


Epidemiology and clinical features of respiratory syncytial virus (RSV) infection in hospitalized children during the COVID-19 pandemic in Gorgan, Iran

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Abstract

Background and Aims: Respiratory syncytial virus (RSV) is a leading cause of acute respiratory infection in infants and young children. Given the altered circulation patterns of respiratory viruses during the coronavirus disease pandemic-2019 (COVID-19), the study aimed to evaluate epidemiology and clinical features of RSV infections in hospitalized children during the COVID-19 pandemic in Gorgan, northeastern Iran. Molecular epidemiology studies on respiratory viral infections are necessary to monitor circulating viruses, disease severity, and clinical symptoms, in addition to early warning of new outbreaks.

Methods: Overall, 411 respiratory swab samples from hospitalized children from October 2021 to March 2022 were collected at Taleghani Children's Hospital, Gorgan, Iran. The incidence of RSV, as well as the circulating subgroups and genotypes, were investigated and confirmed using PCR methods. Additionally, all samples tested for severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2) and influenza, and demographic and clinical data were analyzed using SPSS software.

Results: The share of RSV, SARS-CoV-2, and influenza among hospitalized children with acute lower respiratory infections (ALRI) were 27%, 16.5%, and 4.1%, respectively. The RSV subgroup A (genotype ON1) was dominant over subgroup B (genotype BA9), with more severe clinical symptoms. Compared with the prepandemic era there were high numbers of hospitalized SARS-CoV-2 positive children and low numbers of other respiratory viruses. Despite this, the prevalence of ALRI-related RSV-disease among hospitalized children in our specialized pediatric center was higher than COVID-19 disease in the same cohort.

Conclusions: Studying the epidemiology of respiratory viruses and determining the circulating strains can contribute to effective infection control and treatment strategies.

KEYWORDS

COVID-19, influenza, polymerase chain reaction, respiratory syncytial virus

1 | INTRODUCTION

Respiratory syncytial virus (RSV) causes infections of the lungs and respiratory tract. It is so common that most children have been infected with the virus by age 2. Symptoms of RSV infection vary from mild upper respiratory tract infections to acute lower respiratory tract infection (ALRI), such as bronchiolitis and pneumonia.^{1,2} Globally RSV disease, among hospitalized children under 5, has been estimated to account for 28% of overall ALRI with high (13%–22%) mortality rates. More than 93% of episodes and 99% of the deaths occur in developing countries.³ The risk of severe RSV-disease is highest in premature infants and those with chronic lung disease, congenital heart defects, and immunodeficiency disorders.^{2,4} Severe early-life RSV-disease may even predispose some infants to develop childhood asthma.⁵

There are two main subtypes, RSV A and B, with different genotypes circulating throughout the year,⁶ with a seasonal pattern in temperate climates.^{6,7} To develop and plan strategies for prevention or reducing RSV-associated child morbidity and mortality it is important to understand the epidemiology and the burden of RSV infection.⁸ Understanding the risk factors for severe infection allows us to identify high-risk groups and provide more efficient patient management.^{4,9} In Iran, from 1996 to 2013, the prevalence of RSV-disease was reported to be 18.7%, with the highest number of positive cases between November and March. More recently, from 2018 to 2019, two genotypes of RSV, ON1 (RSV-A) and BA9 (RSV-B), were most prevalent in viral isolates.¹⁰ During the coronavirus disease pandemic-2019 (COVID-19), the circulation of other respiratory viruses changed, probably due to health and care measures taken to prevent severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2) disease. In many countries, RSV surprisingly disappeared between 2020 and 2021, possibly due to lockdown and precautions taken to fight the COVID pandemic.^{11,12} Due to varying reports about RSV spread during the COVID-19 pandemic, we aimed to investigate the prevalence and clinical features of RSV-disease in infants and children in Golestan province, north of Iran, during the COVID pandemic.

