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Research Article

Update of Respiratory Viruses Infection Rate in the Pandemic SARS-CoV-2 Period in Symptomatic Patients (December 2021-April 2023)

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Abstract

Background: The outbreak of COVID-19 caused by SARS-CoV-2 is currently the peak season of common respiratory viral infections. The clinical symptoms of most patients infected with SARS-CoV-2 are not significantly different from those of common respiratory viral infections. Therefore, the use of fast and specific methods, such as BiofilmArray RP2.1plus, play an important role in the early discrimination of respiratory viruses in symphomatic subjects.

Methods: Nasopharyngeal swabs from 1,680 patients, clinically suspected of Acute Respiratory Infections (ARI) from December 2021 to April 2023, were collected to detect SARS-CoV-2 or/and other viruses from common respiratory pathogens via BioFilmArray RP2.1plus. All data was analyzed to unravel the epidemiological patterns.

Results: A total of 1680 patients with suspicion of ARI were included in this study. Among them, 1468 were men, with a mean age of 63.9±14.6 years. The pathogens detected most frequently included SARS-CoV-2 (31,42%, 548/1680) and Rhinovirus (6.72%,113/1680).

Conclusion: This study reveals the epidemiological characteristics of common respiratory viruses and their clinical impact in the last SARS-CoV-2 outbreak in an epidemic area. Possible causes were attributable to the use of masks, or to specific respiratory receptors or lifestyle factors.

Keywords: Respiratory viral pathogens; SARS-CoV-2 virus; Surveillance research; BiofilmArray; COVID-19;

Abbreviations

AdV: Adenovirus;

CoV: Coronavirus;

COVID-19: Coronavirus disease 19;

HCoV: Human Coronavirus;

HMPV: Human Metapneumovirus;

HEV: Human Rinoviruses/Enteroviruses;

Inf A-B: Influenza virus A-B;

MERS: Middle East respiratory syndrome;

NPS: Nasopharyngeal swab;

PCR: Polimerase Chain Reaction;

PIV: influenza virus;

RSV: Respiratory syncytial virus;

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2;

Introduction

Acute respiratory infections (ARI) and severe acute respiratory diseases (SARI) are public health burdens worldwide. Changes in the epidemiology of other respiratory viruses during the COVID-19 pandemic are being reported throughout the world [1].

Respiratory viruses are responsible for high morbidity and mortality among infected individuals imposing a heavy economic burden [2] and causing varying degrees of respiratory diseases among all age groups. They include respiratory syncytial virus (RSV), influenza virus (Inf A-B), parainfluenza virus (PIV), human metapneumovirus (HMPV), rhinovirus (HEV), adenovirus (AdV), and coronavirus (COV) [3-5]. These respiratory viruses can be transmitted through direct or indirect contact, droplets, and aerosols. SARSCoV-2, the cause of the epidemic in Wuhan, China, in December 2019, has spread around the world, causing significant morbidity and mortality. The World Health Organisation (WHO) classified COVID-19 a public health emergency of worldwide significance on January 31, 2020 [6]. The control measures implemented during the COVID-19 pandemic were associated with changes in the prevalence of other respiratory viruses [4].

Furthermore, mask use, social distancing, smart work and closure of schools, as measures implemented by the national policy to contain the spread of SARS-CoV-2 (https://www.governo.it/it/coronavirus-misure-del-governo (accessed on 20 April 2022), have further influenced changes in the positivity rates of most respiratory viruses [7].

In the updated study, a sharp decrease in the number of patients positive for SARS-CoV-2 infection was found in the respiratory virus panel. This reveals that most COVID-19 vaccines reduce, or likely reduce, the percentage of participants with COVID-19 confirmed symptomatic, and for some, there is high certainty evidence that reduces severe or critical disease, particularly in immunocompromised patients [8]. However, the number of patients suffering from other respiratory viruses has increased significantly, particularly the rhinovirus. Adopting this diagnostic approach using the BiofilmArray RP2.1*plus* as a test, the analysis performed in our previous study revealed the epidemiological features of common respiratory viruses and their clinical impact during the ongoing outbreak of COVID-19 in an epidemic area of Italy (Rome) in the period between (March 2020-November 2021) [7].

