ORIGINAL ARTICLE

Epidemiology of community-acquired Mycoplasma Pneumoniae respiratory tract infections among hospitalized Chinese children, including relationships with meteorological factors

Chen ZR[1], Yan YD[1], Wang YQ[1], Zhu H[2], Shao XJ[2], Xu J[2], Ji W[1]

[1]Department of Respiratory Disease

[2]Laboratory of Molecular Biology

Children's Hospital Affiliated with Soochow University, Suzhou, China

Abstract

Background: *Mycoplasma Pneumoniae (M. pneumoniae)* is a common cause of respiratory tract infections (RTIs), especially in children. Combined diagnostic techniques have provided more reliable information about the epidemiology of infections by this pathogen. The relationship between *M. pneumoniae* RTIs and climatic conditions is not well documented in the literature.

Aims: To study the epidemiology of *M. pneumoniae* infections in hospitalized children with RTIs and its association with meteorological factors.

Methods: Samples were obtained from children with RTIs and tested for *M. pneumoniae* by PCR and ELISA. Meanwhile, meteorological factors were recorded.

Results: *M. pneumoniae* was identified in 11.02% of the 8,157 specimens. There were significant differences among the annual distribution of infections (χ^2 =130.13, P<0.0001) and among different seasons (χ^2 =93.59, P<0.0001). Of the total number of patients with *M. pneumoniae* infections, 14.5% were infected with more than one pathogen. *M. pneumoniae* infection strongly correlated with mean temperature. Children with a single *M. pneumoniae* infection had significantly higher neutrophil percentages and CRP levels than children with co-infections.

Conclusions: *M. pneumoniae* is one of the most commonly held pathogens, according to the 5-year surveillance. *M. pneumoniae* infection has its own epidemic season, especially in the summer. Mean temperature is the main meteorological factor affecting the epidemiology of *M. pneumoniae* infections.

Keywords: *Mycoplasma pneumoniae*, Respiratory tract infections, Meteorological factors, Children, Epidemiology.

Corresponding author: Wei Ji, Department of Respiratory Disease, Children's Hospital Affiliated with Soochow University. NO. 303, Jingde Road, Suzhou, Jiangsu, 215003, China, tel: +8613962108901, fax: +8651267786204, e-mail: szdxjiwei@163.com

Introduction

Currently, there are 16 known *Mycoplasma* species isolated from humans, excluding the occasional animal *Mycoplasmas* that have been detected. *Mycoplasmas* are usually found in immunosuppressed hosts, and *Mycoplasma pneumoniae (M. pneumoniae)* was the first disease-causing *Mycoplasma* discovered in humans and is the leading cause of community-acquired pneumonia (CAP) in children. *M. pneumoniae* is the smallest known free-living microbe. The lack of a cell wall in this microbe does not allow for Gram-staining and makes this microbe insensitive to beta-lactam antibiotics. Originally described as benign respiratory commensals, the *Mycoplasmas* have been associated with a widening clinical spectrum and are a common cause of respiratory tract infections (RTIs) in children. *Mycoplasmas* are found in approximately 40% of the children infected with CAP, and of these, 18% of the patients require hospitalization[1].

Clinical diagnosis of *M. pneumoniae* infections is very difficult because its clinical manifestations are similar to those of infections with *M. pneumoniae*, viruses and other pathogens. However, the laboratory diagnosis of *M. pneumoniae* infections is more difficult. The culturing conditions for *M. pneumoniae* are relatively strict, and it generally takes several weeks to culture the pathogen, which makes the clinical application of culturing unfeasible. Serological tests can be persistently positive for weeks to months, so the combination of PCR and serology is recommended as a more reliable diagnostic approach[2,3]. Real-time PCR applied for the identification of *M. pneumoniae* infections has accuracy, efficiency and time-saving advantages, for which it is valued by clinicians[4]. Epidemics of *M. pneumoniae* infections may occur every 3 to 5 years and last for a period of time that allows transmission by respiratory droplets. Epidemic occurrences of *M. pneumoniae* infections may be closely associated with the climate, although the relationships between *M. pneumoniae* and climatic conditions, such as temperature, humidity, rain-

fall, sunlight and wind velocity, are not well documented in the literature. Recently, one study reported that *M. pneumoniae* activity increased as the average temperature and relative humidity increased[5]. Further studies involving large sample populations should be performed to clarify the relationships between *M. pneumoniae* activity and meteorological factors.