2 | MATERIALS AND METHODS

Between October 2021 and March 2022, we recruited 411 hospitalized Iranian children with viral induced ALRI including sneezing, cough, dyspnea, and fever at Taleghani Children's Hospital, Gorgan, Iran. The hospital is the only specialized pediatric center in Golestan province. The samples were collected during the COVID-19 pandemic and examined for SARS-CoV-2 and influenza performed at the Golestan province coronavirus laboratory.^{13,14} Demographic and clinical information of the patients were collected by the caregivers

and relatives of the patients in a questionnaire. This study was approved by the science and bioethics committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1401.023).

Flowchart of the study is shown in Figure 1. For the RSV detection test, viral RNA was extracted from nasopharyngeal swabs using a viral high pure nucleic acid extraction kit following the manufacturer's instructions (BehPrep Viral RNA Extraction kit). The extracted RNA was converted into cDNA using the cDNA reverse transcription kit (Yekta Tajhiz Azma) according to the manufacturer's instructions.

The presence of the RSV genome (N gene) in collected samples was analyzed through the conventional PCR method using specifically designed primers (Table 1). The cDNA (100 ng) was added to 20 μ L of reaction mixtures containing optimal buffers, each deoxynucleoside triphosphate at a final concentration of 200 μ M, 3.0 mM MgCl₂, 0.5 μ M forward and reverse primers, and 0.5 units of Taq DNA Polymerase. The reaction conditions were: 95°C for 10 min followed by 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min, and then 72°C for 5 min. Finally, the amplified product was analyzed by electrophoresis on 1.5% agarose.

The positive samples were tested for the RSV-G gene using nested PCR. Primers were designed to detect the RSV-A and B strains (Table 1). In both steps of the nested PCR for RSV-A and RSV-B, 100 ng of the sample was added to 20 μ L of reaction mixtures containing optimal buffers. The reaction conditions for RSV-A were as follows: for the outer stage, 95°C for 5 min followed by 35 cycles

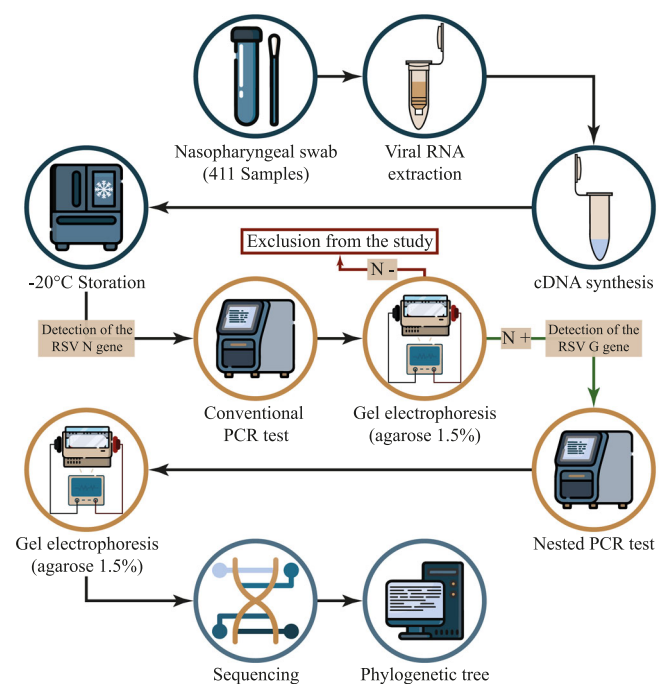


FIGURE 1 Flowchart showing the procedure of study.

TABLE 1 Selected primers for amplification of viral RNA from RSV A and B.

