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Impact of COVID-19 on the Circulation of Respiratory Viruses in Kinshasa: Analysis of the Prevalence and Viral Etiologies of Acute Respiratory Infections

Edith Nkwembe Ngabana[1,2*], Youdhie Ituneme N'ka Flabo[1,2*], Grace Mufwaya Makayi[1,2], Lisa Lebo Nsimba[1,2], Meris Matondo Kuamfumu[1,2], Heritier Yalungu Milo[3], Laurette Imbanda[2], Leonie Manya Kitoto[4], Wally Disuasani[3], Pelagie Babakazo[3], Steve Ahuka Mundeke[1,2]

[1]Service de Microbiologie, Département de Biologie Médicale, Cliniques Universitaires de Kinshasa, Kinshasa, République Démocratique du Congo
[2]Département de Virologie, Institut National de Recherche Biomédicale, Kinshasa, République Démocratique du Congo
[3]Ecole de Santé Publique de l'Université de Kinshasa, Kinshasa, République Démocratique du Congo
[4]Direction de Surveillance Epidémiologique, Ministère de la Santé Publique Hygiène et Prévention, Kinshasa, République Démocratique du Congo
Email: *edithnkwembe1@gmail.com, *itunemeyoudhie@gmail.com

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Abstract

Background: In the Democratic Republic of the Congo (DRC), the sentinel surveillance system for influenza was adapted to monitor SARS-CoV-2 infections during the COVID-19 pandemic, providing an opportunity to expand surveillance to other respiratory viruses responsible for acute respiratory infections (ARI). This study aims to document the extent and circulation of influenza viruses, as well as the viral etiologies of ARIs during the COVID-19 pandemic in Kinshasa. **Methodology:** Between 2020 and 2021, respiratory samples were collected from patients presenting with influenza-like illness (ILI) or severe acute respiratory infection (SARI). Socio-demographic information was gathered using a pre-established form. Virus detection was carried out using molecular biology techniques. The prevalence of the respiratory viruses and the characteristics of the population were analyzed using descriptive statistics, and comparisons between gender and age groups were made using Chi-2 test. **Results:** Eleven respiratory viruses were detected in the analyzed samples. Children under five years of age, individuals over 65 years old, and male patients were particularly affected. The COVID-19 pandemic altered the circulation of influenza viruses. **Conclusion:** In addition to SARS-CoV-2, other respiratory viruses co-circulated during the pandemic, and the circulation of influenza viruses was disrupted.

Keywords

Viral Etiologies, ARI, Pandemic, Surveillance, Kinshasa

1. Introduction

The emergence of SARS-CoV-2 caused significant morbidity and mortality worldwide, leading governments to prioritize non-pharmaceutical interventions (NPIs) to slow the spread of the infection and alleviate the burden on healthcare systems. Since NPIs aim to reduce the transmission of respiratory viruses, notable disruptions occurred in the usual circulation of common respiratory viruses [1]-[4]. This resulted in a reduction in viral activity, particularly of influenza and respiratory syncytial virus (RSV), thus impacting the prevalence of viral respiratory infections [1]-[4]. These changes, which vary by virus type, are multifactorial, influenced in part by the collective implementation of NPIs and by interactions among viruses, including viral interference [5].

Acute respiratory infections (ARIs) are highly contagious, transmitted through direct contact via respiratory secretions or indirectly via contaminated objects or surfaces [6]. They exhibit similar clinical manifestations, ranging from a common cold to severe acute respiratory distress syndrome [6] [7]. The viruses responsible for ARIs belong to different families, exclusively targeting the respiratory system and following a seasonal pattern [6]. These infections can manifest in epidemic or pandemic form, representing one of the leading causes of morbidity and mortality worldwide, particularly among children under five years of age [7] [8].

Approximately 97% of severe ARIs are reported in low-income countries, where around 70% of cases originate from South Asia and sub-Saharan Africa [6]-[8]. In these regions, the causes of respiratory infections are often poorly understood due to the diversity of respiratory pathogens, lack of diagnostic resources, similarity in ARI symptoms, and confusion with tropical febrile illnesses, making their management particularly challenging, especially in children [7]-[10].