The aims of the present study include the following: 1) to investigate the prevalence of acute *M. pneumoniae* infections among children hospitalized for upper or lower RTIs in Suzhou, China; 2) to explore the relationships between meteorological factors and *M. pneumoniae* activity in Suzhou from 2006 to 2010; and 3) to examine the most frequent clinical characteristics and laboratory findings associated with *M. pneumoniae* infections to promote the early detection and prevention of the disease, as well as the immediate treatment of children with *M. pneumoniae* RTIs.

Materials and methods

Patients

The patients in the present prospective study were selected from among children who were hospitalized from 1st January 2006 to 31st December 2010 in the Department of Respiratory Disease in the Children's Hospital affiliated with Soochow University. Children with clinical symptoms of community-acquired RTIs (rhinorrhea, sore throat, cough, fever, wheezing, chest pain, tachypnea, and dyspnea) were eligible for enrollment, while those with a history of chronic lung disease, underlying immunodeficiencies or pre-existing cardiac, renal, neurological or hepatic dysfunction were excluded from the study. A total of 8,157 cases ranging in age from 1 month to 14 years were enrolled: 4,890 males (59.95%) and 3,267 females (40.05%); the ratio of males to females was 1.50:1. The study protocol was approved by the Institutional Review Boards of Soochow University, and informed consent was obtained from the parents or legal guardians of each child before enrollment.

Patient evaluation

Upon hospital admission of the patients, pediatricians completed a questionnaire regarding the age of the patient, the date of onset of the disease, the clinical symptoms, the laboratory findings, and the potential presence of underlying disease. Patients who had clinical symptoms of lower RTIs underwent chest radiography. Chest radiography was performed using standard equipment and radiographic techniques and was reviewed by the radiologists in digital format. Meanwhile, each patient's serum (4 mL) was obtained for complete blood counts, C-reactive protein concentrations, and platelet detections. According to the clinical findings, the children were diagnosed as having the following: 1) upper respiratory tract infections (URIs), with rhinorrhea, sore throat, and a normal chest radiograph when it was performed and without rales; 2) bronchiolitis in patients less than 2 years old, with the first episode of wheezing, with or without moist rales, with chest radiographic evidence of hyperinflation of the lung; 3) bronchitis, with cough and unfixable rales, with a normal chest radiograph; 4) pneumonia, with cough and fixable rales, with an abnormal chest radiograph including flaky, lobar or diffuse pulmonary infiltration; 5) severe pneumonia, with which patients required mechanical ventilation.

Methods

Specimen collection

Nasopharyngeal aspiration (NPA) fluids were collected from all of the subjects within 24 hours of admission before beginning antibiotic therapy; sera samples were obtained on admission, whereas convalescent serum specimens were taken one week later, usually on the day of hospital discharge. The specimens were preserved in standard transport media at 4 ºC and transported to the Department of Clinical Virology and Microbiology Laboratory for study.

DNA extraction and PCR for *M. pneumoniae* gene detection.

