Iranian Journal of Microbiology
Volume 18 Number 3 (June 2026) 367-374
DOI: http://doi.org/10.18502/ijm.v18i3.21663

ORIGINAL ARTICLE

Epidemiology and subtype analysis of respiratory syncytial virus among hospitalized children under five in Isfahan during early 2024

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Received: December 2025**, Accepted:** February 2026

ABSTRACT

Background and Objectives: Respiratory syncytial virus is the most common virus causing acute respiratory infections in children under 5 years old. We aimed to investigate the prevalence and circulating strains of RSV in hospitalized children in Isfahan.
Materials and Methods: Between January and May 2024, children under 5 years of age were enrolled in this study. Nasal swabs were collected from 100 children with acute respiratory infections admitted to the referral pediatric ward at Imam Hossein Children's Hospital in Isfahan, Iran. The prevalence of circulating RSV was investigated using the RSV qPCR detection kit. The virus type was identified by RT-PCR using type A- and B-specific primers.
Results: A total of 51 (51%) samples tested positive for RSV. Among them, typing was done in 33 specimens, of which 66.6% (22/33 cases) were assigned as subtype B and 33.3% (11/33 cases) as subtype A. Infants under 6 months were most severely affected by RSV (47.1%, 24/51). RSV-positive samples peaked in February (43.1%), followed by January (29.4%).
Conclusion: The results of the current study revealed a high prevalence of RSV and co-circulation of subtypes A and B, with subtype B more prevalent among children. This highlights the importance of ongoing surveillance of RSV.

Keywords: Respiratory syncytial virus; Prevalence; Respiratory infections; Epidemiology

INTRODUCTION

Respiratory syncytial virus (RSV) is the primary viral pathogen causing acute lower respiratory infections (ALRI) in infants and children under 5 years of age, particularly those under 6 months old, people aged > 65 years, and military recruits (1-4). Due to high morbidity and mortality, it is considered a threat to public health (5).

While most RSV infections cause self-limiting diseases, about 3.4 million children worldwide experience pneumonia or bronchiolitis (6). According to the last report in 2019, RSV is responsible for 33.1 million cases of LRTI tract infections with 3.6 million hospitalizations, 26300 in-hospital deaths, and 101400 RSV-attributable overall deaths in children before age 5 years worldwide, especially in low and middle-income countries (7). The burden of RSV

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infection in infants aged 0-6 months is estimated to be 6·6 million RSV-associated ALRI cases, 1·4 million RSV-associated ALRI hospitalizations, 13,300 RSV-associated ALRI in-hospital deaths, and 45,700 RSV-attributable overall deaths. RSV was responsible for one in 50 deaths in children aged 0–60 months and one in 28 deaths in those aged 28 days to 6 months (7).

Most annual RSV epidemics occur during winter, with peaks in February and March in temperate climates. In comparison, infections are more variable and occur throughout the year in tropical regions (8).

Prevention of RSV ALRI in infants is an urgent global health priority (9). The current therapeutic interventions are mainly supportive and include hydration, oxygen supply, and mechanical ventilation in severe cases (10). The use of Ribavirin, the only licensed antiviral for RSV disease, is no longer recommended (11). Ziresovir and EDP-323, two candidate antivirals, are currently undergoing clinical trials with significant efficacy, offering a promising therapeutic option for hospitalized children and infants (12).

Another FDA-approved intervention for high-risk infants is the use of monoclonal antibodies, including Palivizumab and RSVpreF (Nirsevimab) (10). In November 2022, the European Medicines Agency (EMA) recommended Nirsevimab administration in all newborn babies and infants during their first RSV season to protect against ALRI caused by RSV (13). Increasing studies demonstrate the noteworthy efficacy of Nirsevimab in preventing RSV infection and hospitalization in countries such as Spain, the UK, and Australia (14-16).

