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Original Article

Epidemiological characteristics of mycoplasma pneumoniae respiratory tract infection in hospitalized children from 2006 to 2013

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Abstract: Mycoplasma pneumoniae (MP) has been reported to be responsible for 10~30% of all cases of community-acquired pneumonia (CAP) among children and shows an even higher proportion during epidemics. Currently, little is known about the association between the prevalence of MP RTIs and climate in China. Nasopharyngeal aspirates obtained from children with acute respiratory tract infections during Jan 2006 to Dec 2013 were tested for MP by real-time fluorescent PCR. The relationship between the detection of MP and metrorological factors was analyzed by linear regression and stepwise regression analysis. The average positive rate of MP was 28.1% (25.1%, 32.6%, 39%, 41%, 19.1%, 7.5%, 27.6%, and 31.3% per year, respectively) during 2006 to 2013. The positive rates of MP infection were 25.3% in spring, 33.7% in summer, 32.8% in autumn and 20.8% in winter. The positive rate of MP showed a moderate correlation with average monthly temperature ($r = 0.3388$, $P = 0.000$) and a low correlation with average monthly sunshine ($r = 0.241$, $P = 0.018$) and rainfall ($r = 0.207$, $P = 0.043$). MP was the common pathogen in respiratory tract infection of hospitalized children in Suzhou. Epidemic outbreaks of MP infection occurred lasting for 4 years and at intervals of 2 years from 2006 to 2013. The prevalence of MP infection was higher during summer and autumn and showed a correlation with average temperature.

Keywords: Respiratory tract infection, mycoplasma pneumoniae, children

Introduction

Mycoplasma is the smallest free-living, self-replicating microorganism, and is a frequent cause of respiratory tract infection in children. Mycoplasma pneumoniae infections can involve both the upper and lower respiratory tract and occur both endemically and epidemically worldwide in persons of all ages. Mycoplasma pneumoniae has been reported to be responsible for 10~30% of all cases of community-acquired pneumonia (CAP) among children and shows an even higher proportion during epidemics [1-3]. MP infection shows a variety of clinical manifestations, ranging from asymptomatic infection to fatal pneumonia or extrapulmonary diseases [4-7]. An estimated 30% or more of MP infections in children aged 5~15 years result in pneumonia, and as many as 18% of these cases require hospitalization [8, 9].

M. pneumoniae infections can occur worldwide, with outbreaks occurring cyclically every 3~5 years, lasting several months to years [10, 11]. In Poland, Kałużewski et al reproted that MP epidemically peak in autumn and winter by seven consecutive years of observation from 2008~2013 [12]. In Korea, 3 to 4-year cycles of MP pneumonia have been observed from the mid-1980s to 2012, peak mainly in the fall or winter seasons, epidemics last approximately 12 to 18 months [13, 14]. However, the result of an epidemiological longitudinal study in Japan from 1979 to 1999 suggested that the survival and spread of M. pneumoniae were highly favored during the spring and early autumn seasons and there might be a positive relationship between M. pneumoniae infections and temperature [15]. Onozuka et al reported that cases of M. pneumoniae pneumonia increased significantly with increased average temperature and relative humidity in

Fukuoka, Japan [16]. du Prel et al reported that Influenza A, RSV, and adenovirus were correlated with temperature and rhinovirus to relative humidity, however, M. pneumoniae was no correlated with meteorological parameters [17]. Therefore, it is assumed that the different epidemic characteristics of M. Pneumoniae are attributed to different regional climates, even though the infections occur during the same season. Currently, little is known about the association between the prevalence of M. pneumoniae RTIs and climate in China. This study used PCR techniques to detect M. pneumoniae and monitored the temperature, humidity, total rainfall, total sunshine and wind velocity in Suzhou, China to determine the prevalence of MP and the relationship between M. pneumoniae infections and climate.

Materials and methods

Patients

This retrospective study was conducted from January 2006 to December 2013 in pediatric patients at the Department of Respiratory Disease of the Affiliated Children's Hospital, Soochow University. The diagnosis of acute respiratory tract infections (ARITs) was based on the following criteria [18]. Upper respiratory tract infection was diagnosed if the patient presented with nasal obstruction, nasal discharge, fever, or sore throat. Lower respiratory tract infection was diagnosed when fever, cough, wheeze, tachypnea, chest retractions, Physical examination revealed abnormal auscultatory findings. Patients with congenital heart disease, immune deficiency, bronchus, or pulmonary dysplasia were excluded in this study. The study was approved by the Medical Ethics Committee of Soochow University (No. Sdfey-201508). The parents of all study participants gave written informed consent before study enrollment. The sample collection procedure and processing the sample is well explained.

