Coronavirus Pandemic

Seasonal patterns and prevalence of respiratory pathogens in children with acute respiratory infections in Wuhan, China

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Abstract

Introduction: Acute respiratory infection (ARI) is the major cause of disease and death in children. This study aimed to analyze the prevalence of common pathogens causing ARI in children aged 6 years or younger in Wuhan and the impact of the Coronavirus Disease 2019 (COVID-19) pandemic on the detection rate of these pathogens.

Methodology: Between March 2019 and January 2023, throat swab specimens were collected from 12,743 hospitalized children with ARI symptoms. Polymerase Chain Reaction (PCR) capillary electrophoresis fragment analysis was performed to detect nucleic acids of 12 respiratory pathogens. Then, we analyzed the seasonal epidemic and mixed infection patterns of various pathogens and assessed the impact of the COVID-19 pandemic on the detection rate of these pathogens.

Results: Of the 12,743 samples collected, 6,200 cases (48.65%) were positive for at least one pathogen. Human rhinoviruses (HRV) had the highest detection rate (2,475 cases, 19.42%). HRV was prevalent mainly in spring and autumn. Respiratory syncytial virus (RSV) was mainly popular in fall and winter. The detection rates of respiratory pathogens were significantly lower than average during the COVID-19 pandemic ($p < 0.01$). Children aged 1-3 years were found to be more susceptible to ARI. Among the positive cases, 405 cases (3.18%) had co-infection with two or more pathogens.

Conclusions: This study presents important findings on the epidemic seasonality and mixed infection patterns of common respiratory pathogens in children aged 6 years or younger with ARI in Wuhan and found that the COVID-19 pandemic had altered the epidemiology of ARI in this population.

Key words: Prevalence; acute respiratory infections; children; co-infection; pathogens.

Introduction

Acute respiratory infection (ARI) is a leading cause of morbidity and mortality in children, particularly in developing countries [1]. Despite a decline in the prevalence of pneumonia-related infections from 13.6 to 6.6 per 1,000 children younger than 5 years between 2000 and 2015 [2], pneumonia remains the leading cause of mortality in this age group [3]. In 2015, the World Health Organization reported the highest rates of pneumonia deaths among children younger than 5 years in Africa (0.5 million), followed by Southeast Asia (0.2 million) [2]. In China, pneumonia-related deaths account for 12.4% of all child deaths in children under 5 years old and 23.1% of all post-natal infant deaths [4]. Thus, ARI has been associated with a significant economic burden on families and societies in both developed and developing countries [5,6].

ARI can result from various bacteria, including *Streptococcus pneumoniae* and atypical bacteria like *Mycoplasma pneumoniae* (Mp), as well as viruses such as influenza A/B (FluA/B), parainfluenza (PIV), respiratory syncytial virus (RSV), and adenovirus (ADV) [7-10]. The emergence of the Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had significant impacts on the medical system and human health in many countries worldwide [11,12]. According to a report published by the Chinese Center for Disease Control and Prevention on February 1, 2023, since December 9, 2022, the number and positive rate of COVID-19 nucleic acid testing in all provinces across China first showed an increasing trend, then decreased [13]. The number of infected people peaked on December 22, 2022 (6.94 million positive cases), then gradually decreased to 2.5% on January 30.

As the prevalence of respiratory pathogens varies

among regions and populations, particularly in developing countries, rapid detection of respiratory pathogens is very important for guiding appropriate ARI treatment. However, there are limited reports on respiratory viruses in China, especially in the Wuhan region. Therefore, this study was designed to investigate the nucleic acid detection results of 12 pathogens from nasopharyngeal swabs of 12,743 children with ARI in Wuhan between March 2019 and January 2023 and compare the results before and after the COVID-19 pandemic to provide crucial epidemiological, clinical, and treatment information on respiratory pathogens in Wuhan.

