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# Respiratory viral infections in children under five during two consecutive autumn–winter seasons

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## Abstract

**Background** Respiratory viral infections are a leading cause of hospitalization in children < 5 years. Understanding viral circulation, co-infections, and disease severity is essential for prevention and clinical management.

**Methods** This retrospective, single-center observational study was conducted between September 2023 and April 2025, and was based on multiplex PCR viral panel results obtained from respiratory samples of children under 5 years of age referred to IRCCS Policlinico. Repeated respiratory samples from the same patient yielding identical results were considered a single episode. When multiple samples collected < 7 days apart showed alternating positive and negative results, only the first positive sample was considered. Descriptive statistics and Fisher's exact test evaluated episode characteristics, virus circulation, and co-infections. Logistic regression analysis identified factors associated with pediatric high-dependency ward or intensive care unit admission.

**Results** A total of 2,512 respiratory episodes in 2,239 patients were analyzed; 1,405 (55.9%) occurred in male patients, with a median(IQR) age of 1.03 (0.26–2.17) years. Pediatric high-dependency ward admission and intensive care unit admissions were reported in 432 (21.7%) and 103 (5.2%) of 1990 episodes with available clinical data, respectively. Human rhinovirus (HRV, 31.4%) was the most frequently detected pathogen, followed by RSV-A/B (13.9%). Co-infections were detected in 598 (37.6%) samples, most commonly HRV plus enterovirus (21.4%). Compared with 2023/2024, the 2024/2025 season showed reduced circulation of RSV (18.4% vs. 13.9%,  $P=0.006$ ) and human metapneumovirus (MPV; 8.9% vs. 5.5%,  $P=0.002$ ), alongside increased influenza A/B (7.4% vs. 11.8%,  $P=0.0005$ ). Logistic regression analysis revealed that pediatric high-dependency ward or intensive care unit admission was positively associated with lower respiratory tract symptoms and with the detection of RSV A/B, HRV, MPV, human enterovirus, human parainfluenza virus, and adenovirus ( $P < 0.05$ ).

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**Conclusion** Respiratory viruses remained prevalent among symptomatic children < 5 years, with HRV predominating across seasons. A reduction in RSV circulation was observed in 2024/2025, temporally coinciding with nirsevimab introduction, even though no direct attribution can be made in the absence of individual-level immunization data. Overall, these findings support continued hospital-based virological surveillance in young children to monitor changes in viral circulation and clinical burden.

**Keywords** Respiratory viral infections, Pediatrics, Respiratory syncytial virus, Seasonality, Disease severity

## Background

Respiratory viral infections (RVI) are the leading cause of healthcare visits and hospital admissions in early childhood and remain a major contributor to morbidity and mortality in children under five years of age [1, 2]. Viral pathogens account for an estimated 50–70% of lower respiratory tract infections (LRTIs) in this population [3], which are among the most common and severe causes of illness in children under five years and represent a substantial proportion of hospitalizations in this age group [4, 5].

A broad range of viruses is known to cause acute respiratory infections (ARIs) in childhood, with respiratory syncytial viruses (RSV-A and -B), human rhinoviruses (HRV), metapneumoviruses (MPV), human parainfluenza viruses (HPIV), human enterovirus (EV), influenza viruses (Flu-A and -B), human coronaviruses (HCoV), adenovirus (ADV), and human bocavirus (HBoV) together responsible for nearly 70% of viral ARIs [6–8]. Among these viruses, RSV is a leading cause of lower respiratory tract infections in infants under one year of age and exhibits a marked seasonal pattern in temperate regions, with annual epidemics peaking in winter [9–12].

ARIs are classified as upper and lower respiratory tract infections, based on the respiratory tract involved. Upper respiratory tract infections (URTIs) typically present with nasal congestion, rhinorrhea, cough, and fever. In contrast, lower respiratory tract infections (LRTIs) more commonly manifest as bronchiolitis or pneumonia. Certain infections, such as respiratory syncytial virus (RSV), may also cause wheezing, respiratory distress, or apnea, particularly in young infants [13].

The increasing use of real-time multiplex polymerase chain reaction (RT-mPCR) assays has markedly improved the identification of viral pathogens in respiratory disease. RT-mPCR enables rapid and sensitive detection of multiple viruses simultaneously with a short turnaround time, supporting a more accurate understanding of viral circulation patterns and contributing to improved prevention strategies in pediatric populations [14, 15].

Although multiplex molecular testing has expanded the identification of respiratory viruses in children, the interpretation of viral co-detections and their relationship with clinical presentation remains complex. Hospital-based studies integrating virological and clinical data may therefore contribute to a better characterization

of respiratory virus circulation and related outcomes in young children. The present single-centre, hospital-based study describes the circulation patterns of respiratory viruses detected in children under five years of age during the 2023–2025 autumn–winter seasons. Using RT-mPCR respiratory pathogen testing, we characterize the respiratory viruses detected and the occurrence of viral co-infections. Moreover, we evaluate the association between specific viral pathogens and clinical severity by integrating epidemiological, clinical, and laboratory data. By providing an updated overview of respiratory virus circulation and outcome predictors in young children, this study aims to support ongoing surveillance activities and inform infection control and prevention strategies in pediatric healthcare settings.