Sputum specimen collection

Nasopharyngeal secretions were collected from each study participant within 24 h after admission. The sample collection procedure and processing the sample is well explained. Briefly, an aseptic plastic sputum catheter was inserted into the nostril to a depth of about 7~8 cm until reaching the pharynx. Approximately 2 ml of nasopharyngeal secretions was collected by applying negative pressure. The sample was mixed with 4~8 ml PBS, and centrifuged for 10 minutes at 300~500 rpm. The supernatant was discarded and the pellet was mixed with 4~8 ml PBS and centrifuged for an additional 10 minutes. The pellet was stored at -80°C until testing began.

Sputum MP-DNA detection and evaluation

DNA lysate (Shanghai Shenyou biotechnology company, Shanghai, China) was added to the sputum pellet following washing with PBS. The sample was heated to at 95°C for 10 min, centrifuged for 5 min at 12000 rpm, and then the supernatant was collected. After extracting the DNA from the sputum specimen, MP DNA was detected by fluorescent real-time PCR (BIO-RAD iCycler, USA). The cyclic temperature settings were 93°C, 2 min; 93°C, 45 s; 55°C, 60 s → 10 cycles; 93°C 30 s → 55°C, 45 s → 30 cycles. The fluorescence collection point was set at the 55°C, 45 s. Ct value was used to quantify the fluorescence quantitative PCR results. The primer sequences and MP probe are: Forward 5'-CCA ACCAAA CAA CAA CGT TCA-3', Reverse 5'ACC TTG ACTGGA GGC CGT TA-3', Probe 5'-FAM-TCA ACT CGA ATA 'ACG GTG ACTTCT TAC CAC TG-3'-TAMRA. The probe binding sequence was located between the upstream and downstream primer. The fluorescent reporter dye at the 5' end of probe was 6-carboxyfluorescein (FAM), and the quencher at the 3' end of the probe was 6-carboxytetramethylrhodamine (TAMRA). The primers and probe were purchased from Guangzhou Daan Gene Ltd. (Guangzhou, China). An MP-negative sample was defined as having an amplification curve that was not S-shaped or a Ct value = 30. Both results indicated that the MP DNA content was below the detection limit. A positive MP sample was defined as having an amplification curve was S-shaped and a Ct value < 30. The DNA content of the sputum was determined by the following criteria. If the sample $C < 5.00 \times 10^2$, the DNA content was $< 2.5 \times 10^3$ gene copies/ml; if $5.00 \times 10^2 \leq C \leq 5.00 \times 10^8$, the DNA content = 5×10^3 gene copies/ml; and if $C > 5.00 \times 10^8$, the DNA content was $> 5 \times 10^3$ gene copies/ml.

Direct immunofluorescence assay to detect seven common viruses

Direct immunofluorescence was used to detect syncytial virus infection (RSV), influenza virus A

(IVA), influenza virus B (IVB), parainfluenza virus (PIV) I, PIV II, PIV III, and adenovirus (ADV). All assay kits were purchased from Chemicon (USA) Immunostained preparations were viewed with a fluorescence microscope (Leica 020-518.500, Germany).

Real-time PCR to detect the human metapneumovirus (hMPV) gene

RNA was extracted from sputum specimens using Trizol (Invitrogen, USA). cDNA was synthesized by reverse transcription. The cyclic temperature settings were 94°C, 30 s; 55°C, 30 s; 68°C, 30 s; amplified by 45 cycles with the last at 68°C for 7 min. hMPV was assayed by fluorescent real-time PCR (BIO-RAD icycle). The cyclic temperature settings were 94°C, 30 s; 56°C, 30 s; 72°C, 30 s; amplified, 40 cycles. The primer sequences for hMPV are: Forward 5'-AACCGTGTACTAAGTGATGCACTC-3', Reverse 5'-CATTGTTTGACCGGCCCCATAA-3'.

Real-time PCR to detect the human bocavirus (hBoV) gene

Sputum DNA was extracted as described above, and hBoV-DNA was detected by real-time fluorescent PCR. The cyclic temperature settings were 94°C, 30 s; 56°C, 30 s; 72°C, 30 s; amplified by 40 cycles. The primer sequences and hBoV probe are: Forward 5'-TGACATTCAACTACCAACAACCTG-3', Reverse 5'-CAGATCCTTTTCCTCCTCCAATAC-3'.