Here we report the results obtained from the analysis conducted during the period December 2021 to April 2023 in conjunction with the declaration of the end of the pandemic period. The results give a clear picture of how the circulation of respiratory viruses, especially rhinoviruses, and SARS-CoV-2 has changed in association with the new health and treatment policies developed and implemented in the last pandemic period.

Materials and Methods

Study Design and Patients

This was an observational ongoing study investigating the rate of infection of common respiratory viruses carried out at the Virology unit of "Tor Vergata" University Covid-Hospital of Rome, Italy from December 2021 to April 2023. The eligibility criteria for the patients enrolled in this study were as follow: available anagraphical data, suspicion of ARIs and diagnosis of respiratory virus by BioFilmArray RP2.1*plus*. The results were tabulated and statistically analysed. The details of the laboratory test findings were recorded on case report forms. The study was in accordance with the Declaration of Helsinki, as revised in 2013.

The Multiplex Platform for Respiratory Pathogen Panel Testing

The FilmArray Respiratory Panel 2.1*plus* (RP2.1*plus*) is a multiplex *in vitro* diagnostic test for the simultaneous and rapid (~45-min) detection of 23 pathogens directly from nasopharyngeal swab (NPS) samples,as previously reported [7]. Testing was carried out according to the manufacturer's instructions (BioFire Defence LLC, and BioFire Diagnostics LLC; Salt Lake City, UT) [9].

Briefly, 300 µL of sample was mixed with buffer and injected into a test pouch containing all the necessary reagents for the extraction of nucleic acids, PCR amplification and detection of the respective targets. The test pouch was inserted into the BioFire FilmArray Torch instrument and run using the software provided. Quality control was performed using BioFire external positive control according to the manufacturer's protocol prior to study testing.

Statistical Analyses

The categorical variables were expressed as numbers and percentages. Positive rates were calculated as the number of positive cases/total number of effected tests. Statistical analysis of group-wise expression levels was performed using a nonparametric Mann Whitney test in case of 2 independent samples. Data were analysed by one-way analysis of variance (ANOVA) and Bonferroni *post hoc* test. Statistical analyses formed using v SPSS 22 and a two-sided p-value of 0.05 were considered statistically significant in this study.

Results

Clinical and epidemiological characteristics of patients enrolled after FilmArray Respiratory Panel 2.1 *plus assay*

A total of 1680 patients (period: December 2021-April 2023) with suspicious of symptoms of respiratory infection were included in this study. Men were 857 and 832 were women, witha mean age of 63.9 years. As reported in **Table 1**, the most frequently detected pathogens included SARS-CoV-2 (31.42%, 548/1680, from December 2021 to April 2023), Rhinoviruses (6.70%, 125/1680), RSV (3.7 %, 67/1680) and InfA-B (2.55%, 43/1680). Considereng the requests from the different departments of the hospital, the majority of them were from the Emergency Department (40.1%, of patients), followed by Infectious Diseases Department (33.3% of total).

Number of samples	**1680**		
Age	63.9±14.6	n.	%
Sex	male	857	51,01
	female	823	48,99
Type on Infection		**n.**	**%**
SARS-CoV-2	Positive Cases	528	31.42
Adenovirus	Positive Cases	18	1,07
Influenza A viruses	Positive Cases	43	2.55
Respiratory Syncytial Virus (RSV)	Positive Cases	62	3.7
Human Metapneumovirus (HMPV)	Positive Cases	16	0.95
Parainfluenzaviruses (PIV)	Positive Cases	21	1.25
Coronaviruses	Positive Cases	16	0.95
Rhinoviruses/Enterovirus (HEV)	Positive Cases	125	7.44
Total	Positive Cases	817	48.63
	Negative Cases	863	51,36