In the Democratic Republic of the Congo (DRC), a surveillance system for severe acute respiratory infections and influenza-like illnesses has been in place since 2006, has provided valuable insights into the epidemiology and extent of influenza [11]. This system, along with the resources of respiratory virus laboratories, has also been used to monitor, study, and respond to SARS-CoV-2 infections.

This study aims to document the impact of the COVID-19 pandemic on the circulation of influenza viruses and the viral etiology of ARIs during this period in Kinshasa, to inform future public health responses.

2. Materials and Methods

2.1. Study Setting and Design

This cross-sectional study was conducted in Kinshasa from January 2020 to

December 2021, as part of the influenza sentinel surveillance system established by the Ministry of Public Health, Hygiene, and Prevention in the DRC since 2006.

2.2. Study Population

The study population included patients who visited one of the four influenza sentinel surveillance sites in Kinshasa for influenza-like illness (ILI), defined as a fever ≥38˚C accompanied by cough and/or runny nose, sore throat, or for severe acute respiratory infection (SARI), defined as dyspnea in addition to respiratory symptoms, requiring hospitalization at one of the surveillance sites. Respiratory samples were collected from these patients.

2.3. Sample Collection, Storage, and Transport

Nasopharyngeal and oropharyngeal samples were collected from outpatients with ILI and inpatients with SARI using sterile polyester swabs. The samples were placed in a tube containing 3 ml of Viral Transport Medium (VTM) (COPAN Italia S.p.A) and stored at +4˚C before being transported to the National Institute of Biomedical Research (INRB) within 72 hours, in accordance with the influenza surveillance protocol in the DRC.

2.4. Nucleic Acid Extraction

Viral nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN) for non-SARS-CoV-2 respiratory viruses and the RNA/DNA Purification Kit, Spin Column (DaAn Gene, China) for SARS-CoV-2, following the manufacturers' instructions.

2.5. qRT-PCR for SARS-CoV-2

SARS-CoV-2 detection was performed using multiplex qRT-PCR with the "Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA PCR-Fluorescence Probing" (DaAn Gene, China) on the ABI 7500 Fast system (Applied Biosystems, USA).

2.6. qRT-PCR for Non-SARS-CoV-2 Respiratory Viruses

qRT-PCR tests were performed on the ABI 7500 Fast system (Applied Biosystems, USA) using the Ag Path One Step RT-PCR Kit (Applied Biosystems by Thermo FISHER Scientific). Influenza viruses A and B were initially detected using the singleplex method, followed by subtyping and genotyping of positive influenza A and B viruses using specific primers and probes according to the CDC protocol from Atlanta. Detection of eight other respiratory viruses, including human metapneumovirus (hMPV), human respiratory syncytial virus (hRSV), human parainfluenza viruses (hPIV) 1, 2, 3, and 4, rhinovirus (RV), and adenovirus (AdV), was performed using singleplex rRT-PCR with specific primers and probes for each virus, following the CDC protocol from Atlanta.

2.7. Data Collection and Statistical Analysis

Data were collected using Access 2020 software before being analyzed. Patients were categorized into five age groups based on their vulnerability to ARIs: infants and young children (0 - 4 years), children (5 - 14 years), adolescents and young adults (15 - 44 years), adults (45 - 64 years), and elderly individuals (≥65 years). The prevalence of viruses and population characteristics were analyzed using descriptive statistics. Comparisons between age groups and gender were made using chi-square tests, with P-values <0.050 considered statistically significant. Statistical analyses were performed with Epi Info software (version 22.0), and the results are presented in tables and graphs.

3. Results

3.1. Description of the Study Population

The median age of the patients was 20 years, ranging from 1 to 42 years. The most represented age groups were 0 - 4 years (36%) and 15 - 44 years (33%). The majority of patients were female, accounting for 53% of the population (858 out of 1655) (**Table 1**).

Table 1. Sociodemographic characteristics of the patients.

