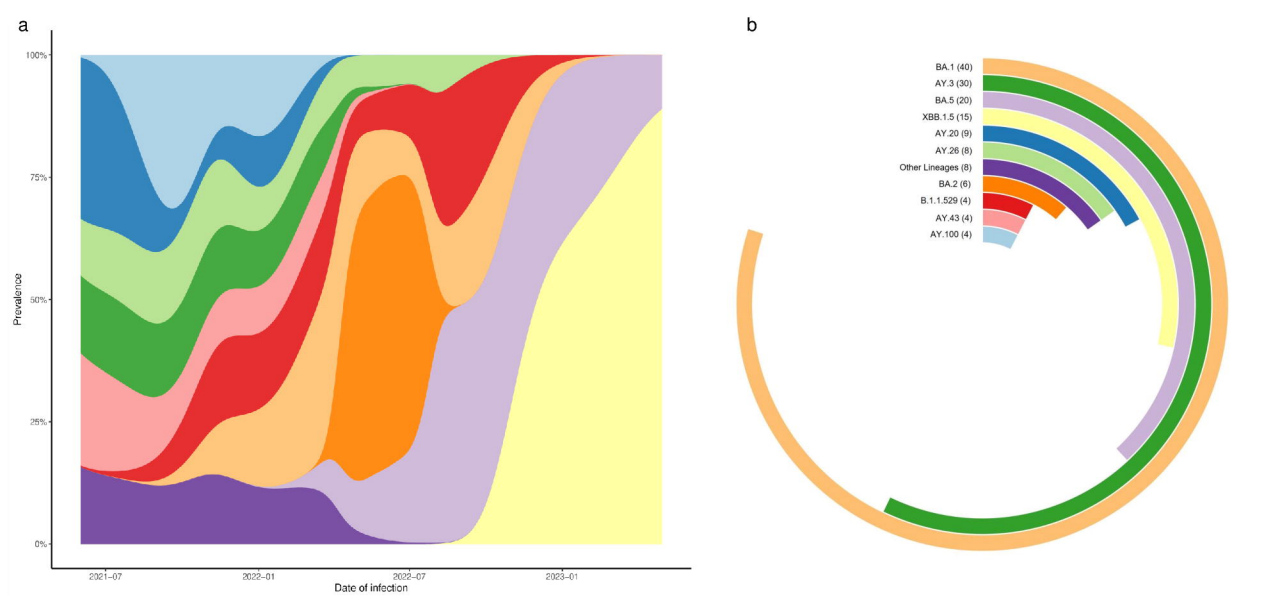
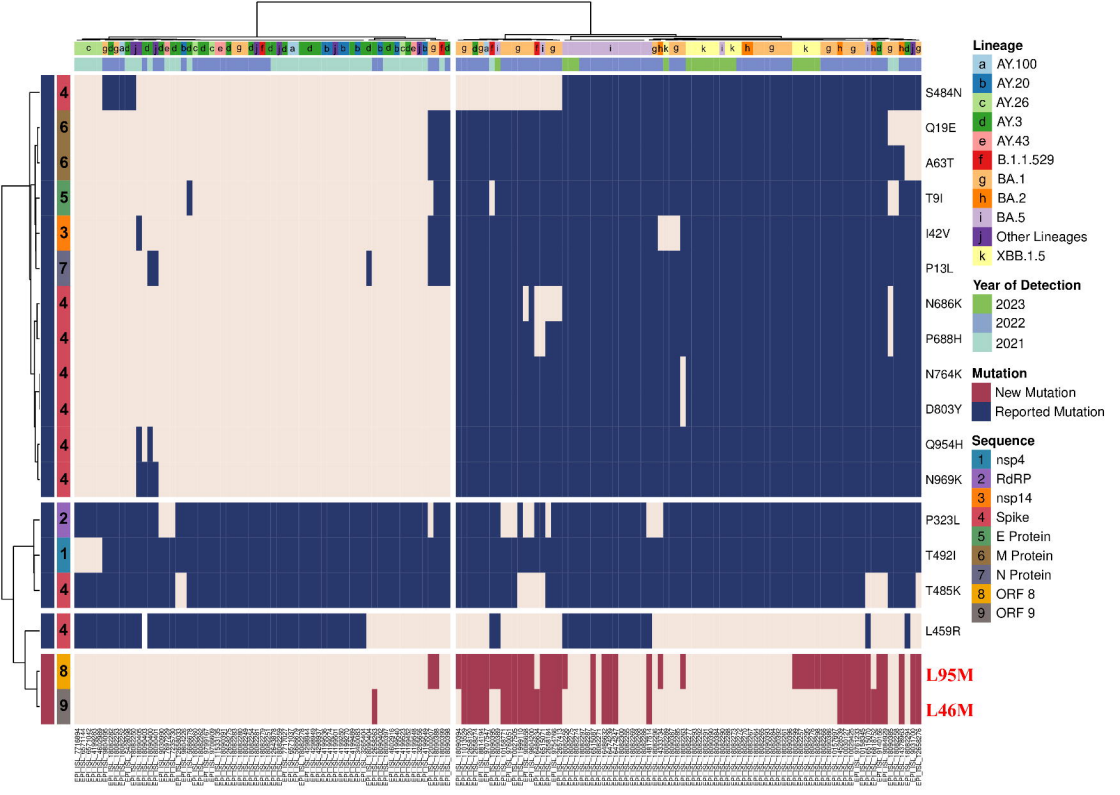
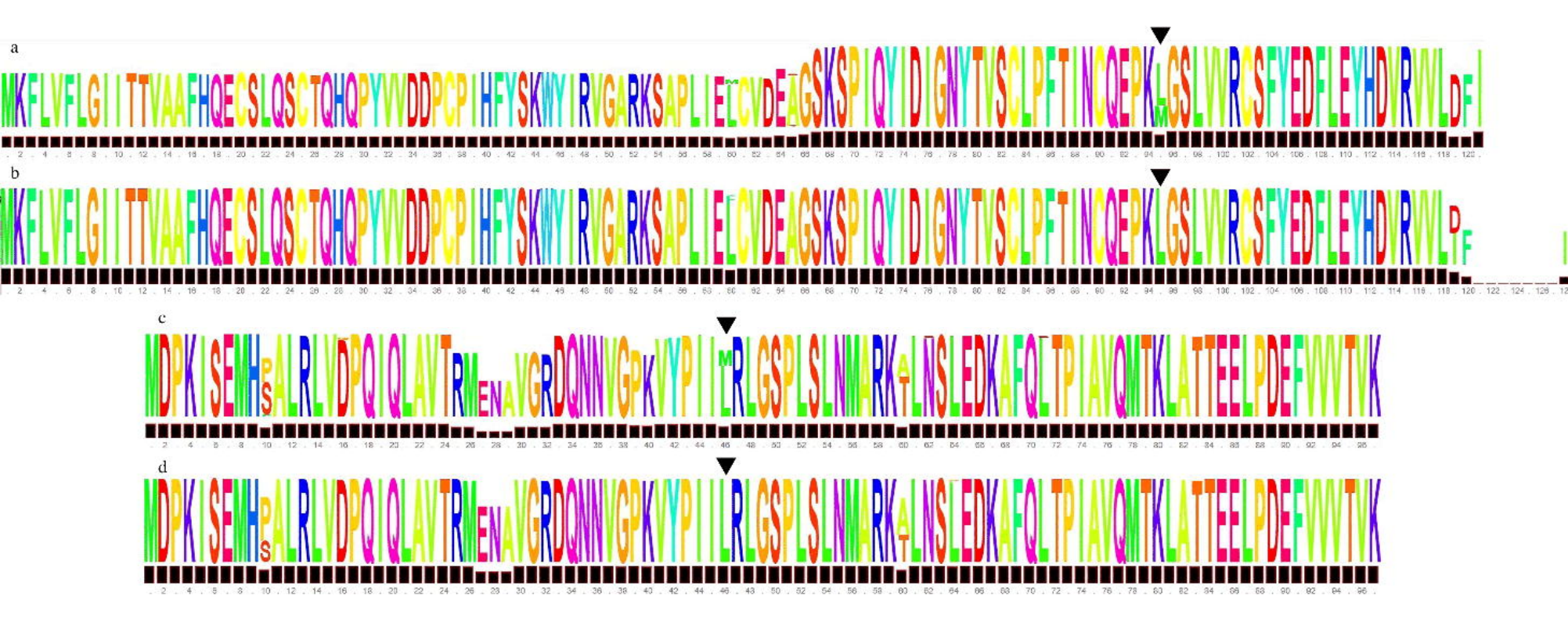
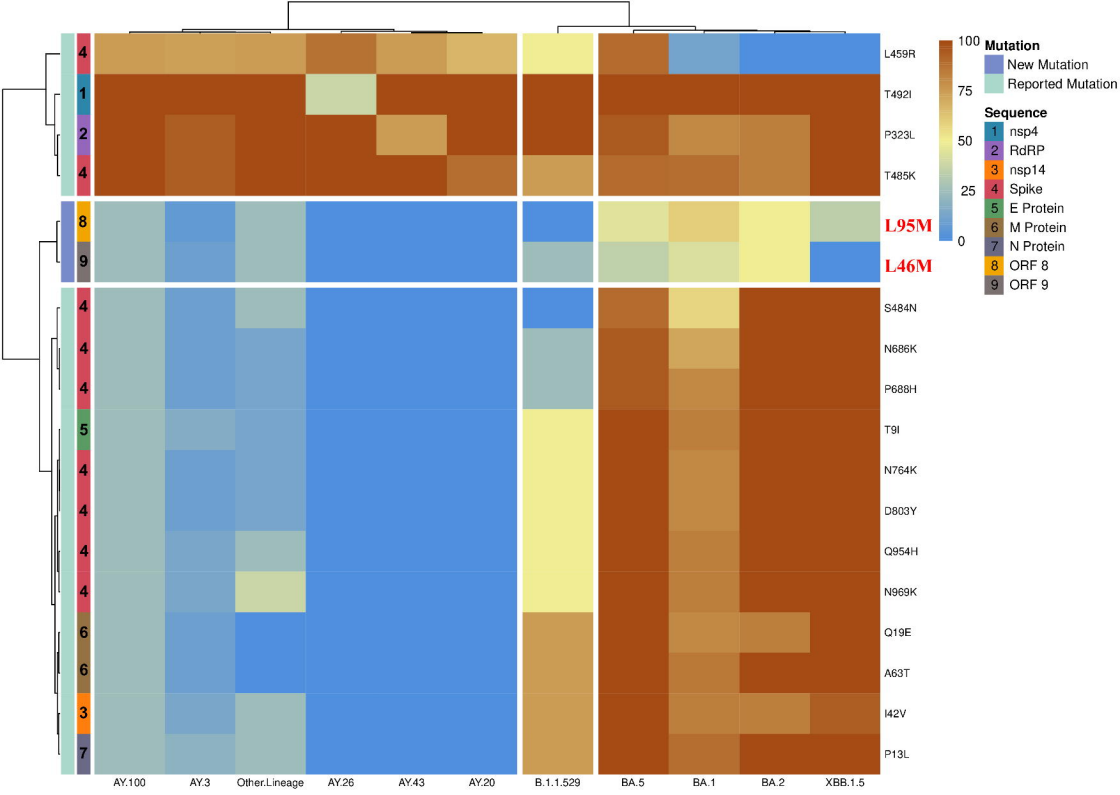
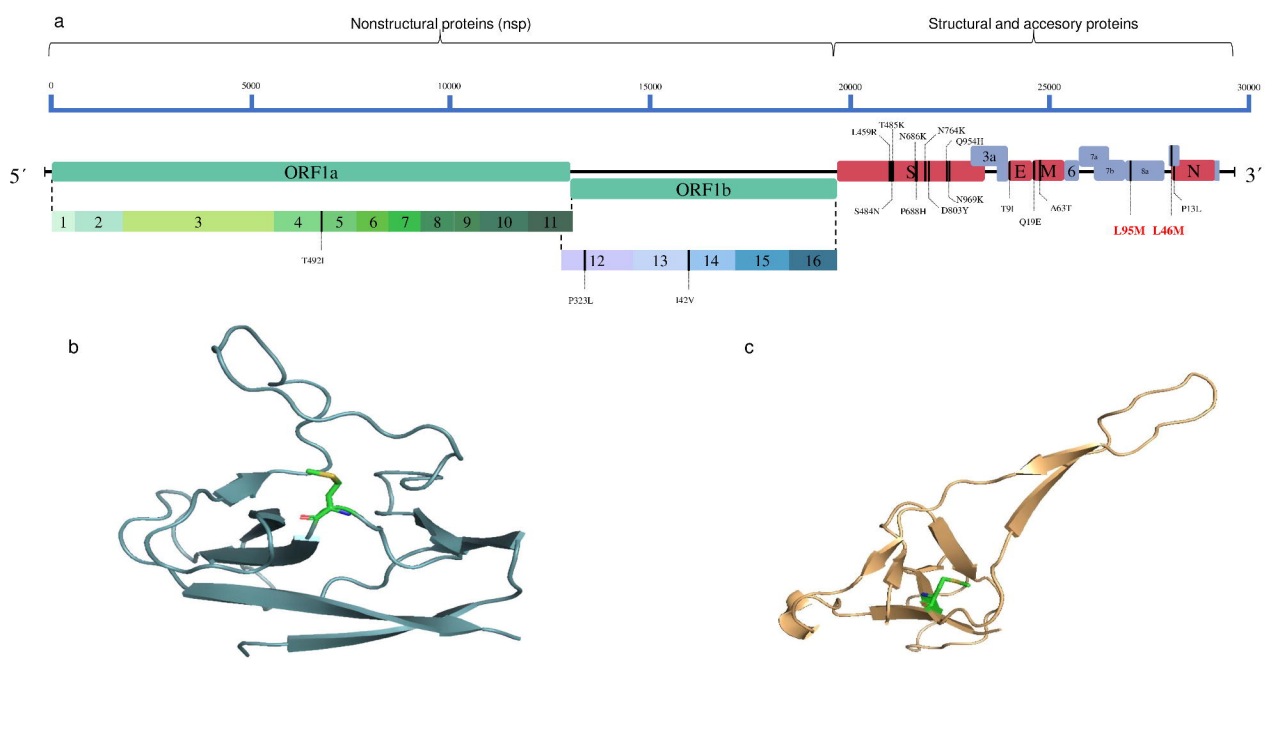

a
100%
75%
50%
25%
0%
Prevalence
2021-07
2022-01
2022-07
2023-01
Date of infection
b
BA.1 (40)
AY.3 (30)
BA.5 (20)
XBB.1.5 (15)
AY.20 (9)
AY.26 (8)
