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# Epidemiology of respiratory infections during the different levels of non-pharmaceutical interventions

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## Abstract

**Objective** This study aimed to describe epidemiological changes of common respiratory pathogens in children with acute respiratory infections (ARIs) during the different levels of non-pharmaceutical interventions (NPIs).

**Methods** In this retrospective study, we analyzed the prevalence of 12 respiratory pathogens from September 2021 through February 2024 in all inpatients with ARIs admitted to Children's Hospital of Soochow University (SCH). We compared the detection rates of these pathogens at different levels of public NPI measures.

**Results** In total, 27,851 respiratory samples were analyzed, including 15,231 boys and 12,620 girls. Under strict NPIs, inpatients with ARIs were more commonly boy and infants ( $p < 0.01$ ). However, after the discontinuation of NPIs, they were more commonly children over 6 years of age ( $p < 0.01$ ). Under the strict NPIs, the most detected respiratory pathogen was HRV (23.6%), followed by HRSV (16.0%), HMPV (10.8%) and HPIV (10.4%). But after the abolition strict NPIs, the most common respiratory pathogen was MP (35.9%), followed by HRV (19.7%), HRSV (17.5%) and HPIV (6.6%). Moreover, the number and detection rate of MP and ADV had significantly increased and remained at a high level ( $P < 0.05$ ). In addition, the number of HRSV detection increased and the epidemic season has changed (transitioning from winter to summer), and detection rate of multiple pathogens was more high after the abolition NPIs ( $P < 0.01$ ).

**Conclusions** Withdrawal of major measures, the disease burden of ARIs in children in Suzhou has sharply increased, mainly attributed to MP, and mixed infections were more common.

**Keywords** Respiratory pathogens, Children, Immune-debt, NPIs

## Introduction

Acute respiratory infections (ARIs) pose a major threat to human health worldwide, leading to high morbidity and mortality in children [1] and causing more than one million deaths annually, especially in developing countries [2]. A range of pathogens are responsible for childhood ARIs; however, viral and atypical pathogens, such as *Mycoplasma pneumoniae* (MP), play particularly important roles [3]. Since 2020, countries worldwide have committed to reducing the adverse effect of coronavirus disease 2019 (COVID-19) on human health. In China, the government implemented strict non-pharmaceutical

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interventions (NPIs), such as the prohibition of social gatherings, maintaining hand hygiene, wearing masks, and cleaning the environment to eliminate COVID-19. Although the NPIs prevented the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), they were also likely to affect the spread of respiratory illnesses in children [4, 5]. With the introduction of the SARS-CoV-2 vaccination and a reduction in its virulence, the NPIs in China were discontinued in December 2022.

After the discontinuation of strict NPIs, several countries such as Germany [6], New Zealand, and Australia [7] reported an increase in inpatients with respiratory infections and altered seasonal epidemic characteristics of respiratory pathogens [8]. Previous studies have associated this phenomenon with “immunity debt” [7, 9], which was defined as the waning of immunity due to long-term low-level exposure to pathogens, making a high proportion of the population prone to infections [10]. In addition, the impact of immunity debt became more pronounced with the extension of NPIs. NPIs in China have lasted for 3 years, and whether the discontinuation of major NPIs affects the population’s immunity to common respiratory pathogens, as well as the prevalence of these pathogens, remains unclear.

This single-center study aimed to compare the detection rates of common respiratory pathogens in inpatients with acute respiratory infections (ARIs) admitted at different levels of public NPI measures. Monitoring the changes in the prevalence of these pathogens can not only provide a reference for public health decision-making but also help prepare for the potential outbreak of certain pathogens.

## Materials and methods

### Setting

This study was conducted at the Children’s Hospital of Soochow University (SCH), the only comprehensive tertiary children’s hospital in Suzhou, China, which has a capacity of 1500 beds and hospitalizes approximately 63,000 patients annually.

