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Epidemiology and clinical impact of co-infection in lower respiratory tract infections attributed to respiratory syncytial virus in infants: A multicenter cross-sectional study

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Abstract

Background

Many respiratory viruses, including respiratory syncytial virus (RSV), can cause lower respiratory tract infections (LRTI) in infants. To date, no clear conclusions have been reached regarding the extent to which RSV LRTI occurs in patients with superinfection, which microorganisms overlap with RSV, or whether superinfection contributes to clinical severity. Therefore, the present study aimed to clarify the impact of co-infection on clinical symptoms of RSV LRTI in infants aged under 1 year, prior to the introduction of new RSV prophylactic drugs in Japan.

Methods

We conducted pathogenic microbial analyses using a multiplex polymerase chain reaction (PCR) panel test in infants with LRTI who visited medical institutions in Chiba City and Ichihara City, Japan, between 2021 and 2023. The subjects were infants with suspected RSV LRTI who underwent RSV rapid antigen testing. Multiplex PCR testing was conducted using residual samples from antigen testing. We examined isolated microorganisms, age, hospitalization rate, length of hospital stay, and need for respiratory support in cases where RSV was detected alone compared with cases where other pathogens were detected simultaneously.

Results

Of the 492 infants included in this study, 252 (51.2%) tested positive for RSV. Two or more viruses were detected in 91/252 RSV positive infants (36.1%), while one or more viruses were detected in 207/240 RSV-negative infants (86.3%). Rhinovirus/enterovirus and adenovirus were detected throughout the year, whereas parainfluenza virus 3 (PIV3) showed a distinct seasonal peak. Detection of several other respiratory viruses was significantly less common in RSV-positive than RSV-negative infants. The risk of severe disease associated with co-infection did not appear to vary substantially with age. Conversely, human metapneumovirus (hMPV) was commonly detected in patients under 6 months of age, and nearly half of these patients required oxygen administration.

Conclusions

Ongoing surveillance is necessary to determine whether infections with viruses, such as PIV3 and hMPV, which were detected significantly more frequently in RSV-negative cases in our cohort, increased in frequency.

Background

Respiratory syncytial virus (RSV) is one of the most important viruses causing lower respiratory tract infections (LRTI) in infants and young children. RSV LRTI carries a high risk of complications in infants [1]. Globally, RSV contributes substantially to morbidity and mortality burden in children aged 0–60 months, especially during the first 6 months of life [2]. Although most RSV-related deaths occur in low-income countries, deaths are comparatively uncommon in high-income settings, including Japan. Nevertheless, hospitalization is common, and the resulting disease burden is considerable [3]. Furthermore, our population-based RSV epidemiological study in Chiba and Ichihara cities, Japan, revealed that RSV has a high disease burden among infants without underlying conditions [4].

Since the onset of the coronavirus 2019 (COVID-19) pandemic, rapid genetic testing methods for various viruses have been developed and are now being used in clinical settings. Among these is FilmArray, a fully automated genetic testing system capable of simultaneously detecting multiple respiratory pathogens [5]. With the widespread use of FilmArray, it has become possible to identify microorganisms that were previously difficult to diagnose in clinical settings. As a result, cases in which multiple microorganisms test positive are often encountered, although data on how this affects clinical outcomes remain limited.

Until 2023, the only prophylactic agent against RSV available in Japan was palivizumab, restricted to children with selected underlying conditions. In 2024, additional options became available domestically, including a maternal vaccine and nirsevimab, a long-acting anti-RSV monoclonal antibody. Maternal RSV vaccination will become a routine immunization in Japan starting April 2026, although vaccination rates may not be predictable. Moreover, eligibility for nirsevimab remains limited to children with certain comorbidities; however, broader implementation of these preventive strategies is anticipated in the coming years. These new preventive medications may lead to future epidemiological changes.

To date, no clear conclusions have been reached regarding the extent to which RSV LRTI occur in patients with superinfection, which microorganisms overlap with RSV, or whether superinfection contributes to clinical severity. The purpose of this study was to clarify the impact of co-infection on clinical symptoms of RSV LRTI in infants under 1 year of age prior to the introduction of new RSV prophylactic drugs.

Methods

Study design, participants, and samples

We conducted additional analyses using the remaining specimens from our previous epidemiological research [4]. The study was conducted at four hospitals in Chiba City and three hospitals in Ichihara City from January 2021 to December 2023. These cities are located near Tokyo and have a combined population exceeding one million.

The study population comprised infants under 1 year of age who visited the participating hospitals and underwent rapid RSV antigen tests. Infants tested at the referral clinic and those who did not undergo rapid testing at the participating hospitals were excluded. The RSV rapid antigen test is an immunochromatographic test kit that enables beside diagnosis within approximately 15 minutes. Typically, a small amount of sample extract remains after testing, and these residual samples were collected for the present study. Each clinician independently diagnosed LRTI, and residual samples from patients with LRTI were further tested using multiplex PCR.

