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# Genomic characterization of SARS-CoV-2 during the COVID-19 pandemic in Villa Clara, Cuba

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## Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 was declared a public health emergency of international concern in March 2020. This virus evolved into different genetic variants that circulated worldwide. Local genomic surveillance was crucial for the pandemic response. The objective of the research consisted in characterize the genomic surveillance of SARS-CoV-2 from confirmed cases at the Villa Clara LBM (Base Laboratory) during the study period. A cross-sectional descriptive study was conducted from December 2020 to May 2023. The study utilized 106 nasopharyngeal swab samples from patients confirmed by RT-PCR at the Villa Clara LBM, which were sent for nucleotide sequencing to the LNR-IVR-IPK (National Reference Laboratory-Insect Vector Department at the Pedro Kourí Institute). Patients infected with the Delta variant presented severe forms of the disease in 43.75% of cases. The Delta variant had the lowest median cycle threshold value (18.50). 100% of patients with the Delta variant had the mutations found in subgroup 3 (D614G, L452R, P681H, T478K). In the phylogenetic tree, the branch grouping the Omicron variant showed high genetic diversity. The use of genomic surveillance during the COVID-19 pandemic was essential for understanding the clinical-epidemiological dynamics linked to each circulating SARS-CoV-2 variant in Villa Clara province.

**Keywords:** Genetic variants; Genomic surveillance; Severe Acute Respiratory Syndrome Coronavirus 2

## 1. Introduction

In late December 2019, a novel coronavirus, named Severe Acute Respiratory Syndrome 2 (SARS-CoV-2), emerged, causing coronavirus disease (COVID-19) (Pastrian, 2020; Dabancha, 2021; Expósito *et al*., 2021). The rapid and progressive spread of the disease in China, and in countries across Asia, the Middle East, the Americas, Oceania, and Africa, led the World Health Organization (WHO) to declare it a Public Health Emergency of International Concern and, on March 11, 2020, a pandemic (Cronista, 2020; WHO, 2020; Dabancha, 2021).

Its causative agent was identified in China on January 8, 2020, and belongs to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae, genus Betacoronavirus (lineage B). It has a spherical shape, with a double lipid envelope and spike proteins (Pastrian, 2020; Dabancha, 2021; Tao *et al*., 2021); its Ribonucleic Acid (RNA) genome encodes for structural and non-structural proteins that are used as targets in molecular assays (Aguilar *et al*., 2021; Pérez *et al*., 2022a, b).

Following the report of the first cases in China, the first complete sequence of the virus's genome was obtained in just ten days and published in the GISAID database (Global Initiative on Sharing All Influenza Data Platform), which provided

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information for the design of diagnostic methods, drugs and vaccines (Aguilar *et al*., 2020; Aguilar *et al*., 2021; Tao *et al*., 2021).

This virus evolved into different genetic variants that circulated worldwide (Bordoy, 2024; Sánchez *et al*., 2024). The SARS-CoV-2 genome accumulates an estimated mutation rate of $10^{-6}$ to $10^{-4}$ per replicative cycle. Mutations affecting the gene encoding the spike antigen select for variants with alterations in their infectivity, pathogenic potential, and resistance to neutralizing antibodies (Aguilar *et al*., 2021; Expósito *et al*., 2021; Pérez *et al*., 2022a). The original Wuhan-Hu-1 virus was replaced by the D614G variant, which predominated globally during 2020. Molecular characterization studies reported the progressive emergence of other variants, some of them with greater pandemic potential due to their high transmissibility, increased severity, or reduced immune response to natural infection or vaccines (Amanat and Krammer, 2020; Tao *et al*., 2021; Monge *et al*., 2022).

