Integrated Surveillance of Respiratory Viral Pathogens During the Post-Pandemic Period at the Molecular Biology Laboratory of Villa Clara, Cuba

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Abstract

Acute respiratory infections (ARI) of viral etiology represent one of the leading causes of morbidity and mortality worldwide. The objective of this research was to determine the circulation of respiratory viruses in the province of Villa Clara, Cuba, during the post-pandemic period. A cross-sectional, descriptive, observational study was conducted in the Molecular Biology Laboratory of the Provincial Center of Hygiene, Epidemiology, and Microbiology (PCHEM) of Villa Clara province, Cuba, from May 2023 to December 2025. The study population consisted of all nasopharyngeal swab samples from primary and secondary care settings with suspected acute respiratory infections (ARIs) or severe ARIs (N=1,206). These collected samples were processed by real-time RT-PCR for SARS-CoV-2 detection using the RIDA®GENE SARS-CoV-2 commercial kit. Nucleotide sequencing was performed to determine circulating variants and subvariants, thus complementing the epidemiological analysis. Influenza virus (FLU B) was the most frequently detected, with 19 confirmed cases (43.2%), followed by CoV-OC43 with 12 cases (27.3%). It should be noted that 6 samples showed coinfection: one of FLU B with CoV-OC43; one of Respiratory Syncytial Virus B (RSV B) with CoV-OC43; two of FLU B with CoV-HKU1; one of CoV-OC43 with CoV-HKU1; and one of Metapneumovirus with CoV-OC43. That of the 44 confirmed samples, a 97.7% concordance rate was obtained with respect to the LNR-IPK. Of the 10% of negative samples submitted, a 100% concordance rate was obtained. It is concluded that there is active and significant circulation of Human Coronavirus OC43, especially in older adults, which was also the most frequent agent and associated with severe forms of the disease.

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1. Introduction

Acute Respiratory Infections (ARI) of viral etiology constitute a constant challenge for global public health [1-3] and represent one of the main causes of morbidity and mortality worldwide, with a significant health and economic burden for health systems, especially in children under five years of age, older adults and people with comorbidities, even in the post-COVID-19 acute emergency era [2, 4, 5].

The SARS-CoV-2 pandemic not only reshaped the epidemiology of other respiratory viruses but also highlighted the critical need to establish integrated surveillance systems capable of detecting multiple pathogens simultaneously and efficiently [4, 6, 7]. The implementation of this type of surveillance based on molecular techniques should not be limited to emergency situations but is essential as a fundamental tool for public health control programs, enabling more precise clinical care, resource optimization, and the rational use of antimicrobials, especially in susceptible populations such as children [1, 2, 8].

The relaxation of non-pharmacological measures, changes in population immunity, and possible virus-virus interactions have generated dynamic and, at times, unpredictable epidemiological patterns for pathogens such as influenza, respiratory syncytial virus (RSV), rhinovirus, human metapneumovirus, and adenovirus, among others [1, 3].

These viruses compete or co-circulate with new SARS-CoV-2 variants [1, 2, 9]. This complex scenario, where clinical symptoms are frequently nonspecific, underscores the importance of accurate etiological diagnosis [5,10,11]. In the post-COVID-19 era, respiratory virus circulation has shown dynamic, complex, and sometimes atypical patterns, with marked seasonal resurgences [1, 2, 6].

In the specific context of Cuba, acute respiratory infections (ARIs) have historically maintained a high incidence within the population's health profile [10, 12, 13]. The post-pandemic period, defined as beginning in May 2023 with the lifting of the international health emergency, has presented its own particularities. The National Health System, strengthened by the massive implementation of Molecular Biology Laboratories (MBLs) during the COVID-19 response, now faces the challenge of reorienting this diagnostic capacity toward comprehensive and sustainable surveillance [11, 13, 14]. This transition is crucial for monitoring not only the endemic persistence of SARS-CoV-2, but also the resurgence of other respiratory viruses that can cause seasonal outbreaks of clinical and epidemiological relevance [5, 10, 14].