Primers	Sequence	Location	Tm	Product size
RSV-N	F: 5'-ATGGCTCTTAGCAAAGTCAAGT-3'	1085-1106	57.97	122 bp
	R: 5'-TGCACATCATAATTRGGAGTRTCA-3'	1206-1183	55.49	
RSV-A: Outer	F: 5'-AGTGTTCAACTTTGTACCTGC-3'	5120-5141	59.7	594 bp
	R: 5'-CTGCACTGCATGTTGATTGAT-3'	5713-5693	58	
RSV-A: Inner	F: 5'-CACCACCAAGCCCACGAAA-3'	5171-5189	60.83	454 bp
	R: 5'-ATTGTTATTTGCCCCATAGTTT-3'	5624-5603	56.34	
RSV-B: Outer	F: 5'-GCAGCCATAATATTCATCATCTCT-3'	4801-4824	56.75	855 bp
	R: 5'-TGCCCCAGRTTTAATTCGTTC-3'	5655-5634	57.29	
RSV-B: Inner	F: 5'-ACAAACAAACYACCCACCAAA-3'	5246-5266	59.54	400 bp
	R: 5'-TGCTTGAGGGATCAYRGTTTC-3'	5645-5626	60.55	

of 95°C for 1 min, 64.7°C for 1 min, 72°C for 1 min, and then 72°C for 5 min; for the inner stage, 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 62°C for 1 min, 72°C for 1 min, and then 72°C for 5 min. The reaction conditions for the outer stage of RSV-B were as follows: 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 1 min, and then 72°C for 5 min; for inner stage, 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min, and then 72°C for 5 min. Finally, the amplified product was analyzed by electrophoresis on 1.5% agarose. RSV-A and B positive samples were sequenced and results were analyzed via the Sanger method and Chromass 2.6.6. software. The phylogenetic tree was drawn by Mega7 software using the Maximum likelihood method with 1000 bootstrap replicates.

The collected data were analyzed using SPSS software. Results were presented as mean and standard deviation and qualitative data as frequency (percentage). χ^2 and Fisher's tests were used to perform statistical analysis. The χ^2 test was used with a large sample size and expected frequency counts greater than 5, while Fisher's exact test was used when the sample size was small, and the expected frequency counts were less than 5. Significant correlations were considered if p values less than 0.05. The STROBE checklist was followed in the manuscript (<https://www.equator-network.org/reporting-guidelines/strobe/>).

3 | RESULTS

Among the 411 samples, 252 (61.3%) and 159 cases (38.7%) were male and female, respectively. The average age of the patients was 20.7 ± 19.3 months, and the highest number of patients was between 6 and 24 months.

Out of 411 samples, 111 were identified with RSV-N gene primers and considered as positive RSV patients (Supporting Information: Figure 1). Upon analysis of the RSV-G gene, the frequency of the RSV was 27%, of which 60.4% (67 cases) were subtype A (Supporting Information: Figure 2) and 14.3% (16 cases) were subtype B (Supporting Information: Figure 3). Moreover, 25.3%

($n = 28$) of positive cases were not identified, possibly due to the low titer of the virus in these samples. The positive cases of both subgroups A and B were explored with sequencing. All RSV-A cases belonged to the ON1 genotype (GenBank accession numbers: OR799687, OR799688, OR799689, OR799690, OR799691), and all RSV-B cases belonged to the BA9 genotype (GenBank accession number: OR799692). The phylogenetic tree is shown in Figure 2. The frequency of SARS-CoV-2 and influenza was 68 (16.5%) and 17 (4.1%), respectively. Eight (7.2%) cases were positive for both RSV and SARS-CoV-2 and one (0.9%) were positive for both RSV and influenza.

There was a nonsignificant gender inequality in the 111 positive samples, 66 (59.5%) were boys, and 45 (40.5%) were girls (Table 2). A significant difference was observed between age-groups and RSV-positivity ($p < 0.05$). RSV-positive cases were predominantly children aged 6–24 months.

Symptoms in positive cases were primarily cough and fever, with more difficulty of breathing, wheezing, as well as lethargy ($p < 0.05$). There was a significant correlation between subgroup A and cough, wheezing, and shortness of breath, and in subgroup B with cyanosis (both $p < 0.05$). Out of 111 RSV-positive cases, 28 cases (25.2%) had an underlying disease (Table 3), but no significant correlation was found between RSV-positivity and their underlying disease. Ten positive cases (9%) were born preterm, but without a significant correlation to severity of symptoms.