As of August 2023, approximately 769 million cases and 7 million deaths had been recorded worldwide, according to WHO data, of which 5 272 occurred in China (WHO, 2023). In the Americas, there were 193 million cases and approximately 3 million deaths (PAHO, 2023). The United States had the highest number of deaths (1.2 million), followed by Brazil (704 795) (WHO, 2023). At the subregional level, COVID-19 cases increased in the Caribbean and Atlantic Islands in 2023 (PAHO, 2023). As of the end of December 2023, Cuba had processed 14,396,562 samples, of which 1,115,183 were positive, with 8,530 deaths according to a report from the Ministry of Public Health (MINSAP, 2023).

Molecular tools for the diagnosis and monitoring of viral infections have undergone rapid development in recent decades, particularly the design and refinement of techniques based on nucleic acid amplification, such as real-time PCR and nucleotide sequencing techniques (Guzmán *et al*., 2022; Machado *et al*., 2024; Sánchez *et al*., 2024).

In Cuba, the molecular diagnosis of SARS-CoV-2 was spearheaded by the Pedro Kourí Institute of Tropical Medicine (IPK), a leading institution in the management and training of infectious and tropical diseases, which was responsible for advising and overseeing the entire network of laboratories established during the pandemic. Another of its functions was the development of genomic surveillance using Sanger sequencing.

During the pandemic, the Molecular Biology Laboratory of the Provincial Center for Hygiene, Epidemiology, and Microbiology in Villa Clara, Cuba, was involved in sending samples as part of territorial genomic surveillance. This facilitated the pandemic response using molecular epidemiology tools, through the identification of mutations with potential biological effects and genetic variants of interest due to their impact on transmissibility and virulence.

In this context, the objective of this research was to characterize the genomic surveillance of SARS-CoV-2 based on confirmed cases at the Villa Clara, Cuba, Molecular Biology Laboratory during the study period.

## 2. Methodology

### 2.1. About the study

A cross-sectional descriptive study was conducted from December 2020 to May 2023, using 106 nasopharyngeal swab samples from patients confirmed by RT-PCR at the Molecular Biology Laboratory in Villa Clara Province, Cuba. These samples were sent to the National Reference Laboratory-IVR-IPK for nucleotide sequencing. The samples selected for sequencing included travelers from various countries, patients with varying clinical severity, cases from areas with a significant increase in cases, and diverse age groups. All samples met the quality attributes required for amplification or sequencing. For comparative analysis and genomic surveillance studies, the Villa Clara sequences available in GISAID were used.

The phylogenetic tree was constructed using molecular phylogenetic analysis with a maximum likelihood method based on Tamura's three-parameter model. The following variables were used: Clinical classification, Deaths, SARS-CoV-2 genetic variants, Cycle threshold (Ct), Mutations with potential biological effects, and Subgroups.

This research is part of the sectoral project (2105017) coordinated by the IPK (Pedro Kourí Institute of Tropical Medicine) "Strengthening and Development of Molecular Diagnostics in Cuba."

### 2.2. Statistical analysis

Summary measures for qualitative variables included absolute and relative frequencies (percentages), and for quantitative variables, measures of central tendency (median) and dispersion (minimum, maximum, quartiles, and interquartile range). Other statistical tests used included the Kruskal-Wallis test, the Chi-square test, and biostage cluster analysis.

### 2.3. Ethical aspects

The study is based on essential ethical principles approved by the Institution's Ethics Committee. The information obtained from the patients was anonymous, confidential, and used only for research purposes, always adhering to the ethical principles of the Declaration of Helsinki (DHAMM, 2024).

## 3. Results

Table 1 shows the sequenced SARS-CoV-2 variants and their relationship to the clinical classification of patients under genomic surveillance (n=106). Patients infected with the Delta variant presented with severe forms of the disease in 43.75% of cases and were classified as critical at the time of diagnosis in 37.50%. In patients infected with the Omicron variant or one of its subvariants, 75.76% were classified as mild and 22.73% as severe. Patients infected with the Beta variant did not show severe cases; 75% presented with mild forms of the disease and 25% were classified as having moderate forms.