For a country like Cuba, the constant monitoring of circulating variants and subvariants is essential, as it allows for the evaluation of the impact of national vaccines, guides potential updates to vaccine formulas, detects the introduction of lineages of interest, and provides valuable data for evidence-based public health decision-making [5, 13, 14]. Integrating this genomic data with epidemiological, clinical, and laboratory information enables a comprehensive understanding of the dynamics of COVID-19 in its post-pandemic phase [4, 8, 14].

The implementation of the Villa Clara, Cuba, Laboratory of Molecular Biology (LBM) using real-time RT-PCR has been a significant strength for epidemiological surveillance programs, making a notable contribution to the monitoring of viruses responsible for Severe Acute Respiratory Infection (SARI) and Influenza-like Illness (ILI) in primary health care (PHC) and hospitals [13, 14, 15]. Syndromic surveillance is insufficient in this context, making molecular etiological diagnosis an indispensable tool for guiding appropriate clinical management, rationalizing antibiotic use, and optimizing healthcare resources, adopting the comprehensive One Health approach that recognizes the links between human, animal, and environmental health [4, 14, 20].

In this regard, molecular biology techniques are indispensable tools for conducting sentinel surveillance of respiratory viruses circulating in the population. Among these techniques, amplification methods such as real-time reverse transcription polymerase chain reaction (RT-PCR) and multiplex PCR are recognized [5, 10, 14]. Internationally, various commercial tests for viral diagnosis have been designed based on the use of different molecular targets and multiplex PCR platforms to simultaneously detect several respiratory viruses, such as Influenza A and B; Respiratory Syncytial Virus A and B; Parainfluenza 1, 2, 3, and 4; Metapneumovirus; Bocavirus; Adenovirus; Enterovirus; Seasonal Coronaviruses; Co OC43; Co NL63; Co HKU1; and Co 229E. with a high potential to improve the identification of these viral pathogens [8, 11, 14].

In this new landscape, SARS-CoV-2 surveillance transcends mere virus detection, and genomic sequencing stands as a fundamental pillar for global health preparedness and response [8, 11, 14]. The World Health Organization (WHO) and other agencies emphasize the need to monitor the virus's evolution to rapidly identify the emergence of variants with potential implications for transmissibility, virulence, or the effectiveness of medical countermeasures, including vaccines and treatments [5, 8, 17].

The objective of the research was to determine the circulation of respiratory viruses in the province of Villa Clara, Cuba in the post-pandemic period.

2. Materials and Methods

2.1. Study area

The research was carried out in Villa Clara province, Cuba, whose provincial capital is Santa Clara municipality and covered the 13 municipalities that comprise it. This provinces located in the central region of the island of Cuba (Latitude: 22º 29'40" N, Longitude: 79º28'30" W), and has the following geographical limits; to the west, with Matanzas province, to the east, with Sancti Spíritus province and to the south, with Cienfuegos province (Figure 1).

2.2. Type of study

A cross-sectional descriptive observational study was carried out in the Molecular Biology Laboratory of the Provincial Center of Hygiene, Epidemiology and Microbiology (PCHEM) of the province of Villa Clara, Cuba in the period from May 2023 to December 2025.

2.3. Study Population

The study population consisted of all nasopharyngeal swab samples from primary and secondary care settings, with suspected ILI or severe ARI (N=1,206). These collected samples were processed by real-time RT-PCR for SARS-CoV-2 detection using the RIDA®GENE SARS-CoV-2 commercial kit. Those that tested negative (n=123) were then analyzed by multiplex PCR using the VIASURE Respiratory Panel IV kit for the detection of 18 respiratory viruses (Influenza A and B; Influenza A H1N1; Respiratory Syncytial Virus A and B; Parainfluenza 1, 2, 3, and 4; Adenovirus; Bocavirus; Metapneumovirus; Rhinovirus; Enterovirus; Coronavirus strains 229E, OC43, NL63, and HKU1).

Confirmed SARS-CoV-2 cases were sent to the National Reference Laboratory for Influenza and other respiratory viruses at the Pedro Kourí Institute of Tropical Medicine (IPK) for quality control. Nucleotide sequencing was performed to determine circulating variants and subvariants, thus complementing the epidemiological analysis.