Among the positive cases, 73 (65.8%) had pneumonia, related to RSV infection and subtype A. Among the hospitalized children there was a significant correlation between those with incomplete (vs. complete) routine vaccination and RSV disease. Eighty-one percent (91/111) and 18/111 (16.2%) had full and incomplete vaccination, respectively. Oxygen saturations (SpO₂) at admittance or during stay were below 93% in 25% of the positive RSV cases, indicating an important correlation of RSV disease with low SpO₂. Forty-seven (42.3%) of the RSV-positive patients needed mechanically assisted ventilation. We found a significant correlation between RSV-positivity and ventilator treatment, as well subgroup A and ventilator treatment. Ten (9%) were hospitalized in our intensive care unit (ICU)

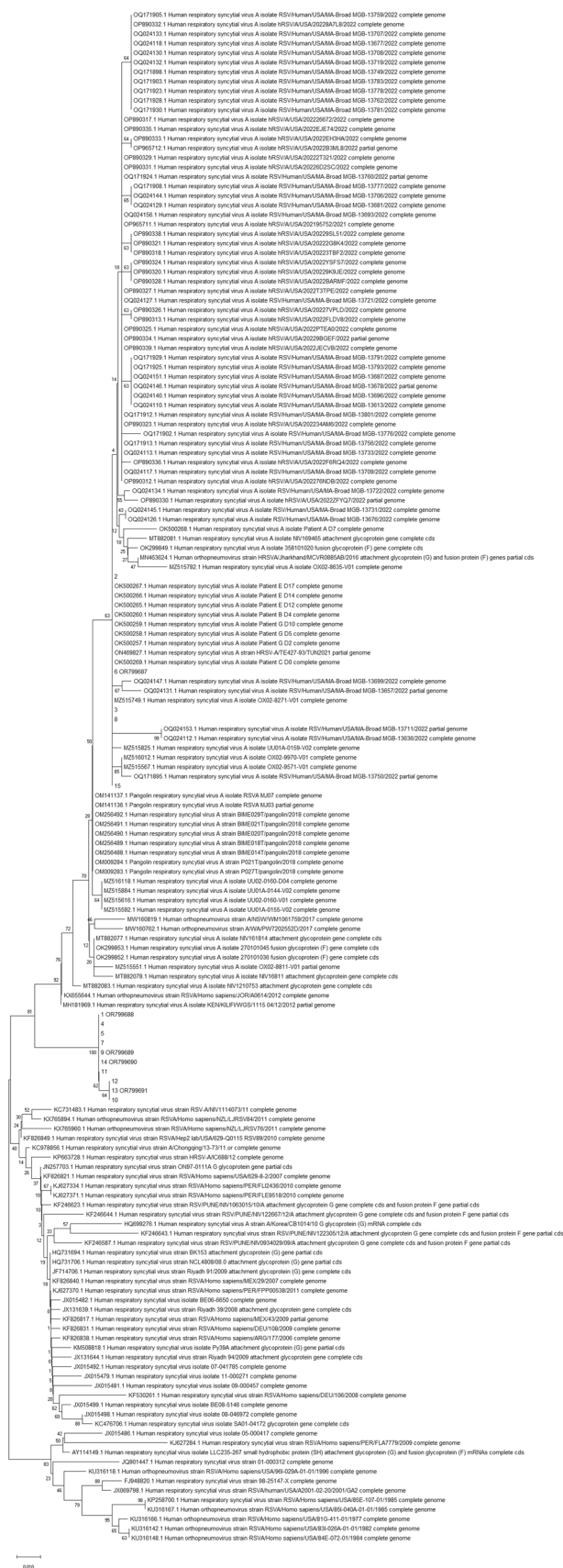


FIGURE 2 The phylogenetic tree drawn by Mega7 software using the Maximum likelihood method with 1000 bootstrap replicates.

and one patient (0.9%) demised. He was a 7-month-old male with spinal muscular atrophy (SMA) and chronic heart disease. His initial respiratory symptoms included cough, fever, shortness of breath, wheezing. He was diagnosed with pneumonia needing ventilator treatment in the intensive care unit due low SpO2 and respiratory distress.