## Materials and methods

### Sample characteristics

This retrospective, single-center observational study was conducted between 1 September 2023 and 30 April 2025 and was based on multiplex PCR viral panel results obtained from respiratory samples of children under five years of age referred to Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. All respiratory samples (i.e., nasopharyngeal swabs, nasopharyngeal aspirates, or bronchoalveolar lavage) were tested using the Allplex Respiratory Panel Assays (Seegene, Seoul, Republic of Korea). This assay enables simultaneous extraction, amplification, detection, and analysis of 16 respiratory viruses, including Flu-A/B, RSV-A/B, ADV, EV, HPIV-1-4, MPV, HBoV, HRV, and seasonal HCoVs (NL63, 229E, OC43). Nucleic acids were extracted from 200 µL of sample using the Seegene STARlet instrumentation with the STARMag™ 96 × 4 Universal Cartridge Kit. The system also automatically prepared the PCR plate by dispensing extracted nucleic acid and PCR master mix. Multiplex RT-PCR was performed on the CFX96 Real-time PCR System (Bio-Rad Laboratories, Hercules, CA, USA), and results were analyzed using Seegene Viewer V3.0 (Seegene Inc., Seoul, Republic of Korea) according to the manufacturer's instructions. Samples were considered positive when the cycle threshold (Ct) value was ≤ 42, in accordance with the assay interpretation criteria provided by the manufacturer, while negative results were defined by the absence of viral amplification with a valid internal control.

Repeated respiratory samples from the same patient yielding the same result were considered a single episode. In cases where multiple samples collected less than 7 days apart from the same patient showed alternating positive and negative results, only the first positive sample was retained for analysis. Information regarding comorbidities was collected for a subgroup of 2,028 episodes. Information regarding symptoms and hospitalization was collected for a subgroup of 1,990 episodes (Supplementary Fig. 1). The following symptom definitions were applied: (i) upper respiratory tract symptoms, including rhinitis, pharyngitis, cough, sore throat, rhinorrhea, and sneezing; (ii) lower respiratory tract symptoms, defined by clinical signs of bronchiolitis, bronchitis, or pneumonia, supported by chest radiography and/or the need for oxygen supplementation; (iii) other symptoms, not related with the respiratory tract. Pediatric high-dependency ward admission, intensive care unit admission, and hospitalization length were also reported.

### Statistical analysis

Based on the results obtained by RT-mPCR, samples were classified as positive or negative for each pathogen. Among the positive results, the percentage of samples with mono-infection and co-infection was determined based on the number of pathogens identified. Co-infection was defined as the presence of more than one virus in the same sample.

Descriptive statistics are expressed as median values and interquartile ranges (IQR) for continuous variables and as number (percentage) for categorical variables. Differences in population characteristics between September 2023–April 2024 and September 2024–April 2025 were assessed using Fisher's exact test for categorical variables and the Mann–Whitney test for continuous variables. To evaluate the distribution of RVI across the two seasons, a Fisher's exact test was performed. Two-tailed  $p$ -values  $< 0.05$  were considered statistically significant. Univariate and multivariate logistic regression models were used to identify factors associated with pediatric high-dependency ward or intensive care unit admission. Variables were selected a priori based on their clinical relevance and availability in the dataset. Accordingly, sex, age, seasonal timepoints, comorbidities, presence of lower respiratory tract symptoms, presence of viral coinfection, and the detection of pathogens were included in the adjusted model. Potential multicollinearity was assessed using variance inflation factors (VIF), with  $VIF > 5$  indicating multicollinearity [16]. Descriptive and statistical analyses were performed using IBM SPSS Statistics Version 29.0.1.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Demographic and clinical characteristics

From September 2023 through April 2025, a total of 2,512 respiratory episodes from 2,239 children under 5 years of age were tested for RVI by RT-mPCR. Epidemiological and clinical characteristics of these episodes are reported in Table 1. Most episodes occurred in male patients (1,405, 55.9%), with a median age of 1.03 years (interquartile range [IQR]: 0.26–2.17). Overall, 1,242 episodes (49.4%) occurred in children aged 12 months or younger. Comorbidity data were available for 2,028 episodes, of which 155 (7.6%) had at least one comorbidity. Nasopharyngeal swabs accounted for most episodes (1,654, 65.8%), followed by nasopharyngeal aspirates (827, 32.9%) and bronchoalveolar lavage (31, 1.2%).

Clinical information regarding symptoms and hospitalization was available for 1,990 episodes. Among these, 1,008 (50.7%) were characterized by only upper respiratory tract symptoms, whereas 907 (45.6%) had lower respiratory tract symptoms. Seventy-two patients (3.6%) reported other symptoms (mainly gastrointestinal). Pediatric high-dependency ward admission and intensive care unit admission were reported in 432 (21.7%) and 103 (5.2%) episodes, respectively, and the median hospitalization length was 6.00 days (interquartile range [IQR]: 4.00–9.00). When comparing seasons, lower respiratory tract symptoms were significantly less frequent in the 2024/2025 season than in the 2023/2024 season (345, 43.2% vs. 461, 50.6%;  $P = 0.002$ ), suggesting a milder clinical presentation in the most recent surveillance period.