Table1: Characterisation of the cohort analysed in the period December 2021-April 2023 and FilmArray Respiratory Panel 2.1 *plus assay* results

Profiles of respiratory viruses and SARS-CoV-2 detected in enrolled patients

In a previous study, we analysed the rate of positive cases traced from October 2019 to November 2021 for common respiratory viruses and from March 2020 to March 2021 for SARS-CoV-2 cases (number of positive cases/number of effected swabs) [7]. As shown in Figure 1, we reported the epidemiological curve of the last analysed period (December 2021- April 23) to get a general overview of the positivity rates of examined viruses. An important change in the the trend of positive SARS-CoV-2 rate trend was represented after June 2022, with a strong decrease of positive cases. (Fig.1, orange line). Then, an increase in the positive SARS-CoV-2 rate was recorded in autum-winter 2022-23, but the percentage was lower than common respiratory viruses (Figure1 blu line).

However, the epidemiological curve for common respiratory viruses was very lower in the first examited period (December 21- August 22) with a significant increase of positive cases in the last period (October 22- April 23). Massive vaccination and other measures determinated an attenuation of the respiratory pathologies caused by SARS-CoV-2.

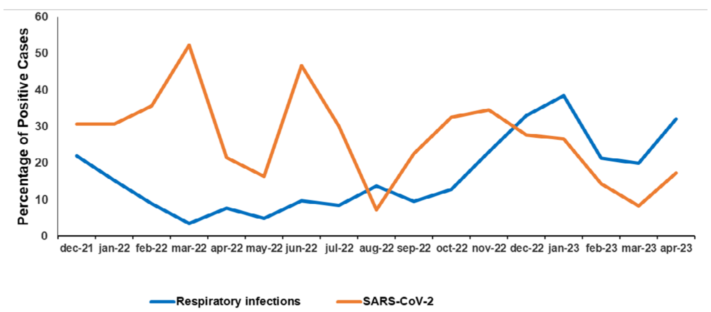

Figure 1: Epidemiological curve of positive cases registered every month from December 2021 to April 2023 of respiratory viruses (blue line) and SARS-CoV-2 (orange line). The BioFilmArray RP2.1plus assay was used on swabs. The percentage of positive cases (ordinate axis) was calculated as number of total number of cases analysed every month.

In this surveillance study, patterns of co-infection were also analized and the information is summarised in **Table 2**. The most prevalent co-infection pattern was SARS-CoV-2/Human Rhino/ Enteroviruses that accounted for 50% (6/12) of all co-infection cases, Table 2.

	n	%
SARS-CoV-2 /Rhinovirus/enteroviruses	6	50
SARS-CoV-2 /Metapneumovirus	1	8.4
SARS-CoV-2/Adenovirus	1	8.4
RSV/ Parainfluenza Type 3	2	16.4
RSV/ InfA-B	2	16.4
Total	**12**	**100**

Table 2: Types of double and triple virus co-infections (period December 2021- April 2023)

The impact of HEV infection positive cases before and during the SARS-CoV-2 pandemic (2019-2023) confirms the influence of SARS-CoV-2

We also analysed in details the epidemiological curve of HEV, starting from the pre-pandemic SARS-CoV-2 period October 2019 to April 2023. As reported in Figure 2, the characteristic seasonal peak of HEV and other common viruses (period October 2019-February 2020) was absent in next winter (October 2020-February 2021), during the full spread of SARS-CoV-2. Interestingly, after the first Italian vaccination campaign, positive rates of HEV infection increased (June 2021-April 2023), differentely of other common respiratory virus as previously reported [7]. We also reported an increase of positive cases in April 2023, hypotizing a selection of routinary seasonal peak.