4 | DISCUSSION

This study aimed to evaluate the epidemiology and clinical features of RSV infection in hospitalized children during the COVID-19 pandemic in Gorgan, northeastern Iran. The prevalence of RSV-disease, as well as the circulating subgroups and genotypes, were investigated and confirmed using PCR methods. During the pandemic, RSV-disease among hospitalized children was more prevalent than that of SARS-CoV-2 and influenza disease, with predominant circulating genotype RSV-A ON1 over RSV-B genotype BA9. Epidemiological knowledge about respiratory viruses and how they circulate, when they cause disease, the severity and symptoms seem important for prevention and mitigation of new outbreaks following the COVID-19 pandemic.

Respiratory infections can affect children at any time of the year, and is more common in temperate climates.¹⁵ In our study, the highest number of cases was observed in January and February 2022 (winter season), similar to Malekshahi et al.¹⁶ This was seen despite worldwide reports of delayed or altered seasonality of RSV infection in several studies during the pandemic.^{12,17,18} Seasonal variations in respiratory viral infections may be related to children staying indoors more often in cold seasons and being exposed to others who may be infected. Moreover, many viruses thrive in low humidity, which makes the nasal passages drier and more susceptible to infection.¹⁹

In this study, more RSV-disease was seen in hospitalized children compared with SARS-CoV-2 and influenza. In contrast, several other studies reported that the prevalence of RSV disease decreased dramatically after the emergence of SARS-CoV-2.^{20–22} A study in the United States found that RSV circulation was historically low during 2020–2021, began earlier and continued longer during 2021–2022 compared with pre-pandemic years and seasons.²³ In China RSV disease showed atypical seasonality during the pandemic, with a peak incidence in May 2020 and a second peak in November 2020, which was different from the typical winter peak observed in previous years.²⁴ In Australia, RSV disease was significantly reduced compared with pre-pandemic years, with no detectable RSV activity from April to September 2020. The RSV peak appeared later than in pre-pandemic years, from October to December 2020.²⁵

In Iran, Malekshahi et al. reported an overall 46% prevalence of RSV-disease in December 2015 to April 2016, among all cases of ALRI less than 2 years,¹⁶ while in the winter season of 2021–2022 we found 27% with RSV-disease among children hospitalized with ALRI. In Italy, during the COVID pandemic, 1.6% of 1213 samples was positive for RSV, with subgroups A (44.4%) and B (55.6%). The peak occurred in December 2020, when the RSV prevalence was 4.6%. All RSV A and B strains belonged to the ON1 and BA

TABLE 2 Distribution of RSV subgroups and genotypes and demographic details for patients infected with RSV.

Variable	RSV	p Value	RSV-A	p Value	RSV-B	p Value
<i>Gender</i>						
Male	66 (59.5%)	0.63	41 (61.2%)	0.98	8 (50%)	0.34
Female	45 (40.5%)		26 (38.8%)		8 (50%)	
<i>Age (month)</i>						
0–2	7 (6.3%)	0.003	4 (6%)	0.01	2 (12.5%)	0.45
2–6	36 (32.4%)		24 (35.8%)		5 (31.3%)	
6–24	40 (36%)		23 (34.3%)		6 (37.5%)	
24–60	21 (18.9%)		13 (19.4%)		3 (18.8%)	
60–86	7 (6.3%)		3 (4.5%)		0	

genotypes, respectively.²⁶ In Japan, comparing RSV activity in 2021 with four previous seasons, they proved a prevalence of 56.8% RSV-positive cases aged ≥ 2 years, higher than the 31.2% reported in the past 5 years. The study also suggested that infants aged < 1 year, supposed to be especially susceptible to RSV infection, were less likely to be infected with RSV because of COVID-19 control measures.²⁷ This reduction may be due to public health and social measures such as facial coverings, mask-wearing, hand hygiene, social distancing, and closure of kindergartens and schools, as well as improved surveillance and response measures. Another explanation could be competition and interference between viruses, although some studies reported high rates of co-infections between RSV and SARS-CoV-2, which might challenge this hypothesis.^{28–30}