Despite extensive efforts to develop an RSV vaccine, there are only three approved vaccines, including ABRYSVO for administration in pregnant women, the elderly, and high-risk patients between 18 -59 years old, AREXVY for administration in the elderly, and the most recently licensed vaccine, mResvia, for use in the elderly aged ≥60 years. No vaccine has yet been approved for infants (13). Maternal vaccination prevents RSV LRTI in young infants, the primary target population for RSV infection, through maternal–fetal antibody transfer (17).

Despite the disease burden of RSV, our knowledge of its epidemiology in the central region of Iran, including Isfahan province, remains limited. Continuous surveillance of RSV epidemiology is crucial to enhance clinical management and vaccine development. This study aims to investigate the prevalence, circulating subtypes, and seasonality of the disease in infants and children in Isfahan Province, Iran.

MATERIALS AND METHODS

Patients and sample. In this descriptive cross-sectional study, 100 nasal swabs were collected from children younger than 5 years of age, hospitalized in the pediatric ward of Imam Hossein Children's Hospital in Isfahan, Iran, between January and May 2024. All children with ARI suspected to RSV infection were included. Cases were excluded from the study if samples were collected too late after symptom onset or if bacterial infection was suspected. Demographic information and clinical symptoms of the studied cases were also gathered through a general questionnaire and are shown in Table 1. Informed consent was obtained from the parents. All samples were immediately placed in the Viral Transport Medium and then rapidly transported to the Bacteriology and Virology Department at Isfahan University of Medical Sciences. This study was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.ARI.MUI.REC.1403.006).

Viral RNA extraction, & RSV detection. Viral nucleic acids were extracted from 200µl of nasal samples using the SinaPure Viral kit (Sinaclon, Iran), according to the manufacturer's instructions. Then, RSV was detected immediately using the One-Step RSV qPCR detection kit (Sinaclon, Iran) according to the recommended protocol. In this reaction, 8 µl of extracted RNA combined with 12 µl of the master mix comprised 10 µl One Step RT qPCR reaction mix, and 2 µl of primer and probe mix. Reverse transcription was achieved at 50°C for 20 min, followed by pre-denaturation at 95°C for 5 min and PCR steps included 45 cycles at 95°C for 20 s and 62°C for 30s. Amplification was performed using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, USA).

cDNA synthesis and conventional RT-PCR for RSV typing. Extracted RNA was converted to cDNA using the cDNA synthesis kit (Pars Tous, Iran) according manufacturer's instruction. Virus typing was performed by conventional hemi-nested RT-PCR

Table 1. Demographic characterization of patients included in this study.

	Total=100 (n)	RSV positive=51 (n,%)	p Value
Sex			
Male	53	26 (50.98%)	0.69
Female	47	25 (49.01%)	
Total	100	51	
Months			
January	32	15 (29.4%)	0.49
February	39	22 (43.1%)	
March	9	3 (5.8%)	
April	15	7 (13.7%)	
May	5	4 (7.8%)	
Total	100	51	
Age groups			
0-5 months	33	24 (47.05%)	0.009
6-11 months	23	8 (15.6%)	
12-24 months	19	6 (11.7%)	
24-60 months	25	13 (25.4%)	
Total	100	51	
Clinical data			
Fever	62 (62%)	28 (54.9%)	
Sore throat	7 (7%)	4 (7.8%)	
Cough	72 (72%)	35 (68.62%)	
Dyspnea	63 (63%)	29 (56.8%)	
Runny nose	15 (15%)	7 (13.7%)	
Nasal congestion	3 (3%)	1 (1.96%)	
Restlessness	2 (2%)	2 (3.92%)	
Sneezing	12 (12%)	6 (11.76%)	
Nausea	16 (16%)	8 (15.68%)	
Anorexia	2 (2%)	0 0%	
Pneumonia	8 (8%)	3 (5.8%)	
Bronchiolitis	9 (9%)	8 (15.68%)	
Respiratory distress	8 (8%)	3 (5.88%)	0.03
Cyanosis	1 (1%)	1 (1.96%)	
Seizure	8 (8%)	4 (7.8%)	
Diarrhea	1 (1%)	1 (1.96%)	
Nursing strike	1 (1%)	1 (1.96%)	