Methodology

Study Design and Population

This retrospective study comprised 12,743 patients with ARI who were treated in a pediatric inpatient unit from March 2019 to January 2023. The inclusion criteria for Cases: patients ≤ 6 years old, acute fever, and respiratory symptoms. Exclusion Criteria were: Children with chronic diseases (such as diabetes, asthma) or immunocompromised conditions (such as HIV infection, undergoing chemotherapy), cases with incomplete medical records or those from which a qualified respiratory specimen was not collected, and duplicate cases. The study cohort consisted of 7,465 males and 5,278 females, aged 15 days to 6 years old, with an average age of 34 months. All pathogenic detection (nasopharyngeal swabs) was conducted within 48 hours of admission to exclude hospital-acquired respiratory tract infections. To better understand the variations in pathogen infection patterns across different developmental stages, the patients were categorized into five age groups based on their growth

and immune-system development: neonatal period (0 to ≤ 1 month, 1,346 cases), infancy (> 1 to ≤ 6 months 791 cases and > 6 months to ≤ 1 year, 1,835 cases), toddlerhood (> 1 to ≤ 3 years, 3633 cases), and preschool age (> 3 to ≤ 6 years 5,138 cases).

Specimen Collection

Nasopharyngeal swab specimens were collected from each child by trained healthcare professionals within 48 hours of admission. Swabs were immediately placed into viral transport medium and transported to the laboratory within 4 hours. Samples that could not be processed immediately were stored at 4°C for up to 24 hours before testing.

Pathogen Detection

The 13 Respiratory Pathogen Multiplex Detection Kit (PCR capillary electrophoresis fragment analysis) from Health BioMed Co was used following the manufacturer's instructions to test for 13 common respiratory pathogens, including FluA (H7N9, H1N1, H3N2, H5N2), Influenza A viruses H1N1(2009) (H1N1) (No H1N1 positive cases were detected in this study; thus, only the results of the additional 12 pathogens tested were analyzed), seasonal H3N2 viruses (H3N2), FluB (Victoria and Yamagata lineages), ADV (groups B, C and E), HBoV viruses (HBoV), Human rhinoviruses (HRV), PIV (types 1, 2, 3 and 4), Human coronaviruses (229E, OC43, NL63 and HKU1) (HCOV), RSV(groups A and B), Human metapneumoviruses (HMPV), Mp and *Chlamydia pneumoniae* (*Chlamydia trachomatis* and *Chlamydia pneumoniae*) (CPn). Among them, the test results for ADV, PIV, HCOV, RSV, and CPn were not typed. All testing procedures were strictly carried out according to

Table 1. Detection characteristics of different pathogens in different sexes and age groups.

	0 ≤ 1 month n = 1346 (769/577)[a] No. %	> 1 to ≤ 6 months n = 791 (518/273) No. %	> 6 months to ≤ 1 year n = 1835 (1121/714) No. %	> 1 to ≤ 3 years n = 3633 (2135/1498) No. %	> 3 to ≤ 6 years n = 5138 (2922/2216) No. %	All ages n = 12743 (7465/5278) No. %
Positive	68 (5.05)	369 (46.65)	956 (52.10)	2070 (56.98)	2737 (53.27)	6200 (48.65)
Male[b]	38 (4.94)	250 (48.26)	608 (54.24)	1271 (59.53)	1571 (53.76)	3738 (50.07)
Female[c]	30 (5.20)	119 (43.59)	348 (48.74)	799 (53.34)	1166 (52.62)	2462 (46.65)
ADV	4 (0.30)	4 (0.51)	83 (4.52)	176 (4.84)	298 (5.80)	565 (4.43)
HBoV	3 (0.22)	5 (0.63)	70 (3.81)	125 (3.44)	44 (0.86)	247 (1.94)
CPn	3 (0.22)	6 (0.76)	2 (0.11)	0 (0)	5 (0.10)	16 (0.13)
H3N2	0 (0)	5 (0.63)	41 (2.23)	59 (1.62)	96 (1.87)	201 (1.58)
HCOV	9 (0.67)	15 (1.90)	62 (3.38)	89 (2.45)	75 (1.46)	250 (1.96)
HMPV	1 (0.07)	34 (4.30)	97 (5.29)	260 (7.16)	323 (6.29)	715 (5.61)
PIV	6 (0.45)	63 (7.96)	176 (9.59)	289 (7.95)	244 (4.75)	778 (6.11)
RSV	22 (1.63)	137 (17.32)	197 (10.74)	390 (10.73)	410 (7.98)	1156 (9.07)
HRV	22 (1.63)	126 (15.93)	315 (17.17)	780 (21.47)	1232 (23.98)	2475 (19.42)
FluA	4 (0.30)	5 (0.63)	48 (2.62)	62 (1.71)	103 (2.00)	222 (1.74)
FluB	0 (0)	5 (0.63)	15 (0.82)	47 (1.29)	114 (2.22)	181 (1.42)
Mp	2 (0.15)	6 (0.76)	36 (1.96)	101 (2.78)	165 (3.21)	310 (2.43)