RNA extraction from all clinical samples was performed using the commercial QIAamp® Viral RNA Mini Kit (QIAGEN) [18]. A real-time RT-PCR amplification program was applied to the RIDA®GENE SARS-CoV-2 kit according to the manufacturer's guidelines [19].

For the VIASURE Respiratory Panel IV kit [20], used for the detection of 18 respiratory viruses, the manufacturer's analytical recommendations were followed, applied to a real-time multiplex PCR program.

2.4. Statistical Analysis

The statistical analysis was performed using SPSS version 15.0. For qualitative variables, absolute and relative frequencies were used, expressed as numbers and percentages. The results are presented in tables and figures.

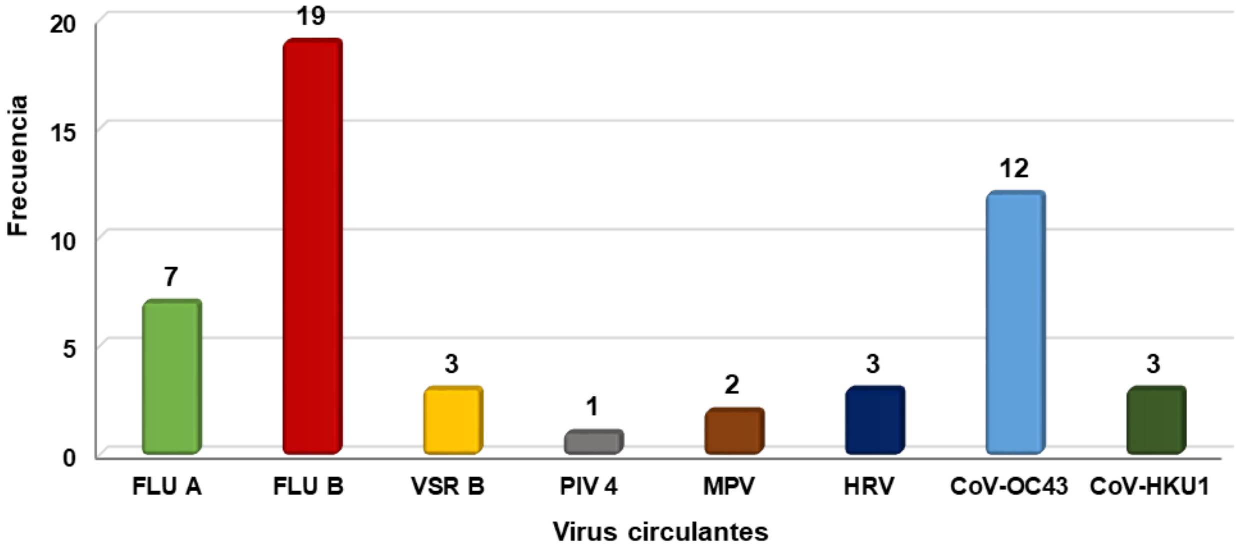
2.5. Ethical aspects

For the conduct of the research, informed consent was taken into account, as well as the ethical standards that made it possible to promote and ensure respect for all participants in the study, so that their criteria/opinions and individual rights were respected, in order to generate new knowledge without violating the ethical principles of privacy and confidentiality of personal information [21].

3. Results

As can be seen in figure 1, of the 123 respiratory samples analyzed by real-time RT-PCR, 44 were confirmed for non-SARS-CoV-2 respiratory viruses at the Molecular Virology Laboratory of Villa Clara. Influenza virus (FLU B) was the most frequently detected, with 19 confirmed cases (43.2%), followed by CoV-OC43 with 12 cases (27.3%). It should be noted that 6 samples showed coinfection: one of FLU B with CoV-OC43; one of Respiratory Syncytial Virus B (RSV B) with CoV-OC43; two of FLU B with CoV-HKU1; one of CoV-OC43 with CoV-HKU1; and one of Metapneumovirus with CoV-OC43.