Statistically significant, *p < 0.05. Demographic characterization consisted of sex, age, monthly distribution, and symptom data of the total study patients and RSV-positive inpatient children.

targeting the hypervariable region 2 (HVR2) of the G protein, using type A- and B-specific primers as described previously (18) (Table 2). In the first round of PCR, a total of 5 µl of cDNA was added to 20 µl of the reaction mixture containing 2.5 µl of 10× One Step RT-PCR buffer,0.5 µl MgCl2, 2 µl of dNTP Mix, 0.5 µl Taq DNA polymerase (5U/ µM), 1µl of each forward and reverse primers at a final concentration of 10 pmol and 12.5 µl of RNase free water. In the hemi-nested PCR, 2.5 µl of the external PCR product were added to the reaction mixture containing the same reagents with the same condition. Amplification were performed by PeqLab thermo- cycler (Germany) under the following conditions: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension cycle of 72°C for 5 minutes. Then the PCR products were verified using electrophoresis on a 1.5% agarose gel.

Statistical analysis. SPSS software, version 26, was used for statistical analyses. Chi-square ($\chi 2$) and Fisher's exact test were performed for the evaluation of qualitative variables, including sex, season, and age groups. Statistical significance was defined as a p-value <0.05.

Ethical permission. This study was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.ARI.MUI.REC.1403.006). The informed consent was completed by the parents.

RESULTS

Demographics of the study population. Between January and May 2024, 100 nasal swabs were collected from children (47% female, 53% male) with respiratory tract infection. Their ages ranged from 0 months to 60 months (median: 9.0 months).

Among the study patients, 33% were younger than 6 months, 23% were aged 6 to 11 months, 19% were aged 12 to 24 months, and 25% were aged 25 to 60 months. Five percent of individuals had underlying

Table 2. Primer sequences targeting G (Glycoprotein) gene of RSV.

Primers	Sequence	Primer usage	Tm
GPA	5′ GAAGTGTTCAACTTTGTACC3′	Initial	52.36
nRSAG	5′TATGCAGCAACAATCCAACC3′	Nested	56.68
F1	5′CAACTCCATTGTTATTTGCC3′	Initial and nested	53.09

conditions (n = 5). There was 1 case of epilepsy, 3 cases of cerebral palsy, and 1 case identified for cerebral palsy and congenital heart disease.

Prevalence of RSV. Out of the 100 nasopharyngeal swabs, a total of 51 samples (51%) were found positive for RSV. To perform RSV typing, a hemi-nested RT-PCR was conducted on all 51 RSV-positive samples. Among them, 33 specimens tested positive, of which 66.6 % (22/33 cases) were assigned as subtype B and 33.3% (11/33 cases) were assigned as subtype A. Some qPCR- RSV positive samples were negative for the hemi nested RT -PCR targeting G gene. This is likely attributable to low RNA concentration that may have arisen from either RNA degradation or late or suboptimal sample collection. The most common symptoms observed among positive cases were cough (68.6%), dyspnea (56.8%), and fever (54.9%). Bronchiolitis was significantly higher in RSV-positive patients compared to those in the RSV-negative group (15.68% vs. 1%) (p= 0.03).

Of all RSV-positive patients, 51% were male, and 49% were female. There was no statistically significant difference in patients with RSV infection by gender (OR = 0.84, 95% CI: 0.38-1.86; p = 0.69).

Fig. 1 illustrates the distribution of RSV infection across age groups. Infants under 6 months were the most severely affected by RSV (47.1%, 24/51) (P = 0.009, Chi-square). 15.6% (n=8) of patients were between 6-11 months, 11.7%(n=6) of patients were between 12-24 months, and 25.4% (n=13) of patients were between 25-60 months.

RSV-positive cases peaked in February (43.1%), followed by January (29.4%); however, this difference was not statistically significant. The monthly prevalence of RSV strains is shown in Fig. 2.