In Japan, nearly all RSV diagnoses in infants are made using rapid antigen tests, which are covered by national insurance for all children under 1 year of age. Consequently, these tests are frequently administered not only to patients with LRTI but also to those with mild or no respiratory symptoms, including upper respiratory tract infections (URTI).

The study period spanned from 1 January 2021 to 31 December 2023. Clinical data collected included sex, age, underlying diseases, eligibility for palivizumab, outpatient/inpatient status, length of hospitalization, presence of respiratory support, and oxygen administration. The infants' guardians provided informed consent for inclusion in this study, which was approved by the Ethics Committee of Chiba University (27th July 2020, approval no. 3894).

Virus detection using multiplex PCR

Samples were stored at -30°C until analysis, which was performed within two months of collection. All samples were analyzed in our laboratory at the Medical Mycology Research Center of Chiba University. RSV detection was performed using FilmArray Respiratory 2.1 panels® (BioMérieux, Lyon, France), according to the manufacturer's instructions. This panel detects RSV, adenovirus (AdV), coronavirus (229E/HKU1/NL63/OC43), human metapneumovirus (hMPV), human rhinovirus/enterovirus, Influenza A/B, parainfluenza virus (PIV 1/2/3/4), *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* through multiplex reverse transcription-nested PCR. In cases of discrepancies between rapid antigen test results and FilmArray results, the latter were considered definitive.

Analysis of RSV co-infection status and its impact on clinical manifestations

We examined isolated microorganisms, age, hospitalization rate, length of hospital stay, and need for respiratory support in cases where RSV was detected alone and in cases where other pathogens were detected simultaneously.

Statistical analysis

Statistical analyses were performed using EZR v1.61 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [6]. In addition, a large language model (ChatGPT, OpenAI) was used as an auxiliary tool to support the selection and description of statistical methods; all calculations and interpretations were verified by the authors. Detection rates of each respiratory pathogen were compared between RSV-positive and RSV-negative infants using 2×2 contingency tables. The chi-square test was applied when all expected cell counts were ≥5, and Fisher's exact test was used otherwise. Age in months and length of hospital days were compared among three groups (RSV only, RSV with one additional virus, and RSV with ≥2 additional viruses) using the Kruskal–Wallis test. When the overall test was significant, post hoc pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction. Hospitalization rates and the distribution of respiratory support categories were compared among the three groups (RSV only, RSV with one additional microorganism, and RSV with ≥2 additional microorganisms) using a chi-square test of independence.

Two-sided p values <0.05 were considered statistically significant.

Results

Sample size and patient characteristics

A total of 1200 infants suspected of having RSV infection underwent a rapid RSV antigen test. Of these, 497 were diagnosed with LRTI, 492 of whom were included in the analysis because five samples were unavailable for multiplex PCR analysis. The cohort comprised 282 male infants (57.3%), with a median age of 6.0 months. LRTI types included bronchitis in 185 cases (37.6%), bronchiolitis in 180 cases (36.6%), and pneumonia in 127 cases (25.8%). Details have been previously reported [4].

Monthly trends in detected microorganisms

As shown in Figure 1, RSV peaked in summer. Rhinovirus/enterovirus was detected throughout the year, while AdV was also constantly detected but at lower frequencies. Among parainfluenza viruses, PIV3 was the most frequently detected, peaking in June and July. hMPV was relatively prevalent in 2023 and was not detected in other years.

Microorganisms detected simultaneously in RSV-positive patients

As shown in Figure 2, Among the 492 infants included in the analysis, 252 (51.2%) tested positive for RSV via PCR testing. Of these RSV-positive infants, RSV alone was detected in 161 cases (63.9%), while two or more microorganisms, including RSV, were found in 91 cases. In cases where only one microorganism was detected along with RSV, rhinovirus/enterovirus was the most common, followed by PIV3. In cases where two microorganisms were detected along with RSV, AdV and rhinovirus/enterovirus were frequently detected, although the total detection number of PIV3 was higher than that of AdV. Only one case involved co-detection of three microorganisms: rhinovirus/enterovirus, AdV, and hMPV. In contrast, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Bordetella pertussis* were not detected in any case.

Microorganisms detected in RSV-negative patients

Among 240 RSV-negative infants, 207 (86.3%) tested positive for one or more other microorganisms, while 33 had no virus detected. Rhinoviruses/enteroviruses were the most commonly co-detected viruses, followed by AdV, coronaviruses, and parainfluenza viruses. The distribution of detected microorganisms by age is shown in Table 1. Compared with other viruses, hMPV was detected more frequently in infants under 6 months of age, and 47.1% (8/17) of them were classified as severe, requiring oxygen administration. As with RSV-positive cases, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Bordetella pertussis* were not detected in any RSV-negative cases.