Characteristics	N = 1655	%
Median age	20 (1 - 42)	
Age groups		
0 - 4 years	589	36
5 - 14 years	128	7.8
15 - 44 years	541	33
45 - 64 years	266	16
65 years+	110	6.7
Sex		
Female	858	53
Male	763	47
Case type		
ILI	1098	66.7
SARI	557	33.3

3.2. Types of Viruses Detected

3.2.1. Overall and Detailed Prevalence of Respiratory Viruses by Case Type

Among the 1655 samples tested, the overall prevalence of respiratory viruses was

45.75% (757 out of 1655) (**Figure 1**). SARS-CoV-2 was detected in 19.5% of the samples (324 out of 1655). Non-SARS-CoV-2 and non-influenza respiratory viruses were detected in 19.5% of the samples (321 out of 1655), distributed as follows: RSV (5.62%, 93 out of 1655), RV (5.32%, 88 out of 1655), PIV3 (3.1%, 51 out of 1655), ADV (1.93%, 32 out of 1655), MPV (1.87%, 31 out of 1655), PIV1 (0.61%, 10 out of 1655), PIV2 (0.39%, 5 out of 1655), and PIV4 (0.66%, 11 out of 1655). Influenza viruses were detected in 6.7% of the samples (112 out of 1655) (**Table 2**).

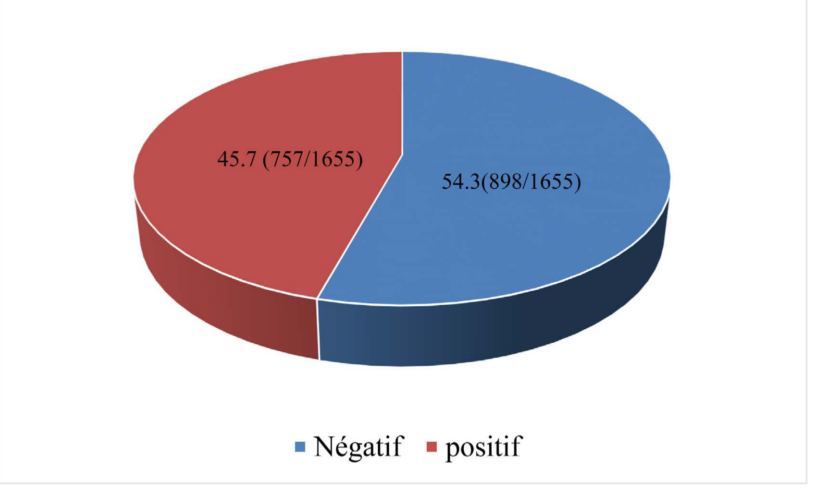

Figure 1. Overall prevalence of viruses responsible for ARIs.

Table 2. Detailed prevalence of respiratory viruses.

Detailed prevalence	N = 757	Case type		Influenza n = 112			
		ILI n = 490	SARI n = 267	Type A		Type B	
Influenza viruses	112 (6.7%)	72 (64.2%)	40 (35.7%)	H1N1p09	12 (11%)	Yamagata	5 (4.6%)
SARS-CoV-2	324 (19.5%)	244 (75%)	80 (25%)	H3N2	55 (49%)	Victoria	22 (20%)
Others respiratory viruses				NST*	18 (17%)		
ADV	32 (1.93%)	18 (56%)	14 (44%)				
PIV3	51 (3.1%)	29 (56.8%)	22 (43.1%)				
PIV4	11 (0.66%)	6 (54.5%)	6 (45.5%)				
RSV	93 (5.62%)	39 (42%)	54 (58%)				
MPV	31 (1.87%)	20 (64.5%)	11 (35.5%)				
PIV1	10 (0.61%)	9 (90%)	1 (10%)				
RV	88 (5.32%)	50 (56.8%)	38 (43.2%)				
PIV2	5 (0.30%)	3 (60%)	2 (40%)				
Total	321 (19.5%)	174 (54.2%)	147 (45.8%)				

(*): Non-subtypeable.

3.2.2. Sociodemographic Characteristics of Confirmed Cases by Type

- **SARS-CoV-2**

Children under 5 years old accounted for 53% of SARI cases, and males were predominantly affected (Table 3).

- **Other Non-SARS-CoV-2 and Non-Influenza Respiratory Viruses**

Children under 5 years (58%), individuals over 65 years (55%), and male patients were particularly affected (Table 4).

- **Influenza Viruses**

Children under 5 years (54%) and elderly individuals over 65 years were also significantly affected, as well as male patients (Table 5).

Table 3. Sociodemographic characteristics of sars-cov-2 confirmed cases by case type.