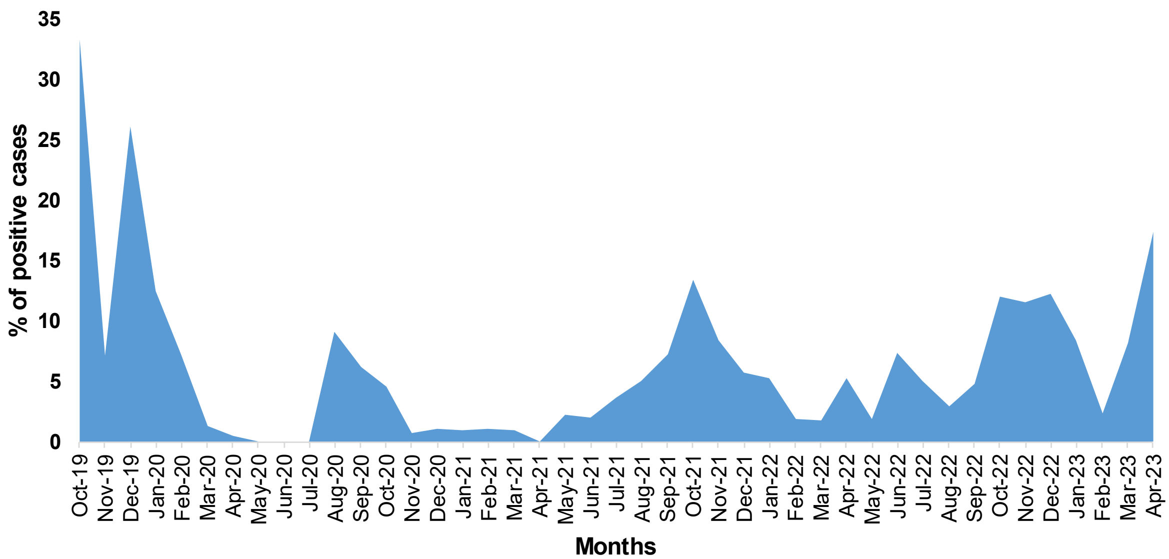

Figure 2: Epidemiological curve of positive cases for HEV infection. As shown, the typical seasonal peak of common respiratory viruses (October 2019-February 2020) was lost during the SARS-CoV-2 pandemic period (March 2020 to April 2021). An increase was recorded after SARS-CoV-2 massive vaccination campaign.

SARS-CoV-2 and other respiratory viruses during the pandemic period: Comparison on Positive rates and Incidence

In our previous work [7] we had analysed the period between Dec 2019 and November 2021, now we compared those data with the new data obtained in the period December 2021-April 2023, analysing the percentages of positive cases and calculated the ratio (Fold Change, FC) between the two periods.

SARS-CoV-2 remains the most frequently detected virus with a constant rate of positivity in the two periods (positivity rate I period *vs* II period: 30% vs. 31.47%, FC =1).

HEVs was the most frequently detected virus after SARS-CoV-2 (I period 108/2300, 4.67 % positivity rate; II period 125/1680, 7.44 %) with a 1.5-fold increase in the second period (FC=1.58). Although the positivity rates for all other viruses remained significantly lower than for SARS-CoV-2, in the II period for some of them we observed a significant increase in positive cases (PIV, FC=2.3; HMPV, FC=3.1; RSV, FC=3.08). Adenoviruses that had a positivity rate of 0.043 % in the first period went up to 1.07% in the second period (FC=24) (Figure 3).

