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ORIGINAL ARTICLE

Etiology of acute bronchiolitis and the relationship with meteorological conditions in hospitalized infants in China

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Background/Purpose: To investigate the prevalence of common viruses and *Mycoplasma pneumoniae* (MP) in hospitalized infants with acute bronchiolitis and study the relationship between bronchiolitis and meteorological conditions.
Methods: A 2-year prospective study was conducted on infants with a first episode of bronchiolitis admitted to Respiratory Department of Suzhou Children's Hospital. Demographic and clinical characteristics and meteorological conditions were obtained and analyzed.
Results: Pathogens were identified in 59.6% of 998 cases analyzed. The most frequent pathogen identified was respiratory syncytial virus (28.7%), followed by human bocavirus (11.6%), MP (9.0%), human parainfluenza virus-3 (7.8%), human metapneumovirus (6.6%), influenza A (3.5%), adenovirus (1.0%), and human parainfluenza virus-1 (0.3%). The clinical scores in children with MP or human metapneumovirus single infections, based on the assessment of severity of acute bronchiolitis, were significantly lower than in children with respiratory syncytial virus single infections. Respiratory syncytial virus had the strongest inverse correlation with mean temperature, followed by influenza A and human metapneumovirus. In addition, MP and human parainfluenza virus-3 showed positive correlations with mean temperature.
Conclusion: Although respiratory syncytial virus was the most frequent pathogen in patients in whom bronchiolitis was diagnosed, other pathogens, including newly identified viruses and MP,

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also play important roles in infants with bronchiolitis. Different respiratory pathogens have different traits in response to certain meteorological conditions.

Introduction

Bronchiolitis is a disorder that most commonly occurs in infants younger than 2 years via viral infection of the lower respiratory tract. It is characterized by acute inflammation, edema, and necrosis of epithelial cells that line the small airways, increased mucus production, and bronchospasm.[1] It is the leading cause of hospitalization of infants younger than 2 years, and more than 80% of children hospitalized are younger than 6 months. Importantly, disease severity is directly related to the size and maturity of the infant.

Several pathogens can cause similar signs and symptoms, including viruses and atypical bacteria. The most common etiology is infection with respiratory syncytial virus (RSV), with the highest incidence of RSV infection occurring between December and March.[2] Ninety percent of children are infected with RSV in the first 2 years of life,[3] and up to 40% of them will develop a lower respiratory tract infection.[4,5] Other viruses identified as causing bronchiolitis are human metapneumovirus (hMPV), human bocavirus (HBoV), influenza (IV), adenovirus (ADV), and human parainfluenza viruses (HPIVs). To date, there have been a limited number of studies on *Mycoplasma pneumonia*e (MP) infections in infants with bronchiolitis. This atypical bacterium may be an important infectious agent that induces the severe illness of acute bronchiolitis.[6]

Several studies have been performed to investigate the possible relationship between meteorological factors and respiratory pathogens, and the use of a single-center study to explore the relationship between bronchiolitis and meteorological conditions is rational given the number of patients seen at this institution each year.

Our study explored the viral and atypical bacterium etiology of hospitalized infants with bronchiolitis. We also aimed to describe the relationship between respiratory pathogens and meteorological conditions. A greater understanding of the etiology of bronchiolitis and the role of meteorological conditions is important for clinicians to master their knowledge base of different pathogens, make correct diagnoses, and avoid overprescribing antibiotic agents.

Materials and methods

Study design

This prospective cross-sectional study of children with bronchiolitis was conducted between January 2009 and December 2010 at the Children's Hospital affiliated to Suzhou University in Suzhou, China. The study was approved by the medical ethics committee of the hospital. Informed consent was obtained from parents or legal guardians. All patients were evaluated by an attending physician.

Patient population

From 2009 to 2010, 3134 children were consecutively hospitalized due to acute respiratory infection at the Respiratory Department of Suzhou Children's Hospital. Of these patients, 89 patients [bronchitis (15), bronchiolitis (33), and pneumonia (41)] were excluded because their parents refused to participate in the study. Nineteen children who presented with a history of chronic lung disease, underlying immunodeficiency states, or preexisting cardiac, renal, neurologic, or hepatic dysfunction, or bronchopulmonary malformation were excluded from the study. A total of 998 children admitted to Children's Hospital were identified with a diagnosis of acute bronchiolitis by an attending physician were enrolled in the study. Acute bronchiolitis was defined as the first episode of cough, rhinorrhea, wheezing, or rales, with chest radiographic findings of over-aeration and peribronchial infiltration with or without atelectasis in children younger than 2 years. Children with recurrent wheezing in whom bronchiolitis had been previously diagnosed and treated by a physician were excluded from the study.