**Table 1** SARS-CoV-2 variants and clinical classification of patients for genomic surveillance in Villa Clara. December 2020 - May 2023

| SARS-CoV-2 variants | Clinical classification | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mild | | Moderate | | Serious | | Critical | | | |
| | Nº | % | Nº | % | Nº | % | Nº | % | Nº | % |
| Variant D614G | 7 | 87.50 | 0 | 0.00 | 0 | 0.00 | 1 | 12.50 | 8 | 100 |
| Beta | 6 | 75.00 | 2 | 25.00 | 0 | 0.00 | 0 | 0.00 | 8 | 100 |
| Delta | 2 | 12.50 | 1 | 6.25 | 7 | 43.75 | 6 | 37.50 | 16 | 100 |
| Omicron and subvariants | 50 | 75.76 | 0 | 0.00 | 15 | 22.73 | 1 | 1.52 | 66 | 100 |
| Others* | 4 | 50.00 | 2 | 25.00 | 2 | 25.00 | 0 | 0.00 | 8 | 100 |
| Total | 69 | 65.09 | 5 | 4.72 | 24 | 22,64 | 8 | 7.55 | 106 | 100 |

Percentage calculated in relation to the total of each row; Fisher's exact test Chi-square = 45.999 p = 0.000; **Source:** record of sequenced cases in LNR-IVR-IPK

The relationship between virus variants and mortality shows that 31.25% of all cases infected with the Delta variant died, compared to 25.00% with the Beta variant and 1.52% with the Omicron variant.

Table 2 and figure 1 show the central tendency and dispersion of the threshold cycle value according to virus variants. Statistical analysis demonstrated significant differences between the median threshold cycle values (Chi-square = 14.587, p = 0.006). The lowest median was observed in the Delta variant (18.50), and the highest in the D614G variant (27.50). The lowest minimum values (14) were observed in the Delta and Omicron variants. The greatest dispersion was shown in the D614G variant, with an interquartile range of 10 values.

**Table 2** Central tendency and dispersion of the threshold cycle value according to virus variants

| Virus variants | Descriptive statistics (threshold cycle value) | | | | | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Median | Quartiles | | Interquartile range |
| | | | | First | Third | |
| Variant D614G | 17 | 32 | 27.50 | 20.50 | 30.50 | 10.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Beta | 20 | 30 | 24.50 | 22.00 | 26.50 | 4.50 |
| Delta | 14 | 25 | 18.50 | 16.50 | 22.50 | 6.00 |
| Omicron and subvariants | 14 | 30 | 23.00 | 20.00 | 28.00 | 8.00 |
| Others* | 18 | 30 | 24.50 | 21.50 | 28.50 | 7.00 |

Kruskal-Walli's test Chi-square=14.587 p=0.006 **Source**: sequenced case registry in LNR-IVR-IPK