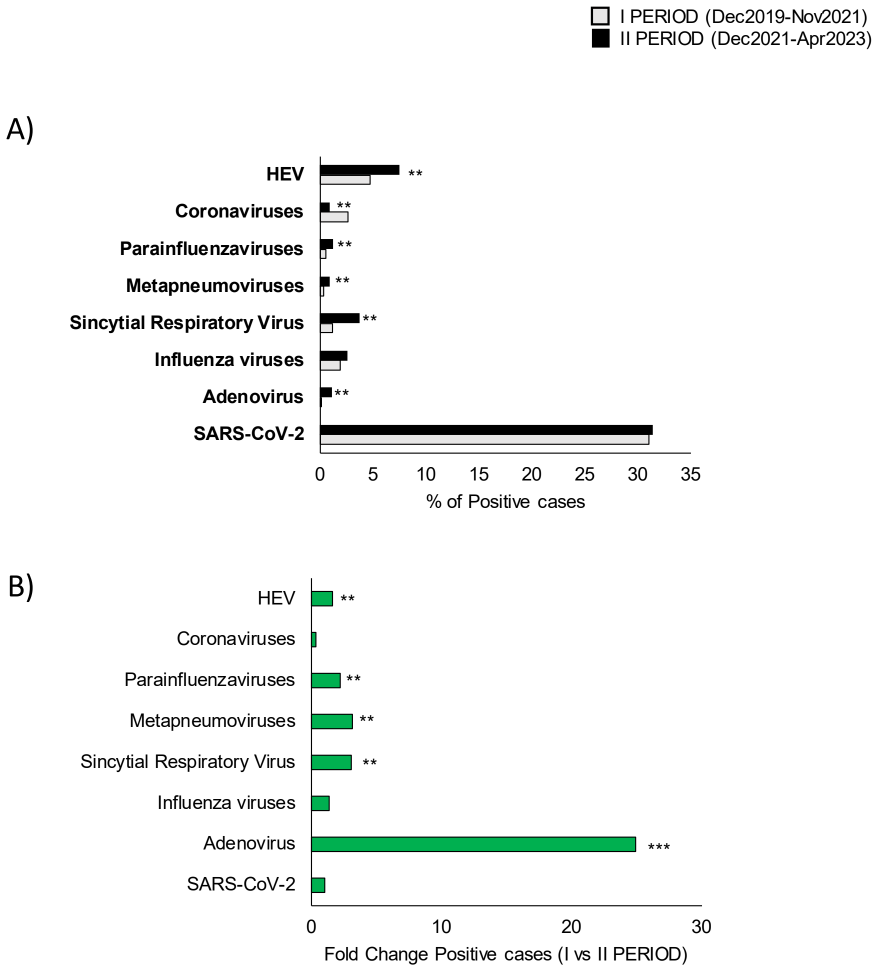

Figure 3: Comparison of SARS-CoV-2 and other respiratory virus positivity rates in the I and II periods of the pandemic. Statistical analysis of group-wise expression levels was performed using a nonparametric Mann Whitney test in case of 2 independent samples.

Finally, the analysis of the incidence of SARS-CoV-2 positivity with respect to other respiratory viruses in the two periods showed a significant reduction in the incidence of SARS-CoV-2 with respect to Adenovirus, PIV, HMPV, RSV and HEV, while the incidence of SARS-CoV-2 increased compared to other coronaviruses. No differences were found between the two periods with respect to Influenza A viruses. Thus, in the second period we found a completely different circulation of respiratory viruses with a 95% increase in adenovirus, 26% increase in Influenza A virus, 67% increase in Respiratory Syncytial Virus, and 68% increase in metapneumovirus and parainfluenza virus. 54% and 36% increase in HEV.