### Prevalence of respiratory viruses and coinfections

A total of 1,592 of 2,512 episodes (63.4%) tested positive for at least one respiratory virus. HRV (790, 31.4%) was the most frequently detected pathogen, followed by RSV-A/B (350, 13.9%), ADV (220, 8.8%), Flu-A/B (209, 8.3%), HBoV (201, 8.0%), HCoV (199, 7.9%), EV (185, 7.4%), MPV (183, 7.3%), and HPIV (148, 5.9%).

Among these 1,592 RVI episodes, 981 (61.6%) involved a single virus infection; 402 episodes (25.3%) involved coinfection with two RVs, 151 episodes (9.5%) involved coinfection with three RVs, and 58 episodes (3.6%) involved coinfection with more than three RVs (Fig. 1A).

The most common coinfection combination was HRV plus EV, observed in 128 (21.4%) coinfection episodes, followed by HRV plus ADV (122, 20.4%) and HRV plus HBoV (118, 19.7%).

### Seasonal trend of RVIs

The distribution of RVI episodes showed a clear seasonal pattern, with most cases occurring during the autumn/winter periods. Specifically, 1139 (45.4%) episodes were recorded between September 2023 and April 2024, 1015

**Table 1** Demographic and clinical characteristics

| Characteristic                                                                      | Overall<br>(n = 2512) | Sep 2023-Apr<br>2024 (n = 1139) | May 2024-Aug<br>2024 (n = 358) | Sep 2024-Apr<br>2025 (n = 1015) | p-<br>value <sup>c</sup> |
|-------------------------------------------------------------------------------------|-----------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------|
| <i>Demographics</i>                                                                 |                       |                                 |                                |                                 |                          |
| Sex, Male (n,%)                                                                     | 1405 (55.9%)          | 644 (56.5%)                     | 196 (54.7%)                    | 565 (55.7%)                     | 0.696                    |
| Age, years (median, IQR)                                                            | 1.03 (0.26–2.17)      | 0.97 (0.26–2.08)                | 1.10 (0.24–2.42)               | 1.06 (0.29–2.23)                | 0.360                    |
| ≤ 1 year (n,%)                                                                      | 1242 (49.4%)          | 584 (51.3%)                     | 171 (47.8%)                    | 487 (48.0%)                     | 0.131                    |
| > 1 year (n,%)                                                                      | 1270 (50.6%)          | 555 (48.7%)                     | 187 (52.2%)                    | 528 (52.0%)                     |                          |
| Presence of comorbidities (n, %) <sup>a</sup>                                       | 155 (7.6%)            | 74 (8.0%)                       | 25 (8.6%)                      | 56 (6.9%)                       | 0.465                    |
| <i>Sample type</i>                                                                  |                       |                                 |                                |                                 |                          |
| NFS (n,%)                                                                           | 1654 (65.8%)          | 755 (66.3%)                     | 225 (62.8%)                    | 674 (66.4%)                     | 0.964                    |
| NFA (n,%)                                                                           | 827 (32.9%)           | 367 (32.2%)                     | 130 (36.3%)                    | 330 (32.5%)                     | 0.890                    |
| BAL (n,%)                                                                           | 31 (1.2%)             | 17 (1.5%)                       | 3 (0.8%)                       | 11 (1.1%)                       | 0.450                    |
| <i>Respiratory symptoms<sup>b</sup></i>                                             |                       |                                 |                                |                                 |                          |
| Upper respiratory tract only (n,%)                                                  | 1008 (50.7%)          | 428 (47.0%)                     | 167 (59.6%)                    | 413 (51.7%)                     | 0.053                    |
| Lower respiratory tract (n,%)                                                       | 907 (45.6%)           | 461 (50.6%)                     | 101 (36.1%)                    | 345 (43.2%)                     | 0.002                    |
| <i>Hospitalization in high-dependency wards or intensive care units<sup>b</sup></i> |                       |                                 |                                |                                 |                          |
| Pediatric High-Dependency ward admission (n, %)                                     | 432 (21.7%)           | 211 (23.2%)                     | 49 (17.5%)                     | 172 (21.5%)                     | 0.450                    |
| Intensive care unit admission (n, %)                                                | 103 (5.2%)            | 43 (4.7%)                       | 11 (3.9%)                      | 49 (6.1%)                       | 0.200                    |
| Hospitalization length, days (median, IQR)                                          | 6.00 (4.00–9.00)      | 6.00 (4.00–9.00)                | 5.00 (3.00–7.00)               | 6.00 (3.00–9.00)                | 0.222                    |

NFS: Nasopharyngeal Swab, NFA: Nasopharyngeal Aspirate, BAL: Bronchoalveolar Lavage, IQR: Interquartile range. <sup>a</sup>Prevalence was calculated on the number of available information (N = 2028). <sup>b</sup>Prevalence was calculated on the number of available information (N = 1990); 72 patients reported other symptoms (mainly gastrointestinal symptoms). <sup>c</sup> The p-value was used to compare the September 2023–April 2024 season with the September 2024–April 2025 season. Fisher exact test (two-sided p-values) and Mann-Whitney test were used as appropriate

(40.4%) between September 2024 and April 2025, and 358 (14.2%) between May and August 2024.