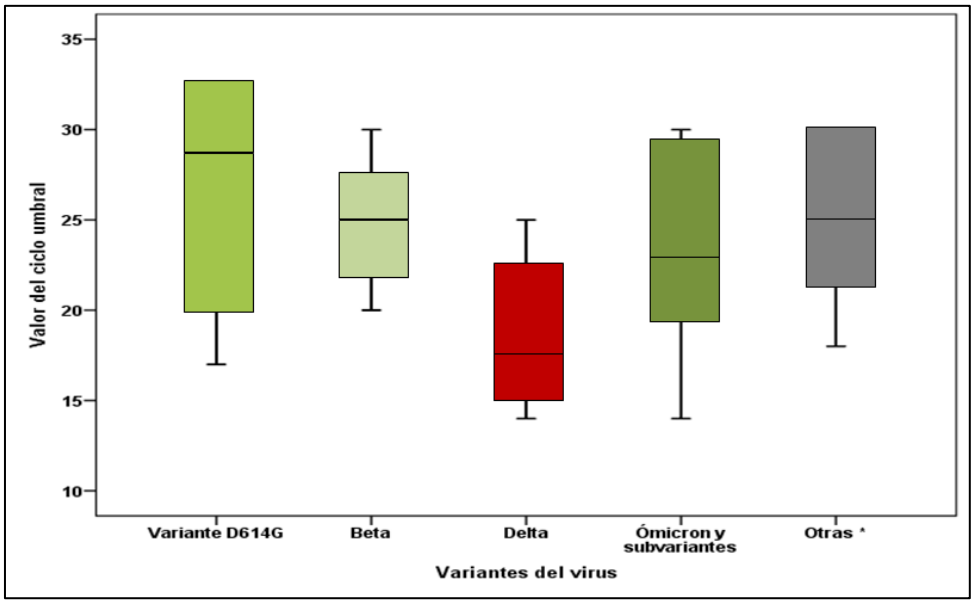


**Figure 1** Central tendency and dispersion of the threshold cycle value according to virus variants

The SARS-CoV-2 mutations with potential biological effects were analyzed using a two-phase clustering procedure. Three similar but distinct natural subgroups (clusters) were identified within these clusters.

The frequency and profile of these subgroups are highlighted as follows:

- Subgroup 1: Highest representation of the D614G, N501Y, E484K, and K471N mutations. N = 30 (28.30%)
- Subgroup 2: Highest representation of the D614G, N501Y, P681H, T478K, and N440K mutations. N = 49 (46.23%)
- Subgroup 3: Highest representation of the D614G, L452R, P681H, and T478K mutations. N = 27 (25.47%)

By establishing the relationship between genetic variants and mutation subgroups with potential biological effects, a significant p-value of 0.000 was observed. 100% of patients with the Delta variant had mutations found in subgroup 3. Cases with the D614G, Beta, and other variants were found in subgroup 1, and most cases with Omicron and its subvariants were found in subgroup 2. This is reflected in Table 3.

**Table 3** Relationship of SARS-CoV-2 variants and mutation subgroups with potential biological effects

| **Virus variants** | **Subgroups of mutations with potential biological effects** | | | | | | **Total** | |
|---|---|---|---|---|---|---|---|---|
| | **Subgroup 1** | | **Subgroup 2** | | **Subgroup 3** | | | |
| | **Nº** | **%** | **Nº** | **%** | **Nº** | **%** | **Nº** | **%** |
| Variant D614G | 8 | 100 | 0 | 0.00 | 0 | 0.00 | 8 | 100 |
| Beta | 8 | 100 | 0 | 0.00 | 0 | 0.00 | 8 | 100 |
| Delta | 0 | 0.00 | 0 | 0.00 | 16 | 100 | 16 | 100 |
| Omicron and subvariants | 8 | 12.31 | 47 | 72.31 | 10 | 15.39 | 66 | 100 |
| Others* | 6 | 66.67 | 2 | 22.22 | 1 | 11.11 | 8 | 100 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Total | 30 | 28.30 | 49 | 46.23 | 27 | 25.47 | 106 | 100 |

Percentage calculated in relation to the row total; Fisher's exact test Chi-square = 92.492 p = 0.000; **Source**: Record of sequenced cases in LNR-IVR-IPK

The phylogenetic analysis of the sequenced Villa Clara samples (Figure 2) revealed that the identified sequences are divided into five major clades, which were grouped according to variants. Two of the sequences (61226 and 61215) of the D614G variant showed longer branches, associated with a greater number of substitutions. Analysis of the clade containing the Beta variant sequences showed that most are genetically similar; only one (32057) exhibited a higher number of mutations. Notably, the Omicron variant branch showed high genetic diversity, giving rise to two new branches, one of which exhibited greater variability. These branches were grouped according to subvariants (BA2, B.1.1.529, BA5, BQ1, XBB1, XBB.1.16, JN1), regardless of when they were identified.