Other Lineages (8)
BA.2 (6)
B.1.1.529 (4)
AY.43 (4)
AY.100 (4)


S484N
Q19E
A63T
T9I
I42V
P13L
N686K
P688H
N764K
D803Y
Q954H
N969K
P323L
T492I
T485K
L459R
L95M
L46M
Lineage
a AY.100
b AY.20
c AY.26
d AY.3
e AY.43
f B.1.1.529
g BA.1
h BA.2
i BA.5
j Other Lineages
k XBB.1.5
Year of Detection
2023
2022
2021
Mutation
New Mutation
Reported Mutation
Sequence
1 nsp4
2 RdRP
3 nsp14
4 Spike
5 E Protein
6 M Protein
7 N Protein
8 ORF 8
9 ORF 9


a
b
c
d


L459R
T492I
P323L
T485K
L95M
L46M
S484N
N686K
P688H
T9I
N764K
D803Y
Q954H
N969K
Q19E
A63T
I42V
P13L
AY.100
AY.3
Other.Lineage
AY.26
AY.43
AY.20
B.1.1.529
BA.5
BA.1
BA.2
XBB.1.5
100
75
50
25
0
Mutation
New Mutation
Reported Mutation
Sequence
1 nsp4
2 RdRP
3 nsp14
4 Spike
5 E Protein
6 M Protein
7 N Protein
8 ORF 8
9 ORF 9


a
Nonstructural proteins (nsp)
Structural and accesory proteins
0
5000
10000
15000
20000
25000
30000
5′
3′
ORF1a
ORF1b
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
T492I
P323L
I42V
L459R
T485K
N686K
N764K
Q954H
S484N
P668H
D803Y
N969K
T9I
Q19E
A63T
L95M
L46M
P13L
3a
S
E
M
6
7a
7b
8a
N
b
c