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Respiratory syncytial virus infection in children and its correlation with climatic and environmental factors

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Abstract

Objective: In this study, we aimed to investigate the clinical epidemiology of lower respiratory tract infections with different respiratory syncytial virus (RSV) subtypes in hospitalized children in Suzhou and their correlation with climatic and environmental factors.

Method: In this retrospective cross-sectional study, we collected nasopharyngeal secretion samples from children hospitalized with acute lower respiratory tract infection. We collected the clinical data of children with RSV infection, and compared and analyzed their epidemiological characteristics.

Results: RSV-B was the dominant strain in 2016. In 2018, RSV-A was the dominant strain. The positive detection rate of RSV-A was negatively correlated with monthly mean temperature, monthly mean wind speed, total monthly rainfall, and O_3 concentration and positively correlated with PM2.5, PM10, and NO_2, SO_2, and CO concentrations. The positive detection rate of RSV-B was negatively correlated with monthly average temperature, monthly total rainfall, monthly sunshine duration, and O_3 concentration and positively correlated with CO concentration.

Conclusions: RSV-A was the main subtype detected in this study. The positive detection rate of RSV-A was related to temperature, wind speed, rainfall, PM2.5. PM10, and NO_2, SO_2, CO, and O_3

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concentrations. The positive detection rate of RSV-B was related to temperature, rainfall, sunshine time, and O_3 concentration.

Keywords
Climate factor, environmental factor, clinical manifestation, lower respiratory tract infection, respiratory syncytial virus, China

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Introduction

Respiratory syncytial virus (RSV) belongs to the *Paramyxoviridae* family of respiratory viruses. Its envelope is composed of four proteins related to the lipid bilayer: matrix protein, hydrophobic small protein, and two glycosylated surface proteins (F and G). When F protein binds to the cell surface, conformational changes occur, resulting in fusion of the virus with the host cell membrane. The G protein is a type II glycoprotein comprising approximately 300 amino acids (depending on the virus strain), with a single C-terminal hydrophobic domain and a large number of glucans.[1,2] According to the genetic characteristics of the G protein and its response to antibodies, RSV can be divided into two different subtypes: RSV-A and RSV-B.[2] The clinical severity of RSV infection varies. Capillary bronchitis with wheezing is the main manifestation; however, severe pneumonia, pleural effusion, and respiratory failure may occur in severe cases. In this a retrospective study, we aimed to retrospectively analyze the clinical data of children with RSV infection from 2016 to 2018 by subtyping sputum samples. We sought to summarize the clinical epidemiological characteristics of the two subtypes and the correlation between the different subtypes with climate and environmental factors. Our findings could provide additional scientific evidence for the prevention and control of RSV as well as development of an effective vaccine against RSV infection.

Methods

Patients

In this study, we selected patients according to the following inclusion criteria: (1) age 1 to 15 years, and (2) clinical diagnosis of lower respiratory tract infection (LRTI).[3] Patients with the following conditions were excluded: immunodeficiency disease, congenital heart disease, severe malnutrition or anemia, failure to detect the RSV subtype, and incomplete baseline data. From among children meeting the criteria for selection, 389 were excluded owing to failure to detect the RSV subtype and incomplete baseline data. We randomly selected RSV-positive children with detailed data, such as general characteristics and clinical manifestations, and compared the demographic and clinical characteristics between patients infected with two subtypes.

This study was approved by the ethics committee of Children's Hospital of Soochow University (No: 2018LW016: 2018-08-30). After being informed about the study procedures, parents of the included children provided signed informed consent for their child's participation in the

study and nasopharyngeal secretion sampling. The reporting of this study conformed to the STROBE guidelines.[4]

Meteorological data collection

Suzhou is located in the northern subtropical monsoon marine climate zone. It is warm, humid, and rainy, with an obvious monsoon season and four distinct annual seasons. Winter and summer are long whereas spring and autumn are short. The annual average temperature is approximately 17°C, annual precipitation is approximately 1300 mm, the frost-free period is around 230 days, and there are approximately 2000 hours of sunshine in Suzhou. We divided the whole year into four seasons: winter from January to March, spring from April to June, summer from July to September, and autumn from October to December. As the main seasonal characteristics, the rainy season in Suzhou occurs during the transition from spring to summer, and typhoons are more likely from the end of summer to early autumn; the precipitation from March to August accounts for 63% of the annual rainfall. The meteorological data from 2016 to 2018 used in this study were obtained from the Suzhou Meteorological Bureau. The geographic location of the meteorological observatory is 120°E and 31°N. The data included monthly average temperature (°C), monthly relative humidity (%), total monthly rainfall (mm), monthly sunshine (hours), monthly average wind speed (m/s), and the concentrations of the following air pollutants: particulate matter of aerodynamic diameter less than 2.5 μm (PM2.5; $\mu g/m^3$), particulate matter of aerodynamic diameter less than 10 μm (PM10; $\mu g/m^3$), nitrogen dioxide (NO_2; $\mu g/m^3$), sulfur dioxide (SO_2; $\mu g/m^3$), ozone (O_3; $\mu g/m^3$), and carbon monoxide (CO; mg/m^3).