During this period, other respiratory viruses also circulated in the province: Rhinovirus (HRV), Parainfluenza type 4 (PIV4) and seasonal coronaviruses CoV HKU1 and CoV OC43.

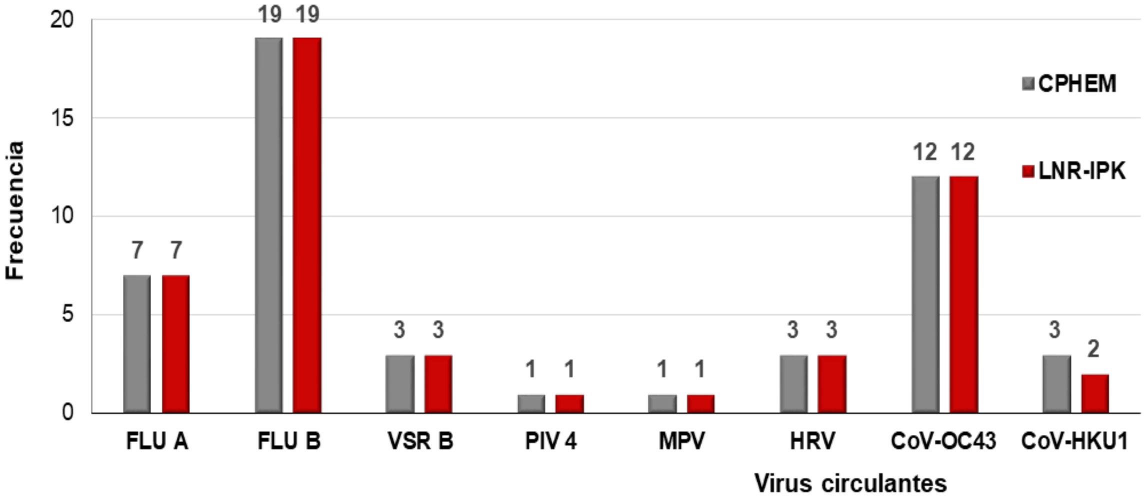


Source: Respiratory virus surveillance Database

Fig 1: Circulation of non-SARS-CoV-2 respiratory viruses in the post-pandemic period. CPHEM, Villa Clara. May 2023 - December 2025

Figure 2 shows that of the 44 confirmed samples, a 97.7% concordance rate was obtained with respect to the LNR-IPK. Of the 10% of negative samples submitted, a 100% concordance rate was obtained.

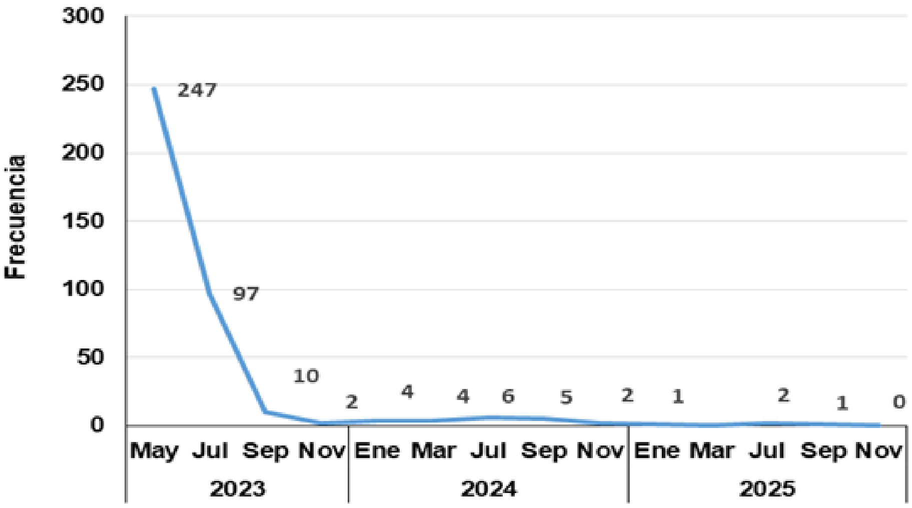
Only one case of CoV-HKU1 detected at the Villa Clara Molecular Biology Laboratory did not match the LNR-IPK results. This misclassified sample may have been related to RNA degradation during sample storage and transport, as well as the use of different platforms for nucleic acid extraction and amplification.