The prevalence of coinfections varies from country to country. Among hospitalized children, we report 7% RSV-positive cases with co-infections of RSV and SARS-CoV-2, while only a single case (0.9%) was detected with both RSV and influenza. According to a retrospective review study in United States 2021, only 1.4% were co-infected with RSV and SARS-CoV-2,³¹ while in UK, between February 2020 and December 2021, 3.2% were co-infected with RSV and SARS-CoV-2.³² Studies from Italy, Poland, and South Africa did not report such co-infections,^{33–35} however, in Iran and Brazil they found high rates of co-infections with SARS-CoV-2 and influenza, ranging from 9.7% to 22.3%.^{36,37} We speculate that co-infections with RSV and other respiratory viruses may increase the risk of developing severe acute respiratory infections.^{6,38} However, we proved no significant correlation between RSV-related ALRI severity and other respiratory viruses such as Rhinovirus, Enterovirus, and Metapneumovirus, possibly due to the small sample size.

In our cohort RSV subtypes A and B were prevalent, with subtype A being predominant with or without co-infection. These findings are consistent with reports from studies both during the COVID-19 pandemic and in the prepandemic era, suggesting a novel strain-related outbreak after lifting preventive measures and restrictions.²⁸ In this study, all RSV-A and B cases belonged to the ON1 and BA9 genotypes, respectively, consistent with Malekshahi et al. (2015–2016) and Tavakoli et al. (2018–2019) in Tehran, Iran.^{10,16} Also in Saudi Arabia, all positive RSV-A cases belonged to the ON1

genotype, while all positive RSV-B cases belonged to the BA9 genotype.³⁹ The ON1 and BA9 genotypes contain 72 and 60 bp in the second variable region of the G gene, respectively. This lengthening modification in glycoprotein G is likely an evolutionary advantage that could be the reason for the dominance of these genotypes. In addition, glycosylation of the C-terminal end of the G protein can affect the expression of epitopes and hide them from detection by antibodies.^{40,41}

The RSV disease severity varies among individuals due to several virus- and host-associated factors.^{42,43} In this study the relationship between RSV disease severity and subgroups, indicate that severity was associated with subtype A. Significant correlations was seen between RSV-A positivity and cough, wheezing, shortness of breath, and respiratory distress among the hospitalized children. Previous studies confirmed these severe symptoms in RSV disease, but without a significant correlation between RSV disease severity and subgroups.^{41,44} Furthermore, there was a significant correlation between hospitalized RSV-positive cases and pneumonia, whereas no correlation was seen with bronchiolitis, in contrast to Tabatabaei et al. and Tran et al.^{41,45} Additionally, we proved a significant correlation between RSV subgroup A, and low SpO₂ levels with a need for mechanical ventilation, in accordance with a study from Brazil by Vianna et al.⁴⁶

The number of RSV-infected individuals was higher than those infected with SARS-CoV-2 and influenza. In accordance with Tran et al.,⁴⁵ the majority of RSV-diseased children were between 6 and 24 months. This underscores the vulnerability and severity of RSV disease in the youngest children, in line with others^{41,47,48} and may explain why RSV infections, especially subgroup A, are more prominent in this cohort compared with SARS-CoV-2 and influenza. Several studies have indicated a higher prevalence of RSV disease in males.^{6,12} In contrast, we found no gender differences, but RSV infection tended to be more prevalent in males than females, with predominance of subtype A, whereas RSV subtype B were equally distributed among male and females.