Collection of nasopharyngeal secretion samples

For each patient, a disposable aseptic sputum aspiration tube was inserted through the nasal cavity at least 6 cm below the pharynx to collect samples of nasopharyngeal secretions. The samples were diluted with 2 mL of sterile normal saline, mixed with oscillation, and then sent for testing. After centrifugation, the pellet was retained and suspended for later use. Qualified specimens were considered those with >25 white blood cells/low-power field and >10 squamous cells/low-power field, using a fluorescent microscope (Leica, Germany). All nasopharyngeal secretions were tested within 24 hours of hospital admission.

Detection of RSV subtype and other viruses

An RNA extraction kit was used (Beijing Tiangen Biochemical Technology Co., Ltd.) to extract nucleic acid from nasopharyngeal secretion samples. The extracted RNA was reverse-transcribed to DNA with reference to the reverse transcription reaction system for nucleic acids. The total reaction conditions were as follows: predenaturation at 65°C for 5 minutes, denaturation at 37°C for 60 minutes, and termination at 70°C for 15 minutes. Then, real-time reverse transcription polymerase chain reaction (RT-PCR) was used for nucleic acid amplification. The primers and probes (Shanghai Sangon Bioengineering Co., Ltd.) were as follows.

RSV-AF: 5-AGATCACTTCTGTCATCCAGCAA-3′,
RSV-AR: 5-TTCTGCACATCATAATTAGGAG-3′,
RSV-A probes: CACCATCCAACGGAGCACAGGAGAT,
RSV-BF: 5-AAGATGCAAATCATAAATTCACAGGA-3′,

RSV-BR: 5-TGATATCCAGCATCT TTAAGTA-3′,
RSV-B probes: TTTCCCTTCCTAACC TGGA-CATA.

The reaction conditions of PCR amplification were as follows: initial denaturation at 95°C for 5 minutes, 95°C for 15 s, and 55°C for 30 s for 40 cycles, followed by 37°C for 5 s. The subtype A gene amplification target fragment was 277 bp, and the subtype B gene target fragment was 863 bp. LightCyler 480 software was used for real-time data collection and subtype analysis (Roche, Basel, Switzerland). The positive determination criterion was an amplification curve with a typical S-type. The number of cycles when the fluorescence signal in the reaction tube reached a set threshold was ≤ 40.

Other common respiratory viruses were detected in nasopharyngeal aspirate specimens using RT-PCR; these included influenza virus A, influenza virus B, parainfluenza virus (PIV) I, PIV II, PIV III, and adenovirus, human metapneumovirus, rhinovirus, and human bocavirus.

Statistical analysis

We used IBM SPSS 22.0 software for statistical analysis of the data (IBM Corp., Armonk, NY, USA). Categorical data are expressed as n (%), and the chi-square test was used. Measurement data are expressed as mean ± standard deviation or median with interquartile range. The *t*-test was used when the data had a normal distribution, and the nonparametric test was used when the data had a non-normal distribution. Pearson correlation analysis was used to investigate correlations with climate and environmental factors that had a normal distribution; Spearman correlation analysis was used with a non-normal distribution. We analyzed the interaction of meteorological factors and the inclusion/exclusion of independent variables using multiple stepwise regression. A P value < 0.05 indicated statistical significance.

Results

Epidemiological characteristics of patients with RSV subtype infection

In this study, 1157 children (718 boys [62.06%] and 439 girls [37.94%]) hospitalized with RSV-induced LRTI at the Children's Hospital of Soochow University, Suzhou, China from January 2016 to December 2018 were included. The sex ratio was 1.64:1, and the average age was 5 (2–13) months. Among the 1157 children with RSV infection from January 2016 to December 2018, 699 had RSV-A, accounting for 60.41%; 458 had RSV-B, accounting for 39.59%. Among them, RSV-B was the dominant strain in 2016 (21.6% vs. 45.6%, $\chi^2 = 12.834$, $P = 0.01$). No statistically significant difference was found in the distribution of the two subtypes in 2017 (28.8% vs. 39.5%, $\chi^2 = 2.677$). In 2018, RSV-A was the dominant strain (49.6% vs 14.8% $\chi^2 = 27.920$, $P < 0.001$). Overall, the RSV-A subtype showed an upward trend, and the RSV-B subtype showed a downward trend. Both subtypes had a higher incidence in spring and winter (91.09%) and the difference in the distribution between RSV-A and RSV-B in spring, summer, autumn, and winter was not statistically significant ($\chi^2 = 1.657$, $\chi^2 = 0.205$, $\chi^2 = 0.082$, and $\chi^2 = 2.422$, respectively), as shown in Table 1.

The age group with the highest incidence of RSV-A and RSV-B infection was ≤ 3 years, particularly age ≤ 6 months. No statistically significant difference was observed in the distribution of RSV-A and RSV-B subtypes for each age group, as shown in Table 2.

A total of 353 children with LTRI and a definite RSV subtype classification from

Table 1. Comparison of the distribution of respiratory syncytial virus (RSV) subtypes RSV-A and RSV-B in children during different seasons.

	RSV-A (n = 699)	RSV-B (n = 458)	χ^2	*P* value
Spring, n (%)	265 (37.91)	214 (46.72)	1.657	0.252
Summer, n (%)	13 (1.86)	13 (2.84)	0.205	1.000
Autumn, n (%)	45 (6.44)	32 (6.99)	0.082	1.000
Winter, n (%)	376 (53.79)	199 (43.45)	2.422	0.157

Table 2. Comparison of the distribution of RSV-A and RSV-B infection among children of different ages.