### Participants

This retrospective longitudinal study assessed the detection rates of common respiratory pathogens in children admitted to the SCH during the COVID-19 pandemic and after the pandemic at different levels of public NPIs. NPIs included the prohibition of social gatherings, maintaining hand hygiene, wearing masks, and cleaning the environment. The period was divided into two cohorts: during- NPIs (from September 2021 to December 2022) and after-abolition of NPIs (from January 2023 to February 2024). During the observation period between September 1, 2021, and February 29, 2024, patients who presented with ARIs and regularly underwent 12

respiratory pathogens assay were included. Data regarding participants’ sex, age, sputum test time, discharge diagnosis, and results for 12 respiratory pathogens were extracted from the electronic medical system. This retrospective study was approved by the Ethics Committee of Children’s Hospital of Soochow University, China.

### Specimen collection

Nasopharyngeal aspirates were obtained within 24 h of admission for inpatients with ARIs. The specimens were collected and stored at 2–8 °C for examination within 30 min.

### Respiratory pathogen assays using PCR capillary electrophoresis fragment analysis

The 12 respiratory pathogens examined herein included chlamydia (Ch), MP, human metapneumovirus (HMPV), human syncytial virus (HRSV), human coronavirus (HCOV; Kit untyping assay 229E, NL63, HKU1, and OC43), adenovirus (ADV), rhinovirus (HRV), bocavirus, human parainfluenza virus (HPIV), influenza A virus subtype H3N2 (H3N2), influenza A virus subtype H1N1 (H1N1), and influenza virus B (InfB).

The nasopharyngeal aspirates were mixed with DNA extract (Ningbo Haishi Gene Technology Co., Ltd., Ningbo, China), added to PCR mixtures containing primers targeting highly conserved sequences of the 12 respiratory pathogens (Ningbo Haishi Gene Technology Co., Ltd., Ningbo, China), and centrifuged at 2000 rpm for 10 s. Real-time PCR was performed with the LightCycler 480II system (Roche, Basel, Switzerland) using the following procedure: incubation at 25 °C for 5 min, 50 °C for 15 min, and 95 °C for 2 min, and 6 cycles of 94 °C for 30s, 65→60 °C for 30s, and 72 °C for 60s; this was followed by 29 cycles of 94 °C for 30s, 60 °C for 30s, and 72 °C for 60s, and finally incubation at 72 °C for 10 min. The results were evaluated by measuring the fluorescence intensity using capillary electrophoresis. All procedures were performed according to the manufacturers’ instructions.

### Statistical analysis

Data are presented as number (%) or mean ± standard deviation. Categorical variables were compared using the chi-squared test. A two-sample t-test was used for continuous normally distributed variables. All statistical analyses were conducted using IBM SPSS Statistics and mapped using GraphPad Prism version 9. Statistical significance was set at  $p < 0.05$ .

## Results

### Characteristics of the enrolled patients

In total, 27,851 respiratory samples were analyzed, including 10,204 samples collected between September 2021 and December 2022 (before the discontinuation of

**Table 1** Demographics of the enrolled patients

Parameters	Before (n= 10204)	After (n= 17647)	P
Sex(male)	5796(56.8)	9435(53.5)	<0.01
Age(years)			
≤ 1	3445(33.7)	3195(18.1)	<0.01
1–≤3	2982(29.2)	3092(17.5)	<0.01
3–≤6	2384(23.4)	4429(25.1)	<0.01
>6	1393(13.7)	6931(39.3)	<0.01
	<0.01	<0.01	

Notes: The data presented as n (%) and mean±standard deviation. The chi-square test for categorical variables, and t-test for continuous variables

strict NPIs) and 17,647 samples collected between January 2023 to February 2024 (after the discontinuation of strict NPIs). As shown in Table 1, the sex and age distributions of the participants before and after the discontinuation of strict NPIs ( $p < 0.01$ ) were significantly different. Under strict NPIs, inpatients with respiratory sample assays were more commonly men and infants ( $p < 0.01$ ). After the discontinuation of strict NPIs, inpatients were more commonly children over 6 years of age ( $p < 0.01$ ).