Source: Respiratory virus surveillance Database

Fig 2: Sentinel surveillance of respiratory viruses. Molecular Virology Laboratories of Villa Clara and LNR-IPK. May 2023 - December 2025

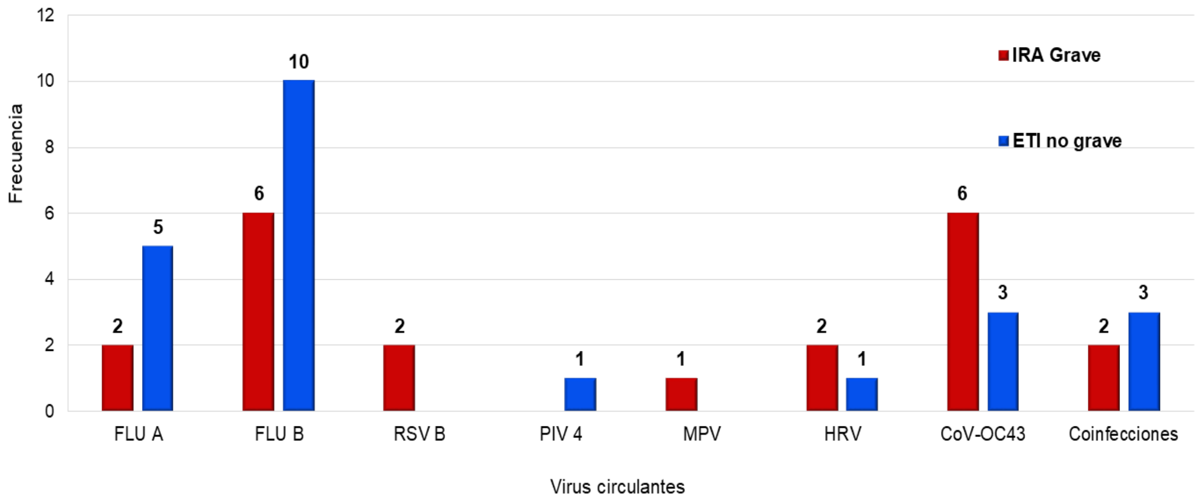
As can be seen in figure 3, in the post-pandemic period there was a sustained decrease in the number of confirmed cases of SARS-CoV-2, with May 2023 being the month with the highest reported number of cases with 247 confirmed cases, followed by June 2023 with 97 cases.



Source: Respiratory virus surveillance Database

Fig 3: Confirmed cases of SARS-CoV-2 in the post-pandemic period. CPHEM, Villa Clara. May 2023-December 2025