Comparing September 2023–April 2024 to September 2024–April 2025, no difference was found in the prevalence of at least one respiratory infection (743, 65.2% vs. 640, 63.1%,  $P=0.322$ ). Looking at the single viruses (Fig. 1B), HRV was the most frequently detected virus in both seasons, accounting for 348 (30.5%) and 315 (31.0%) episodes, respectively. RSV was the second most frequently detected virus in both seasons; however, a significant decrease from 210 (18.4%) to 141 (13.9%) cases was observed between September 2023–April 2024 and September 2024–April 2025 ( $P=0.006$ ) (Fig. 1B). This reduction was mainly driven by lower RSV-A detections in September 2024–April 2025 compared with September 2023–April 2024 (7.8% vs. 12.9%,  $P=0.0001$ ).

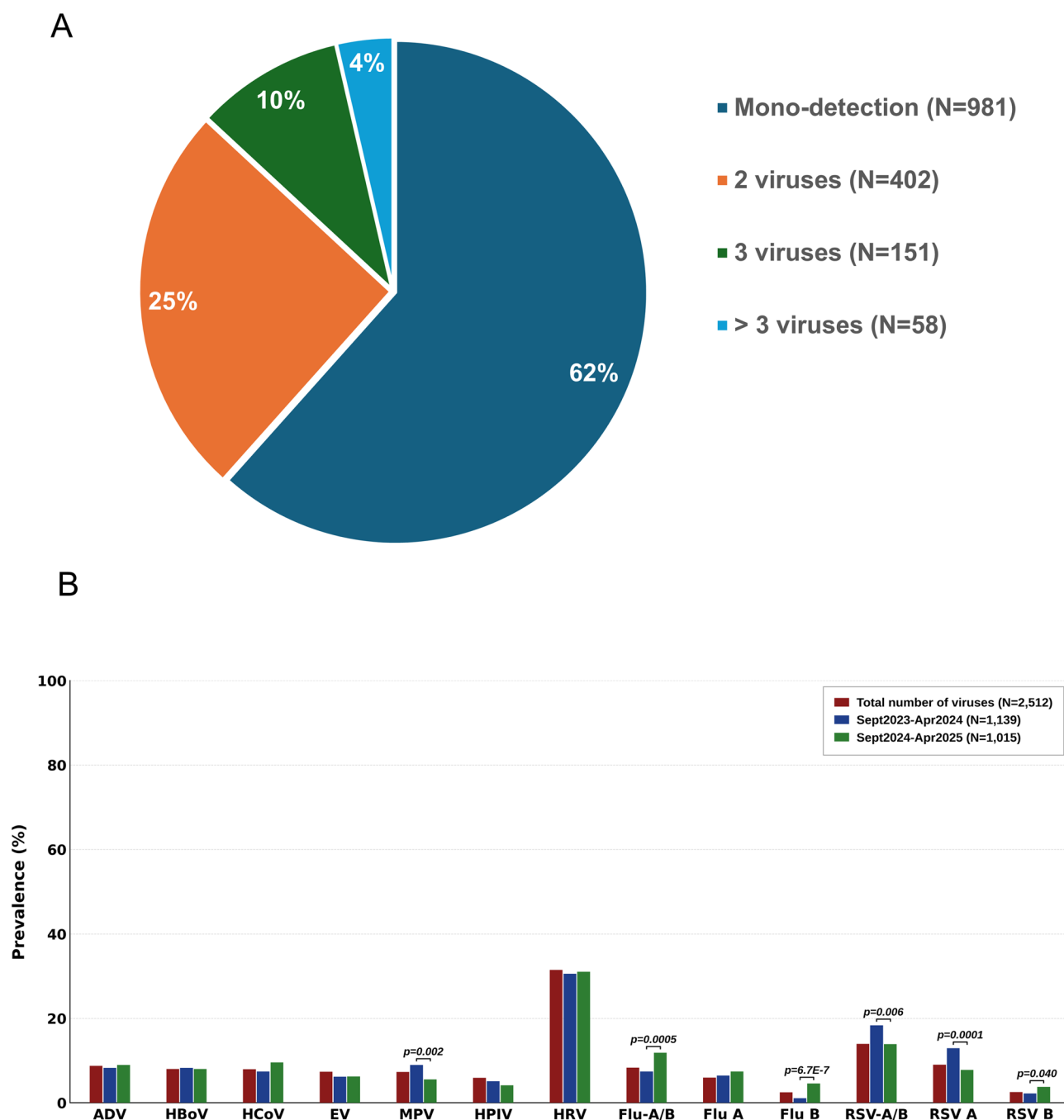
Significant differences in viral circulation between the two seasons were also observed for MPV, which decreased from 8.9% to 5.5% ( $P=0.002$ ), and for FluA/B, which showed higher circulation in the 2024–2025 season compared with 2023–2024 (11.8% vs. 7.4%,  $P=0.0005$ ). Of note, while FluA circulation did not differ significantly between seasons (6.4% vs. 7.4%,  $P=0.39$ ), FluB showed a statistically significant increase (1.1% vs. 4.5%,  $P=6.7E-7$ ). The month-by-month distribution of respiratory viral infections across the study period is shown in Fig. 2.