Clinical data

During the hospital stay, the trained attending physician administered a study questionnaire and collected the clinical information. The severity of disease was assessed by clinical score using seven criteria (Table 1).

Specimens

We prospectively collected nasopharyngeal aspiration (NPA) fluids from all patients within 24 hours of admission

Table 1 Seven-component bronchiolitis clinical score system for assessing the severity of acute bronchiolitis.

Score	0	1	2
Heart rate (beats/min)	<120	120–160	>160
Wheezing	None	Audible wheezing with auscultation	Audible wheezing without auscultation
Respiratory rate breaths/min	20–40	40–60	>60
Dyspnea	Nil	Yes, without cyanosis	Yes, with cyanosis
Respiratory support	Nil	Oxygen tent	Respirator
Feeding difficulties	Absent	Mild	Serious
Duration of hospital stay (days)	<6	6–10	>10

by blindly passing a suction catheter through the nose with the intent of passing it into the lower part of the pharynx. The depth of penetration for the NPA catheter was set at 5—10 cm, which was measured as the length from the tip of the nose to the earlobe. The specimens were preserved in standard transport media (BBL Microbiology Systems, Cockeysville, MD, USA) and transported to the Department of Clinical Virology and Microbiology Laboratory for pathogen detection and nucleic acid (NA) amplification.

Direct immunofluorescence assay for seven respiratory viruses

One of the equally divided samples of NPA was vortexed and centrifuged at 500*g* for 10 minutes. The cell pellet was resuspended in phosphate-buffered solution (PBS) and centrifuged again at 500*g* for 10 minutes. The sample was finally resuspended in PBS to form a slightly cloudy suspension. NPA fluid samples were sent for direct immunofluorescence assay (DFA) to detect the presence of RSV, IV-A and -B, HPIV-1, -2, and -3, and ADV using a D^3 Ultra DFA Respiratory Virus Screening and ID Kit (Diagnostic HYBRIDS, Athens, GA, USA).

Nucleic acid extraction and polymerase chain reaction methods for detection of hMPV, HBoV, and MP

Another equally divided sample of NPA was diluted in 2 mL of normal saline before being centrifuged at 500 × g for 10 minutes. The resultant cell pellet was resuspended and then separated into two aliquots for DNA and RNA extraction. DNA and RNA were separately extracted from a 200 μL sample of supernatant using DNA-EZ Reagents (Sangon Biotech (Shanghai) Co. Ltd., New York, NY, USA) and TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions before performing the PCR. The method for the reverse transcription-polymerase chain reaction (RT-PCR) assay was described in our previous study[7] for hMPV using the RT-PCR Kit (Promega, Madison, WI, USA) and the fluorescent real-time PCR assay for detection of HBoV and MP using GoTaq qPCR Master Mix (Promega, Madison, WI, USA). For hMPV detection, primers were designed to specifically amplify the *N* gene (213 base pairs). The forward and reverse primers were 5′-AACCGTGTACTAAGTGATGCACTC-3′ and 5′-CATTGTTT-GACCGGCCCCATAA-3′, respectively. The fluorescent reporter dye at the 5′ end was 6-carboxy-fluorescein (FAM), and the quencher at the 3′ end was 6-carboxy-tetramethyl-rhodamine (TAMRA) for both real-time PCR assays. For HBoV detection, the PCR was performed with forward (TGACATTCAACTACCAACAACCTG) and reverse (CAGATCCTTTTCCTCCTCCAATAC) primers to amplify the *NP-1* gene. The sequence of the probe used for HBoV detection was FAM-5′-AGCACCACAAAACACCTCAGGGG-3′-TAMRA. Another fluorescent real-time PCR was performed to identify the *P1* adhesion protein gene of MP as previously described.[8] The forward and reverse primers were 5′-CCAACCAAA CAACAACGT TCA-3′ and 5′-ACCTTGACTG-GAGGCCGTTA-3′, respectively, and the probe sequence was (FAM)—5′-TCAACTCGAATAACGGTGACTTCTTACCACTG-3′-TAMRA.