Characteristics	N = 324	ILI	SARI	p-value
		N = 244	N = 80	
Median age	35 (8, 53)	36 (20, 53)	4 (1, 53)	
Age groups				<0.001
0 - 4 years	76 (24%)	36 (47%)	40 (53%)	
5 - 14 years	13 (4.1%)	13 (100%)	0 (0%)	
15 - 44 years	116 (36%)	103 (89%)	13 (11%)	
45 - 64 years	84 (26%)	69 (82%)	15 (18%)	
65 years+	35 (11%)	23 (66%)	12 (34%)	
Sex				0.046
Female	182 (56%)	140 (77%)	42 (23%)	
Male	142 (44%)	104 (74%)	36 (26%)	

Table 4. Sociodemographic characteristics of confirmed cases of other Non-SARS-CoV-2 and Non-Influenza Viruses by case type.

Characteristics	N = 321	ILI	SARI	p-value
		N = 170	N = 151	
Median age	2 (1, 23)	6 (1, 26)	1 (0, 6)	
Age groups				<0.001
0 - 4 years	190 (59.1%)	80 (42%)	110 (58%)	
5 - 14 years	28 (8.7%)	24 (86%)	4 (14%)	
15 - 44 years	70 (21.8%)	48 (69%)	22 (31%)	
45 – 64 years	20 (6.2%)	12 (60%)	8 (40%)	
65 years+	13 (4.04 %)	6 (45%)	7 (55%)	

Continued

Sex				0.001
Female	193 (60%)	112 (58%)	81 (42%)	
Male	128 (39%)	58 (45%)	70 (55%)	

Table 5. Sociodemographic characteristics of Influenza Virus confirmed cases by case type.

Characteristics	N = 112	ILI	SARI	p-value
		N = 72	N = 40	
Median age	6 (1, 29)	16 (2, 33)	1 (1, 4)	
Age groups				<0.001
0 - 4 Years	54 (49%)	25 (46%)	29 (54%)	
5 - 14 Years	12 (11%)	9 (75%)	3 (25%)	
15 - 44 Years	35 (32%)	31 (89%)	4 (11%)	
45 - 64 years	8 (7.1%)	6 (75%)	1 (25%)	
65 years+	3 (2.7%)	1 (33%)	2 (67%)	
Sex				0.047
Female	62 (55%)	44 (73%)	18 (27%)	
Male	50 (45%)	28 (55%)	22 (45%)	

3.3. Influenza Virus Circulation During the COVID-19 Pandemic, 2020-2021

In 2020, influenza activity was intense, with the circulation of influenza viruses A/H3N2, A/H1N1p09, and the B/Victoria and B/Yamagata lineages from Week 1 (W1) to Week 8 (W8), the pre-pandemic period. After the detection of SARS-CoV-2 in Week 9 (W9), a halt in influenza activity was observed until Week 46, followed by a resurgence of influenza activity with the A/H3N2 virus until Week 52 (W52) (**Figure 2**).

In 2021, influenza activity continued with the A/H3N2 virus co-circulating with B/Victoria from Week 1 to Week 9 (W9). Influenza activity was marked by the B/Victoria virus from Week 15 to Week 23 (W23) and by the A/H3N2 virus from Week 49 to Week 52 (W52) (**Figure 3**).

From 2020 to 2021, the monthly circulation of SARS-CoV-2 and Influenza viruses shows that SARS-CoV-2 dominated in terms of the number of positive cases compared to influenza viruses over the observed period, especially during peak times. However, influenza viruses continued to circulate, albeit more sporadically, indicating co-circulation with SARS-CoV-2 (**Figure 4**).