Real-time PCR to detect the human rhinovirus (hRV) gene

The primer sequences and hRV probe are: sense, 5'-TGG ACA GGG TGT GAA GAG C-3'; antisense, 5'-CAA AGT AGT CGG TCC CAT CC-3', hRV PROBE FAM-TCC TCC GGC CCC TGA ATG-TAMRA. RT-PCR of hRV RNA. Viral RNA was extracted with Trizol reagent (Invitrogen). PCR assays were performed in an automatic PCR cycler (Perkin Elmer, USA) under the following conditions: 40 cycles of denaturation at 95°C for 5 min, annealing at 95°C for 15 s and extension at 60°C for 30 s.

Bacteria culture

Sample were cultured in trypticase soy broth containing 5 μg/ml gentamicin, on enriched chocolate agar plates, and on selective sheep blood agar plates containing 5 μg/ml gentamicin. Isolates were identified as S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus and so on were identified according to the laboratory's standard operating procedure.

Meteorological data collection

Meteorological data including average monthly temperature (°C), average monthly humidity (%), total rainfall (mm) and total bright sunshine (h) were provided by the Meteorological Bureau of Suzhou. The weather observing station is located atlongitude 120°.6' east, latitude 31°.3' north.

Statistical analysis

Statistical analysis was performed using SPSS version 10.0 software (SPSS, Inc., Chicago, IL, USA). The enumeration data were compared using the chi-square test, and a normality and homogeneity of variance test was performed on the measurement data. Normally distributed data were compared by the Pearson correlation analysis, and other data were compared by the Spearman non-parametric tests. Univariate regression analysis with the Pearson correlation coefficient was used to analyze the relation between MP incidence and meteorological factors, whereas independent associations were analyzed by multiple linear regression using the forward stepwise method. All tests were two tailed and *p*-values less than 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 15098 patients aged 1 month to 14 years with ARITs were studied. There were 9349 (61.9%) male and 5749 (38.1%) female patients. 4884 (36.6%) were younger than 6 months of age, 2900 (34.7%) were 6 months~ 12 months of age, 3798 (28.6%) were 13~36 months of age, 1958 were 37~60 months of age. 1558 were > 60 months of age. The youngest patient was 1 month old, and the oldest patient was 14 years of age.

Pathogen detected in clinical specimens

Pathogens were identified in 8727 of 15098 specimens (57.8%). Among 15098 clinical specimens the most commonly detected patho-

gen was: MP 28.1% followed by RSV 15.1%, Streptococcus pneumoniae 11.2%, hBoV6.7%, Haemophilus influenzae 4.6%, hMPV 4.3%, Staphylococcus aureus 3.7%, Moraxella catarrhalis 3.4%, Pinf III-3%, Inf-A1.9%, Pseudomonas aeruginosa 1.5%, Escherichia coli 1.4%, Klebsiella pneumoniae 1.3%, ADV 1.2%, Haemophilus parainfluenzae 0.5%, Acinetobacter baumannii 0.4%, Pinf-I 0.4%, Inf-B 0.4%, Stenotrophomonas maltophilia 0.3%, Enterobacter cloacae 0.3%, Serratia marcescens 0.1% and Pinf-II 0.1%.

Of the 4246 MP positive patients, 1142 were co-infected with other respiratory pathogens of which Streptococcus pneumoniae was the most common at 22.3% (255/1142) followed by RSV 19.7% (225/1142), hBoV 18.5% (212/1142), Pinf-III 14.3% (164/1142), hRV 11.1% (123/1142), Haemophilus influenzae 7.8% (89/1142), hMPV 7% (80/1142), Inf A 3.7% (42/1142), Escherichia coli 2.6% (30/1142), Inf B 2.5% (29/1142) and ADV 1.4% (16/1142).

Gender and age distribution of MP infected children

The MP infection incidence in male and female patients was 24.7% (2309/9349) and 33.7% (1937/5749), respectively. The positive rate in female was higher than in male patients (χ^2 = 142.49, P < 0.01). The positive rates of the age groups of < 6 months, 7~12 months, 13~36 months, 37~60 months, and > 60 months were 10.2%, 21.6%, 34.8%, 45.1% and 59.9%, respectively. The positive rates increased with age, with a statistical significance for age distribution. The MP infection rate was highest among the > 60 months group (59.9%) and lowest among the < 6 months group (10.2%). This observed difference in MP prevalence by age was statistically significant (χ^2 = 1990.49, P < 0.01).