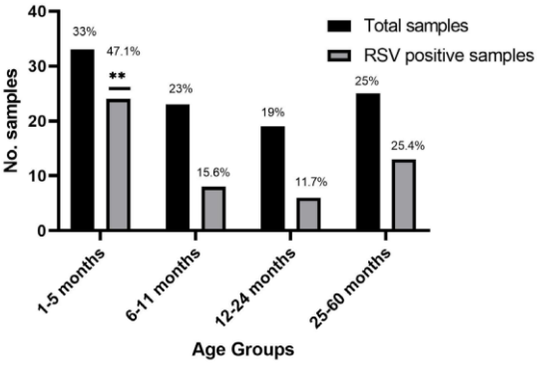

Fig. 1. Age distribution of the total study patients and RSV-positive children.

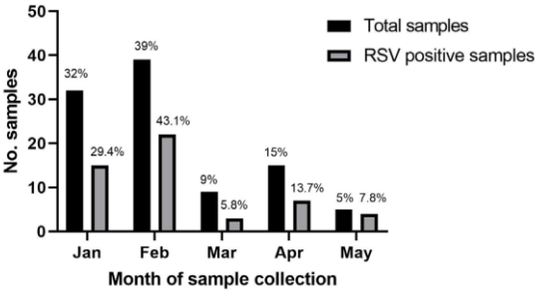

Fig. 2. Monthly distribution of the RSV strains detected in early 2024 in Isfahan, Iran.

DISCUSSION

The present study emphasized the prevalence and clinical features of RSV infection among hospitalized children aged less than 5 years after the COVID-19 pandemic in 2024 in Isfahan, Iran. The prevalence rate of RSV in the 2024 outbreak was 51%, which was higher than those observed in previous similar studies focusing on RSV prevalence in the prepandemic era. These studies have reported varying prevalence estimates, ranging from 46.1% in 2015-2016 to 33.95% in 2018-2019, among the pediatric population in Tehran, the capital of Iran (18, 19).

RSV nearly disappeared in many countries between 2020 and 2021, possibly due to COVID-19-related restrictions and precautions. A study conducted between October 2021 and March 2022 reported a 27% RSV detection rate in hospitalized Iranian children in Gorgan (20). Other study, conducted from December 2021 to March 2022 among 92 SARS–CoV–2–negative inpatients, reported a RSV detection rate of nearly 2.2% (21). In early spring 2022, an unexpected RSV prevalence of 53.33% was reported in a children's hospital in Dezful, a city in Southwest Iran (22). This finding indicates a delayed, unprecedented off-season RSV return, as described in other countries (23-26). The high RSV positivity rate is likely due to an immunity gap in young children and the accumulation of susceptible individuals resulting from reduced RSV circulation during the COVID-19 pandemic (27).

Moreover, RSV prevalence peaked in February, followed by January, which supports previous findings in Iran before the COVID-19 pandemic (18, 19). This demonstrates the consistency of RSV's sea-

sonal pattern across the country. In summary, these findings confirm a pattern of initial decline in 2020, followed by a gradual off-season resurgence in virus prevalence during 2021-2022, which ultimately returned to the pre-COVID-19 seasonal circulation pattern during 2022-2023, consistent with other reports worldwide (28, 29).