Coinfections were identified in 265 (23.25%) episodes during September 2023–April 2024 and in 250 (24.63%)

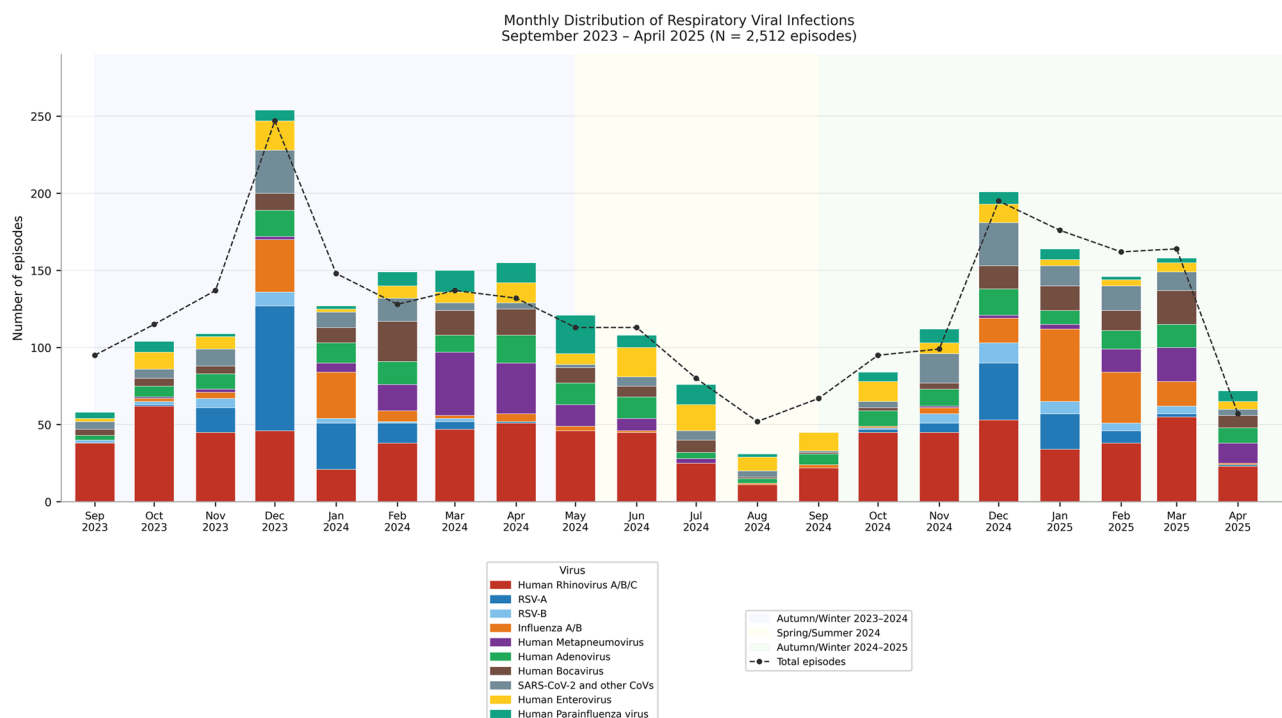
episodes during September 2024–April 2025. Among coinfection episodes, HRV was the most frequently involved in both seasons, accounting for 182 (68.7%) and 183 (73.2%) episodes, respectively. During September 2023–April 2024, the viruses most frequently involved in coinfections after HRV were HBoV (87 cases, 32.8%), RSV (83 cases, 31.3%) and ADV (77, 29.5%). Similarly, during September 2024–April 2025, the most frequent viruses after HRV were ADV (73 cases, 29.2%), HBoV and RSV (72 cases each, 28.8%).

#### Factors associated with pediatric high-dependency ward or ICU admission

Univariate and multivariate logistic regression analyses identified several factors independently associated with pediatric high-dependency ward or intensive care unit admission (Table 2). Lower respiratory tract symptoms showed the strongest association with the outcome in the adjusted model (AOR 3.40, 95% CI 2.69–4.29;  $P=7.16E-25$ ). Among respiratory viruses, HRV showed the strongest association with the outcome (AOR 2.32, 95% CI 1.83–2.94;  $P=2.39E-12$ ), followed by EV (AOR 1.75, 95% CI 1.19–2.58;  $P=0.004$ ), RSV-A/B (AOR 1.65, 95% CI 1.23–2.20;  $P=7.48E-04$ ), HPIV (AOR 1.62, 95% CI 1.07–2.45;  $P=0.023$ ), MPV (AOR 1.61, 95% CI 1.12–2.33;  $P=0.011$ ), and ADV (AOR 1.58, 95% CI 1.10–2.27;  $P=0.012$ ). Viral coinfection was associated with the outcome in the univariate model, but this association was not confirmed after adjustment (AOR 0.90, 95% CI