Annual and seasonal distribution of hMPV-infection

MP infection was observed in each year of the 8-year period from January 2006 to December 2013. The annual MP-positive rates were 25.1% (487/1938) in 2006, 32.6% (671/2057) in 2007, 39% (790/2023) in 2008, and 41% (842/2052) in 2009, 19.1% (319/1667) in 2010, 7.5% (151/2001) in 2011, 27.6% (486/1762) in 2012, 31.3% (500/1598) in 2013, and there was a statistically significant difference between the eight years, (χ^2 = 811.8, P < 0.01). The detection rates in 2008 and 2009 were higher than the other six years while the rates in 2012 and 2013 were the lowest. The positive rates increased with years, with a statistical significance for year distribution from 2006 to 2009, the positive rates in 2009 were the highest. However, they deceased significantly in 2010 and 2011 and increased in 2012 and 2013 with a lowest positive rate in 2011 during the 8 years.

The positive rates of MP infection were 25.3% in spring (March-May), 33.7% in summer (June-August), 32.8% in autumn (September-November) and 20.8% in winter (December-February), and the incidence rates of MP infection was significantly higher during summer and autumn than those in spring and winter (χ^2 = 212.61, P < 0.01). However, variations in this trend were observed from one year to another. The incidence rates of MP infection was significantly higher during autumn in 2006, spring, summer and autumn in 2007, summer and autumn in 2008, 2009, 2012, and 2013, summer in 2010, autumn and winter in 2011.

MP was detected throughout the year with an epidemic peaks observed each year between July and September. The lowest MP-positive rates were January to February and November to December each year. The highest rates occurred in November 2006 (39.2%), April 2007 (41.1%), August 2008 (50.6%), June 2009 (55.4%), July 2010 (38.9%), November 2011 (20.1%), October 2012 (48.6%) and July 2013 (48.2%), The lowest MP-positive rates were Feburary 2006 (10%), Feburary 2007 (21.6%), Feburary 2008 (21.2%), Feburary 2009 (23.3%), December 2010 (9.2%), January 2011 (2.3%), March 2012 (13.7%) and March 2013 (24%).

MP-infection and correlation with meteorological factors

The Suzhou area with its typical temperate climate had a monthly average temperature of 17.45 ± 8.9°C, humidity of 69.8 ± 5.9%, and wind velocity 1.9 ± 0.4 (m/s) monthly total rainfall of 88.1 ± 62.6 mm, and monthly total bright sunshine duration of 154.3 ± 50.9 h (**Table 1**). Univariate analysis showed that the incidence of MP infection was strongly positively correlat-

Table 1. MP incidence and meteorological parameters in Suzhou from January 2006 to December 2013