Differences in detection rates of each virus between RSV-positive and RSV-negative cases

As shown in Table 2, the detection rates of several other respiratory viruses were significantly lower in RSV-positive infants than in RSV-negative infants. Rhinovirus/enterovirus was detected in 73/252 (29.0%) RSV-positive cases and in 139/240 (57.9%) RSV-negative cases ($p < 0.001$; odds ratio [OR] 0.30, 95% confidence interval [CI] 0.20–0.43). Similarly, PIV3 (9/252 [3.6%] vs 38/240 [15.8%]; $p < 0.001$; odds ratio 0.20, 95% CI 0.09–0.42), PIV4 (3/252 [1.2%] vs 15/240 [6.2%]; $p = 0.003$; odds ratio 0.18, 95% CI 0.05–0.63), hMPV (5/252 [2.0%] vs 31/240 [12.9%]; $p < 0.001$; odds ratio 0.14, 95% CI 0.05–0.36), and human coronavirus (HCoV)-OC43 (1/252 [0.4%] vs 10/240 [4.2%]; $p = 0.005$; odds ratio 0.09, 95% CI 0.01–0.72) were less frequently detected in RSV-positive than RSV-negative infants. For adenovirus, PIV1 and PIV2, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and *Bordetella parapertussis*, detection rates did not differ significantly between the two groups (all $p \geq 0.05$).

Differences in age by number of microorganisms detected in RSV-positive cases

As shown in Figure 3, the median age was 3.0 months (interquartile range [IQR] 1.0–8.0) in infants with RSV alone (n = 161), 5.0 months (IQR 2.0–9.0) in those with RSV plus one virus (n = 74), and 7.0 months (IQR 5.0–8.0) in those with RSV plus ≥2 viruses (n = 17). Infants with RSV alone were significantly younger than those with RSV plus one virus ($p = 0.038$) and those with RSV plus ≥2 viruses ($p = 0.016$), whereas no significant difference was observed between the latter two groups ($p = 0.36$). These findings suggest that the likelihood of co-infection with pathogens other than RSV increases with age.

Correlation between multiple infections and severity

Table 3 shows comparison of hospitalization rates based on the number of microorganisms detected along with RSV. Hospitalization occurred in 123/161 (76.4%) in infants with RSV alone, 53/74 (71.6%) in those infected with RSV plus one microorganism, and 12/17 (70.6%) in those infected with RSV plus ≥2 microorganisms. Hospitalization rates did not differ significantly among the three groups ($p = 0.68$). Pairwise comparisons using Fisher's exact test also showed no significant differences (all $p \geq 0.5$).

Figure 4 shows comparison of length of hospital stay based on the number of microorganisms detected along with RSV. The median length of hospital stay was 6 days (IQR 5–8) in infants with RSV alone (n = 123), 7 days (IQR 5–9) in those with RSV plus one virus (n = 53), and 7 days (IQR 5.5–8.5) in those with RSV plus ≥2 additional viruses (n = 12). No statistically significant difference in length of hospital stay was observed among the groups ($p = 0.11$).

Figure 5 shows respiratory support requirement based on the number of microorganisms detected along with RSV. No respiratory support, oxygen administration, non-invasive positive pressure ventilation, and intubation with mechanical ventilation were required in 89 (55.3%), 51 (31.7%), 16 (9.9%), and 5 (3.1%) of infants in the RSV-only group (n = 161); in 35 (47.3%), 28 (37.8%), 11 (14.9%), and 0 (0.0%) in the RSV plus one microorganism group (n = 74); and in 9 (52.9%), 8 (47.1%), 0 (0.0%), and 0 (0.0%) in the RSV plus ≥2 microorganisms group (n = 17), respectively. No statistically significant differences in respiratory support requirement were observed among the three groups ($p = 0.24$).

Because the RSV-only group was younger than other groups, we further examined whether the severity of respiratory support differed between infants with RSV alone and those with RSV plus ≥1 additional microorganism within each age stratum (0–2, 3–5, 6–8, and 9–11 months). In each age group, no significant differences were observed in the distribution of respiratory support categories (no respiratory support, oxygen

administration, non-invasive positive pressure ventilation, and intubation with mechanical ventilation) between the two groups (0–2 months, p = 0.41; 3–5 months, p = 0.16; 6–8 months, p = 0.41; 9–11 months, p = 0.28).

Discussion

The aim of this study was to clarify the clinical implications of co-infection in infants with RSV LRTI. Rhinovirus/enterovirus and AdV were detected throughout the year, whereas PIV3 showed a distinct seasonal peak, and these viruses were identified in relatively high numbers. Although the target populations and clinical settings differed somewhat, previous surveillance studies in Japan similarly showed that rhinovirus was the most frequently detected pathogen between 2020 and 2022, with little or no clear seasonality [7]. Additionally, in Hokkaido, northern Japan, increased detection of AdV among infants aged 0 years in 2021 and 2023, and predominance of PIV3 among individuals ≤ 16 years of age has been reported [8, 9]. The viruses commonly reported in these studies were also frequently detected in our cohort, although the regions differed. However, it remains unclear whether these viruses are prone to co-infection with RSV. In our study, rhinovirus/enterovirus was detected less frequently in RSV-positive infants than in RSV-negative infants, whereas none of the other viruses were detected more frequently in the RSV-positive group. These findings suggest that the higher detection counts simply reflect viruses concurrently circulating widely in the community, rather than viruses specifically associated with RSV infection. Notably, previous cohort studies have also described a negative association between RSV and rhinovirus detection, interpreted as a possible viral interference between these pathogens [10, 11], consistent with our observations. On the other hand, SARS-CoV-2 has rarely been detected as a causative virus of LRTIs in infants, even during the COVID-19 pandemic.