Age	RSV-A (n = 699)	RSV-B (n = 458)	χ^2	*P* value
≤6 months, n (%)	379 (54.22)	277 (60.48)	0.734	0.475
≤1 year, n (%)	115 (16.45)	73 (15.94)	0.000	1.000
≤3 years, n (%)	181 (25.89)	96 (21.96)	0.439	0.620
≤5 years, n (%)	21 (3.00)	10 (2.18)	0.205	1.000
>5 years, n (%)	3 (0.43)	2 (0.44)	0.000	1.000

RSV, respiratory syncytial virus.

2016 to 2018 were randomly selected. Among them, 202 (57.22%) had RSV-A and 151 (42.78%) had RSV-B. We compared the demographic and clinical characteristics between the two subtypes. No statistically significant differences were observed in sex, age, and personal history, such as birth mode, term, eczema history, wheezing history, and allergy history among children with different RSV subtypes, as shown in Table 3.

Comparison of clinical manifestations

All children with confirmed RSV infection had clinical manifestations, such as cough, wheezing, and fever. No statistically significant difference was observed in clinical manifestations, prehospital course, and the duration of hospitalization between children with the two RSV subtypes (Table 3).

To further analyze the RSV subtypes and their correlation with environmental factors, we used Suzhou Bureau of Meteorology climate and environmental data for the Suzhou area during 2016 to 2018 (temperature, humidity, rainfall, sunshine, wind speed, PM2.5, PM10, and O_3, SO_2, NO_2, and CO concentrations). The monthly distribution of infections with the two RSV subtypes and climate and environmental factors are shown in Tables 4, 5, and 6.

The monthly RSV-A positive rate was negatively correlated with monthly mean air temperature, monthly mean wind speed, monthly total rainfall, and O_3 concentration ($r = -0.586$, $P < 0.001$; $r = -0.612$, $P = <0.001$; $r = -0.500$, $P = 0.002$; and $r = -0.644$, $P < 0.001$, respectively) and positively correlated with PM2.5, PM10, and concentrations of NO_2, SO_2, and CO ($r = 0.664$, $P < 0.001$; $r = 0.609$, $P < 0.001$; $r = 0.725$, $P < 0.001$; $r = 0.496$, $P = 0.002$; and $r = 0.632$, $P < 0.001$, respectively). However, monthly RSV-A positivity was not correlated with monthly relative humidity and sunshine hours.

We then used multiple stepwise regression analysis to examine the effect of interaction between various climate and environmental factors. The results suggested that monthly mean temperature (adjusted $R^2 = 0.551$, regression coefficient

Table 3. Comparison of general data and clinical manifestations in children infected with respiratory syncytial virus (RSV) subtypes RSV-A and RSV-B.

Characteristics	RSV-A (N = 202)	RSV-B (N = 151)	χ^2 /Z value	P value
Age (months), mean (range)	4 (2.00–11.25)	3 (2.00–7.00)	−1.684	0.092
Birth weight (kg), mean (range)	3.3 (2.89–3.65)	3.3 (2.95–3.65)	−0.496	0.62
Sex (male/female), n	129/73	104/47	0.968	0.364
Cesarean delivery, n	84	74	1.925	0.194
Full term, n	177	134	0.103	0.748
History of eczema, n	105	77	0.034	0.914
History of shortness of breath, n	27	23	0.273	0.645
History of allergy, n	28	19	0.122	0.754
Cough, n	200	147	0.615	0.655
Wheezing, n	148	102	1.134	0.291
Fever, n	51	32	0.79	0.447
Duration before admission (days)*, mean (range)	5.0 (4.0–8.0)	5.0 (4.0–7.0)	−0.457	0.648
Length of hospital stay (days), mean ± SD	8.24 ± 2.09	8.39 ± 2.79	−0.081	0.935

*Duration of clinical manifestations, such as cough, wheezing, fever, vomiting, and shortness of breath before hospitalization.
SD, standard deviation.

−0.927, $t = -0.358$, $P = 0.001$) and SO_2 concentration (adjusted $R^2 = 0.895$, regression coefficient 0.527, $t = 2.704$, $P = 0.011$) had a significant effect on the RSV-A positive detection rate. The positive RSV-B detection rate was negatively correlated with monthly mean temperature, total monthly rainfall, monthly sunshine duration, and O_3 concentration ($r = -0.573$, $P < 0.001$; $r = -0.366$, $P = 0.028$; $r = -0.402$, $P = 0.015$; and $r = -0.595$, $P < 0.001$, respectively) and positively correlated with the CO concentration ($r = 0.554$, $P < 0.001$), but not with monthly relative humidity, average wind speed, PM2.5, PM10, and NO_2 and SO_2 concentrations. We conducted multiple regression analysis to investigate the effect of interaction between various climatic factors on the results, which suggested that the monthly mean temperature (adjusted $R^2 = 0.572$, regression coefficient −1.051, $t = -1.457$, $P < 0.001$) and O_3 concentration (adjusted $R^2 = 0.505$, regression coefficient −0.659, $t = -2.683$, $P = 0.011$) had a significant effect on the positive detection rate of RSV-B. The results of regression analysis for the two RSV subtypes showed that monthly mean temperature had a significant influence on positive detection rates of the two RSV subtypes, as shown in Table 7.