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2006 MP incidence (%)	14.6	10	16.4	25.2	27.2	30.6	26.3	20.9	31.2	29.8	39.2	29
Temperature (°C)	5.3	5.5	11.6	16.9	21.2	26.2	29.6	30.3	23.4	21.8	14.8	5.2
Rainfall (mm)	150.9	75.7	37.9	115	100.8	66.8	142.4	86.7	81.8	4.4	132.7	15.1
Relative humidity (%)	73	69	64	67	69	74	75	70	74	72	73	71
Sunshine (h)	90.1	88	172.7	162.8	157.8	153.2	178.5	263	130.4	147.8	102.8	140.8
Wind velocity (m/s)	1.9	2.3	2.5	2.6	2.7	2.5	2.9	2.8	1.8	1.7	1.8	1.6
2007 MP incidence (%)	24	21.6	30.5	41.1	36.6	40	37.5	28.8	38.1	37.8	30.6	24.1
Temperature (°C)	5	9.4	11.9	16	23.2	25.1	30.3	29.8	24.9	19.9	13.1	8.4
Rainfall (mm)	55.9	64.6	92.7	88.2	61	79	104.1	69.3	169	146.2	17.3	48.7
Relative humidity (%)	72	73	70	63	63	76	70	69	73	68	66	74
Sunshine (h)	101.8	152.3	144.2	187.9	216.4	93	170.6	232.6	127.4	150.9	142.1	83
Wind velocit (m/s)	1.8	2	2.6	2.3	2.7	2.2	2	3.2	1.9	1.7	1.3	1.4
2008 MP incidence (%)	21.2	29.7	31.5	37.4	37.7	43.6	47.6	50.6	40.5	42.6	49.4	39.4
Temperature (°C)	3.2	3.4	11.5	15.9	21.9	24	30.4	28.3	25.6	20.5	12.7	7.3
Rainfall (mm)	65.3	145.	29.3	19.5	18.5	326.1	61.4	81.2	110.1	65	61.2	27.5
Relative humidity (%)	73	66	63	67	63	76	67	71	71	70	67	59
Sunshine (h)	61	171	179.8	108.4	243.1	76.6	240.7	185.8	153.1	128.1	120.2	153.4
Wind velocit (m/s)	1.5	1.3	1.9	2.2	2.2	1.9	2.6	1.8	1.7	1.4	1.2	1.2
2009 MP incidence (%)	30	23.3	33.1	42	45.6	55.4	53.5	37.6	40.1	53.4	55.2	27.3
Temperature (°C)	3.5	8.6	10.5	16.6	22.4	26.4	29.1	28.2	25	21.2	10.9	6.0
Rainfall (mm)	51.9	123.7	76.7	79.3	51.9	156.1	210.9	145.3	66.7	4.6	116.2	67.6
Relative humidity (%)	64	76	67	63	56	70	69	76	73	63	73	68
Sunshine (h)	119.1	48.7	119.1	196.6	224.7	159.8	204	133.6	124.8	205.5	94.3	138.5
Wind velocit (m/s)	1.2	1.8	1.7	2.1	1.9	2	2	1.8	1.6	1.3	1.5	1.2
2010 MP incidence (%)	21.1	19.1	13.4	6.9	11.3	28.2	38.9	23.1	13.1	16.1	12.9	9.2
Temperature (°C)	4.9	7.1	9.1	13.1	20.9	24.3	28.6	30.9	26	18.6	13.3	7.6
Rainfall (mm)	40.9	58	185.9	81	66.5	59.5	185	51.2	65.4	53.8	2.9	41.4
Relative humidity (%)	66	75	70	69	69	74	77	68	74	69	64	57
Sunshine (h)	120.4	95.6	124.2	125.1	151.1	106.7	160.5	266.4	169.1	143	161.6	171.2
Wind velocit (m/s)	1.4	1.5	2.1	1.8	2.1	1.7	2	2.1	1.8	1.5	1.2	1.8
2011 MP incidence (%)	2.3	2.6	5.1	3.1	4.7	2.6	6.8	7.5	8	12.1	20.1	14.5
Temperature (°C)	1.1	5.8	9.4	16.3	21.9	24.5	29.8	28.2	24.5	19	16.3	6
Rainfall (mm)	5.8	17	48.5	55.1	53.2	295.2	108.9	181.5	8.7	61.8	22.7	26
Relative humidity (%)	58	69	58	60	61	83	75	79	71	70	77	69
Sunshine (h)	129.6	110.5	186.9	206.1	187.6	95.4	180	126.6	170.1	143.2	113.5	89.2
Wind velocit (m/s)	1.3	1.4	1.7	1.9	1.9	1.7	2.2	2.3	2.3	2	1.9	1.8
2012 MP incidence (%)	16	14	13.7	20.5	33.9	40.6	36.5	32.9	33.5	48.6	28.3	16
Temperature (°C)	4.3	4.1	9.7	18	21.9	25.2	30	29.1	23.8	19.7	11.9	5.7
Rainfall (mm)	71.6	85.3	147.9	56.5	133.4	110.8	154	215.5	92.1	37.3	117.6	86.1
Relative humidity (%)	72	75	73	70	72	81	77	85	74	67	70	71
Sunshine (h)	84.7	77.8	131.5	189.4	190.6	136.5	261.5	213.2	185.2	200.1	149	109.2
Wind velocit (m/s)	1.9	2	2.3	2.4	2.2	2.4	2.4	3.4	2.4	2	2	2.3
2013 MP incidence (%)	30.5	26.5	24	29.8	28.9	29.5	48.2	45.9	29.8	28.5	31.5	23.8
Temperature (°C)	4.2	6.4	11.2	16	21.8	24.6	32.3	31.5	25.2	19.7	13.7	16.3
Rainfall (mm)	29.8	113.8	82.7	51.7	126.2	229.3	74.7	89	85.7	257.1	19.3	47.2
Relative humidity (%)	76	82	70	60	74	81	63	69	69	68	62	62
Sunshine (h)	125.6	82.9	175.7	241.5	178.3	110.2	321.5	256.3	179.9	177.8	165	159
Wind velocity (m/s)	1.7	2.4	2.4	2.6	2.7	2.2	2.5	2.9	2.5	2.4	1.6	1.4