In the present study, infants with co-infection were older than those with RSV infection alone. Several studies have been conducted to examine the relationship between age and the proportion of viral co-infections. Hayek et al. reported that the proportion of co-infections was significantly higher in children aged 0–4 years than in older age groups (5–17, 18–64, and ≥ 65 years) [12]. Similarly, Arimura et al. showed that the risk of co-infection was higher in children aged ≤ 6 years [13]. Although these studies suggest that younger age is associated with a higher frequency of co-infection, they included broad age categories. To the best of our knowledge, no prior work has focused exclusively on infants younger than 1 year during the COVID-19 pandemic. In our study, the frequency of co-infection increased with age in months, suggesting that, among infants under 1 year of age, co-infections are more likely to occur after maturation rather than immediately after birth.

Our results suggest no relationship between disease severity and co-infection. A meta-analysis of 27 literature articles on RSV co-infection showed a higher rate of intensive care unit admission in patients with hMPV co-infection but no difference in the incidence of rhinovirus co-infection, although only three articles on hMPV were included [14]. In contrast, a retrospective study of 75 patients at a single institution showed no significant differences in length of hospital stay or need for assisted ventilation [15]. Thus, while some reports suggest that co-infection increases disease severity, others indicate no difference [16]. One possible explanation for these conflicting findings is that most studies rely on molecular diagnostic tests that cannot distinguish true co-infection from mere co-detection, making it unclear how common genuine co-infections are.

In the present study, although the proportion of co-infections increased with age in months, no differences in disease severity were observed according to co-infection status. To address the possibility that differing age distributions across groups might have obscured associations, comparisons stratified by 3-month age categories were additionally performed; however, no significant differences were detected. Because stratification inevitably reduced the sample size within each group, the possibility that the limited statistical power influenced these results cannot be ruled out. In contrast, hMPV was frequently detected in patients under 6 months of age, and nearly half of these patients required oxygen administration. If prevention of RSV-associated lower respiratory tract infection in early infancy becomes feasible, human metapneumovirus (hMPV) infection—which is currently lacking both preventive measures and specific treatments—may become a more important clinical concern. [17].

In Japan, RSV vaccines and long-acting anti-RSV monoclonal antibodies are expected to become available by 2024. From April 2026, the RSV vaccine will become a routine vaccination for pregnant women, and anti-RSV monoclonal antibodies will continue to be administered to high-risk children. A recent French cohort study showed that long-acting anti-RSV monoclonal antibodies may be more effective than maternal RSV immunization in preventing hospitalization due to RSV infection in infants [18]. It is therefore necessary to continue precise epidemiological surveys of infant RSV infection, including co-infections, in Japan and to evaluate RSV prophylactic medicines.

Our study has some limitations. First, we used nucleic acid tests and, as in previous studies, were unable to strictly distinguish co-detection from true co-infection. Because this study included patients with clinically suspected RSV LRTI, when multiple viruses were detected, it was highly likely that RSV represented the acute-phase infection. However, the exact order and timing of infection with each virus could not be determined. Second, we did not assess concomitant infections with common bacterial pathogens that cause pneumonia, such as *Streptococcus pneumoniae* or *Haemophilus influenzae*. Therefore, the impact of bacterial co-infection on the severity of RSV infection could not be evaluated [19].

In conclusion, among infants younger than 12 months, the risk of viral co-infection increased with age in months; however, this was not accompanied by longer hospital stays or greater oxygen requirements, and the risk of severe disease associated with co-infection did not appear to vary substantially with age. As RSV preventive agents become more widely implemented, these epidemiological patterns may change. Ongoing surveillance of the prevalence of RSV and other viruses should continue, in order to identify the impact of immunization strategies.

Abbreviations

respiratory syncytial virus: RSV, lower respiratory tract infections: LRTI, polymerase chain reaction; PCR, the coronavirus 2019: COVID-19, upper respiratory tract infections: URTI, adenovirus: AdV, human metapneumovirus: hMPV, parainfluenza virus: PIV, human coronavirus: HCoV

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Chiba University (27th July 2020, approval no. 3894). The infants' guardians provided informed consent to participate. This research was conducted in accordance with the Declaration of Helsinki.

Consent for publication: Not applicable

Clinical trial number: Not applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Ataru Igarashi received research funding from Sanofi and AstraZeneca, Japan. Takeshi Arashiro is an employee of Sanofi. Naruhiko Ishiwada was the principal investigator of the study. The other authors declare no conflicts of interest.