Discussion

RSV infection is a common cause of LRTI in infants and young children.[5] It is also the leading cause of hospitalization for respiratory virus infections among children aged less than 5 years worldwide. [6,7] In this study, among children hospitalized with RSV infection from 2016 to 2018, a high proportion were aged under 1 year. The age group <6 months accounted for the highest proportion of patients hospitalized owing to RSV infection. With increasing age, the proportion of hospitalized children with RSV infection was significantly decreased, indicating that RSV infection occurred mostly in the age group <6

Table 4. Monthly distribution of different respiratory syncytial virus (RSV) subtypes and correlation with climatic factors from 2016 to 2018.

Year, month	Cases, N	RSV-A n (%)	RSV-B n (%)	Average temperature (°C)	Relative humidity (%)	Rainfall (mm)	Sunshine time (hours)	Average wind speed (m/s)
2016								
1	74	31 (41.9)	16 (21.6)	4.2	73	71.9	80.1	2.7
2	56	30 (53.6)	21 (37.5)	7.2	64	30	157.9	2.9
3	39	12 (30.8)	16 (41.0)	11.4	67	46.3	121.3	3
4	6	4 (66.7)	2 (33.3)	17.4	74	186.9	126.3	3
5	2	1 (50.0)	1 (50.0)	20.8	76	192.5	122.3	3
6	0	0 (0.0)	0 (0.0)	24.4	83	400.8	87	2.6
7	1	0 (0.0)	0 (0.0)	30.1	75	283.6	176.5	2.7
8	12	3 (25.0)	3 (25.0)	29.9	69	31.9	238.7	3
9	28	7 (25.0)	13 (46.4)	24.7	76	260	130.3	2.5
10	61	9 (14.8)	36 (59.0)	19.9	83	282.9	42.6	2.5
11	108	21 (19.4)	57 (52.8)	12.8	80	131.8	87	2.3
12	102	33 (32.3)	44 (43.1)	8.9	74	55.5	120.5	2.4
2017								
1	82	18 (22.0)	34 (41.4)	6.4	74	67.3	104.5	2.5
2	87	12 (13.8)	65 (74.7)	7	65	28.9	130.7	2.5
3	44	9 (20.5)	30 (68.2)	10.7	67	74.1	129.2	2.9
4	6	2 (33.3)	3 (50.0)	18	63	90.4	169.6	2.9
5	0	0 (0.0)	0 (0.0)	22.7	65	83	186.9	3.1
6	0	0 (0.0)	0 (0.0)	24.5	75	201.8	84.5	2.6
7	0	0 (0.0)	0 (0.0)	32.1	66	46.4	210.6	2.6
8	1	0 (0.0)	1 (100.0)	29.7	75	283.7	169.4	2.9
9	18	2 (11.1)	8 (44.4)	24.3	79	230.8	93.6	2.3
10	52	30 (57.7)	14 (26.9)	18.6	76	53.3	102.6	2.6
11	95	50 (52.6)	13 (13.7)	13.4	72	48.7	95.2	2.3
12	122	78 (63.9)	13 (9.8)	6.9	65	16.2	140.3	2.2
2018								
1	107	31 (29.0)	14 (13.1)	4.2	76	29.8	125.6	1.7
2	72	42 (58.3)	10 (13.9)	6.4	82	113.8	82.9	2.4
3	78	45 (57.7)	8 (10.3)	11.2	70	82.7	175.7	2.4
4	18	3 (16.7)	7 (38.9)	16	60	51.7	241.5	2.6
5	5	3 (60.0)	0 (0.0)	21.8	74	126.2	178.3	2.7
6	0	0 (0.0)	0 (0.0)	24.6	81	229.3	110.2	2.2
7	0	0 (0.0)	0 (0.0)	32.3	63	74.7	321.5	2.5
8	11	5 (45.5)	4 (36.4)	31.5	69	89	256.3	2.9
9	36	28 (77.8)	3 (8.3)	25.2	69	85.7	179.9	2.5
10	64	47 (73.4)	11 (17.2)	19.7	68	257.1	177.8	2.4
11	52	36 (69.2)	2 (3.8)	13.7	62	19.3	165	1.6
12	96	72 (75.0)	9 (9.4)	16.3	62	47.2	159	1.4

months. However, no statistically significant difference was found in the distribution of RSV-A and RSV-B infections among children in the five age groups. According to a report by Xiaoqing et al,[8] among 128 children with LRTI from 2006 to 2007 in Fuzhou, the largest proportion of RSV-positive patients was those aged < 1

Table 5. Monthly distribution of different RSV subtypes and correlation with environmental factors from 2016 to 2018.