#### Number of respiratory specimens per month before and after the discontinuation of strict NPIs

The mean number of respiratory specimens per month before and after the discontinuation of major NPIs measures were  $637 \pm 296$  and  $1260 \pm 456$ , respectively. As shown in Fig. 1, after the discontinuation of strict NPIs, the number of respiratory specimens per month

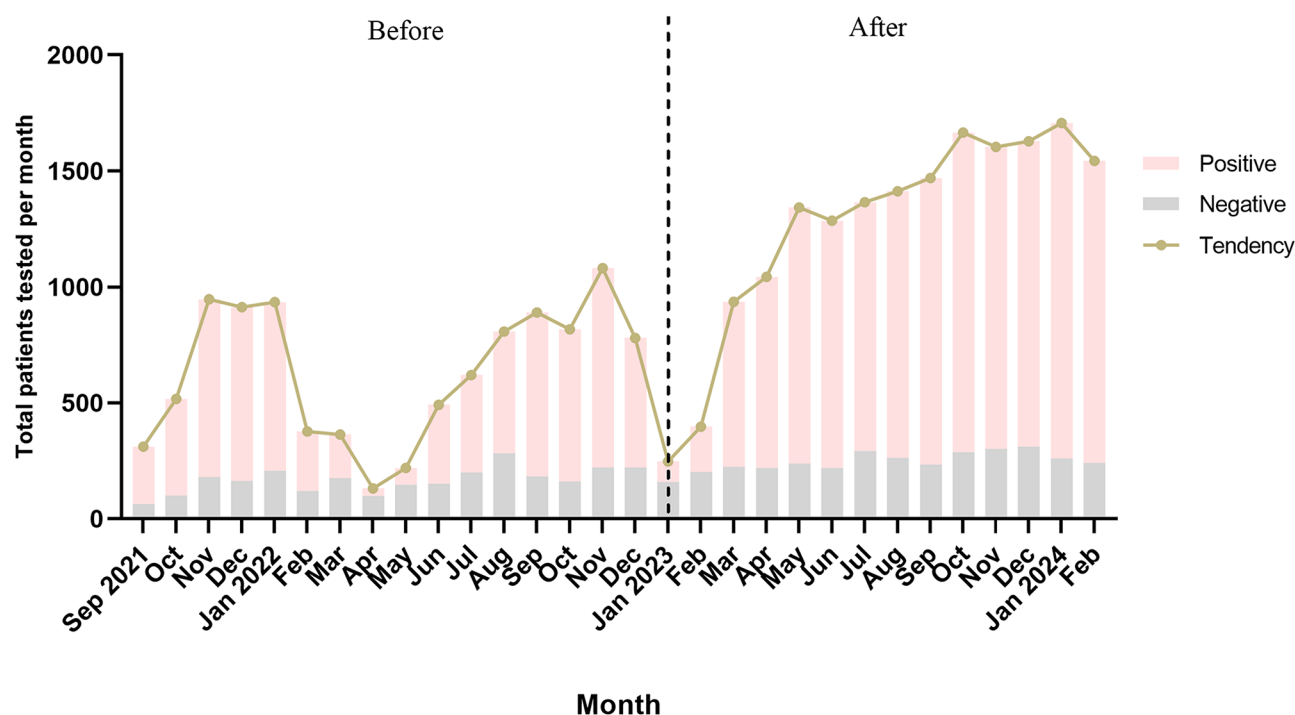
significantly increased ( $p < 0.01$ ). In addition, the number of respiratory specimens per month showed seasonal behavior, with the peak months being October, November, and December under the major NPIs. However, even after the discontinuation of strict NPIs, the number of respiratory specimens remained high.