ADV: human adenovirus; HBoV: HBoV viruses; CPn: *Chlamydia pneumoniae*; H3N2: H3N2 viruses; HCOV: human coronaviruses; HMPV: human metapneumoviruses; PIV: parainfluenza; RSV: respiratory syncytial virus; HRV: human rhinoviruses; FluA: influenza A; FluB: influenza B; Mp: *Mycoplasma pneumoniae*. [a](Male cases / Female cases); [b]the denominator is male cases; [c]the denominator is female cases.

the manufacturer's instructions for the reagents. During the PCR capillary electrophoresis fragment analysis testing process, the following quality control steps were taken to ensure the accuracy of pathogen detection: Positive and negative control samples were included in each batch of tests. Reagents and equipment were regularly calibrated and validated. Laboratory personnel underwent regular training and assessment to ensure consistency and accuracy in their operations.

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using SPSS version 23.0. The detection rate of respiratory pathogens, co-infection rates, and age-based distribution were calculated. Chi-square tests were applied to compare detection rates among different groups (e.g., between genders, age groups, and before and after the COVID-19 pandemic). A $p < 0.05$ were considered statistically significant.

Results

Prevalence of respiratory pathogens

Respiratory pathogens were assessed in the 12,743 collected samples, and one or more respiratory pathogens were identified in 48.65% (6200/12743) of the patients (Table 1). The infection rate in boys (50.07%, 3738/7465) was significantly higher than that of girls (46.65%, 2462/5278) ($\chi^2 = 14.54$, $p < 0.01$). HRV (19.42%, 2475/12743) and RSV (9.07%, 1156/12743) were the most commonly identified respiratory pathogens in the hospitalized children, followed by PIV (6.11%, 778/12743), HMPV (5.61%, 715/12743) and ADV (4.43%, 565/12743).

Distribution of respiratory pathogens according to age groups

Analysis of the 12 pathogenesis in each age group (Table 1) showed that the highest infection rate was found in the 1-3 years age group (56.98%, 2070/3633), followed by the 3-6 years (53.27%, 2737/5138), while the lowest detection rate was observed in the < 1 month age group (5.05%, 68/1346) ($\chi^2 = 551.67$, $p < 0.01$). Among the < 1 month age group, RSV and HRV were the most detected pathogens, with 22 cases (1.63%). In the 1-6 months age group, RSV had the highest detection rate, with 137 cases (17.32%). HRV was the most detected pathogen in the 6 months-1year, 1-3 years and 3-6 years age groups, demonstrating a detection rate of 17.17% (315 cases), 21.47% (780 cases) and 23.98% (1232 cases).