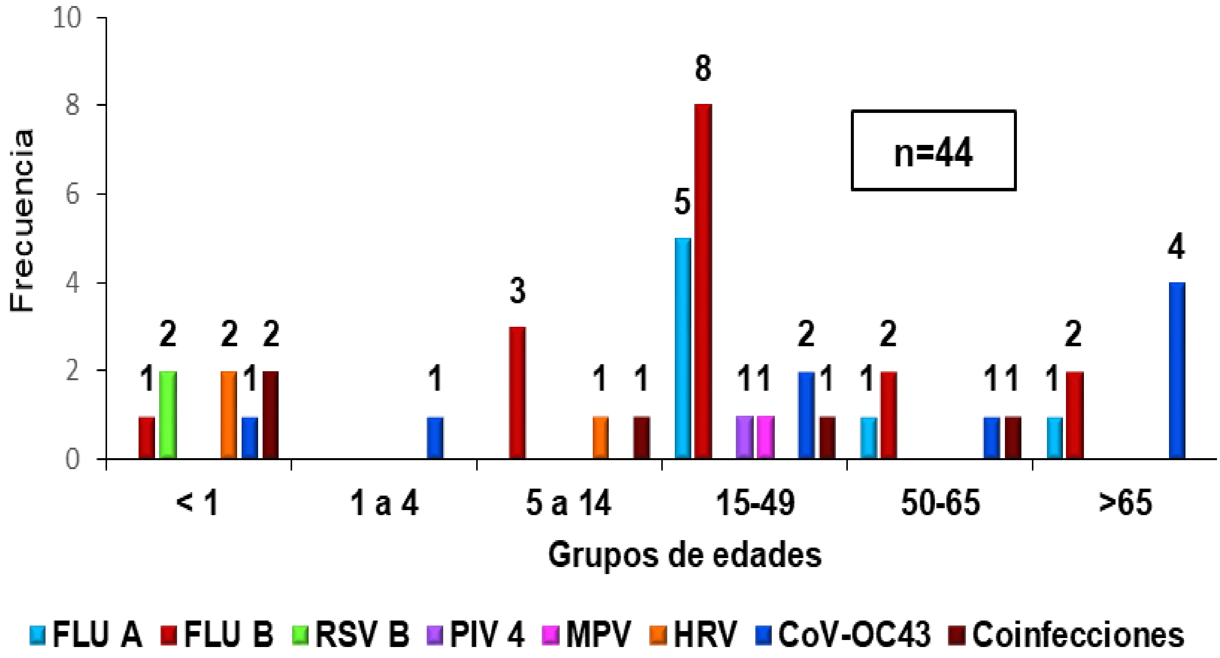
When analyzing the frequency of detected respiratory viruses in relation to age groups (Figure 4), it was observed that in the under-1-year age group, RSV B was detected more frequently in 3 cases (15%); in the 5–14-year, 15-49 year, and 50–65-year age groups, FLU B predominated in 3, 8, and 2 cases (60%, 44.4%, and 40%), respectively; likewise, in patients over 65 years of age, a greater number of samples were confirmed positive for CoV-OC43 (4, or 57.1%). It is important to note that the highest number of coinfections occurred in children under 1 year of age (2 cases: one of FLU B with CoV-OC43 and another of CoV-HKU1 with CoV-OC43 as well).



Source: Respiratory virus surveillance Database

Fig 4: Surveillance of respiratory viruses by age group in samples processed at the Molecular Virology Laboratory of CPHEM, Villa Clara. May 2023-December 2025

When analyzing the frequency of the respiratory viruses detected in relation to the epidemiological classification of the processed samples (Figure 5), there was a higher frequency of non-severe ILIs, predominantly Influenza A (71.4%); however, severe ARIs were predominantly Influenza B (37.5%) and CoV-OC43 (66.7%) with 8 and 6 confirmed cases respectively, followed by RSV with the 2 cases reported in children under 1 year of age and 2 of the detected coinfections.



Source*:* Respiratory virus surveillance Database

Fig 5: Surveillance of respiratory viruses according to epidemiological classification in samples processed at the Molecular Virology Laboratory of the CPHEM. March to December 2019

As can be seen in figure 6, which shows the Omicron subvariants and lineages identified during the post-pandemic period, there was a predominance of the XBB.1.16 subvariant, which circulated between May and February 2023. Subsequently, the presence of JN.1 was detected (February-June 2024), followed by KP3 (June-July 2024), and finally the LP.1 and XFC lineages, which remained in circulation from July 2024 to August 2025.