Year, month	Cases, N	RSV-A n (%)	RSV-B n (%)	PM2.5 ($\mu g/m^3$)	PM10 ($\mu g/m^3$)	NO_2 ($\mu g/m^3$)	SO_2 ($\mu g/m^3$)	O_3 ($\mu g/m^3$)	CO (mg/m^3)
2016									
1	74	31 (41.9)	16 (21.6)	70.5	108	57	28	56	1.1
2	56	30 (53.6)	21 (37.5)	58.8	95	42	21	78	0.92
3	39	12 (30.8)	16 (41.0)	59.1	97	51	24	97	0.89
4	6	4 (66.7)	2 (33.3)	50.5	85	51	20	123	0.93
5	2	1 (50.0)	1 (50.0)	44.4	79	38	16	124	0.87
6	0	0 (0.0)	0 (0.0)	40	53	34	14	114	0.81
7	1	0 (0.0)	0 (0.0)	31.9	57	31	16	123	0.81
8	12	3 (25.0)	3 (25.0)	22.6	43	26	17	124	0.65
9	28	7 (25.0)	13 (46.4)	33.1	58	36	16	113	0.78
10	61	9 (14.8)	36 (59.0)	24.7	46	33	15	74	0.71
11	108	21 (19.4)	57 (52.8)	47.4	82	50	20	57	0.98
12	102	33 (32.3)	44 (43.1)	66.9	100	54	21	60	1.03
2017									
1	82	18 (22.0)	34 (41.4)	59	47	49	18	46	0.96
2	87	12 (13.8)	65 (74.7)	63	39	47	17	28	0.92
3	44	9 (20.5)	30 (68.2)	52	31	48	16	80	0.85
4	6	2 (33.3)	3 (50.0)	48	76	51	16	94	0.91
5	0	0 (0.0)	0 (0.0)	33	74	38	13	102	0.7
6	0	0 (0.0)	0 (0.0)	30	47	37	9	87	0.68
7	0	0 (0.0)	0 (0.0)	13	49	33	10	86	0.62
8	1	0 (0.0)	1 (100.0)	25	40	29	9	87	0.74
9	18	2 (11.1)	8 (44.4)	28	43	43	10	66	0.78
10	52	30 (57.7)	14 (26.9)	28	44	41	13	62	0.76
11	95	50 (52.6)	13 (13.7)	47	76	65	16	40	0.97
12	122	78 (63.9)	13 (9.8)	70	96	74	19	29	1.13
2018									
1	107	31 (29.0)	14 (13.1)	94	124	58	43	45	1.3
2	72	42 (58.3)	10 (13.9)	56	72	34	22	66	0.7
3	78	45 (57.7)	8 (10.3)	61	98	52	35	89	0.8
4	18	3 (16.7)	7 (38.9)	56	92	44	40	134	0.7
5	5	3 (60.0)	0 (0.0)	48	73	34	29	112	0.7
6	0	0 (0.0)	0 (0.0)	48	72	34	25	103	0.7
7	0	0 (0.0)	0 (0.0)	33	64	32	30	134	0.6
8	11	5 (45.5)	4 (36.4)	32	61	28	28	129	0.7
9	36	28 (77.8)	3 (8.3)	36	61	34	28	104	0.8
10	64	47 (73.4)	11 (17.2)	46	74	40	27	95	0.8
11	52	36 (69.2)	2 (3.8)	84	138	60	47	68	1
12	96	72 (75.0)	9 (9.4)	134	174	69	64	48	1.4

RSV, respiratory syncytial virus; PM2.5, particulate matter of aerodynamic diameter less than 2.5 μm; PM10, particulate matter of aerodynamic diameter less than 10 μm; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; O_3, ozone; CO, carbon monoxide.

Table 6. Average values of various meteorological factors from 2016 to 2018.

Climate and environmental factors	Mean ± standard deviation
Monthly average temperature (°C)	18.03 ± 8.68
Monthly relative humidity (%)	71.44 ± 6.51
Total monthly rainfall (mm)	122.37 ± 98.67
Monthly sunshine duration (hours)	146.70 ± 57.74
Monthly mean wind speed (m/s)	2.54 ± 0.39
PM2.5 ($\mu g/m^3$)	49.28 ± 22.98
PM10 ($\mu g/m^3$)	74.11 ± 30.40
NO_2 ($\mu g/m^3$)	43.81 ± 12.02
SO_2 ($\mu g/m^3$)	22.56 ± 11.68
O^3 ($\mu g/m^3$)	85.47 ± 30.81
CO (mg/m^3)	0.85 ± 0.18

PM2.5, particulate matter of aerodynamic diameter less than 2.5 μm; PM10, particulate matter of aerodynamic diameter less than 10 μm; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; O_3, ozone; CO, carbon monoxide.

Table 7. Correlation between different RSV subtypes and climate and environmental factors from 2016 to 2018.

	RSV-A		RSV-B	
Climate and environmental factors	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Monthly average temperature (°C)	−0.586	<0.001	−0.573	<0.001
Monthly relative humidity (%)	−0.207	0.225	0.095	0.580
Total monthly rainfall (mm)	−0.500	0.002	−0.366	0.028
Monthly sunshine duration (hours)	−0.181	0.290	−0.402	0.015
Monthly mean wind speed (m/s)	−0.612	<0.001	−0.119	0.490
PM2.5 ($\mu g/m^3$)	0.664	<0.001	0.210	0.219
PM10 ($\mu g/m^3$)	0.609	<0.001	−0.076	0.661
NO_2 ($\mu g/m^3$)	0.725	<0.001	0.289	0.087
SO_2 ($\mu g/m^3$)	0.496	0.002	0.117	0.496
O_3 ($\mu g/m^3$)	−0.644	<0.001	−0.595	<0.001
CO (mg/m^3)	0.632	<0.001	0.554	<0.001

RSV, respiratory syncytial virus; PM2.5, particulate matter of aerodynamic diameter less than 2.5 μm; PM10, particulate matter of aerodynamic diameter less than 10 μm; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; O_3, ozone; CO, carbon monoxide.

year (35%). In that study, no significant difference was found in the distribution of different subtypes, with the RSV-A subtype as the dominant strain. Ping et al. reported no differences in the distribution of the two RSV subtypes according to sex and age among 500 children from the Chongqing Three Gorges Central Hospital between 2014 and 2015.[9] RSV infections occur more frequently in younger age groups because infants and young children, especially premature infants, are in a continuous stage of development of all organ systems, the speed of which is slow and differs for each system. Infants and young children have relatively few antibodies against RSV