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Authors' contributions: Igarashi A, Nagasawa K, Hagiwara Y, and Ishiwada N designed the study and analysed the data. Nagasawa K, Ishiwada N, Shizuno K, Kurihara E, Yoshida M, Kodama T, Abe K, Arii J, Takeshita K, Hishiki H, Ota S, Takahashi Y, Omata Y, Nakazawa T, Someya T, and Hoshino T collected the samples and clinical information. Ohata M, Ohkusu M, and Takeuchi N analysed the samples. Arashiro T, Hagiwara Y, Nagasawa K, Ishiwada N, and Igarashi A drafted the manuscript. All authors contributed to the revision of the manuscript and approved the final submitted version.

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Tables

Table 1. Isolation status of microorganisms by age among RSV-negative group

	Rhino /Entero	**PIV3**	**hMPV**	**AdV**	**PIV4**	**OC43**	***B. parapertussis***	**SARS-CoV-2**	**NL63**	**PIV1**	**PIV2**	**HKU1**
Number (%)	139 (100%)	38 (100%)	31 (100%)	24 (100%)	15 (100%)	10 (100%)	6 (100%)	6 (100%)	4 (100%)	3 (100%)	2 (100%)	1 (100%)
0M (n=5)	1 (0.72)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1M (n=20)	12 (8.6)	5 (13.2)	5 (16.1)	2 (8.3)	1 (6.7)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2M (n=21)	10 (7.2)	2 (5.3)	2 (6.5)	1 (4.2)	2 (13.3)	2 (20)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
3M (n=14)	7 (5.0)	1 (2.6)	5 (16.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
4M (n=11)	3 (2.2)	0 (0)	1 (3.2)	1 (4.2)	1 (6.7)	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
5M (n=15)	7 (5.0)	3 (7.9)	3 (9.7)	0 (0)	2 (13.3)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
6M (n=28)	18 (12.9)	4 (10.5)	2 (6.5)	2 (8.3)	5 (33.3)	1 (10)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
7M (n=14)	9 (6.5)	2 (5.3)	3 (9.7)	2 (8.3)	0 (0)	1 (10)	1 (16.7)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
8M (n=21)	14 (10.1)	3 (7.9)	0 (0)	4 (16.7)	0 (0)	2 (20)	1 (16.7)	1 (16.7)	0 (0)	2 (66.7)	0 (0)	0 (0)
9M (n=24)	14 (10.1)	3 (7.9)	2 (6.5)	2 (8.3)	0 (0)	0 (0)	0 (0)	1 (16.7)	2 (50)	0 (0)	0 (0)	0 (0)
10M(n=29)	19(13.7)	3 (7.9)	5 (16.1)	5 (20.8)	2 (13.3)	1 (10)	1 (16.7)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
11M(n=38)	25 (18.0)	12 (31.6)	2 (6.5)	5 (20.8)	2 (13.3)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	1 (33.3)	1 (50)	1 (100)

*The numbers in parentheses indicate the detection rates (%) of each virus within each age-in-months group.

Table 2. Detection rates of respiratory viruses and bacteria among RSV-positive and RSV-negative infants

Virus/Bacteria	RSV-positive (n = 252), n (%)	RSV-negative (n = 240), n (%)	OR (95% CI)	p-value	Test
Rhino/Entero	73 (29.0)	139 (57.9)	0.30 (0.20–0.43)	<0.001	χ^2
Adv	16 (6.3)	24 (10.0)	0.61 (0.32–1.18)	0.139	χ^2
PIV1	0 (0.0)	3 (1.2)	0.13 (0.01–2.62)	0.115	Fisher
PIV2	0 (0.0)	2 (0.8)	0.19 (0.01–3.96)	0.237	Fisher
PIV3	9 (3.6)	38 (15.8)	0.20 (0.09–0.42)	<0.001	χ^2
PIV4	3 (1.2)	15 (6.2)	0.18 (0.05–0.63)	0.003	χ^2
hMPV	5 (2.0)	31 (12.9)	0.14 (0.05–0.36)	<0.001	χ^2
OC43	1 (0.4)	10 (4.2)	0.09 (0.01–0.72)	0.005	χ^2
NL63	0 (0.0)	4 (1.7)	0.10 (0.01–1.94)	0.056	Fisher
HKU1	0 (0.0)	1 (0.4)	0.32 (0.01–7.80)	0.488	Fisher
SARS-CoV-2	1 (0.4)	6 (2.5)	0.16 (0.02–1.30)	0.063	Fisher
B. parapertussis	1 (0.4)	6 (2.5)	0.16 (0.02–1.30)	0.063	Fisher

Data are shown as n (%). Odds ratios (ORs) are calculated with RSV-negative children as the reference group. p-values are based on chi-square (χ^2) or Fisher's exact test, as indicated.