Meteorological data

Data concerning monthly mean temperatures, relative humidity, rainfall level, total sun exposure, and mean velocity were obtained from the local weather bureau, which is located at 120°E 31°N. This information was obtained by the primary investigator from the Suzhou Weather Bureau, Suzhou, China.

Statistical analysis

Values were expressed as percentages for discrete variables, as mean and standard deviation, or as a 95% confidence interval for continuous variables. Clinical characteristics of acute bronchiolitis associated with single RSV were compared with those associated with single infections of HBoV, MP, HPIV-3, IV-A, hMPV, and coinfections using the Student *t* test, Mann-Whitney *U* test, χ^2 test, and Fisher exact test. The relationship of meteorological conditions to the number of cases due to different pathogens was determined using Spearman rank correlations. A two-sided value of $p < 0.05$ was considered statistically significant. All analyses were performed using the statistical package for SAS for Windows, Version 8.2 (SAS Inc., Cary, NC, USA).

Results

Bronchiolitis hospitalizations and etiology

Acute bronchiolitis was diagnosed in 998 patients. Overall, a respiratory virus or atypical bacterial pathogen was

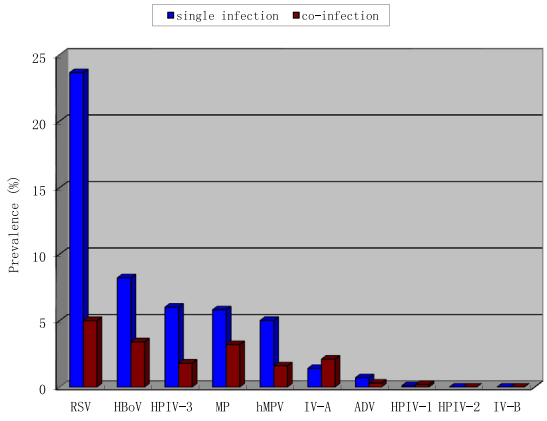

Figure 1 Pathogen prevalence (%) of single infections and coinfections. The most common pathogen in single infections (50.9% of the total number of cases) was RSV. The most common pathogen associated with coinfection was IV-A (60.0%), followed by MP (35.6%), ADV (30.0%), HBoV (29.3%), hMPV (24.2%), HPIV-3 (23.1%), and RSV (17.5%). ADV = adenovirus; HBoV = human bocavirus; hMPV = human metapneumovirus; HPIV-3 = human parainfluenza virus-3; IV-A = influenza A; MP = *Mycoplasma pneumoniae*; RSV = respiratory syncytial virus.

identified in 59.6% ($n = 595$) of the cases. The pathogen most commonly identified was RSV (28.7%; $n = 286$), followed by HBoV (11.6%; $n = 116$), MP (9.0%; $n = 90$), HPIV-3 (7.8%; $n = 78$), hMPV (6.6%; $n = 66$), IV-A (3.5%; $n = 35$), ADV (1.0%; $n = 10$), and HPIV-1 (0.3%; $n = 3$). The most common pathogens in single infection cases (50.9% of all samples; $n = 508$) were RSV (23.7%), HBoV (8.2%), HPIV-3 (6.0%), MP (5.8%), and hMPV (5.0%). More than one pathogen was identified in 87 (8.72%) of all patients. The most common pathogens associated with coinfection were IV-A (60.0%; 21/35) and MP (35.6%; 32/90) (Fig. 1).

Demographic and clinical characteristics

Children with acute bronchiolitis were age 1—24 months (mean 255.6 days; 95% CI 240.4—270.9 days). Detailed demographic and clinical characteristics are described in Table 2. Importantly, the characteristics assessed were comparable between RSV single infections and single infections associated with the other prevalent viruses or MP (Table 2).

Seasonality and the correlation between meteorological conditions and pathogen activity

RSV, HPIV-3, MP, hMPV, and IV-A exhibited strong seasonal patterns, with the number of RSV, hMPV, and IV-A cases peaking during the winter and spring seasons (December to May), and the number of MP, HPIV-3, and ADV cases peaking during the spring and summer seasons (March to August). In addition, HBoV cases prevailed throughout the year (Fig. 2).