Seasonality of respiratory pathogens

Next, the results were divided into four seasons by combining astronomical seasons with climatic seasons in Wuhan, China: spring (March, April, and May), summer (June, July, and August), fall (September, October, and November) and winter (December, January, and February). Among the DNA viruses (Figure 1A), epidemics of ADV were observed in the summer of 2019 and 2022, while HBoV was predominantly detected in the summer and fall of 2019 and the summer of 2021. Among the RNA viruses (Figure 1B), RSV was mainly epidemic in the winter.

Figure 1 A. influenza virus (FluA, FluB and H3N2); **B.** DNA virus (ADV and HBoV); **C.** atypical pathogen (Mp and CPn); **D.** RNA virus (HCOV, HMPV, PIV, RSV and HRV).
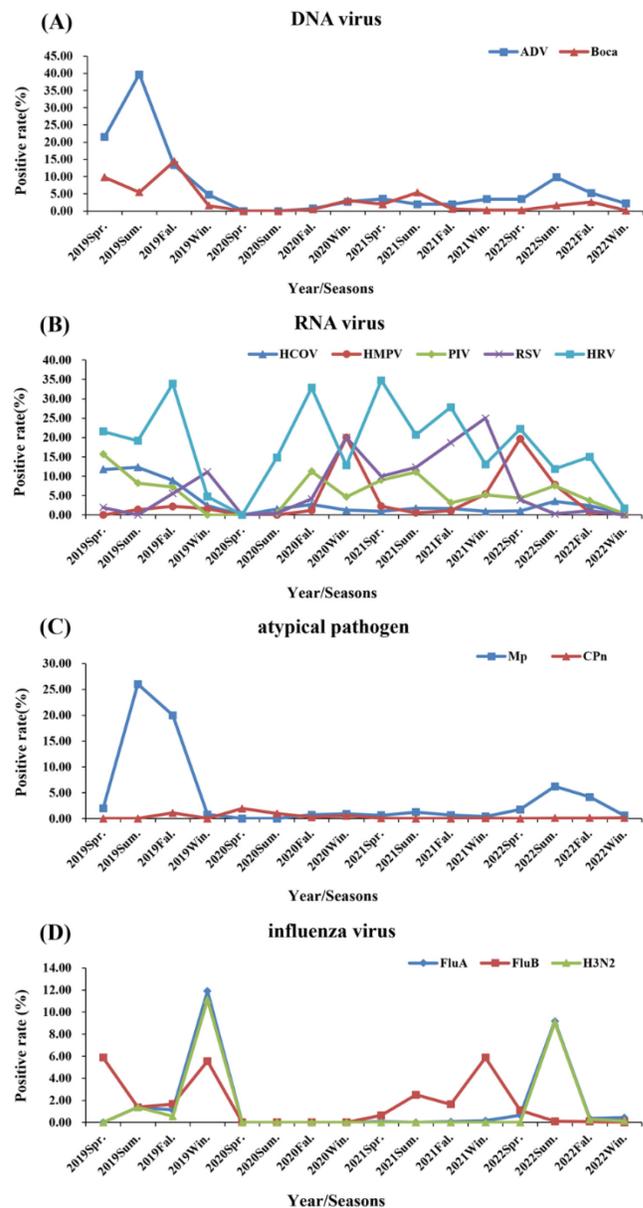
HRV was mainly epidemic in the spring and fall. Comparatively, PIV and HMPV seasonality was not obvious. The detection rate of HCOV was higher in the spring and summer of 2019 and the summer of 2022. Mp (Figure 1C) was mainly prevalent in summer and fall, and the detection rate of CPn was low throughout the year. Among the influenza viruses (Figure 1D), FluA and H3N2 had the highest detection rate in the winter of 2019 and the summer of 2022, while the seasonality of FluB was not obvious.