Table 3. Comparison of hospitalization rates based on the number of microorganisms detected with RSV

Detected microorganisms	Age months (median; IQR)	Number of cases	Number of hospitalized case	Hospitalization rate
RSV only	3.0 (1.0-8.0)	161	123	76.4%
RSV +1microorganism	5.0 (2.0-9.0)	74	53	71.6%
RSV +≥2microorganisms	7.0 (5.0-8.0)	17	12	70.6%

Figures

Fig1

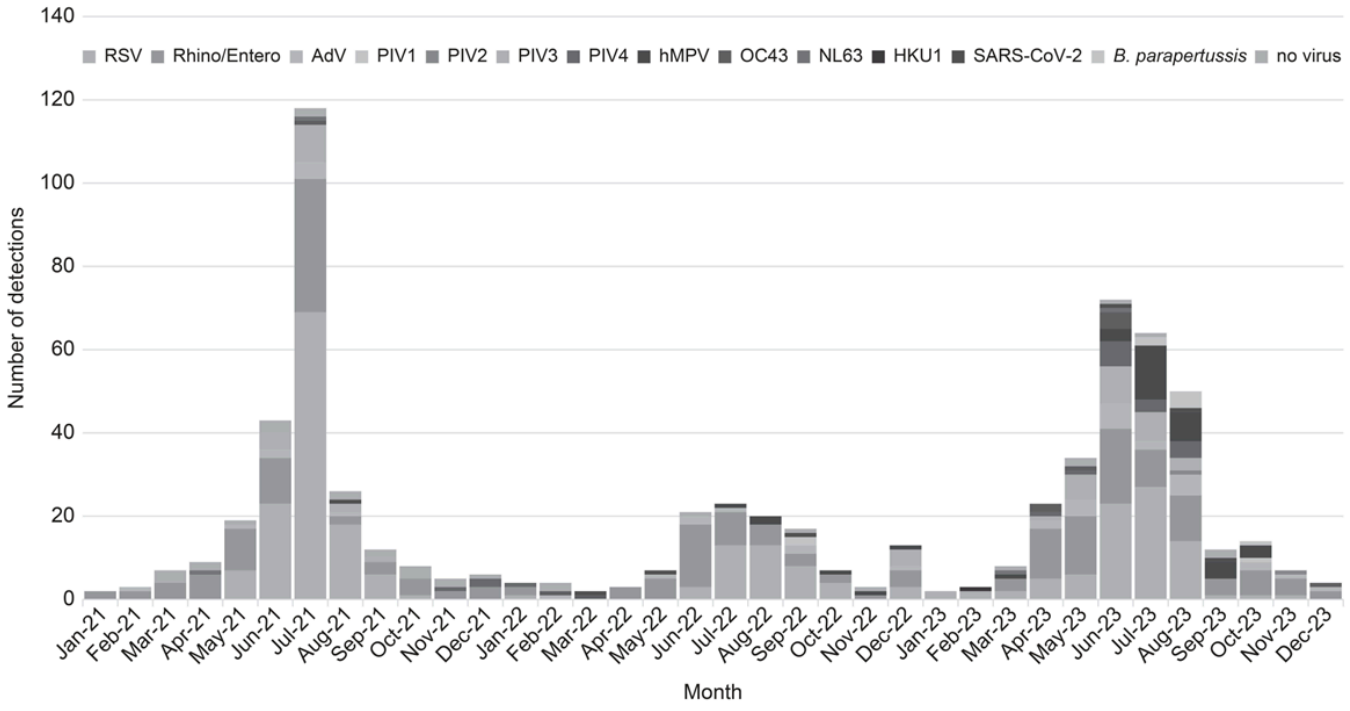
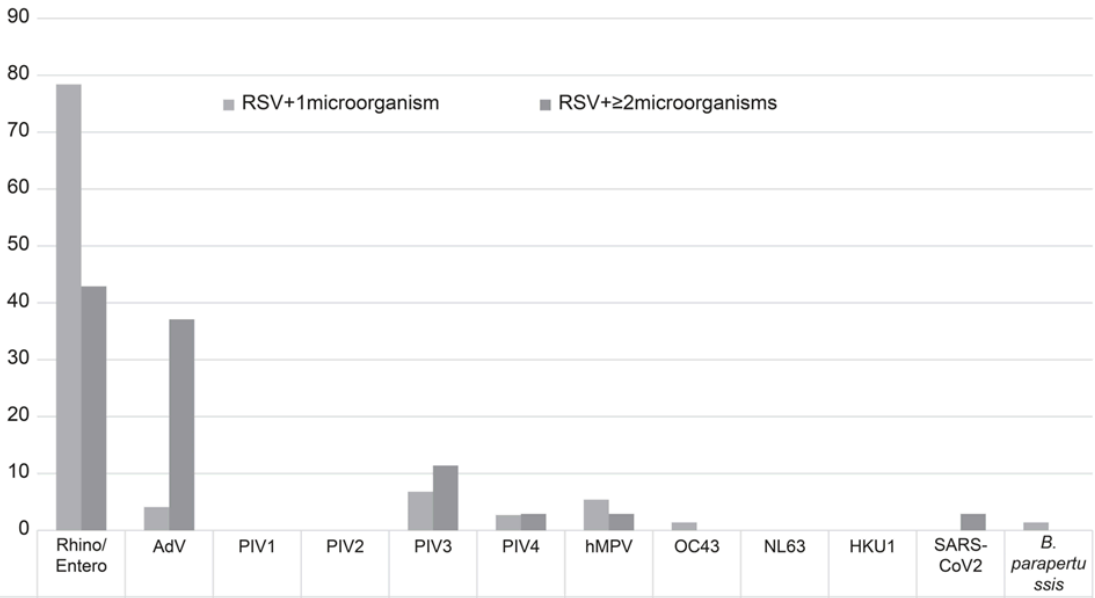


Figure 1

Monthly trends in detected microorganisms.