G and F glycoproteins, such as immunoglobulin G (IgM), IgG, and secretory sIgA. Fetal immunity is mainly derived from maternal IgG transmitted via the placenta, which reacts with RSV antigen and exerts the role of immune regulation by recruiting and activating various inflammatory cells. However, IgG antibodies can decline to a minimum within 3 to 5 months after birth, resulting in low immune defense among children of this age, which greatly increases the possibility of infection with various pathogenic agents. A recent epidemiological investigation showed that the regional prevalence of RSV alternated between the two subtypes, and one subtype was dominant during each epidemic season.[10] Jian et al. summarized the prevalence of RSV subtypes in Suzhou from 2006 to 2012. The results showed that November to March of the following year (winter and spring seasons) was the period with the highest incidence of RSV infection. During the high-incidence season, subtype A was dominant in all years, accounting for 95.98% of infections in 2011 to 2012, except for 2009 to 2010 when subtype B accounted for 87.50% of infections.[11] Our study showed that the RSV epidemic in the Suzhou area from 2016 to 2018 was characterized by the two subtypes alternating with each other; the RSV-A subtype was dominant in 2018. In an investigation of the prevalence of RSV infection during five epidemic seasons from 1998 to 2003 in Yokohama, Japan, Saikusa et al. reported that, except in 2000/2001 and 2002/2003, the B subtype was the dominant strain; the A subtype predominated in the remaining years.[12] Schnabel et al. conducted epidemiological studies in New York over a period of 15 years and found that, apart from 4 years in which both subtypes were prevalent and 2 years in which subtype B was dominant, subtype A predominated in the remaining 9 years. This indicates that both RSV subtypes were prevalent in this region, with RSV-A the dominant subtype.[13] All these findings are consistent with the results of our study. However, research has also been conducted of RSV-B epidemics and cross-circulation of the two subtypes in China and abroad. The results of Zhou's study indicated that subtype B was the dominant strain in RSV infections among children in Chongqing from 2014 to 2015.[9] The RSV-B subtype has been reported in Uruguay, Denmark, and Turkey.[14–16] Hibino et al. also reported that 744 samples from RSV-positive children were collected from 2012 to 2015, among which 400 (53.7%) were the RSV-A subtype and 172 (23.1%) were the RSV-B subtype; the two subtypes were alternately dominant during the following seasons.[17] Muelenaer et al. reported that the antibody response to RSV-A after infection was more cross-reactive than the response to RSV-B.[18] Previous studies have shown that RSV-A homologous reinfection is more frequent, in comparison with RSV-B, which is owing to the higher degree of differentiation between the genotype of RSV-A and the specificity of RSV-B-induced subpopulations; the immune response is more complete and durable than that of RSV-A.[19–22] Some scholars believe that overall, the RSV-A subtype is an epidemic strain, which might be related to the pathogenicity of the two subtypes. Subtype B often presents as a subclinical condition or asymptomatic infection whereas subtype A is more pathogenic than subtype B. However, scholars also believe that many genetic variations exist within the genotype of RSV-A, especially in relation to the role of amino acid substitution in the F and G glycoproteins, which leads to the A subtype becoming an epidemic strain in most regions. More detailed typing of the RSV subtype depends on the G-protein gene, that is, on the highly variable C-terminal region of the nucleotide sequence of the G-protein gene.[23] The G protein of the

two subtypes induces different protective antibodies and there is no cross-protection among these antibodies; the amino acid sequences of different genotypes of the same subtype are also different, resulting in different pathogenicities that induce different immune defense mechanisms. Therefore, the same RSV subtype can appear repeatedly or even become epidemic.

The relationship between the RSV subtype and severity of clinical illness has been a focus of research for some time, and viewpoints differ regarding variation in the severity of disease by subtype in different regions. Our study findings suggested that there were no significant differences in the clinical manifestations and severity of disease between the two subtypes in Suzhou. However, these results may be biased owing to the small sample size and lack of information on children with severe pneumonia, which are limitations of this study. Reports from cohort studies on the association between RSV subtypes and clinical severity have also been related to the age range of children. Older children might experience a second or third recurrent RSV infection rather than a first primary infection; older children are also less likely to develop more severe clinical illness.[7,24] Changes in the coding proteins of the virus itself (except the G and F proteins) might also have an impact on pathogenicity and severity, but their importance is still under investigation. Further studies are needed to determine whether changes in the amino acid sequences of RSV proteins contribute to changes in the antigenic characteristics of the virus, leading to different degrees of disease severity.

Analysis of the correlation between different RSV subtypes with climate and environmental factors showed that the detection rates of different RSV subtypes were correlated with temperature, wind speed, rainfall, and sunshine duration, to some extent. In particular, temperature had a significant influence on the positive detection rates of RSV. This was consistent with the results of previous studies.[25–27] Suzhou is located in the transitional area between subtropical and warm temperate zones. Both of these zones have southern and northern climate characteristics, that is, large differences in temperature between the four seasons. In summer, the high temperatures, dry climate, and long sunshine duration may inactivate RSV or make virus-laden aerosols less likely to spread.[26] Ultimately, the virus can easily colonize the respiratory mucosa and reproduce. However, low temperatures can cause vasoconstriction in the nasal mucosa, which is more conducive to the discharge of airway secretions. We found that relative humidity had no correlation with RSV subtype, which was different from results reported by Jiansheng et al. in Wuanzhou, Fujian Province, China.[28] This might be because Fujian is located in the subtropical marine monsoon climate zone whereas Suzhou is in the East Asian monsoon climate zone, with little change in annual relative humidity ($71.44 \pm 6.51\%$).