Children with underlying diseases are more susceptible to developing severe RSV infections. Chronic lung disease, congenital heart disease, immunodeficiency, and preterm birth (less than 37

TABLE 3 Distribution of RSV subgroups and multivariable details for patients infected with RSV.

Characteristics	Total (%)	RSV (%)	p Value	RSV-A (%)	p Value	RSV-B (%)	p Value
<i>Clinical symptoms</i>							
Cough	312 (75.9)	90 (81.1)	0.13	60 (89.6)	0.00	15 (93.8)	0.13
Fever	305 (74.2)	79 (71.2)	0.39	46 (68.7)	0.25	10 (62.5)	0.25
Difficult breath	115 (28)	40 (36)	0.02	24 (35.8)	0.11	5 (31.3)	0.77
Wheezing	112 (27.3)	40 (36)	0.01	26 (38.8)	0.02	7 (43.8)	0.15
Lethargy	129 (31.4)	26 (23.4)	0.03	15 (22.4)	0.08	2 (12.5)	0.09
Vomit	109 (26.5)	24 (21.6)	0.17	13 (19.4)	0.14	3 (18.8)	0.57
Hysteria	59 (14.4)	16 (4.4)	0.98	5 (7.5)	0.07	2 (12.5)	1
Fast breath	31 (7.5)	13 (11.7)	0.05	10 (14.9)	0.01	2 (12.5)	0.34
Cyanosis	26 (6.3)	10 (9)	0.32	2 (35)	0.42	5 (31.3)	0.00
Chest pain	4 (1)	1 (0.9)	0.38	1 (1.5)	0.81	0	–
<i>Underlying diseases</i>							
Neuromuscular disease	36 (8.8)	9 (1.8)	0.77	3 (4.5)	0.17	0	–
Icterus	25 (6.1)	6 (5.4)	0.72	3 (4.5)	0.78	2 (12.5)	0.25
Chronic heart disease	11 (2.7)	2 (1.8)	0.22	7 (10.4)	1.77	0	–
Congenital heart disease	11 (2.7)	2 (1.8)	0.73	0	–	1 (6.3)	0.35
Blood disease	6 (1.5)	2 (1.8)	0.66	2 (3)	0.25	0	–
Kidney disease	5 (1.2)	3 (2.7)	0.12	1 (1.5)	0.59	0	–
Genetic disease	3 (0.7)	1 (0.9)	1	0	–	0	–
Respiratory failure	3 (0.7)	1 (0.9)	1	0	–	0	–
UTI	5 (1.2)	1 (0.9)	1	0	–	0	–
Down syndrome	2 (0.5)	1 (0.9)	0.46	0	–	0	–
Premature	40 (9.7)	10 (9)	0.65	5 (7.5)	0.64	1 (6.3)	0.85
<i>Respiratory involvement</i>							
Pneumonia	231 (56.2)	73 (65.8)	0.01	46 (68.7)	0.02	11 (68.8)	0.3
Bronchiolitis	37 (9)	12 (10.8)	0.43	9 (13.4)	0.16	1 (6.3)	1
Asthma	11 (2.7)	3 (2.7)	1	1 (1.5)	1	1 (6.3)	0.35
<i>Vaccination</i>							
Complete vaccination	357 (86.9)	91 (82)	0.02	57 (85.1)	0.3	12 (75)	0.68
Incomplete vaccination	52 (12.7)	18 (16.2)		10 (14.9)		4 (25)	
Unknown	2 (0.5)	2 (1.8)		0		0	
<i>SPO2</i>							
>93%	347 (84.4)	85 (76.6)	0.02	52 (77.6)	0.23	11 (68.8)	0.19
<93%	54 (13.1)	21 (18.9)		13 (19.4)		4 (25)	
Unknown	10 (2.4)	5 (4.5)		2 (3)		1 (6.3)	
<i>Medical support</i>							
Ventilation	136 (33.6)	47 (42.3)	0.02	30 (44.8)	0.03	6 (37.5)	0.73
<i>Disease severity</i>							
ICU	34 (8.3)	10 (9)	0.74	4 (6)	0.45	1 (6.3)	1