The impact of the COVID-19 pandemic on the infection rate

We compared the detection rates of various pathogens before the COVID-19 pandemic (pre-COVID-19, before January 2020) and during the COVID-19 pandemic (January 2020 - March 2020 and December 2022 to January 2023) (Figure 2). The detection rates of respiratory infection pathogens during the pandemic were significantly lower than those pre-COVID-19 (8.21% vs. 71.82%, $p < 0.01$) (Table 2), particularly for ADV (20.30% vs. 2.21%), HBoV (10.91% vs. 0.25%), HCoV (9.39% vs. 0.61%), PIV (8.18% vs. 0.25%), RSV (4.55% vs. 1.23%), HRV (26.97% vs. 1.84%), and Mp (16.97% vs. 0.61%).

Co-detection of respiratory pathogens

At least two pathogens were detected in 405 cases (3.18%), among which 364 (2.86%) cases were infected by two pathogens, 39 (0.31%) were infected by three pathogens, and 2 (0.02%) were infected by four pathogens (Table 3). HRV and RSV co-infection was the most frequent, accounting for 0.48% of all respiratory infection cases. Co-infections involving three or more pathogens were rare, with only 39 cases (0.31%) involving combinations of three viruses and 2 cases (0.01%) involving four pathogens.

Figure 2. Impact on detection rates of different pathogens during the COVID-19 pandemic.
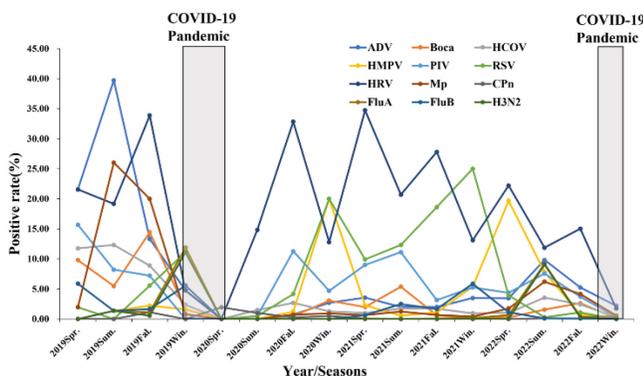
Discussion

To determine the prevalence of common respiratory pathogens in children aged 0-6 years in Wuhan, we analyzed 12 common respiratory pathogens in 12,743 children with ARI. The results showed that the total positive detection rate of the 12 pathogens was 48.65%, which was similar to some previous Chinese literature reports (44.8-46.9%) [14,15]. The overall detection rate was associated with the detection method and the number of pathogens detected, and variability in the detection results was also observed in different regions and climatic conditions [16]. Consistent with previous studies [17,18], single pathogen infection was more common than two or more pathogens among the 12 investigated respiratory pathogens (45.48% vs. 3.18%, $p < 0.01$). HRV was found to be the most common pathogen causing single infections, with a detection rate of 19.42%, which differed from previous studies conducted in Chengdu (23%) [19] and Fuzhou (17.6%)

Table 3. The co-detection of 13 respiratory pathogens in respiratory infection cases.

Co-infections	Co-infection viruses	n (%)
2 pathogens	HRV + RSV	61 (0.48)
	HRV + PIV	55 (0.43)
	HRV + HMPV	33 (0.26)
	HRV + HBoV	31 (0.24)
	HRV + ADV	30 (0.24)
	HRV + Mp	18 (0.14)
	RSV + HMPV	18 (0.14)
	FluA + HCOV	14 (0.11)
	HRV + HCOV	13 (0.10)
	PIV + ADV	9 (0.07)
	PIV + HMPV	8 (0.06)
	HMPV + ADV	7 (0.05)
	RSV + HBoV	6 (0.05)
	Mp + ADV	6 (0.05)
	FluA + Mp	5 (0.04)
	RSV + ADV	5 (0.04)
	RSV + HCOV	5 (0.04)
	HRV + FluA	5 (0.04)
	PIV + HBoV	5 (0.04)
	HRV + FluB	4 (0.03)
	RSV + PIV	4 (0.03)
	PIV + HCOV	4 (0.03)
	HMPV + HCOV	4 (0.03)
	PIV + Mp	3 (0.02)
	Other	11 (0.09)
3 pathogens	HRV + HBoV + ADV	4 (0.03)
	RSV + HRV + HBoV	3 (0.02)
	RSV + HRV + PIV	3 (0.02)
	ADV + HCOV + Mp	3 (0.02)
	RSV + HRV + ADV	3 (0.02)
	PIV + HRV + HCOV	2 (0.02)
	PIV + HRV + ADV	2 (0.02)
	PIV + HRV + HBoV	2 (0.02)
	Other	17 (0.13)
4 pathogens	ADV + HBoV + RSV + HRV	1 (0.01)
	ADV + RSV + HRV + Mp	1 (0.01)
Total		**405 (3.18)**