The analysis of the relationship of different subtypes with climatic and environmental factors indicated that the RSV-A positive detection rate was positively correlated with PM2.5, PM10, and NO_2, SO_2, and CO concentrations in the air, but negatively correlated with the O_3 concentration. The positive RSV-B detection rate was more negatively correlated with the O_3 concentration but had no significant correlation with other air pollutants. In particular, O_3 and SO_2 concentrations had a significant impact on the RSV detection rate. It is speculated that this might be owing to the protective effect of O_3 in the atmosphere, which absorbs ultraviolet light and converts it into heat energy to maintain the stratospheric temperature. High temperatures are not conducive to RSV survival. Additionally, as a strong oxidant, O_3

might react directly with RSV and other viruses, destroying the genetic material of the virus and various enzymes, thereby preventing virus replication. After long-term exposure, SO_2 causes strong irritation of the respiratory mucosa, mucosal congestion, edema, and inflammatory exudation. This results in increased airway secretion discharge and destruction of the mucosal structure, which is conducive to the adhesion and replication of RSV. PM2.5 and PM10 are fine particulate matter in the air that can be inhaled and deposited in the lungs. After long-term exposure to PM2.5 and PM10, the integrity of the mucosal surface in the respiratory tract is destroyed, leading to increased probability of a respiratory inflammatory response. The deposition of air pollutants mainly causes damage to the host's immune system, interfering with the immune system's ability to fight pathogens. Shi et al.[29] studied pathological changes and inflammatory responses in the lung tissue of rats with airway exposure to PM2.5. The trachea and alveoli of the mice showed a large amount of inflammatory cell infiltration, accompanied by considerable thickening of the alveolar wall. Levels of immune proteins in bronchoalveolar lavage fluid (BALF), including monocyte chemoattractant protein-1 and macrophage inflammatory protein-1, as well as lactate dehydrogenase (LDH) activity, were also significantly increased. Increased levels of total protein and LDH activity in BALF revealed increased cytotoxicity and lung permeability in alveolar epithelial cells. Those authors also reported that PM2.5 induced DNA methylation, which affects a series of cellular biological processes and aggravates the pathophysiological process of airway inflammation by mediating cellular signal transduction.[29]

Vaccine research can be said to be at its most active at present. Vaccines currently being developed can be roughly divided into two categories: non-replicating vaccines (live-attenuated vaccines and DNA vaccines) and replicating vaccines (formalin-inactivated vaccines and subunit vaccines).[30] Clinical applications have shown that after vaccination with live-attenuated vaccines, infants aged 1 to 2 months mainly produce serum and nasal antibodies, limited to IgA, which mainly interact with G protein; however, the neutralizing activity does not increase. Among infants older than 6 months, these vaccines have a wide range of immunogenicity. Live-attenuated vaccines do not cause fever or lower respiratory tract diseases; however, the virus must be properly diluted and attenuated. Additionally, the genetic stability of these vaccines needs to be further improved.[31] Nevertheless, these studies provide important evidence for the feasibility of immunizing infants and young children with live virus vaccines.

One limitation of this study is that climate data prior to hospital admission for RSV were not analyzed using statistical modeling, so only associations could be discerned. Additionally, sample size calculation was not performed; the limited sample size may affect the statistical significance of the results.

Conclusion

The clinical epidemiological characteristics of RSV subtypes in Suzhou have rarely been reported. In this study, we obtained the clinical data of children with RSV infection and explored differences between the two RSV subtypes in terms of epidemiological characteristics, clinical manifestations, and disease severity. Our findings can provide a clinical basis for prevention and control of RSV in the Suzhou region. The main treatment after RSV infection is symptomatic support management. A few drugs are currently used to prevent or treat RSV infections. Many drugs are still in clinical trials, and no safe and effective vaccine is

available for clinical use. Hence, controlling RSV remains a formidable challenge. Investigation of the etiological characteristics, immune mechanisms, epidemiological characteristics, and correlation with climatic and environmental factors of RSV subtypes can provide a scientific basis for the early prevention and control of RSV infection in children.

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The authors declare that there is no conflict of interest.