RSV has the strongest inverse correlation with mean temperature, followed by IV-A and hMPV, for which a bimodal relationship was observed. Meanwhile, MP and HPIV-3 showed positive correlations with mean temperature. There were statistically significant but weaker inverse correlations for the relationship between sun exposure and RSV and hMPV. Mean wind velocity also presented a strong correlation with RSV and HPIV-3 activity, which was inversely correlated with RSV and positively correlated with HPIV-3 (Table 3).

Discussion

Bronchiolitis is usually a viral-associated condition that is defined as the first episode of acute respiratory wheezing in infants younger than 2 years and is a major cause of hospitalizations around the world. According to the literature published to date, few studies have examined atypical bacterial bronchiolitis in this age group. Most current information pertains to adults or older children, and MP infection may promote the exacerbation of asthmatic symptoms or be accompanied by wheezing in children without asthma.[9—11] However, little is known about the role of MP among young children with acute bronchiolitis.

In our study, viral or atypical pathogens were identified in 59.6% of the cases. The detection rate in our study was similar to that of previous studies (35—78%),[12—14] and lower than that of the studies performed by Bezerra et al and Calvo et al.[15,16] This could be because we did not detect human rhinoviruses, enteroviruses, and coronaviruses, which may play an important role in children with bronchiolitis. RSV was the most common pathogen detected in hospitalized children with acute bronchiolitis. Manoha et al[17] also reported an RSV infection rate of 28.5% (265 of 931), which is consistent with our findings. However, Antunes et al[18] reported a rate of 58.1%, but this is most likely due to the fact that the study period was from November 1, 2007 to April 30, 2008, which represents the RSV epidemic season. In addition, 9.0% of the patients in our study had a positive pathogen of MP approximated to other common viruses as well as HBoV (11.6%), HPIV-3 (7.8%), and hMPV (6.6%). Regarding emerging viruses, such as human bocavirus and metapneumovirus, the HBoV detection rate in this study was lower than that reported by Uršič et al (18.4%).[19] On one hand, the aim of this study only focused on children with bronchiolitis, whereas the study by Uršič et al assessed all respiratory infections in children. On the other hand, detected genetic differences may lead to biased results. The human metapneumovirus result is consistent with the results from a South Korean study.[20] Importantly, the findings of our study indicate that the role of MP in bronchiolitis cannot be ignored. Some researchers from Thailand have also reported results that are consistent with this study.[6]

In addition, we studied the pathogen circulation for 2 consecutive years, which compensates for annual variations in some pathogen circulations. This study describes, for the first time, the relationship between meteorological conditions and pathogen activity in children with bronchiolitis. Our findings showed that RSV, HPIV-3, MP, hMPV, and IV-A exhibited strong seasonal patterns, with the number of RSV, hMPV, and IV-A cases peaking during the winter and spring seasons and the number of MP, HPIV-3, and ADV cases peaking during the spring and summer seasons. Importantly, these results are consistent with previous studies.[14,17,21,22] There are several reasons for the seasonality of certain bronchiolitis pathogens, including the effects of meteorological conditions on the survival and spread of infectious pathogens in the environment, the variable susceptibility of the population to bronchiolitis, and the varying possibility of pathogen transmission by the host behavior due to the different meteorological conditions.[23—25] The study by du Prel et al,[26] which assessed 19 pathogens of acute respiratory infection in patients in Germany, showed the same results as ours, with the exception of HPIV-3 and MP. In that study, the authors showed that the number of HPIV-3 and MP cases was inversely correlated with temperature, whereas we found that the number of those cases was positively correlated with temperature. We hypothesize that the different climates at the locations of the two studies contributed to these differences. The city of Mainz in Germany has a marine climate with very little change in temperature throughout the year, whereas our city has a subtropical monsoon climate that has a wide range of temperatures through the year. Therefore, further studies are needed to explain these observed differences. Our study also presented an inverse correlation between pathogen activity and total sunshine exposure. It is hypothesized that ultraviolet light (UVB) radiation could interfere with the spread of RSV and hMPV by inactivating the virus in nature. UVB could also indirectly affect RSV and hMPV activity by

Table 2 Comparison of demographic and clinic characteristics between children with a single respiratory syncytial virus infection and those with other single pathogen or coinfections.[a,b]