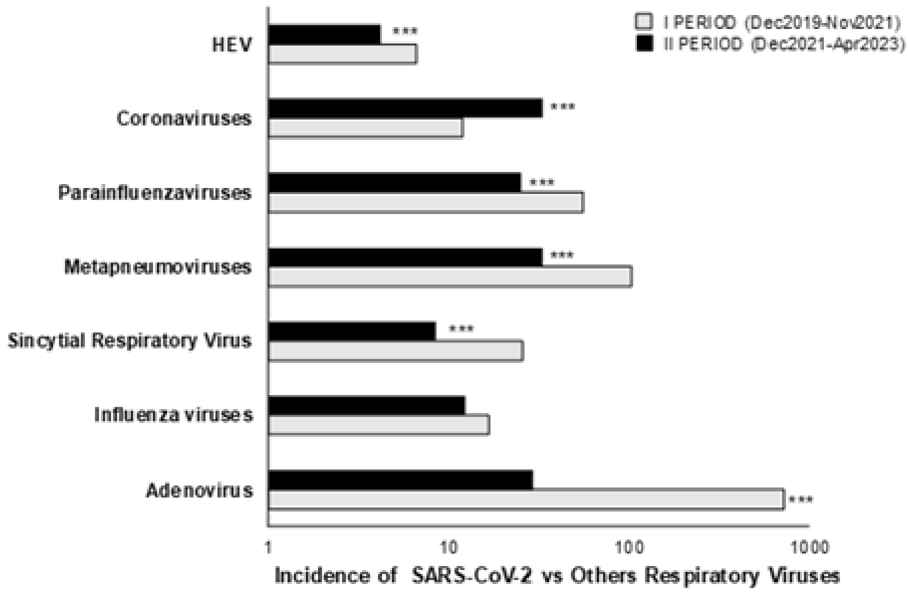

Figure 4: Incidence of SARS-CoV-2 in other Respiratory Infection in the I and II period analysed. Statistical analysis of group-wise expression levels was performed using a nonparametric Mann Whitney test in case of 2 independent samples.

Discussion

The end of the COVID-19 pandemic caused by SARS-CoV-2 was declared by the WHO on May 5, 2023 although serious concerns about patient management and long-term sequelae remain. Diagnostic work done in recent years has contributed to more rapid diagnosis and specificity [9-13]. The ability to differentiate diseases caused by these viruses was essential for patient management and control of SARS-CoV-2 infection, as well as public health surveillance and responses. Unfortunately, these viruses can cause infections that can present very similar symptoms, making clinical differentiation very difficult [14]. In addition, understanding the possible influence of public health and social measures (PHSM) during the COVID-19 pandemic on the incidence of other respiratory viral diseases gives a broader picture of the public health impact of the COVID-19 pandemic [15].

Therefore, we have continued to monitor until April 2023 simultaneously the positivity rates of common respiratory viruses and SARS-CoV-2 in our hospital , one of the largest in the Lazio region, allowing us to monitor in real time the developments of the pandemic and confirm what has been recorded in other parts of the world [16-19].

We observed an important change of the positive rate of SARS-CoV-2 after June 2022, with a strongly decrease of positive cases, while in autum-winter 2022-23 a new increase of positive SARS-CoV-2 rate was registered, but with a rate lower than that of the common respiratory viruses. The latter ones showed low positive rates in the first examited period (December 2021- August 2022) with a significant increase of positive cases in the last period (October 2022- April 2023). Massive vaccination and other preventive measures determinated an attenuation of the gravity of respiratory pathologies caused by SARS-CoV-2, but comparing the data obtained in the previous work [7] with the data from the new analysed period it was found that SARS-CoV-2 remains constant as a percentage of positivity (FC=1), while all other respiratory viruses, very little circulating until November 2021 returned.

Regarding RSV, we have observed a three-fold increase in the II period (FC=3.08) with 67% incidence of SARS-CoV-2. In 2022, an increase in RSV infections has been demonstrated in several countries. When comparing clinical characteristics with case histories from 2009-2015, no differences were found associated with survival and mortality rates suggesting that the RSVs currently circulating are not virulent anymore [20]. Although in a study conducted in Egypt in the 2022-2023 winter season influenza caused a higher infection rate than RSV, while RSV caused more severe symptoms than influenza [18]. Similarly both in the USA and in Germany there have been an unprecedented number of paediatric and adult hospitalisations for RSV infections [21] confirming what was predicted by an in silico model that predicted how reemerging RSV outbreaks would affect larger swathes of populations than typical seasonal ones [22]. Thus, the current epidemics of RSV reflect a stronger dependence of infection susceptibility on immunity from previous exposure, and the Public Health interventions to counter the spread of COVID-19 have had an impact on the transmission of respiratory viruses and related diseases. The absence of circulating viruses could lead to a lack of immunity and increased susceptibility to more serious infections [23].