The concurrent circulation of both RSV-A and RSV-B, with alternating predominance in subsequent seasons, is well established (30). This phenomenon may be attributed to gap immunity against the dominant subgroup in the preceding year. The circulating subgroups in the country from 2007 to 2013 were as follows: RSV-A was observed in each of the seven seasons, while RSV-B was detected in only 2009 and 2013, and was absent in the other five seasons (31). In the 2015-2016 study, all positive samples belonged to subgroup A and genotype ON1. In the study conducted before the emergence of COVID-19, RSV-B was dominant in 2018 (60%) and RSV-A in 2019 (84%) (18, 19). During the 2021-2022 pandemic era, subgroup A was predominant (60%) (20). According to some reports, the predominance of RSV subtypes has fluctuated more pronouncedly since the emergence of SARS-CoV-2. The current study revealed that both RSV subgroups A and B were circulating, with subgroup B predominance (66.6% vs 33.3%). However, this occurrence was not unexpected, given that subgroup A had prevailed over B in the country during the 2021-2022 period. For instance, Korsun et al. (2024) reported RSV-A predominance from 2020 to 2022 and RSV-B predominance in 2022-2023 (32). A similar predominance pattern from 2020 to 2023 is also shown in other studies (20, 33, 34). Our findings also correspond with a report from Ethiopia, which showed a 68.8% RSV-B dominance in 2024 (35). The predominance of RSV-B since 2022 may be attributed to the hypothesis that reduced RSV circulation during COVID-19-related restrictions led to genetic bottlenecks that favored the dominance of RSV-B strains (32, 36, 37). In other words, RSV-B predominance may be related, on one hand, to the beneficial fitness advantage of subgroup B over A in low-rate circulation of RSV that caused rapid predominance. On the other hand, a divergent RSV-B strain may have emerged, as has been documented in Italy, the United States, and the Philippines (36-38). Further studies are needed to investigate circulating RSV-B strain lineages and potential alterations in viral transmission and pathogenesis. The strong fluctuation in RSV subtype circulation after the COVID-19 pandemic underscores the need to match circulating subtypes with virus strain vaccines (35).

The significance of gender differences is controversial. In this study, no substantial gender disparity in RSV positivity and hospitalization rates was observed among males and females < 5 years old with RSV infection. This finding contrasts with most previous studies, which have reported a higher incidence rate and severity of respiratory pediatric infections, including RSV, in males (18, 19, 22). Several other studies, however, have reported results that parallel those of the current study (18, 33). In this study, bronchiolitis was observed as a significant symptom among RSV-positive patients, which is in line with the observations of Huang et al. (39). This study found a higher prevalence of RSV infection in infants under 6 months than in other age groups, a finding consistent with numerous other studies indicating the greatest burden of RSV infection falls on infants under 6 months , accounting for over 50% of hospitalizations within the first trimester (40, 41).

RSV accounts for the primary etiological agent of bronchiolitis, both in terms of frequency and severity of infections (42). RSV hospitalization has a substantial economic and health burden. It demands a prolonged hospital stay, supplementary oxygen, intensive care units (ICU), and antibiotic use, which affects hospital resources, resulting in a severe impact on healthcare services (43). Ongoing RSV surveillance and prioritizing prevention strategies over treatment may reduce these burdens. The economic and health burden associated with RSV highlights the importance of immunization in children under 6 months of age. Regarding the current FDA-approved RSV vaccine for pregnant women and our finding indicating increased RSV prevalence during cold months, data from expanded national studies estimating RSV burden in the country, could inform vaccination strategies for pregnant women, thereby reducing virus burden and hospitalization.

The exclusive inclusion of hospitalized patients without consideration of outpatients is a study limitation that could lead to potential bias. Focusing on just one city and center, as well as the relatively small sample size, are other limitations. Future research should prioritize larger, multicenter cohorts that include both inpatient and outpatient populations over a more extended study period to enhance understanding of the epidemiology of RSV and im-

prove preparedness against RSV epidemics. Another limitation is that due to low RNA concentration of some specimens, typing could not be performed on specimens tested negative for the G gene.

CONCLUSION

The study provides evidence of high RSV prevalence in children with acute respiratory infections in Isfahan, Iran, which underscores the necessity of robust prophylactic and therapeutic interventions to reduce the significant burden of RSV, as well as continuous surveillance of RSV among young children for tracking epidemic-circulating strains.

ACKNOWLEDGEMENTS

The Research reported in this publication was supported by the Research Deputy at Isfahan University of Medical Sciences (1402395). The authors gratefully acknowledge the staff of the pediatric ward of Imam Hossein Children's Hospital in Isfahan, Iran.

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