Characteristics[c]	Total (n = 998)	RSV (n = 236)	HBoV (n = 82)	HPIV-3 (n = 60)	MP (n = 58)	hMPV (n = 50)	IV-A (n = 14)	Coinfection (n = 87)
Demographic data								
Sex, male	672 (67.3)	170 (72.0)	57 (69.5)	48 (80.0)	35 (60.3)	37 (74.0)	8 (57.1)	50 (57.5)
Days	255.6 ± 184.9	207.9 ± 167.7	**340.5 ± 168.5******	224.0 ± 168.7	**381.7 ± 186.2******	242.0 ± 176.7	211.8 ± 160.9	251.5 ± 198.1
Prematurity	79 (7.9)	24 (10.2)	7 (8.5)	6 (10)	7 (12.1)	3 (6)	1 (7.1)	9 (10.3)
Clinical presentation								
Hypoxia (SatO2 <94%)	315 (31.5)	131 (55.5)	49 (59.8)	**18 (30.0)******	**11 (19.0)******	**10 (20.0)******	**3 (21.4)***	51 (58.6)
Respirator	46 (4.6)	15 (6.4)	5 (6.1)	1 (1.7)	1 (1.7)	0 (0)	1 (7.1)	6 (6.9)
Fever (%)	328 (32.9)	62 (26.3)	39 (47.6)***	20 (33.3)	**31 (53.4)******	20 (40.0)	**8 (57.1)******	26 (29.9)
Duration of fever (d)	3.3 ± 1.9	3.1 ± 1.4	3.2 ± 1.4	3.3 ± 1.2	3.8 ± 1.9	2.8 ± 1.6	3.25 ± 2.1	3.4 ± 1.8
Audible wheezing without auscultation	481 (48.2)	147 (62.3)	52 (63.4)	**26 (43.3)****	**23 (39.7)****	**19 (38.0)****	**5 (35.7)***	63 (72.4)
Dyspnea	166 (16.6)	72 (30.5)	18 (22.0)	**7 (11.7)****	12 (20.7)	**4 (8.0)****	1 (7.1)	19 (21.8)
Cyanosis	172 (17.2)	24 (16.1)	9 (11.0)	4 (6.7)	**5 (5.2)***	**2 (4.0)***	1 (7.1)	8 (9.2)
Gastrointestinal symptoms (%)	72 (7.2)	52 (22.0)	**8 (9.7)***	9 (15.0)	**6 (10.3)***	11 (22.0)	2(12.6)	11(14.3)
Duration of hospitalization (d)	8.4 ± 2.5	8.3 ± 2.4	9.0 ± 3.2	7.9 ± 2.0	8.1 ± 2.5	8.2 ± 1.8	8.1 ± 1.5	9.2 ± 3.15
Clinic Score (Mean, 95% CI)	4.76 (3.85−5.59)	5.12 (4.65−5.63)	5.24 (4.4−6.08)	4.27 (3.41−5.13)	**3.55 (2.75−4.36)****	**3.78 (2.97−4.59)***	3.54 (1.44−5.64)	5.25 (4.43−6.07)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

[a] Significant differences are presented in bold font.

[b] Data are presented as mean age ± SD and values in parentheses are in percentage.

[c] HBoV = human bocavirus; hMPV = human metapneumovirus; HPIV-3 = parainfluenza virus 3; IV-A = influenza virus A; MP = *Mycoplasma pneumoniae*; RSV = respiratory syncytial virus.