#### Positivity of respiratory pathogens per month before and after the discontinuation of strict NPIs

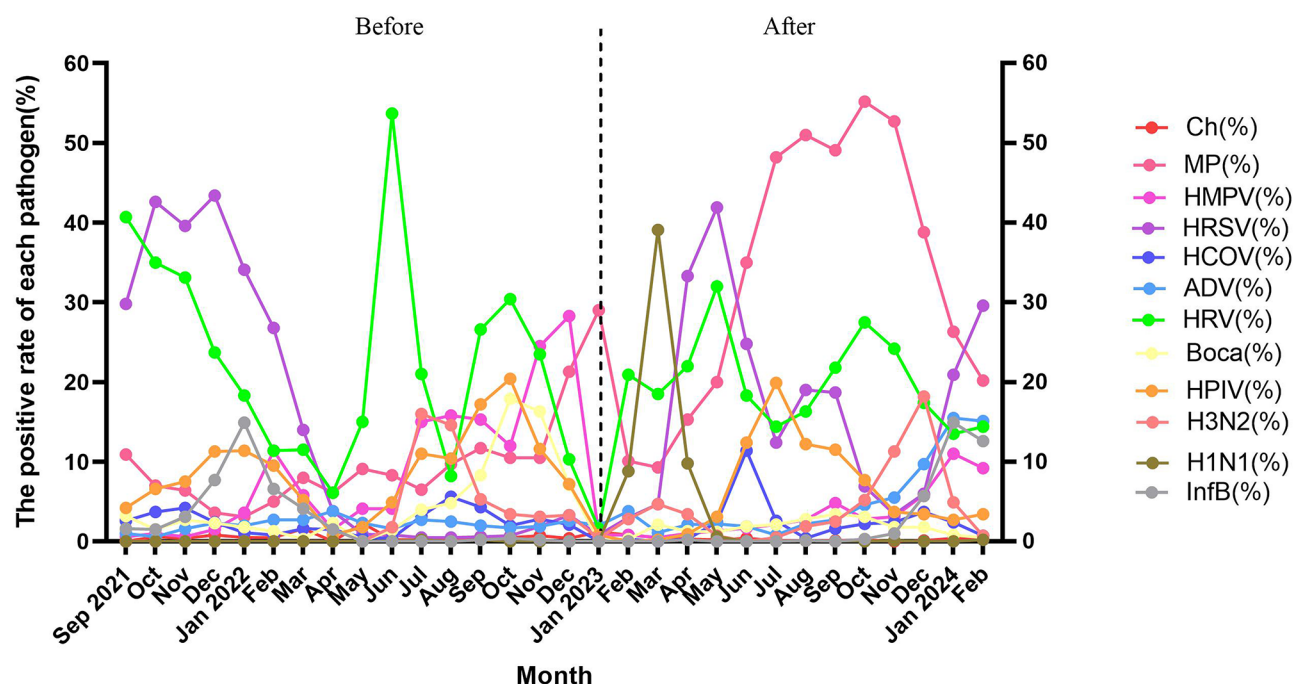
Under strict NPIs, the respiratory pathogens were seasonally prevalent, reaching their peak between September and December. The most frequently detected respiratory pathogen was HRV (23.6%), followed by HRSV (16.0%), HMPV (10.8%), and HPIV (10.4%) (Fig. 2). After the discontinuation of strict NPIs, respiratory pathogens showed no obvious seasonality and high prevalence, and the most common respiratory pathogen was MP (35.9%), followed by HRV (19.7%), HRSV (17.5%), and HPIV (6.6%).

#### The number and positivity rates of respiratory pathogens per month before and after the discontinuation of strict NPIs

As shown in Fig. 3, the detection rates of Ch, MP, HCOV, and ADV per month remained low prior to the discontinuation of strict NPIs. After the discontinuation of strict NPIs, the number and detection rates of MP and ADV increased significantly and remained high ( $p < 0.05$ ), whereas the detection rates of Ch and HCOV remained



**Fig. 1** The number of respiratory specimens and positive rate of respiratory pathogens between September 2021 and February 2024



**Fig. 2** The positive rate of respiratory pathogens between September 2021 and February 2024

low. In addition, the detection rate of HRSV before and after the discontinuation of strict NPIs ( $p > 0.05$ ) were not significantly different; however, the number of HRSV detections increased post-NPI discontinuation, and the epidemic season changed from winter (November, December, and January) to summer (April to June). Neither the number nor the epidemic season of HMPV changed post-NPI discontinuation, while the detection rates decreased after NPI discontinuation.