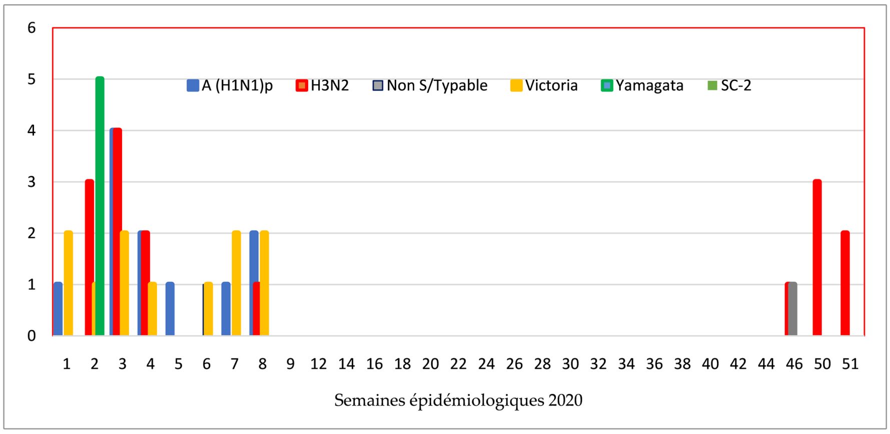

Figure 2. Weekly circulation of Influenza Viruses in Kinshasa, 2020.

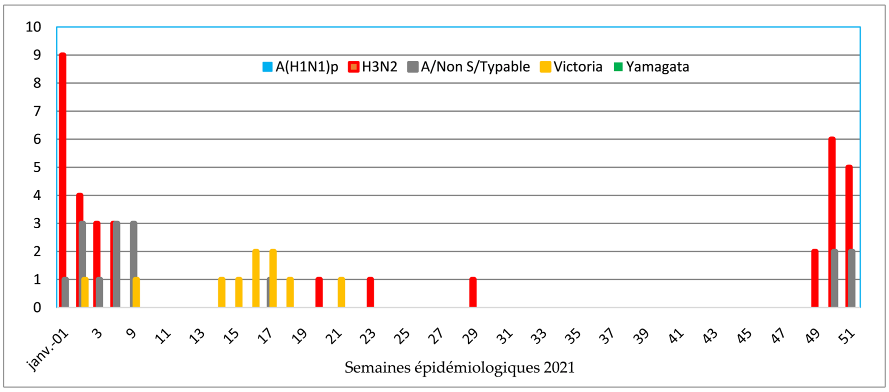

Figure 3. Weekly circulation of Influenza Viruses in Kinshasa, 2021.

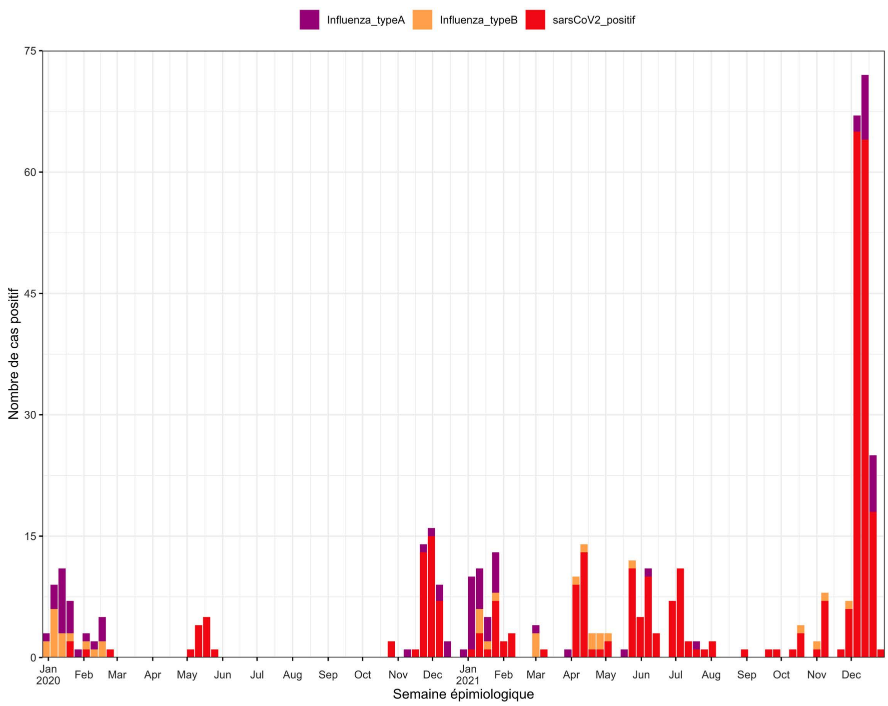

Figure 4. Monthly circulation of SARS CoV-2 and Influenza Viruses in Kinshasa, 2020-2021.