This figure shows the number of microorganisms detected each month from 2021 to 2023. If multiple microorganisms were detected in a single specimen, each microorganism was counted as a single sample.

Fig2



	Rhino/ Entero	AdV	PIV1	PIV2	PIV3	PIV4	hMPV	OC43	NL63	HKU1	SARS-CoV2	*B. parapertussis*
RSV+1microorganism	78.4	4.1	0	0	6.8	2.7	5.4	1.4	0	0	0	1.4
RSV+≥2microorganisms	42.9	37.1	0	0	11.4	2.9	2.9	0	0	0	2.9	0

Figure 2

Microorganisms detected simultaneously in RSV-positive patients.

The figure shows the proportion (%) of microorganisms other than RSV detected in cases where one or two or more additional microorganisms were identified in addition to RSV.

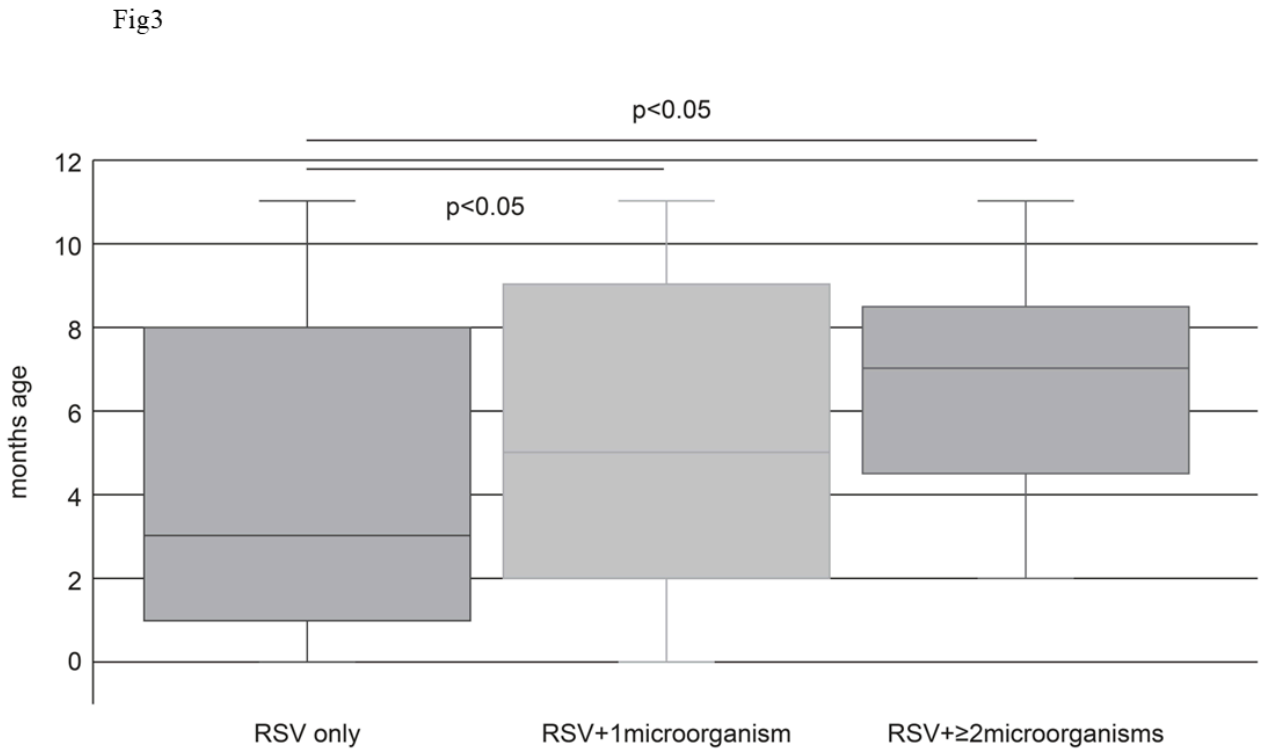


Figure 3

Differences in age based on the number of microorganisms detected in the RSV-positive group.

The vertical axis represents age in months.