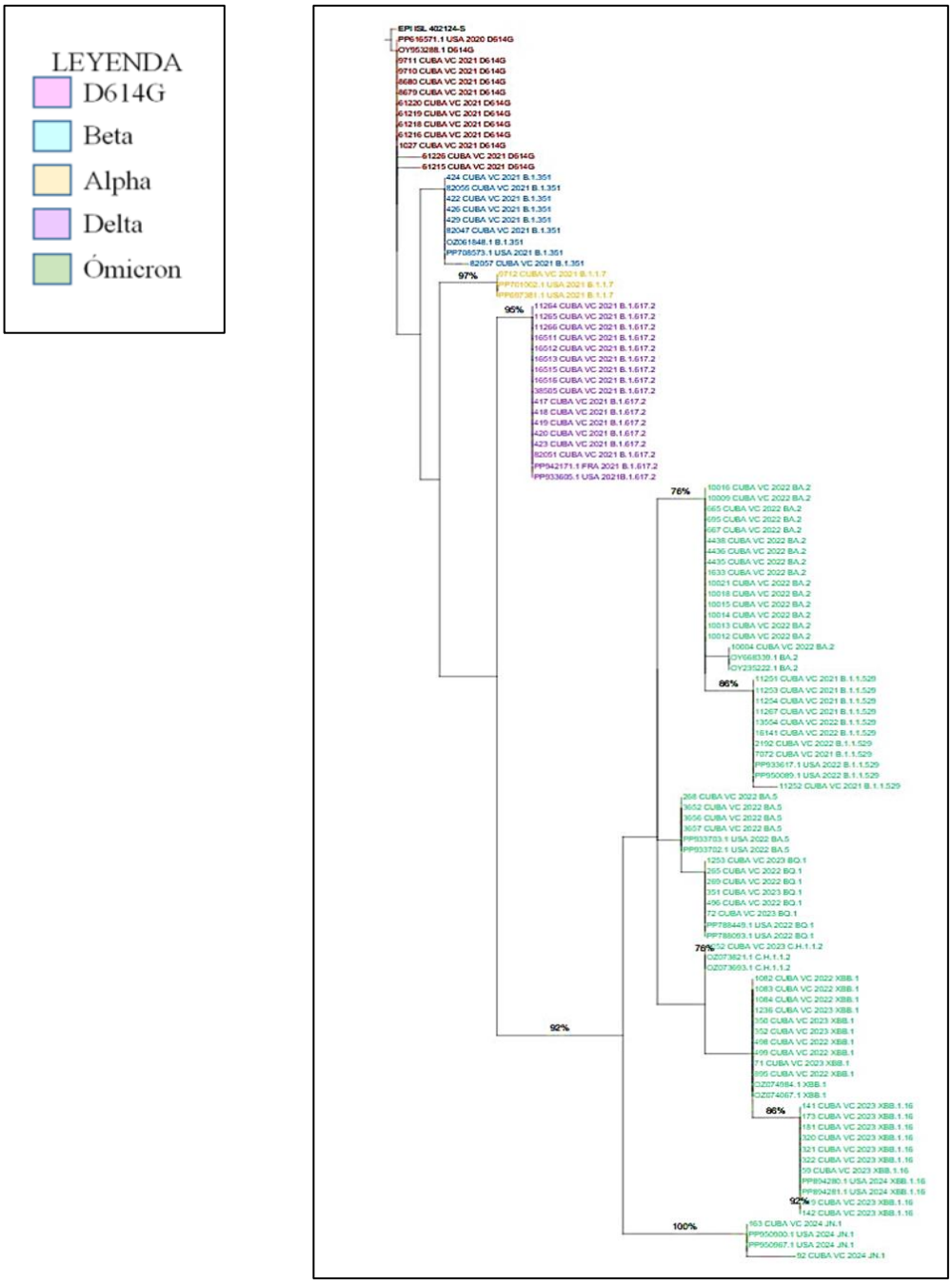


**Figure 2** Phylogenetic tree of SARS-CoV-2

**Source:** record of sequenced cases in LNR-IVR-IPK.

## 4. Discussion

Genomic surveillance is an effective tool for monitoring and understanding the evolution of SARS-CoV-2, and it allows for the early detection of new circulating variants (Aguilar *et al*., 2021; Expósito *et al*., 2021; Guzmán *et al*., 2024).