As shown in Fig. 4, the positive HRV and HPIV rates remained high before and after the discontinuation of strict NPIs. However, the peak HPIV detection rate observed in summer (June to August 2023) had shifted by 3–4 months, in relation to the COVID-19 pandemic. Under strict NPIs, the detection rates of bocavirus, H3N2, H1N1, and InfB remained low. After the discontinuation of strict NPIs, the detection rates of H3N2, H1N1, and InfB showed seasonal epidemic peaks; however, bocavirus detection rate remained at a low level, with no detectable peaks.

#### The proportion of single and co-detection pathogens before and after the discontinuation strict NPIs

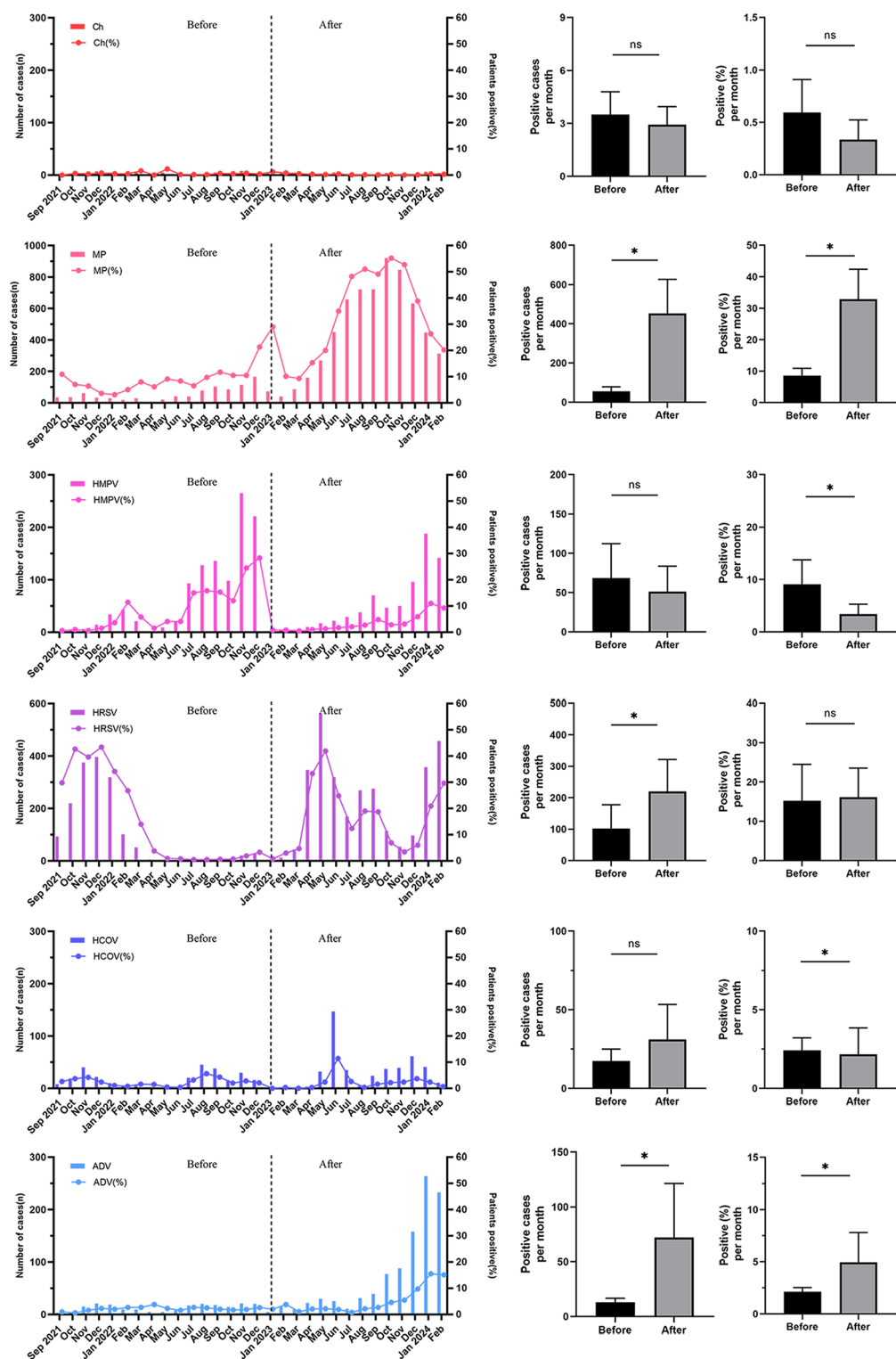
As shown in Table 2, respiratory pathogens were identified in 73.7% (7521 of 10204) of patients with ARI before the discontinuation of strict NPIs. In this cohort, single-pathogen detection was significantly more frequent (6261/7521, 61.4%) than co-detection (1260/7521, 12.3%) ( $p < 0.01$ ). After the discontinuation of strict NPIs, the detection rate of respiratory pathogens was 80.3%