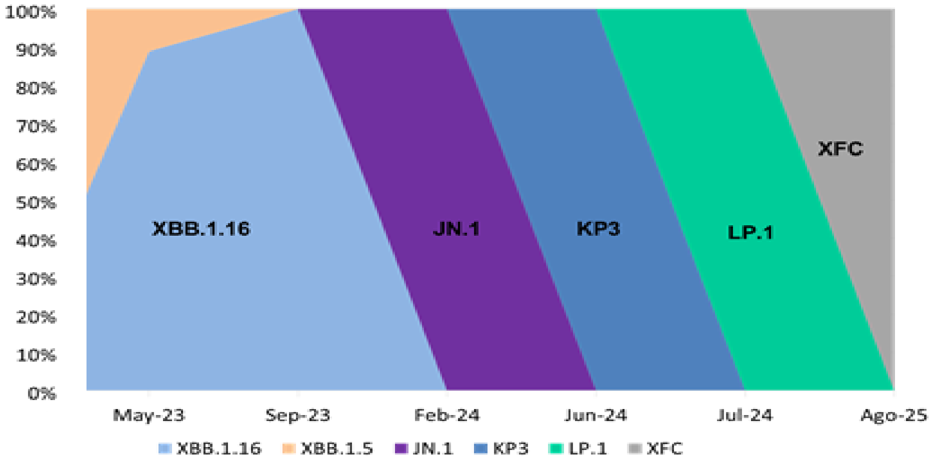


Fig 6: Genomic surveillance of SARS-CoV-2 in the post-pandemic period. CPHEM, Villa Clara. May 2023-December 2025

4. Discussion

This study provides crucial information on the dynamics of respiratory viruses in Villa Clara province during the post-pandemic period (May 2023–December 2025). The findings reflect a complex epidemiological scenario, where SARS-CoV-2 circulation decreases and other seasonal respiratory viruses re-emerge strongly, exhibiting specific distribution patterns according to age and clinical severity [1,2,4].

The gradual decline in SARS-CoV-2 detection throughout the study period, with a peak in May 2023, reflects the transition to an endemic phase of the virus, aligning with global trends reported for the same period [1,22,23]. This pattern suggests the consolidation of population immunity due to vaccine effectiveness and the possible influence of reduced diagnostic pressure specifically targeting this pathogen outside of hospital settings [6,10,17]. However, its persistent circulation underscores the need for continued surveillance, given its capacity to generate new variants.

Characterization of non-SARS-CoV-2 respiratory virus circulation revealed a clear predominance of Influenza B virus, followed by Human Coronavirus OC43. This finding is particularly relevant, as it marks a divergence from the immediate pandemic period, when influenza circulation was suppressed globally [22-24]. The reemergence of Influenza B as the most frequently detected virus in our sample coincides with international reports describing a return of influenza viruses with atypical or out-of-season circulation patterns in the post-COVID-19 period [23-25]. The notable presence of HCoV-OC43, a seasonal coronavirus, highlights the full resumption of circulation of traditional respiratory pathogens, whose transmission was drastically reduced by pandemic containment measures [25-27].

However, the results achieved by several authors found minimal or no circulation of Influenza virus during the post-pandemic period, at historically low levels after the lifting of health containment measures and the reopening of borders [28-33].

According to the authors, it is important to point out that influenza B, as a cause of respiratory infection outbreaks, represents a significant public health challenge due to its impact on vulnerable populations such as children [11,34,35]. Although historically considered less severe than influenza A, its outbreaks can trigger a substantial burden on healthcare systems. Therefore, a multidisciplinary approach to sentinel surveillance is necessary to monitor cases in hospitals and establish measures to prevent community and healthcare-associated outbreaks, starting within secondary healthcare settings [24,25,36].

Analysis by age group revealed a distinct infection profile. Respiratory Syncytial Virus (RSV), detected exclusively in infants under one year of age, is consistent with the known epidemiology of this virus, which causes the greatest burden of disease in infants [11,34,35]. In contrast, Influenza B was the predominant pattern across all age groups from 5 to 65 years, demonstrating a broad spectrum of infection in the economically active and school-aged population. In adults over 65 years of age, HCoV-OC43 was the most frequent agent [1,11,37]. This finding warrants attention, as, although seasonal coronaviruses are usually associated with common colds, in older adults and people with comorbidities they can cause severe lower respiratory tract infections, as evidenced in these results.

Regarding clinical severity, while most non-severe Acute Respiratory Infections (ARI) were associated with Influenza B, this virus, along with HCoV-OC43, was also the main agent identified in cases of severe ARI. This reinforces the fact that Influenza B should not be underestimated as a cause of severe morbidity and hospitalization, a widely documented fact [22,25,38].

It should also be noted that viral coinfections are frequently not taken into consideration in the etiological diagnosis of viral infectious diseases. This coexistence of two or more viruses responsible for a respiratory condition in the same individual can directly affect key clinical variables such as the duration of the illness, its progression to severe forms, or mortality; especially at the extremes of age, such as in children, in whom the immaturity of their immune system compared to adults contributes to a higher risk of coinfections [2,11,35].