The global prevalence of the D614G variant during 2020 reached approximately 100%, due to the genetic selection pressure of SARS-CoV-2 (Abu-Raddad *et al*., 2022; Bordoy, 2024; Ortega *et al*., 2024).

In Villa Clara, the D614G variant circulated from December 2020 to April 2021. This epidemic period had the lowest incidence, which may have been due to the strict lockdown, airport closures, and other epidemiological measures implemented by government and health authorities.

In Cuba, as of November 2020, only one SARS-CoV-2 variant, D614G, was detected in more than 90% of the samples analyzed by sequencing (Guzmán *et al*., 2022; Pérez *et al*., 2022b; Guzmán *et al*., 2024). In December 2020, the Alpha variant entered the country and co-circulated with D614G (Guzmán *et al*., 2022; Guzmán *et al*., 2024; Machado *et al*., 2024). This new variant was not associated with an increase in cases or deaths (Guzmán *et al*., 2022; Guzmán *et al*., 2024). Villa Clara reported a situation similar to that of Cuba during the same period, both in terms of the circulation of variants and the incidence of cases and deaths, even though the borders were open and vaccination had not yet begun (Miranda, 2021; Sánchez *et al*., 2024).

In the US and European countries, the circulation of the Alpha variant was associated with increased transmissibility, a higher risk of reinfection, and reduced vaccine efficacy. Mortality increased to 50% (Amanat and Krammer, 2020; Farid and Amin, 2023; Bordoy, 2024).

The Beta variant was first detected in South Africa in October 2020 (Grubaugh *et al*., 2020; Aguilar *et al*., 2021; Avello *et al*., 2023). It was associated with a higher risk of severe or critical illness or death (Abu-Raddad *et al*., 2022; Avello *et al*., 2023; Ortega *et al*., 2024).

In Cuba, the Beta variant was initially detected in Havana in late December 2020 and gradually spread to the rest of the country (Guzmán *et al*., 2022; López *et al*., 2022). Globally, the Beta B 1351 lineage has been associated with significant increases in the number of cases, decreased response to neutralizing antibodies against different vaccine formulations, increased risk of hospitalization, clinical severity, and mortality (Pérez *et al*., 2022b; Machado *et al*., 2024).

In Villa Clara, in 2021, the Beta variant was detected co-circulating with the Delta variant. This period saw the greatest increase in cases and deaths, similar to findings reported by other researchers (Guzmán *et al*., 2022; Sánchez *et al*., 2024).

RT-PCR threshold cycle values showed significant differences among the circulating variants, with the Delta variant exhibiting the lowest median. A low Ct value indicates a high viral load and is associated with more severe cases of the disease, while higher Ct values are typically observed in patients with mild or asymptomatic cases. However, the usefulness of Ct as a predictor of severity is limited due to the influence of other factors such as sample quality and individual patient characteristics (Pérez *et al*., 2022b; Ortega *et al*., 2024). These findings suggest the importance of considering Ct as a complement to clinical assessment, rather than as a sole indicator (Reina and Fraile, 2021; Tao *et al*., 2021; Avello *et al*., 2023).

The Delta B.1.617.2 variant was responsible for the second deadly wave of COVID-19 infections in April 2021 in India (Grubaugh *et al*., 2020; Aguilar *et al*., 2021; Masa and Celis, 2021). The variant's increased infectivity is due to a combination of key mutations that give the spike protein a higher affinity for binding to ACE2. This results in reduced vaccine efficacy and higher viral loads in infected individuals (Amanat and Krammer, 2020; Miranda, 2022; Monge *et al*., 2022).