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References

1. Wertz GW and Moudy RM. Antigenic and genetic variation in human respiratory syncytial virus. *Pediatr Infect Dis J*. 2004; 23 (1 Suppl): S19–S24.
2. Biowas D, Ysdav K, Borkakoty B, et al. Molecular characterization of human respiratory syncytial virus NA1 ang GA5 genotypes detected in Assam in northeast India, 2009–2012. *J Med Virol*. 2013; 85: 1639–1644.
3. Zaifang J, Kunling S and Ying S. *Zhu Futang's Practical Pediatrics [M]*. Beijing: People's Medical Publishing House, 2015: 1251–1288.
4. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med*. 2007; 147: 573–577.
5. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. *Lancet* 2013; 381: 1405–1416.
6. Mazur NI, Martinón-Torres F, Baraldi E, et al. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *Lancet Respir Med*. 2015; 3: 888–900.
7. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; 375: 1545–1555.
8. Xiaoqing W, Yanan W, Xiuya Z, et al. Detection and subtype analysis of respiratory syncytial virus in children with acute respiratory infection in Fuzhou area. *Journal of Practical Medicine* 2008; 24: 50–52.
9. Ping Z, Xian Q, Zhongyou T, et al. Epidemiological characteristics and clinical manifestations of respiratory syncytial virus subtypes A and B in children. *Chinese Medicine* 2016; 13: 115–118.
10. Mufson MA, Belshe RB, Orvell C, et al. Respiratory syncytial virus epidemics: Variable dominance of subgroups A and B among children, 1981-1986. *J Infect Dis*. 1988; 157: 143–148.
11. Jian J, Xuejun S, Xuelan Z, et al. Detection and analysis of respiratory syncytial virus subtypes in nasopharyngeal secretions of children with acute respiratory infections. *Journal of Clinical Pediatrics* 2014; 32: 375–378.
12. Saikusa M, Kawakami C, Noguchi Y, et al. An epidemiological study of respiratory syncytial virus infection in Yokohama, Japan for five years. *Kansenshogaku Zasshi*. 2005; 79: 381–387.
13. Hall CB, Walsh EE, Schnabel KC, et al. Occurrence of groups A and B of respiratory syncytial virus over 15 years: Associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. *J Infect Dis*. 1990; 162: 1283–1290.
14. Arbiza J, Delfraro A, Frabasile S. Molecular epidemiology of human respiratory syncytial virus in Uruguay: 1985-2001– a review. *Men Inst Oswaldo Cruz*. 2005; 100: 221–230.

15. Johansen J, Christensen LS, Hornsleth A, et al. Restriction pattern variability of respiratory syncytial virus during three consecutive epidemics in Denmark. *APMIS*. 1997; 105: 303–308.
16. Guney C, Kubar A, Yapar M, et al. An outbreak of respiratory infection due to respiratory syncytial virus subgroup B in Ankara, Turkey. *Jpn J Infect Dis*. 2004; 57: 178–180.
17. Hibino A, Saito R, Taniguchi K, et al. Molecular epidemiology of human respiratory syncytial virus among children in Japan during three seasons and hospitalization risk of genotype ON1. *Plos One*, 2018; 13: e0192085.
18. Muelenaer PM, Henderson FW, Hemming VG, et al. Group specific serum antibody responses in children with primary and recurrent respiratory syncytial virus infections. *J Infect Dis*. 1991; 164: 15–21.
19. Parveen S, Broor S, Kapoor SK, et al. Genetic diversity among respiratory syncytial viruses that have caused repeated infections in children from rural India. *J Med Virol*. 2006; 78: 659–665.
20. Scott PD, Ochola R, Sande C, et al. Comparison of strain-specific antibody responses during primary and secondary infections with respiratory syncytial virus. *Med Virol*. 2007; 79: 1943–1950.
21. Zlateva KT, Vijgen L, Dekeersmaeker N, et al. Subgroup prevalence and genotype circulation patterns of human respiratory syncytial virus in Belgium during ten successive epidemic seasons. *J Clin Microbiol*. 2007; 45: 3022–3030.
22. Yamaguchi M, Sano Y, Dapat IC, et al. High frequency of repeated infections due to emerging genotypes of human respiratory syncytial viruses among children during eight successive epidemic seasons in Japan. *J Clin Microbiol*. 2011; 49: 1034–1040.
23. Panayiotou C, Richter J, Koliou M, et al. Epidemiology of respiratory syncytial virus in children in Cyprus during three consecutive winter seasons (2010-2013): age distribution, seasonality and association between prevalent genotypes and disease severity. *Epidemiol Infect*. 2014; 142: 2406–2411.
24. Iwane MK, Farnon EC and Gerber SI. Importance of global surveillance for respiratory syncytial virus. *J Pediatr Infect Dis*. 2013; 208: 165–166.
25. Wei J, Zhengrong C, Hongbo G, et al. Epidemic characteristics of respiratory virus and its correlation with climatic factors in children in Suzhou Children's Hospital. *Chinese Journal of Preventive Medicine* 2011; 45: 205–210.
26. Shenghui T and Yuqing W. Research on the relationship between respiratory syncytial virus infection and climatic factors in children. *Journal of Pediatric Pharmacy* 2013; 19: 1–3.
27. Fengguo W, Xuelan Z, Xuejun S, et al. Epidemic characteristics of respiratory syncytial virus in hospitalized children in Suzhou area. *Journal of Clinical Pediatrics* 2013; 3: 723–725.
28. Jiansheng L, Zaoxin R, Zongming S, et al. Epidemic characteristics of eight respiratory pathogens and their relationship with meteorological factors in Quanzhou region in 2013. *Journal of Clinical Laboratory Medicine (electronic edition)*, 2015; 4: 897–903.
29. Shi Y, Zhao T, Yang X, et al. PM2.5-induced alteration of DNA methylation and RNA-transcription are associated with inflammatory response and lung injury. *Sci Total Environ*. 2019; 650 (Pt 1): 908–992.
30. Su X, Chen G. Prevention of respiratory syncytial virus. *Chemistry of Life*. 2006; 26: 2551–2553.
31. Wright PF, Karron RA, Belshe RB, et al. Evaluation of a live, cold-passaged, temperature sensitive respiratory syncytial virus vaccine candidate in infancy. *Infect Dis*. 2000; 182: 1331–1342. DOI:10.1086/315859.