**Fig. 1** Viral detection patterns and seasonal distribution of respiratory viruses. **(A)** Distribution of respiratory viruses among positive respiratory samples ( $N=1592$ ), classified as single-virus detection, dual detection, triple detection, and > 3 viral co-detections. **(B)** Prevalence of respiratory viruses among all tested samples ( $N=2,512$ ) and across the two autumn–winter seasons (Sep2023–Apr2024,  $N=1,139$ ; Sep2024–Apr2025,  $N=1,015$ ). The prevalence of respiratory viruses between May 2024 and August 2024 was not shown. Significant two-sided  $p$ -values by the Fisher exact test are reported by square. RSV: Respiratory Syncytial Virus; Flu A/B: Influenza Virus A/B; HRV: Human Rhinovirus; HPIV: Human Parainfluenza Virus; MPV: Human Metapneumovirus; EV: Human Enterovirus; HCoV: Human Coronavirus; HBoV: Human Bocavirus; ADV: Human Adenovirus



**Fig. 2** Monthly distribution of respiratory viral infections. Stacked bars show the monthly distribution of respiratory viruses detected from September 2023 to April 2025 (N = 2,512 episodes). The dashed line indicates the total number of respiratory episodes tested per month. Shaded areas represent the three study periods: Autumn/Winter 2023–2024, Spring/Summer 2024, and Autumn/Winter 2024–2025. HRV: Human Rhinovirus; RSV: Respiratory Syncytial Virus; CoVs: Coronaviruses

**Table 2** Univariate and multivariate logistic regression analyses of factors associated with pediatric high-dependency ward or intensive care unit admission

| Variable                                 | Univariate analysis |          | Multivariate analysis |          |
|------------------------------------------|---------------------|----------|-----------------------|----------|
|                                          | OR (95% CI)         | p-value  | AOR (95% CI)          | p-value  |
| Demographics                             |                     |          |                       |          |
| Sex, Male                                | 0.97 (0.80–1.18)    | 0.769    | 0.98 (0.79–1.22)      | 0.862    |
| Age (> 1 year vs. ≤1 year)               | 1.24 (1.02–1.52)    | 0.032    | 1.07 (0.86–1.35)      | 0.534    |
| Seasons                                  |                     |          |                       |          |
| May2024–Aug2024                          | Ref.                | Ref.     | Ref.                  | Ref.     |
| Sep2023–Apr2024                          | 1.42 (1.03–1.95)    | 0.033    | 1.30 (0.91–1.85)      | 0.154    |
| Sep2024–Apr2025                          | 1.40 (1.01–1.94)    | 0.041    | 1.17 (0.94–1.46)      | 0.171    |
| Comorbidities (yes vs. no)               | 0.51 (0.32–0.82)    | 0.005    | 0.47 (0.28–0.77)      | 0.003    |
| Symptoms                                 |                     |          |                       |          |
| LRTS (yes vs. no)                        | 3.74 (3.03–4.63)    | 2.10E-34 | 3.40 (2.69–4.29)      | 7.16E-25 |
| Respiratory viruses                      |                     |          |                       |          |
| Human Adenovirus                         | 1.75 (1.27–2.41)    | 6.40E-04 | 1.58 (1.10–2.27)      | 0.012    |
| Human Bocavirus                          | 2.31 (1.68–3.18)    | 3.01E-07 | 1.43 (0.99–2.05)      | 0.054    |
| Sars-CoV-2 and other Human Coronaviruses | 1.12 (0.79–1.59)    | 0.538    | 1.08 (0.71–1.65)      | 0.724    |
| Human Enterovirus                        | 2.30 (1.64–3.22)    | 1.53E-06 | 1.75 (1.19–2.58)      | 0.004    |
| Human Metapneumovirus                    | 2.17 (1.56–3.01)    | 3.92E-06 | 1.61 (1.12–2.33)      | 0.011    |
| Human Parainfluenza virus                | 1.78 (1.23–2.58)    | 0.002    | 1.62 (1.07–2.45)      | 0.023    |
| Human Rhinovirus A/B/C                   | 2.56 (2.08–3.14)    | 5.12E-19 | 2.32 (1.83–2.94)      | 2.39E-12 |
| Influenza virus A/B                      | 0.74 (0.51–1.07)    | 0.107    | 1.48 (0.99–2.23)      | 0.058    |
| RSV-A/B                                  | 2.11 (1.63–2.73)    | 1.14E-08 | 1.65 (1.23–2.20)      | 7.48E-04 |
| Viral coinfection (yes vs. no)           | 2.82 (2.27–3.50)    | 6.29E-21 | 0.90 (0.61–1.33)      | 0.611    |

CI: confidence interval; OR: odds ratio; AOR: adjusted odds ratio. RSV: Respiratory Syncytial Virus

0.61–1.33;  $P=0.611$ ). Conversely, comorbidities were inversely associated with pediatric high-dependency ward or intensive care unit admission (AOR 0.47, 95% CI 0.28–0.77;  $P=0.003$ ). No evidence of meaningful multicollinearity was observed in the final model, with VIF values ranging from 1.02 to 1.16 (Supplementary Table 1).

## Discussion and conclusion

This two-year retrospective study provides a comprehensive characterization of respiratory viral infections in children younger than 5 years of age. The use of multiplex PCR throughout the study period enabled a highly sensitive, rapid, and accurate evaluation of respiratory viral infections.