In Matanzas, Cuba, the Delta variant was detected in late April 2021 and spread rapidly to the rest of the provinces within two months (Expósito *et al*., 2021; Guzmán *et al*., 2022; Sánchez *et al*., 2024). The Delta variant, which had been co-circulating with Beta in Villa Clara since May 2021, was established as the only variant in August and circulated until December 2021 (Guzmán *et al*., 2024; Sánchez *et al*., 2024).

In December 2021, the Omicron variant was detected in Villa Clara, definitively displacing the Delta variant. The arrival of Omicron B.1.1.529 altered the clinical-epidemiological scenario; despite the fact that 91.8% of the province's

population had a complete vaccination schedule with Abdala and Soberana, cases increased in January 2022 compared to November and December 2021 (Pérez *et al*., 2022a, b; Sánchez *et al*., 2024).

Research conducted in Cuba detected the Omicron variant in 83.0% (239/288) of confirmed cases during the period (Pérez *et al*., 2022a; Guzmán *et al*., 2024). 65.3% of these cases were fully vaccinated.

The presence of the Omicron variant also caused the highest rates of COVID-19 incidence ever recorded worldwide (Farid and Amin, 2023; Monge *et al*., 2022; Guzmán *et al*., 2024). The explanation for the increased incidence could be related to the greater number of mutations acquired by Omicron, compared to other variants (Monge *et al*., 2022; Vogt *et al*., 2023; Bordoy, 2024).

Epidemiological and genomic surveillance are essential to adapt public health strategies and maintain the effectiveness of control measures (Tao *et al*., 2021; Monge *et al*., 2022; Machado *et al*., 2024).

Genomic surveillance identifies mutations with potential negative biological effects and is crucial for managing cases at the community level. It also allows for understanding biodiversity dynamics and the adaptive advantages the virus can develop. These mutations influence transmissibility, disease severity, and the effectiveness of COVID-19 vaccines and treatments (Amanat and Krammer, 2020; Monge *et al*., 2022; Machado *et al*., 2024). They also affect the accuracy of diagnostic tests, complicating early detection and subsequent transmission control (Jiménez *et al*., 2021; Heudobler *et al*., 2022; Bordoy, 2024).

Analysis of mutations with potential negative biological effects identified in the Villa Clara population revealed three subgroups: 1, 2, and 3, based on the frequency of mutations found in the sequenced samples. A significant relationship was observed between the identified subgroups and the virus's genetic variants, results that coincide with those obtained by other authors, both in Cuba and in different geographical locations (Adebali *et al*., 2020; Michelon, 2021; Tao *et al*., 2021; Machado *et al*., 2024).

In the study "Genetic diversity of Cuban SARS-CoV-2 isolates during the period 2020-2022," López *et al*. (2022) demonstrate that the Alpha genetic variant is associated with 17 mutations, with "N501Y" being the most prominent. In the Beta variant, three mutations were detected in the RBD: K417N, E484K, and N501Y. The Delta variant exhibits a diversity of mutations, predominantly L452R, P681R, D614G, and T487K. Omicron differs from other variants due to the numerous mutations in its genome, results that coincide with those obtained by other authors on this subject (Harvey *et al*., 2021; Monge *et al*., 2022; Gupta, 2023).

A study conducted in Brazil (Michelon, 2021), based on data from the genomic network of the Fiocruz Institute, found circulation of the Alpha variant with seven mutations in the S protein, including N501Y, associated with a greater affinity of the virus for the ECA2 receptor, and the Delta variant identified 13 mutations, including L452R and E484Q, results that coincide with this research.