(14179 of 17647), with the frequency of single-pathogen detection (58.9%) being higher than that of co-detection (21.4%) ( $p < 0.01$ ). In addition, the co-detection rate of multiple pathogens was significantly higher among inpatients undergoing respiratory pathogen testing after the discontinuation of strict NPIs than before the discontinuation ( $p < 0.01$ ).

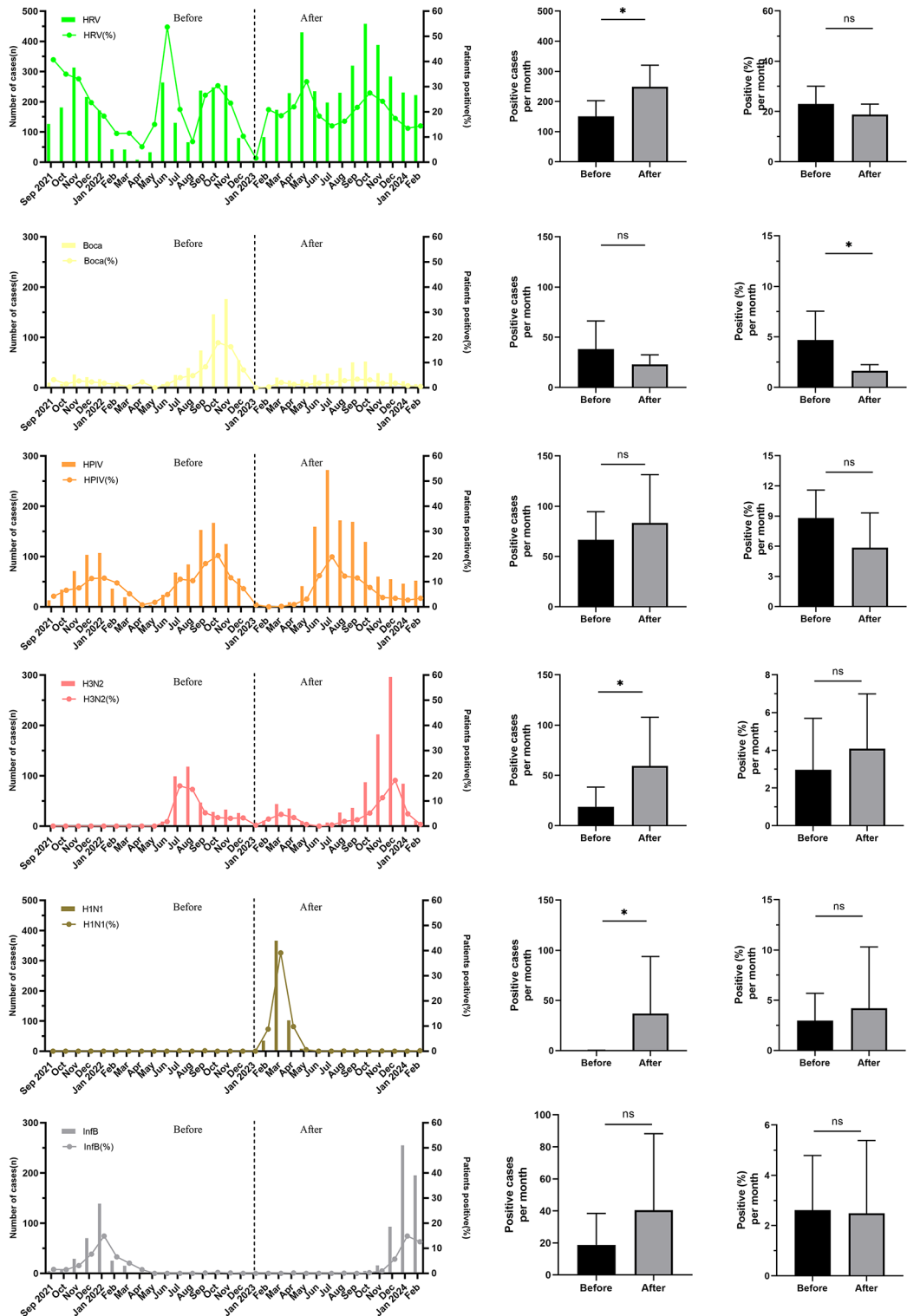
#### Discussion

Herein, the number of inpatients who underwent respiratory pathogen testing sharply increased after the discontinuation of strict NPIs, which has previously been observed in several other countries, including the US, France, Australia, and Canada [11]. Interestingly, during the COVID-19 epidemic, inpatients with ARIs were more commonly infants. However, following the discontinuation of strict NPIs, the trend shifted toward school-aged children (6–16 years). This might be because young children had poor compliance with NPIs, while older children were more socially active after the discontinuation of NPIs.

During the COVID-19 pandemic, the detection rate of HRV was higher than that of other pathogens, which is consistent with previous studies [12, 13]. This was attributed to non-enveloped viruses being able to penetrate surgical masks [14] and being relatively resistant to ethanol-containing disinfectants [15]. After the discontinuation of NPIs, the detection rate of MP markedly increased, becoming the dominant pathogen in children with ARIs instead of HRV. A study from Shenzhen, China