Fig4

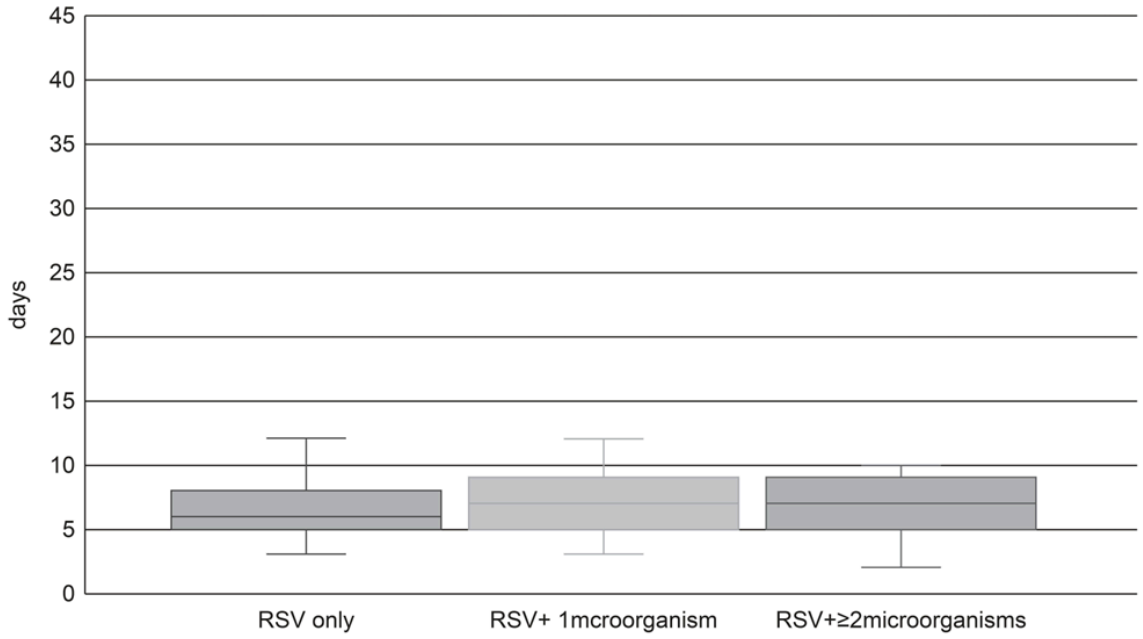


Figure 4

Comparison of hospitalization days based on the number of microorganisms detected along with RSV.

The vertical axis represents the length of hospital stay (days).

Fig5

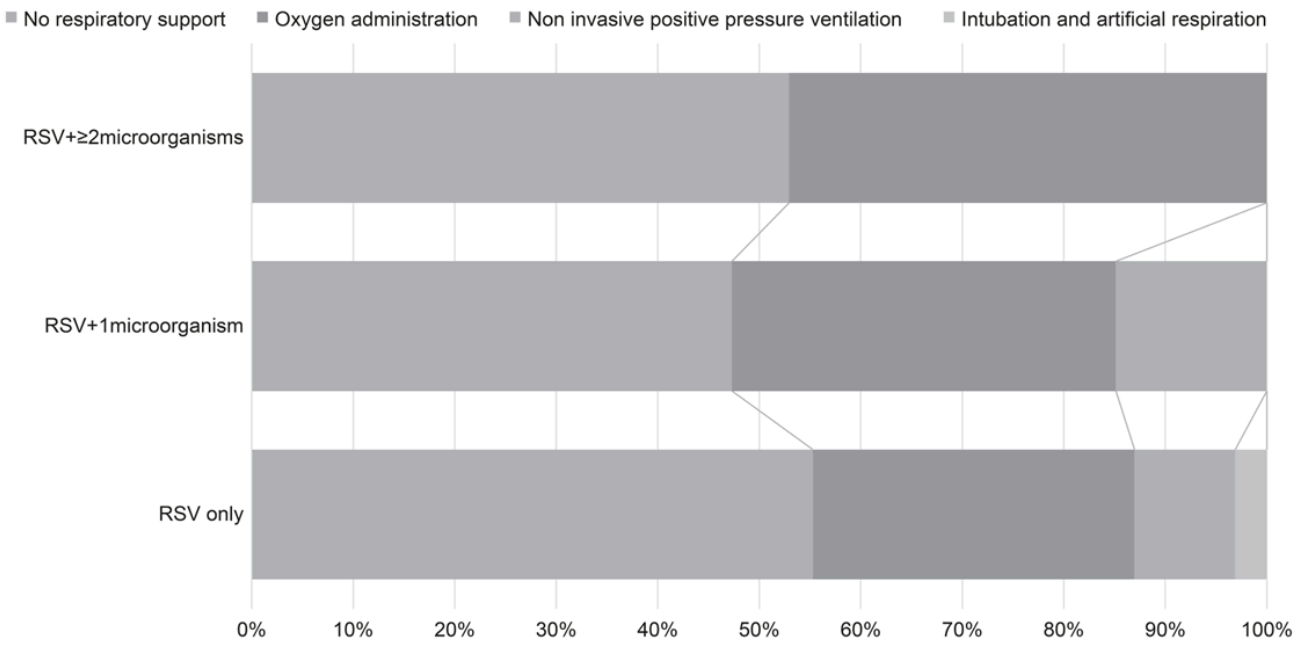


Figure 5

Respiratory support based on the number of microorganisms detected along with RSV.

The horizontal axis represents percentage.