Another aspect to consider in a comprehensive approach to pandemic management is the result of phylogenetic studies (Adebali *et al*., 2020; Feghali *et al*., 2021; Jiménez *et al*., 2021). Their fundamental purpose is to understand the evolution and spread of the virus at global, regional, and local levels. Through genomic sequencing and the comparison of different viral variants, key mutations and transmission patterns are identified, influencing the dynamics of the pandemic (Catalá and Palacios, 2020; Jiménez *et al*., 2021; Reina and Fraile 2021). Phylogeny also provides a detailed view of the evolutionary mechanisms of viruses, which has important implications for the design of vaccines and epidemiological control strategies (Grubaugh *et al*., 2020; Feghali *et al*., 2021; Monge *et al*., 2022). These phylogenetic tools were applied to studies of the SARS-CoV-2 virus.

Phylogenetic analysis of sequences from Villa Clara samples detected the presence of a wide variety of clades. The sequences were grouped according to the main variant of each branch of the phylogenetic tree. The first samples analyzed presented the D614G mutation; subsequently, clades with sequences of the Beta and Delta variants appeared. Omicron was detected in 62.3% of cases and presented the greatest genetic diversity (Sánchez *et al*., 2024).

Studies conducted in several countries describe how the virus was introduced, its dispersal in each territory, and the genetic diversity of circulating variants (Adebali *et al*., 2020; Papanikolaou *et al*., 2020; Tao *et al*., 2021; Guzmán *et al*., 2024).

A Cuban study, conducted by Pérez *et al*. (2022a) in the LNR-IVR-IPK shows the phylogenetic analysis of 34 sequences of the S gene, and the presence of the D614G mutation was observed in 32 sequences. Similar results are obtained by

other researchers who report that at the beginning of the pandemic in Cuba a low level of diversity of the viral genome was evident, due more to random genetic drift than to the selective pressure of SARS-CoV-2 (Papanikolaou *et al*., 2020; Pérez *et al*., 2022a).

Genetic characterization of Cuban SARS-CoV-2 viral isolates obtained at the NSB3 laboratory of the Scientific Research Center of Civil Defense shows high genetic diversity, with a predominance of the Omicron variant (Expósito *et al*., 2021; Pérez *et al*., 2022a; Guzmán *et al*., 2024). Another study in Ecuador (Jiménez *et al*., 2021) identified nine clades and 160 sublineages of the Omicron variant that evolved dynamically throughout 2022. The analysis also reveals 37-point mutations, 19 of which lead to amino acid changes in the S glycoprotein, an example of the genetic diversity of this variant (Barton *et al*., 2021; Pérez *et al*., 2022a; Guzmán *et al*., 2024). All these results coincide with the report of the sequenced samples in the province of Villa Clara.

There were some limitations in the present research on health systems and services, conducted in Villa Clara, which should be mentioned. These included the need to incorporate personnel without experience in the molecular diagnosis of SARS-CoV-2 at the beginning of the pandemic; the use of different diagnostic tools and platforms for nucleic acid extraction and amplification depending on availability in the country during the pandemic; the variation in COVID-19 management protocols at the territorial, national, and international levels as experience was gained; the reuse of blocks and magnets for the extraction process due to the international scarcity of resources caused by the pandemic; and the lack of a genomic sequencing laboratory in the province, which limited the possibility of conducting real-time studies and the number of samples processed.

However, the comprehensive work developed by the LBM of the CPHEM of Villa Clara during the COVID-19 pandemic strengthened the technical and professional training of the researchers and improved the diagnostic and technological capacity of the laboratory, which allows not only the maintenance of SARS-CoV-2 diagnosis in the province, but also the detection of new emerging viral agents.

## 5. Conclusion

The use of genomic surveillance proved essential to understanding the clinical-epidemiological dynamics linked to each variant of SARS-CoV-2 circulating in the province of Villa Clara during the COVID-19 pandemic.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest exists among the Authors.

### *Authors' Contributions*

All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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