ADV: human adenovirus; HBoV: HBoV viruses; HCOV: human coronaviruses; HMPV: human metapneumoviruses; PIV: parainfluenza; RSV: respiratory syncytial virus; HRV: human rhinoviruses; FluB: influenza B; Mp: *Mycoplasma pneumoniae*.

[20].

This study found that children aged 1-3 years had the highest proportion to ARI (56.98%), which is consistent with that reported by Furuse *et al.* [21] and Snoeck *et al.* [22], which we believed might have resulted due to congenital immune deficiency and immature immune system causing higher susceptibility to respiratory pathogens in this age group [23]. HRV was observed to be the most common reason in all age groups [24], which is consistent with previous studies. The detection rate of PIV, HBoV, HCOV, FluA, and H3N2 observed in the 6 months to 1 year old age group could be associated with decreased maternal antibodies and weak acquired immunity. Detection rate to ADV, Mp, and FluB in the 3-6 years old age group could be attributed to the pathogens' ability to cause cross-infection in densely populated locations such as schools, particularly in kindergartens. In contrast, HRV, RSV, and HMPV were found to be prevalent in all age groups except neonates (< 1 month old).

In this study, the infections of various respiratory pathogens showed certain seasonality, with the peak of the total infection rate occurring in the summer and fall of 2019, the winter of 2020, and the spring of 2021. In the spring and winter of 2020 and the winter of 2022, the pathogen detection rate in Wuhan was significantly lower than the average level (1.94% and 5.57% vs. 48.65%, $p < 0.01$) due to the COVID-19 pandemic. Additionally, we observed that the COVID-19 pandemic had significantly changed the epidemiology of ARI in children aged 0-6 years. The obvious decline in the detection rate of 12 pathogens during the COVID-19 pandemic could be associated with the strict public health measures implemented by the government during the COVID-19 pandemic, such as wearing masks, emphasis on hand hygiene, home isolation, and reduced personnel gathering (including the temporary closing of kindergartens) [25]. The detection rates of ADV and HBoV were slightly higher in summer than in other seasons. Xie *et al.* [26] reported that ADV was mainly prevalent in summer, while Fillatre *et al.* [27] reported that ADV and HBoV were prevalent in winter and spring. HRV was observed to be mainly prevalent in spring and fall, consistent with the report of Korsun *et al.* [28]. RSV is mainly prevalent in fall and winter, similar to those reported by Toivonen *et al.* [29] and Zhang *et al.* [30]. Comparatively, PIV showed no obvious seasonality variability and was sporadic in spring, fall and winter. We observed that HMPV had a high detection rate in winter, which differed from that reported by Korsun *et al.* [28], who found that PIV and HMPV mainly prevailed in winter and spring. Although

we found that the detection rate of HCOV was high in spring and summer, Lei *et al.* [31] reported that HCOV was mainly prevalent in fall. FluA and H3N2 were mainly prevalent in summer and winter, while the seasonality of FluB was not obvious. Zhu *et al.* [32] reported that FluA and FluB were mainly prevalent in winter, and Duarte *et al.* [33] reported that FluA and FluB were mainly prevalent in spring and summer. The detection rate of Mp was mainly high in summer and fall, which differed from the report of Gao *et al.* [17], in which Mp was detected in all seasons and was highest in spring. We hypothesized that the epidemic seasonality of some respiratory viruses observed in this study differed from that of other reports due to differences in regional climatic conditions.