Table 2. Correlation between MP incidence and meteorological factors

Meteorological factors	Univariate regression analysis		Multivariate regression analysis	
	Pearson coefficient	*P*-value	Standardized beta coefficient	*P*-value
Temperature (°C)	0.338	0.000***	0.318	0.000***
Relative humidity (%)	-0.030	0.770	-0.092	0.352
Rainfall (mm)	0.207	0.043*	0.015	0.884
Sunshine (h)	0.241	0.018*	0.070	0.550
Windvelocity (m/s)	0.043	0.679	0.127	0.178

P-values < 0.05* and < 0.01*** were considered statistically significant.

ed with monthly average temperature (r = 0.338, P = 0.000), we found statistically significant but weaker correlations with monthly total rainfall (r = 0.207, P = 0.043), and monthly total bright sunshine duration (r = 0.241, P = 0.018). Because different meteorological parameters may also be correlated with each other, associations between meteorological factors and MP detection rates were then further analyzed by multivariate analysis, temperature was found to be the only independent associating factor, MP detection rates were positively correlated with temperatures (β = 0.380, t = 3.983, P = 0.000) (**Table 2**).

Discussion

In this study, MP was the frequently identified ARI pathogen in inpatients, found in 28.1% of the cases, in Suzhou, China. The detection rate of MP was higher than that in previous reports. Zhao H et al used PCR to monitor the M. pneumoniae infection and reported an MP-positive rate of 19.13% in children with ARI in Beijing from 2007 to 2013 [19]. Patrı´cia et al reported that MP was detected in 10% of children with ARI and 17% of children admitted to hospital with pneumonia in children under 5 years [20].

Most studies have shown that the incidence of infection with MP is highest among children aged 5 years or older [21, 22]. Our study was therefore consistent with other studies that indicate that older children are more susceptible to MP. Moreover, we found that the MP detection in 1~3 years and 3~5 years group were not low, at 34.8% and 45.1%, respectively. Our result indicating that MP infection was also common in toddle group and preschool group. More attention should be given to these children. Current studies shows MP infection has a trend of younger age. Chen et al reported that the positive percentages of MP infection were 13.04% in infants group; 35.43% in toddlers group; 55.33% in preschool group; 53.57% in school group [23]. Defilippi et al used PCR test 102 ARI cases, the positive of MP infection were 38.23% in preschool children, 41.76% in school children, children < 3 years accounted for 20.58%, < 8 month accounted for 7.8% [24].

Mycoplasma pneumoniae infections can occur both endemically and epidemically worldwide, and epidemics, lasting several months to years, periodically occur every 3 to 7 years [25]. In Korea, 3 to 4-year cycles of MP pneumonia have been observed from the mid-1980s to 2012 [26]. Serological studies performed in Denmark showed a pattern of M. pneumoniae infections over a 50-year period from 1946 through 1995 with endemic disease transmission punctuated with cyclic epidemics every 3-5 years [10]. Ito reported that the epidemic peaks recurred regularly at 4-year intervals in Japan from January 1979 to December 1999 [27]. The long incubation period, relatively low transmission rate, and persistence of the organisms in the respiratory tract for variable periods following infections may explain in the prolonged duration of epidemics of M. pneumoniae infections. So far, this is the longest and the largest sample size of MP infection epidemiological studies in China. 8 years of observation show that MP can be detected all the year round, the annual detection rates were 28.1%. From the trend, MP detection rate increased year by year since 2006, and reached the highest detection rate in 2009. However, MP detection rate significantly decline in 2010, 2011, detection rate in 2011 was the lowest (7.5%), MP detection rates back up to 27.6% in 2012 and was a certain degree of popularity, Our results suggest that the popularity of MP infection was 4 years for a cycle, at intervals of 2 years in the region from 2006 to 2013.