TABLE 3 (Continued)

Characteristics	Total (%)	RSV (%)	p Value	RSV-A (%)	p Value	RSV-B (%)	p Value
<i>The outcome of the disease</i>							
Death	8 (1.9)	1 (0.9)	0.68	1 (1.5)	1	0	–

Note: The χ^2 test was used when the sample size was large, and the expected frequency counts were greater than 5, while Fisher's exact test was used when the sample size was small, and the expected frequency counts were less than 5.

weeks gestation) are associated with an increased risk of RSV related ALRI.⁴⁸ Our study found no correlation between RSV disease and underlying diseases and preterm birth, maybe because of the small number of patients with underlying disease. Six out of eight patients who died had underlying diseases, including chronic lung disease, kidney disease, progressive neurological disease, chronic heart disease, and Down's syndrome. Among the demised cases, one was a 7-month-old male detected with RSV-A infection who was admitted to our intensive care unit needing ventilatory support.

Our study found no relationship between RSV severity and prior routine vaccination. We speculate that interrupted routine vaccination and less exposure to respiratory viruses during the COVID-19 pandemic may have affected the population's immunity against endemic viruses like RSV. This may have delayed regional outbreaks and increased susceptibility to severe disease when pandemic restrictions were lifted.^{49,50} However, there are several controversies due to complex phylogeographic dynamics, viral genotype, and host phenotype interactions in different regions.⁴⁹

Our findings highlight the severity and burden of RSV infection in hospitalized children and underscores the necessity of providing healthcare facilities and efficient preventive and therapeutic approaches. Our study has several limitations. First, the sample size was relatively small and exclusively including hospitalized children. This limits the generalizability of the findings to the broader population as epidemiological information and clinical manifestations of RSV were investigated only in a single center, although the only specialized pediatric center in Golestan province. Second, the prevalence of viral respiratory disease, clinical findings, and the circulation of the virus were not recorded in nonhospitalized children. Third, cases were collected during the winter months, which may have affected the overall prevalence of RSV disease in our region and genotypes detected. Lastly, the molecular technique used to determine the subtypes of the virus was a nested-PCR, which may not be as accurate as more advanced tests. Therefore, the results of this study should be interpreted with caution and further research is needed to confirm these findings.

5 | CONCLUSION

In this study, the prevalence of RSV disease in hospitalized children, during the COVID-19 pandemic, was higher than that of SARS-CoV-2 and influenza. The predominant RSV subtype A with circulating genotype ON1 dominated in hospitalized children,

whereas subtype B (genotype BA9) was less frequent and not correlated with severity. This aligns with findings from various global studies. Continuous molecular epidemiology studies on respiratory viral infections are vital and beneficial for developing therapeutic strategies and maintaining preparedness against novel outbreaks of viral infections.

AUTHOR CONTRIBUTIONS

Mahnaz Ramzali: Data curation; formal analysis; investigation; writing—original draft. **Vahid Salimi:** Validation; writing—review and editing. **Fatemeh Cheraghali:** Conceptualization; methodology; writing—review and editing. **Seyedeh Delafroz Hosseini:** Formal analysis; investigation; methodology. **Mohammad Yasaghi:** Data curation; formal analysis; investigation. **Saeed Samadizadeh:** Formal analysis; validation; writing—review and editing. **Mostafa Rastegar:** Data curation; software. **Britt Nakstad:** Conceptualization; writing—review and editing. **Alireza Tahamtan:** Conceptualization; methodology; project administration; supervision; validation; visualization; writing—review and editing. All authors have read and approved the final version of the manuscript, had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

TRANSPARENCY STATEMENT

The lead author Alireza Tahamtan affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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