In this study, the most common pattern of multiple infections was a combination of two pathogens, with HRV + RSV being the most frequent, which was consistent with that reported by Conto *et al.* [34], suggesting a possible interaction between these two viruses that warrants further investigation. Co-infections involving more than three pathogens are extremely rare. Nevertheless, for patients who do experience such complex co-infection scenarios, timely and accurate diagnosis along with personalized treatment plans are crucial to prevent potential severe complications. Additionally, studying these rare cases can help the medical community better understand the interaction mechanisms among pathogens, thereby promoting the development of new therapeutic approaches. Mixed infection in children with ARI can occur due to various reasons, such as the damage of airway mucosal cells caused by a certain pathogen, poor physical condition, low immune function, long course of disease, contact with other children during hospitalization, and increased chance of cross-infection. Therefore, it is crucial to implement corresponding isolation measures during hospitalization to prevent cross-infection. The patterns of mixed infection can be influenced by various factors, such as the prevalence of pathogens in different regions, different detection methods, and the intrinsic characteristics of the pathogens themselves, as some may be more prone to mixed infection [35,27]. This highlights the importance of paying more attention to the infection of such pathogens to reduce the incidence of secondary infection by other pathogens, which can worsen these children's illnesses.

In conclusion, this study analyzed the distribution of common respiratory viruses in Wuhan ARI children from 2019 to 2023 using PCR capillary electrophoresis fragment analysis. The results provide important

insights into understanding the common respiratory pathogens and their prevalence in children with ARI from Wuhan and an important basis for the etiological diagnosis of ARI children. Additionally, the findings of this study have several important public health implications, particularly for the management of respiratory infections in children. Healthcare providers can leverage these insights to improve diagnostic and treatment strategies, as well as to enhance preventive measures. For example, diagnostic strategies can be enhanced through targeted testing. With the knowledge that certain pathogens, such as ADV, HBoV, HCoV, PIV, HRV, and Mp, had lower detection rates during the COVID-19 epidemic, healthcare providers can prioritize testing for SARS-CoV-2 while also maintaining vigilance for other common respiratory pathogens. This can help in making more informed clinical decisions and avoiding unnecessary use of antibiotics. Understanding the trends in pathogen prevalence can inform the timing and focus of vaccination campaigns. For example, hospitals and public health agencies can implement targeted vaccination programs based on the seasonal patterns of certain pathogens to reduce the incidence of these infections.

However, it is important to acknowledge the limitations of this study. First, only 13 common respiratory pathogens were analyzed, which may not fully capture the prevalence of all respiratory pathogens in Wuhan. Additionally, this study was conducted at a single center, which may limit the generalizability of the findings to other settings. Thus, larger cohort studies and multicenter investigations are needed to fully determine the seasonality and mixed infection patterns of respiratory infections in children in this age group. The behavior of pathogens may change in unexpected ways in a post-COVID environment. Continuous surveillance is crucial to monitor these changes and to adapt public health strategies accordingly.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Renmin Hospital and Zhongnan Hospital of Wuhan University. Written informed consent was obtained from the legal guardians of all participating children.

Authors' contributions
Zegang Wu and Chenglin Ye had primary responsibility for protocol development, patient screening, and enrollment. Guolei Wang and Jun'e Ma participated in enrollment and analytical framework for the study. Li Yuan participated in preliminary data analysis and outcome assessment. Xuan Xiao and Chengliang Zhu supervised the design and execution of the study and contributed to the writing of the manuscript.

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Conflict of interests
No conflict of interests is declared.

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