In this regard, multiplex PCR is crucial for the patient, the physician, and the laboratory because it allows the detection of multiple pathogens in a single reaction, optimizing turnaround time, accuracy, and diagnostic resources, especially in laboratories with limited resources [5,10,39].

The study by Sanza *et al*. in Spain [35] is consistent with the present research in reporting a higher number of coinfections in children under 14 years of age.

Nationally, the results of this study are similar to reports from the Cuban Surveillance System, which has documented persistent but decreasing circulation of SARS-CoV-2 and the resurgence of influenza and other viruses since 2023 [12-14]. The predominance of Influenza B observed in Villa Clara coincides with partial reports from the Pedro Kourí Institute of Tropical Medicine (IPK), which identified this lineage as the most prevalent in certain post-pandemic seasons [13,14]. The exclusive detection of RSV in infants is a classic pattern also observed in Cuban studies prior to the pandemic [13,14].

Internationally, this research aligns with PAHO/WHO observations and studies in Europe and North America, which report changes in post-pandemic immunity with untimely and, at times, high-intensity circulation of RSV and influenza, sometimes with changes in traditional seasonality [36,37,40]. The co-circulation of multiple viruses, evidenced by the six coinfections detected in this report, is also a phenomenon reported more frequently in the post-COVID-19 era, potentially complicating the clinical picture [2,6,35].

Selective pressure from multiple factors has led to the persistence of the Omicron variant of SARS-CoV-2, along with its subvariants and lineages, reaching endemic status. Cuba and the province of Villa Clara are no exception to this trend [14, 41, 42]. Sequencing confirmed SARS-CoV-2 samples from Villa Clara, beginning in May 2023, at the National Reference Laboratory for Influenza and other respiratory viruses of the IPK (Pedro Kourí Institute of Tropical Medicine), demonstrated that the Omicron variant was the only one detected during that period. This finding aligns with international reports indicating a clear predominance of Omicron lineages in the global epidemiological context [40-42]. The analysis by Markov *et al.* [39] highlights that the current post-pandemic phase is dominated by the continuous evolution and diversification of these lineages, with shifts and no return to previous ones, as was the case in this study.

It should be noted that in Villa Clara, the XBB.1.5 (kraken) and XBB.1.16 (Arcturus) subvariants, classified in 2023 as subvariants of interest and recombinants derived from two sublineages, BA.2.10.1 and BA.2.75, were dominant until the end of 2023, at which point they were displaced by JN.1, designated as a sublineage of interest due to its high transmissibility.

This sublineage was present until June 2024, when the KP1, KP2, and KP3 subvariants emerged. These subvariants, all derived from JN.1, are under monitoring and are not classified as subvariants of interest or concern.

Subsequently, the detection of the Omicron XFG subvariant in June 2025 falls within a global context in which multiple SARS-CoV-2 recombinants have been identified simultaneously or independently in different regions, coinciding with a global increase in cases [24,26,40]. This is also the case with the XFC sublineage, detected in Cuba in August 2025. This finding reflects the country's capacity to maintain active genomic surveillance aligned with international trends.

5. Conclusion

The post-pandemic transition phase of SARS-CoV-2 was confirmed, with a decreasing detection curve beginning in 2023 in Villa Clara province, where active and significant circulation of Human Coronavirus OC43 was observed, especially among older adults. This virus was also the most frequent agent and was associated with severe forms of the disease. Respiratory Syncytial Virus (RSV) maintained its classic pattern of severe illness restricted to infants and newborns. The convergence with the global circulation of subvariants demonstrates that the virus's behavior in Cuba is not isolated, but rather part of the global dynamics of SARS-CoV-2 evolution. This underscores the importance of continuing to strengthen early detection and international scientific cooperation to anticipate potential public health impacts.

Institutional Review Board Statement

Not applicable.

Transparency

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study, and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Competing Interests

The authors declare that they have no competing interests.

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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