**Fig. 3** Compare the number and detection rate per month of Ch, MP, HMPV, HRSV, HCOV and ADV before and after the abolition strict of NPIs



**Fig. 4** Compare the number and detection rate per month of HRV, Boca, HPIV, H3N2, H1N1 and InfB before and after the abolition strict of NPIs



**Table 2** The rate of pathogens identified in hospitalized children with acute respiratory infection before and after the abolition of NPIs

Pathogen assay	Before ( <i>n</i> = 10204)	After ( <i>n</i> = 17647)	<i>P</i>
Negative	2683(26.3)	3468(19.7)	<0.01
Single pathogen	6261(61.4)	10,395(58.9)	<0.01
Multiple pathogen	1260(12.3)	3784(21.4)	<0.01
<i>P</i>	<0.01	<0.01	

Notes: The data presented as *n* (%). The chi-square test for categorical variables

showed that over 90% of inpatients were infected with MP [9]. This reflects the increased disease burden of inpatients with ARIs caused by MP since December 2022.

The detection rates of non-seasonal respiratory pathogens, such as Ch, MP, and ADV, were low during strict NPIs. After the discontinuation of NPIs, the detection rates of MP and ADV showed a sharply increasing trend, whereas that of Ch did not. It's reported that MP outbreaks occur every 3 to 7 years and each outbreak last for 1–2 years. Although the cause of recent global resurgence of MP in late 2023 remains unclear, multiple countries have reported that they are associated with natural epidemiologic patterns of MP [16]. From 2021 to 2022, the prevalence of ADV always maintained a relatively low and had no obvious detection peak. However, a sharply increased prevalence of ADV was observed in late 2023, which was consistent with previous studies [17]. In spite of that some studies have shown that, during the COVID-19 pandemic, adenovirus (ADV) continued to circulate within the community and was cultured from hospitalized patients [18]. The detection rate of Ch remained low, possibly because it is not a common respiratory pathogen in children [5].