HRV emerged as the predominant pathogen in the study cohort, accounting for 30.5% of detections in 2023–2024 and 31.0% in 2024–2025, indicating stable circulation across both autumn–winter seasons. As a frequent cause of both upper and lower respiratory tract infections in children, this high detection rate is in line with previous pediatric surveillance reports [3, 17, 18]. A similar predominance has also been described in adults, where HRV represented approximately 40% of detected respiratory viruses among hospitalized patients across recent seasons [19], suggesting that the high frequency of HRV detections may reflect a broader epidemiological pattern across age groups.

In our cohort, HRV was also the virus most frequently involved in co-infections, remaining high across both seasons (68.7% of co-infection episodes in 2023–2024 and 73.2% in 2024–2025). Consistently, Ito et al. [20] investigated HRV infection and co-infection in children during the COVID-19 pandemic and reported a comparable interannual distribution of co-infection cases, with similar proportions observed in 2020 (49.53%) and 2021 (51.47%), supporting the stability of co-infection seasonality across consecutive periods despite major epidemiological changes [21, 22]. In our multivariate analysis, rhinovirus infection was also independently associated with pediatric high-dependency ward or intensive care unit admission (AOR 2.32, 95% CI 1.83–2.94,  $P=2.39E-12$ ). This finding is consistent with recent evidence from hospitalized cohorts showing that HRV is associated with substantial morbidity across age groups, including increased need for respiratory support, and severe clinical presentation [23].

Following HRV, RSV was the second most frequently detected pathogen and remained independently associated with pediatric high-dependency ward or intensive care unit admission, confirming its major clinical relevance in children under 5 years of age [24–27]. Notably, a significant reduction in overall RSV circulation was observed in the 2024–2025 season compared with

2023–2024, primarily driven by a marked decrease in RSV-A detections, despite a modest increase in RSV-B. The observed reduction in RSV circulation temporally coincided with the introduction of RSV immunoprophylaxis with the long-acting monoclonal antibody nirsevimab [28]. In Italy, Lombardy represents a particularly vulnerable setting for respiratory virus transmission due to its large and densely populated population, the presence of major urban centers such as Milan, and a substantial proportion of high-risk individuals, including young children and clinically fragile residents. In this region, a nirsevimab immunoprophylaxis campaign was launched in October 2024 and offered to all infants born during the RSV epidemic season, as well as to those expected to experience their first RSV season during the 2024–2025 autumn–winter period [29]. In line with this observation, when considering only the children aged  $\leq 1$  year, the reduction in RSV observed in the overall population was confirmed in the 2024–2025 autumn–winter season (20.7% vs. 13.1%,  $P=0.001$ , data not shown). Although this study was not designed to directly evaluate the impact of nirsevimab, the temporal overlap between its implementation and the observed decline in RSV detections is consistent with early reports from other settings.

In our study, MPV was detected in 183 (7.3%) cases and remained independently associated with pediatric high-dependency ward or intensive care unit admission, consistent with data from the literature [30, 31]. In our cohort, MPV circulation decreased during the 2024–2025 autumn–winter season with respect to the 2023–2024 season. However, we cannot determine whether MPV subsequently regained prevalence in the months following April 2025, as these were not included in the study. In a different setting, Zhu et al. [32] reported that in 2024–2025 MPV circulation returned to a typical late winter to early spring pattern, with increased activity from late December 2024 through April 2025 and a peak detection rate of 14.46%. Similar observations have been reported in other countries. This heterogeneity suggests that the direction and magnitude of MPV fluctuations may vary according to local epidemiological conditions and prevention strategies. In this context, García-García et al. [33], in a prospective study conducted across two hospitals in Madrid, Spain, reported that the introduction of nirsevimab was significantly associated with reduced hospitalization and severity not only for RSV but also for other respiratory viruses, and they observed a significant reduction in MPV (–36.6%). Although nirsevimab is RSV-specific and is not expected to directly affect MPV, these observations are compatible with an indirect population-level effect, whereby a marked reduction in RSV circulation may be accompanied by changes in the observed burden of other respiratory viral infections. In our cohort, the reduction in MPV occurred in

parallel with the decline in RSV. These findings suggest that changes in RSV circulation may be accompanied by changes in MPV circulation in pediatric respiratory infections. This observation warrants further, in-depth investigation.

Flu A/B was detected in 209 (8.3%) cases in our cohort, and its circulation increased in 2024–2025 compared with the previous season. One possible contributing factor is influenza vaccine uptake in children. In Italy, Zaino et al. [34] reported that influenza vaccination coverage in children increased during the COVID-19 pandemic and declined in the seasons following this peak through 2023–2024. Rigamonti et al. [35] further reported lower influenza vaccination uptake among children residing in northern Italy and suggested that uptake may also be lower in high-income urban settings. Since our study was conducted in Lombardy, these observations provide context for the increase in influenza A/B observed in 2024–2025.

Among the other commonly detected viruses, adenovirus (ADV) was identified in 8.8% of cases and was independently and positively associated with pediatric high-dependency ward or intensive care unit admission in the adjusted model. This finding is in line with previous pediatric studies supporting the clinical relevance of adenovirus in respiratory illness [36]. Similarly, EV and HPIV were also independently and positively associated with pediatric high-dependency ward or intensive care unit admission.