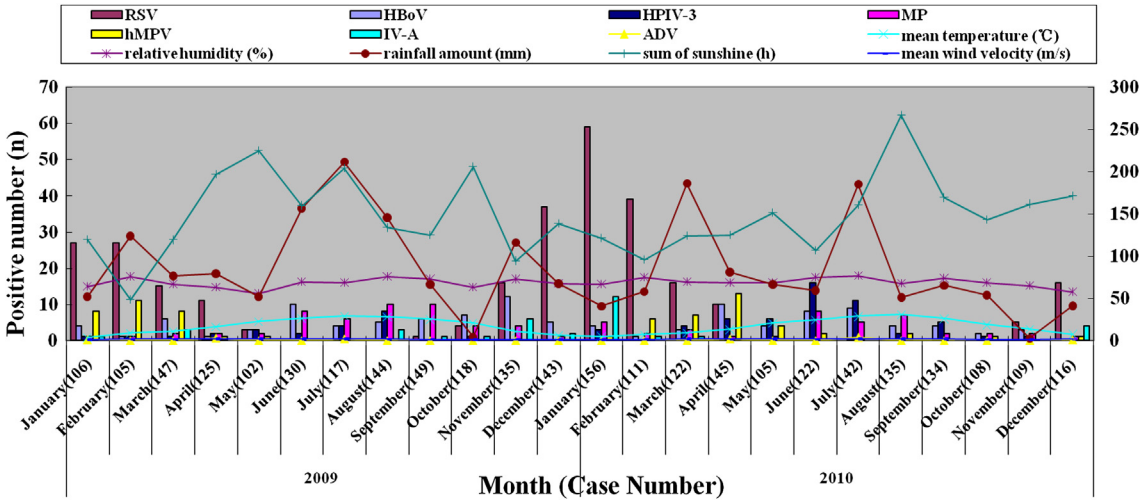

Figure 2 The monthly incidence of bronchiolitis in children due to seven common pathogens. The number of RSV, hMPV, and IV-A cases peaked during the winter and spring seasons (December to May), and the number of MP, HPIV-3, and ADV cases peaked during the spring and summer seasons (March to August). The incidence of HBoV was constant throughout the year. ADV = adenovirus; HBoV = human bocavirus; hMPV = human metapneumovirus; HPIV-3 = human parainfluenza virus-3; IV-A = influenza A; MP = *Mycoplasma pneumoniae*; RSV = respiratory syncytial virus.

Table 3 Spearman rank correlation coefficients for the associations between meteorological parameters and respiratory pathogens responsible for hospitalizations of children with bronchiolitis.

Pathogen	Mean temperature		Relative humidity		Rainfall amount		Sum of sunshine		Mean wind velocity	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
All pathogens	−0.67	0.0005	0.19	0.37	0.2	0.35	−0.72	<0.0001	−0.36	0.08
RSV	−0.92	<0.0001	−0.269	0.2	−0.187	0.38	−0.49	0.013	−0.53	0.008
HBoV	0.34	0.11	0.29	0.16	0.31	0.14	−0.16	0.47	−0.08	0.71
HPIV-3	0.52	0.01	0.3	0.15	0.32	0.12	0.18	0.39	0.59	0.0025
MP	0.65	0.0005	0.36	0.08	0.21	0.33	0.09	0.68	0.18	0.41
hMPV	−0.48	0.02	−0.08	0.71	−0.05	0.81	−0.42	0.04	0.09	0.66
IV-A	−0.54	0.007	−0.13	0.55	−0.12	0.57	−0.4	0.051	−0.51	0.01
ADV	0.04	0.84	−0.15	0.47	0.27	0.21	0.26	0.23	0.27	0.2

ADV = adenovirus; HBoV = human bocavirus; hMPV = human metapneumovirus; IV-A = influenza virus A; MP = *Mycoplasma pneumoniae*; HPIV-3 = human parainfluenza virus-3; RSV = respiratory syncytial virus.

stimulating vitamin D metabolism in the host,[27] which can increase the expression of some antiviral proteins as well as cathelicidin and defensins in the host to defend against virus invasion.[28]

The clinical characteristics of bronchiolitis are similar for RSV infections and other pathogens, but there are still some important differences. HBoV and MP bronchiolitis presented more frequently in older infants than RSV, which is consistent with previous findings.[15,29] Moreover, RSV bronchiolitis caused more severe hypoxia and audible wheezing without auscultation than MP, hMPV, HPIV-3, and IV-A bronchiolitis. Chan et al[30] reported that RSV infections in younger children led to higher oxygen demand than hMPV infections, and these findings were confirmed in our study. No significant difference was found in the severity of disease between RSV and HBoV or coinfections. The role of viral coinfections in the pathogenesis of disease is still unclear, and different opinions regarding the severity can be found in the literature.[16,31,32] In light of these conflicting reports in infants with bronchiolitis, additional larger scale and multicenter studies are needed to identify the significance of this issue.

Our study has a number of limitations. First, we only detected nine viruses and one atypical bacterium using DFA and PCR methods, and did not detect other important viruses, such as HRV.[16,32] Second, the 2-year study period was shorter than other long-term investigations.

According to our study, respiratory pathogens have seasonality differences as well as different responses to certain meteorological conditions, particularly temperature. In addition, the clinical manifestations of RSV bronchiolitis were more severe than those of hMPV, HPIV-3, IV-A, and MP bronchiolitis.

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