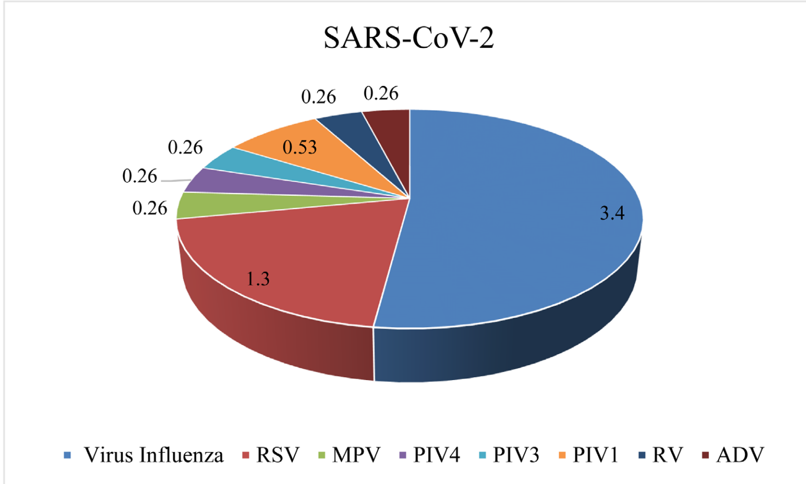

Figure 5. Co-infection of SARS-CoV-2 and other respiratory viruses.

3.4. Co-Infections of SARS-CoV-2 and Other Respiratory Viruses

Co-infections of SARS-CoV-2 with other respiratory viruses accounted for 6.6% (50 out of 757) of the total cases. Among these, 3.4% were associated with influenza virus, 1.3% with RSV, 0.53% with PIV1, and 0.26% each for MPV, PIV3, PIV4, RV, and ADV (**Figure 5**).

4. Discussion

The objective of our study was to document the impact of COVID-19 on the circulation of respiratory viruses and the etiology of acute respiratory infections (ARIs) in Kinshasa during the pandemic.

In our study, a higher proportion of samples was collected from outpatients (66.3%) compared to hospitalized patients (33.7%), particularly among adults. This trend may reflect increased concern among adults who are worried about COVID-19 and seek testing quickly at the onset of symptoms.

The overall prevalence of respiratory viruses in our study was 45.7%. Several respiratory viruses were co-circulated during the pandemic in Kinshasa, which aligns with findings from other studies [2]-[4]. This prevalence is higher than that observed in Southeast China (32.5% [12]) and Madagascar (19% [13]), but lower than the rate reported in Taiwan region (75.3% [14]). The differences in prevalence may be attributed to variations in susceptibility to respiratory viruses across populations, influenced by factors such as behavior, culture, demographics, and environmental conditions [15].

We observed a higher prevalence of SARS-CoV-2 at 19.5% compared to other non-SARS-CoV-2 respiratory viruses. Indeed, SARS-CoV-2 was the predominant virus during the 2020 pandemic, affecting anywhere from 0 to over 40% of the global population, which was naive to SARS-CoV-2 [16]. Furthermore, the prevalence of other respiratory viruses, excluding SARS-CoV and influenza viruses, was also 19.5%.

Severe cases of ARI associated with SARS-CoV-2 were less common (25%)

compared to mild cases (75%). Although SARS-CoV-2 was the predominant virus during the pandemic, infecting a significant portion of the global population [16], its severity is often associated with factors such as comorbidities and older age [17]. Our study revealed that children under 5 years old were significantly impacted, particularly after the emergence of the Delta variant, in contrast to the early stages of the pandemic when severe cases in this age group were rare [18]. These findings could be attributed to the circulating variant, along with factors such as comorbidities, limited healthcare access, and challenging socio-economic conditions.

For other non-SARS-CoV-2 respiratory viruses, the overall prevalence was 19.5%, with RSV (5.7%) and RV (5.3%) being the most common. Many studies have reported the impact of COVID-19 on the prevalence of respiratory viruses [1]-[3] [19] [20]. The absence of pre-pandemic data limits our ability to assess these effects precisely, but low rates of respiratory viruses were observed during the pandemic [21] [22]. Preventive measures such as non-pharmaceutical interventions (NPIs), lockdowns, restrictions, and COVID-19 vaccination efforts helped protect against other respiratory viruses.