Furthermore, HMPV, strongly associated with RSV, increased approximately three times (FC = 3.16, 68%), confirming the data of other groups that observed an an unusual outbreak of respiratory infections caused by human metapneumovirus in children during the sixth wave of COVID-19, which was greater than usual and had higher pulmonary severity associated with longer hospitalization and need for intensive care in Spain [24] and also a resurgence of HMPV associated with no-pharmaceutical reduction interventions to prevent SARS-CoV-2 transmission [25].

HEV are already very present also in the first pandemic period (Dec 2019-Nov 2021) and increased one and a half times in the second period (FC=1.58; 36%). Rhinoviruses and/or enteroviruses were the most common group of respiratory viruses detected in all age groups and in both emergency department and infectious diseases department. In the first year of the COVID-19 pandemic, other respiratory viruses showed reduced circulation, while HEVs rapidly reemerged and remained a major factor in ARI, accounting for approximately three-quarters of viral detections [26].

Our results confirmed the high circulation of respiratory viruses and considering the increase in respiratory virus activity to pre-pandemic levels, and related hospitalizations especially among children under the age of 2 years, it should be recommended that surveillance for influenza, RSV, and SARS-CoV-2 be strengthened and integrated, and necessary measures be taken to prevent and control severe outcomes, ensuring high vaccination coverage in high-risk groups, adequate clinical and health service management, and especially strict observation of infection prevention control measures, appropriate therapies, and the ability to intervene promptly in case of the onset of complications.Moreover, adequate supplies of antivirals and timely treatment of associated complications [27,28].

The removal of COVID-19 containment measures, resulting in the return of further respiratory viral infections, could be rapid and significant. It will be critical for public health professionals to recognise that the return of further respiratory viral infections could be rapid and significant; therefore, public health authorities must be alerted early to the possible spread of respiratory viral diseases. Additionally, the use of PHSM against COVID-19 decreased the circulation of influenza infections during winter compared with previous seasons by reducing their incidence as with SARS-CoV-2 [15,18]. This lack of seasonality will need to be monitored in the future to see whether it is a transient or permanent phenomenon. In addition, the emergence of a major new circulation of respiratory viruses suggests that the implementation of PHSMs in the post-COVID-19 era will be a useful control tool for influenza and respiratory diseases worldwide.

Conclusions

We concluded that in the second part of the pandemic period, particularly in the seasonal peaks of ARI in 2022-2023, we have observed a rapid increase of respiratory viruses with a concomitant slowing down of SARS-CoV-2 incidence with respect to ARI due to removal of restriction measures or the use of masks outdoors, the vaccination effects, and the reduction of required monitoring of SARS-CoV-2 infection. We would like to suggest routine use in diagnostics of multiplex PCR assays, which will allow for timely diagnosis and for surveillance purposes. Additionally, during periods of increased respiratory virus activity, the use of masks could greatly assist healthcare personnel and frail individuals in controlling any outbreaks associated with ARI.

Authors' Contributions

'Conceptualisation, GC, SG, CM, AM, FM, PP. and DM.;

methodology, GC, SG, SDC, MM, LDT, PP, CM, AM.;

software, SDC, MM, GC.;

validation, GC, MM and LDT.; formal analysis, GC.;

investigation, GC, PP, CM, AM, SG, ; resources, SG.;

Data curation, GC, SDC, AM.;

Writing: Original draught preparation, GC, PP, DO, FM, AM, MC, SDC, VG, , SB, EB, LP, VP, EA, MF,CM and SG .;

Writing: Review and editing, GC, PP, DO, FM, AM, MC, SDC, VC, , , SB, EB, CM AND SG.;

supervision, SG, CM, PP, MC, DO, EB, AB, LP;

project administration, SG.;

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Informed Consent Statement:

Written informed consent has been obtained from the patient) to publish this document and the study was in accordance with the Declaration of Helsinki and the local ethics committee guidelines.

Data Availability Statement: All data generated or analysed during this study are included in this article. Further enquiries can be made in the following be directed to the corresponding author.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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