In this study, viral coinfection involving two or more respiratory viruses was observed in 37.6% of the analyzed cases. A high prevalence of viral co-infections in pediatric respiratory infections has been consistently reported in the literature, particularly in studies using multiplex molecular diagnostics [26, 37, 38]. In our study, viral coinfection was not independently associated with pediatric high-dependency ward or intensive care unit admission in the multivariable model (AOR 0.90, 95% CI: 0.61–1.33,  $P = 0.611$ ). This finding is consistent with Bermúdez-Barrezueta et al. [39], who reported that an increasing number of concurrently detected viruses did not consistently translate into more severe clinical presentation, supporting the notion that disease severity is more strongly driven by the specific viral pathogen and host-related factors than by viral multiplicity alone. A comparable concept has been described in adults with haematological diseases, where severe outcomes were primarily associated with RSV infection and clinical vulnerability rather than with the presence of viral coinfection [6].

From a seasonal perspective, RVI episodes showed a clear concentration during the two autumn–winter periods, with the majority of detections occurring between September and April of both study years. This temporal

distribution is consistent with the known seasonality of respiratory viruses in pediatric populations, which typically peak during colder months, as reported in epidemiological surveillance studies from different geographic settings [40, 41]. Our study has some limitations. First, its single-center design may limit the generalizability of the findings to pediatric populations in different clinical and epidemiological settings. Second, clinical information on symptoms was not available for the entire study population, and comorbidity data were available only for a subgroup of episodes, which may have limited the interpretability of the findings. Moreover, the absence of quantitative viral load data may have partially limited the interpretation of the clinical associations observed in our study, particularly in samples with low viral load. Finally, individual-level information on RSV immunoprophylaxis with nirsevimab and influenza vaccination was not available, preventing a direct evaluation of their contribution to the observed inter-season changes in RSV, MPV, and FluA/B circulation.

In conclusion, this two-year analysis provides a comprehensive overview of respiratory viral epidemiology and factors associated with pediatric high-dependency ward or intensive care unit admission in children under 5 years of age. Rhinovirus emerged as the most prevalent pathogen, with a prominent role in viral co-infections, confirming its central contribution to the pediatric respiratory viral burden. In contrast, RSV and MPV were consistently associated with pediatric high-dependency ward or intensive care unit admission, underscoring their clinical relevance despite lower overall prevalence. The reduction in RSV circulation observed during the 2024–2025 season, temporally coinciding with the introduction of nirsevimab in Lombardy, highlights the importance of continued integrated surveillance to monitor changes in RSV circulation in the context of evolving prevention strategies. Overall, these findings support the adoption of integrated strategies that encompass RSV prophylaxis, enhanced virological surveillance, and reinforced infection control measures, aimed at reducing the clinical and healthcare burden of respiratory viral infections in young children.

Abbreviations

|          |                                                |
|----------|------------------------------------------------|
| ADV      | Adenovirus                                     |
| ARI      | Acute respiratory infection                    |
| AOR      | Adjusted odds ratio                            |
| CI       | Confidence interval                            |
| COVID-19 | Coronavirus disease 2019                       |
| Ct       | Cycle threshold                                |
| FluA/B   | Influenza A/B virus                            |
| HBoV     | Human bocavirus                                |
| HCoV     | Human coronavirus (seasonal; 229E, NL63, OC43) |
| EV       | Human enterovirus                              |
| HPIV     | Human parainfluenza virus                      |
| HRV      | Human rhinovirus                               |
| ICU      | Intensive care unit                            |
| IQR      | Interquartile range                            |

|         |                                               |
|---------|-----------------------------------------------|
| LRTI    | Lower respiratory tract infection             |
| MPV     | Human metapneumovirus                         |
| OR      | Odds ratio                                    |
| RT-mPCR | Real-time multiplex polymerase chain reaction |
| RSV-A/B | Respiratory syncytial virus A/B               |
| SPSS    | Statistical Package for the Social Sciences   |
| URTI    | Upper respiratory tract infection             |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-026-13696-7>.

Supplementary Material 1

Supplementary Material 2

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## Author contributions

NH: Writing – original draft, Data curation, Formal analysis. PB: Investigation, Data collection. APA: Methodology. MC: Data collection, Data curation. FG: Data collection. GDP: Data collection. AL: Data collection. CP: Data collection. MS: Methodology. GP: Methodology. GG: Data curation, Data collection. CT: Data collection. AM: Data collection. GC: Data collection. MF: Data collection. FS: Data collection. SB: Data collection. AB: Writing – review & editing, Supervision. ACA: Writing – review & editing, Supervision. CA: Writing – review & editing, Supervision, Funding acquisition.

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## Data availability

All relevant data are in the paper. The datasets analysed during this study are available from the corresponding author, [NH], upon reasonable request.

## Declarations

### Ethical approval

The study protocol was approved by the Territory Research Ethics Committee of Lombardy Region number 3 (CET 3) (prot. 6565\_12.11.2025\_P) and was conducted in accordance with the principles of the 1964 Declaration of Helsinki. The requirement for informed consent was waived by the Territory Research Ethics Committee of Lombardy Region number 3 (CET 3), in accordance with Article 110 of Italian Legislative Decree No. 196/2003, as amended by Law No. 56/2024.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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