Moreover, our study found that 45.8% of ARI cases caused by respiratory viruses other than SARS-CoV-2 and influenza were associated with severe illness (SARI). This finding highlights the significant burden of non-SARS-CoV-2 and non-influenza respiratory viruses in causing severe respiratory infections during the pandemic. While SARS-CoV-2 was the primary focus, other respiratory pathogens continued to contribute to severe cases, underscoring the need for comprehensive surveillance and management strategies that account for a broad range of viral agents. These results suggest that the co-circulation of multiple respiratory viruses can exacerbate the severity of infections, potentially overwhelming healthcare systems, particularly in resource-limited settings like Kinshasa.

ARIs due to respiratory viruses, particularly RSV, were more frequent among hospitalized patients (SARI), especially in children under 5 years ($p<0.001$) and the elderly. These findings are consistent with other studies indicating that RSV is a major pathogen of lower respiratory tract infections, particularly among young children and the elderly [13] [14] [18] [23]. Children and older adults are more vulnerable to ARIs due to immune immaturity or the presence of chronic illnesses [24] [25]. Other modifiable factors such as prematurity, malnutrition, and indoor air pollution also play a role [26].

We also observed an increased susceptibility of men to severe forms of acute respiratory infections (ARI), while women, although more frequently infected, tend to develop less severe cases. This sexual dimorphism could be attributed to hormonal, chromosomal, and behavioral factors, as well as comorbidities [27].

The extent of influenza during the pandemic (6.8%) was lower than before the pandemic [11] [12], which could be explained by a disruption in influenza virus circulation. No B/Yamagata lineage was detected during this period, supporting the idea of an interruption in the transmission of this lineage, as reported elsewhere [28]. Social distancing measures and movement restrictions may have reduced the transmission of the B/Yamagata lineage due to its slower growth phase and shorter transmission chains.

The COVID-19 pandemic led to the implementation of various control measures, including non-pharmaceutical interventions, which significantly impacted the transmission of other respiratory viral infections, particularly influenza and RSV [2] [4] [11]. The seasonal dynamics of influenza viruses, well known in our region, were disrupted during the pandemic. Detection of influenza viruses dropped after SARS-CoV-2 was detected on March 10, 2020, only resuming at the end of 2020. In 2021, influenza virus circulation was weak and sporadic, as observed in other studies [12] [29]. Changes in behavior, such as social distancing, mask-wearing, hygiene measures, and travel restrictions, were the main factors contributing to the reduction in influenza incidence. Additionally, viral interference between SARS-CoV-2 and influenza viruses may have limited co-infections [5].

This study has certain limitations, including The absence of data on the prevalence of respiratory viruses before the pandemic limits the ability to compare changes and understand the specific impacts of COVID-19 on virus circulation; The concentration of samples at sentinel surveillance sites in Kinshasa may introduce bias, as these sites may not be representative of the entire population or rural areas.; Despite The two-year study period, although covering a significant phase of the pandemic, may not capture all seasonal variations or long-term trends in respiratory viruses. The relatively low frequency of detected co-infections may not fully reflect the possible interactions between SARS-CoV-2 and other respiratory viruses, due to limitations in detection methods or the reduced number of co-infections.

Despite the limitations presented, this study has several strengths the Comprehensive Analysis of Viral Circulation who offers a detailed assessment of the prevalence and circulation of various respiratory viruses during the COVID-19 pandemic, including influenza, non-influenza viruses, and SARS-CoV-2.; the Impact of COVID-19 show how the pandemic altered the dynamics of respiratory viruses, with a notable disruption in the circulation of influenza viruses and a decrease in acute respiratory infections unrelated to SARS-CoV-2. By focusing on Kinshasa, the study provides valuable data on the sociodemographic characteristics of patients and prevalence variations by age group and gender, which is crucial for local public health policies. and The use of the sentinel surveillance system established since 2006 demonstrates the effectiveness of this system in adapting surveillance to new epidemiological challenges, such as the COVID-19 pandemic.

5. Conclusion

Our study revealed the impact of COVID-19 on the circulation of influenza viruses and clarified the role of other respiratory viruses in ARIs. These findings underscore the crucial importance of maintaining an integrated disease surveillance system to better understand and monitor respiratory viruses of public health interest.

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Conflicts of Interest

The authors of this paper declare no conflicts of interest in this research.

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