It has been described in earlier studies that MP infections have a seasonal distribution. In

Korean, MP outbreaks occurred during the summer in the earlier epidemics up until 1996, while the later epidemics peaked in the fall or early winter between 1986 to 2004 [11]. In contrast, Ito and colleagues reported that MP was prevalent all year round in Japan, with a relatively higher incidence in summer and autumn than in winter, it appeared most frequently in August [15]. Other reports suggest that MP may also be circulating throughout the year [27]. In Warsaw, epidemics of M. pneumoniae infections began in the third quarter of the year, peaked in the fourth quarter, and ended in the second quarter of the following year from 1970 to 1997 [28]. Data from the present study show that the epidemic MP season in the Suzhou region is in summer and autumn, but MP epidemic peak was different in the different years. In 2008, 2009, 2010, 2012 and 2013, the peak seasons were in June-August, indicating that MP was prevalent in summer. In 2006 and 2011, the peak of MP activity occurred in September-November, indicating that MP was prevalent in spring. However, in 2007, the peak of MP activity occurred in April, indicating that MP was prevalent in spring. The lowest MP-positive rates were December to February each year. Contrary to this finding, Hadil et al reported a higher prevalence of MP in autumn and only a few cases in winter and spring [29]. MP was not detected in summer. Defilippi et al reported the first MP peak in June, and a second peak in December and January [24]. Sidal et al reported the highest prevalence of MP was in winter [27]. Overall, the available studies suggest that the epidemiology of MP differs from region to region because of differences in climate.

Currently, little is known about the association between the prevalence of M. pneumoniae RTIs and climate. A laboratory-based study suggested that the survival of airborne M. pneumoniae was found to be a function of both temperature and relative humidity. The survival of airborne M. pneumoniae was found to be best at 27°C and, 25% or 90% relative humidity, while the most lethal relative humidity levels were at 60% and 80% [30, 31]. The present study has shown that in Suzhou, M. pneumoniae infections are positively correlated with monthly average temperatures, especially in the summer and autumn when the temperature and rates of M. pneumoniae infections are higher. Japanese researchers Onozuka et al analyzed 13,056 cases of M. pneumoniae pneumonia in children in Fukuoka City for 9 years [32]. The results showed that when the weekly temperature rose by 1°C, the incidence of M. pneumoniae pneumonia in children increased by 16.9%, which is similar to the results of our study. Their study also showed that the weekly number of M. pneumoniae pneumonia cases was associated with relative humidity. When the relative humidity increased by 1%, the M. pneumoniae pneumonia incidence in children increased by 4.1%. Another study conducted by German researchers reported that in the city of Mainz, M. pneumoniae infection was inversely associated with temperature but positively correlated with relative humidity [17]; these findings are not in agreement with those of our study. We presume that different climates may account for the different results. Suzhou and Fukuoka have the same subtropical climate with more variable temperatures than the oceanic climate in Mainz. However, the relative humidity is relatively constant and lower in Suzhou. The consistency of the humidity in Suzhou is why this factor had no significant influence on the epidemic of M. pneumoniae infections; the same trend was not observed in Fukuoka and Mainz. With high temperature and humidity, pathogenic microorganisms can form large aerosols [33]. Aerosol formation allows the M. pneumoniae microbe to survive longer, which increases the chances of infection and leads to M. pneumoniae infection epidemics.

More and more attention was attached on MP mixed with other pathogens infection in recent years. Korppi et al reported co-infections with Streptococcus pneumoniae and Chlamydia pneumoniae were present in over 30% and 15%, respectively, of mycoplasmal CAP cases [8]. Chen et al reported that 51.2% of cases with MP pneumonia had mixed infections. Most commonly caused by Chlamydia pneumonia (25.9%) followed by viruses (14.4%) [34]. In this study, MP mixed infection rate was 29.88%, lower than Korppi and Michelow reports [8, 35], this may be related that we did not detect chlamydia pneumonia in this study. RSV and Streptococcus pneumoniae were the common pathogen which co-infection with MP.

In conclusion, our study revealed MP as the important cause of respiratory tract infection of hospitalized children in Suzhou, China. MP

infections occurred throughout the year, with peaks of occurrence during the summer and autumn. Epidemic outbreaks of MP infection occurred lasting for 4 years and at intervals of 2 years from 2006 to 2013. The epidemic of MP showed a correlation with average temperature.

The major limitation of this study was the absence of outpatients with respiratory tract infection, the conclusions have certain limitations.

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Disclosure of conflict of interest

None.

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