The number of detected cases of seasonal viruses, such as HRV, HRSV, and InfA, increased after the discontinuation of NPIs, which was consistent with previous studies [5, 19, 20]. The seasonal peak of HRV detection exhibited no change; however, HRSV showed an “off-season” epidemic pattern after the discontinuation of NPIs. The off-seasonal (transitioning from winter to summer) increase in RSV detection was consistent with studies from multiple countries such as Thailand, France, South Africa, and the United States [21–24]. This phenomenon may be explained by several mechanisms, including virus-virus interactions such as the surge of the HRSV detection rate in the absence of Inf or HRV [25], immunity debt [26, 27], and changes in the RSV spectrum [28]. The influenza virus was identified as the leading pathogen in 2018 and 2019 in China. However, its prevalence declined dramatically during the COVID-19 epidemic [29]. Herein, the “upsurging” pattern was observed for influenza infections after the relaxation of NPIs. In addition, several studies indicated that many seasonal and encapsulated

respiratory viruses showed markedly reduced circulation during the pandemic and began to re-emerge globally in a staggered fashion starting in late 2021, which trends were well documented and reflect complex, multifactorial dynamics beyond local NPI measures alone [30].

In contrast, herein, the detection rates of seasonal viruses such as HPMV, HPIV, HCOV, and bocavirus did not change immediately after NPI discontinuation in children with ARIs. However, the peak HPIV detection rate in the summer of 2023 clearly advanced by 3–4 months, in relation to the COVID-19 pandemic. Changes in epidemic patterns have also been observed in Germany and the United States [6, 24]. It is believed that viruses interfere with other viruses at both population and host levels [31]. However, the detection rate of HMPV strongly adhered to certain seasonalities both before and after the implementation of NPIs. This is not well explained by immune debt or viral interference but can be explained by changes in social life during the winter [6]. Contrary to the findings of Terliesner et al., the detection rates of HCOV and bocavirus remained low herein [4].

Co-detection with multiple pathogens has been frequently observed in children with ARIs since the COVID-19 pandemic [10, 32]. Herein, the co-detection rate of multiple pathogens increased dramatically after NPIs discontinuation, illustrating that the weakened immunity resulting from strict NPIs may have rendered children susceptible to multiple pathogens [33].

This study has some limitations. As a single-center study, it included only a limited number of hospitalized patients. Multi-center studies are needed to more accurately assess the impact of NPI measures on respiratory pathogen infections. Additionally, extending the duration of surveillance in future studies is necessary to elucidate the epidemiological patterns of these pathogens. Finally, further analysis of the correlation between pathogen infections and disease severity is warranted.

In summary, the discontinuation of major NPIs was followed by a sharp increase in the number of inpatients with ARIs in Suzhou, which were more commonly older children. A surge in the number of infections by pathogens such as MP, ADV, HRV, HRSV, and influenza, especially MP, causes a substantial disease burden in children. Moreover, co-detection with multiple pathogens should be given more attention in the future.

#### Abbreviations

ARIs	Acute Respiratory Infections
NPIs	Non-Pharmaceutical Interventions
SCH	Children's Hospital of Soochow University
COVID-19	Coronavirus Disease 2019
Ch	Chlamydia
MP	Mycoplasma Pneumoniae
HMPV	Human Metapneumovirus
HRSV	Human Syncytial Virus
HCOV	Human Coronavirus
ADV	Adenovirus

HRV	Rhinovirus
Boca	Bocavirus
HPIV	Human Parainfluenza Virus
InfA	Influenza A
InfB	Influenza virus B

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### Author contributions

Ting Shi and Linlin Huang collected data; Ting Shi and Linlin Huang analyzed and interpreted data. Ting Shi wrote the main manuscript text. Linlin Huang prepared Figs. 1, 2, 3 and 4; Table 1, and 2. Jianmei Tian revised critically the manuscript. All authors reviewed the manuscript.

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### Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request (2231365607@qq.com; shiting0915@126.com).

### Declarations

#### Ethics approval and consent to participate

This study adheres to the ethical principles of the Declaration of Helsinki. It was approved by the Ethics Committee of Children's Hospital of Soochow University (No.2023CS059). For the presented retrospective data, the requirement to obtain informed consent was waived in accordance with the vote of the Ethics Committee.

#### Consent for publication

Consent to publication is not applicable in this case.

#### Competing interests

The authors declare no competing interests.

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