Two mL samples were obtained from the children and then shaken and centrifuged. The supernatant was taken from the samples, and lysis buffer was added to extract the DNA, which was then amplified by PCR primers and probes. Real-time PCR was performed on the samples to identify the P1 adhesion protein gene of *M. pneumoniae*, according to a previous study[6]. The forward and reverse primers were 5'-CCA ACC AAA CAA CAA CGT TCA-3' and 5'-ACC TTG ACT GGA GGC CGT TA-3', respectively, and the probe sequence was (FAM)–5'-TCA ACT CGA ATA ACG GTG ACT TCT TAC CAC TG-3'-TAMRA. The fluorescent reporter dye at the 5' end of the probe was 6-carboxy-fluorescein (FAM), and the quencher at the 3' end was 6-carboxy-tetramethyl-rhodamine (TAMRA). A 21 μL PCR master mixture (Shenyou Bio Technology, Shanghai, China) containing the primers and probes was combined with 3 μL of the sample DNA and 1 μL of the GoTaq® DNA Polymerase (Promega, Wisconsin, USA) for the PCR reactions. Real-time PCR was performed using the iQ5TM BIO-icycler (BIO-RAD, California, USA), and the cycling conditions were as follows: 2 min at 37ºC; 10 min at 94ºC; 40 cycles of 10 s at 94ºC, 30 s at 55ºC and 40 s at 72ºC. Quantification curves were plotted using several concentrations of control plasmids consisting of the target gene.

Serology of *M. pneumoniae*

The specific IgM and IgG antibodies against *M. pneumoniae* were detected in the serum samples of patients using a commercial ELISA kit (Serion ELISA classic *M. pneumoniae* IgG/IgM, Institute Virion\Serion, Würzburg, Germany), according to the manufacturer's instructions. Evidence of acute *M. pneumoniae* infections was defined as either a single positive serum IgM titer (cut-off 13 U/mL) or a 4-fold increase in the IgG titer of convalescent serum.

Virus detection

With the same NPA samples, a direct immunofluores-

cence assay was used to detect respiratory syncytial virus (RSV), influenza virus A, B (IV-A, IV-B), parainfluenza virus 1, 2, 3 (PIV-1, PIV-2, PIV-3) and adenovirus (ADV) antigens. The reagent was purchased from Chemicon Company, Temecula, California, USA and used according to the manufacturer's instructions; the results were judged according to the positive criteria. For RT-PCR detection of human metapneumovirus (hMPV), the primers were as follows: hMPV-F: 5'-AACCGTGTACTAAGTGATGCACTC-3'; hMPV-R: 5'-CATTGTTTGACCGGCCCCATAA-3'. Real-time PCR was applied for detection of human bocavirus (HBoV) using the following primers: HBoV-F: 5'-TGACATTCAACTACCAACAACCTG-3', HBoV-R: 5'-CAGATCCTTTTCCTCCTCCAATAC-3'. The fluorescent probe sequence was HBoV-Probe: AGCACCACAAAACACCTCAGGGGTAMRA. Trizol was provided by Invitrogen (California, USA); M-MLV reverse transcriptase and Taq enzyme were provided by Promega (Madison, WI, USA); 6-random primers were synthesized by Shanghai Biological Engineering Technology Services Co. Ltd, Shanghai, China.

Meteorological data collection

Climate data were provided by the Weather Bureau of Suzhou, including the monthly mean temperature (ºC), the monthly mean relative humidity (%), the total rainfall (mm), the monthly total sunshine (h), the monthly mean wind velocity (m/s) and other relevant meteorological data. The meteorological station is located at east longitude 120° and north latitude 31°.

Analysis

Values are expressed as percentages for discrete variables or as the mean and standard deviation or 95% confidence intervals for continuous variables. Clinical characteristics and laboratory variables were compared using the Student t-test, the Mann–Whitney U-test, the χ^2 test and Fisher's exact test. The relationship between meteorological factors and the number of cases caused by different pathogens was determined using Spearman rank correlations. Meanwhile, stepwise regressions were conducted independently to determine the relative contribution of different meteorological factors. A two-sided value of $p < 0.05$ was considered statistically significant. All of the analyses were performed using the Statistical Package for SAS for windows, version 8.2 (SAS Inc., Cary, NC, USA).

Results

Distribution of *M. pneumoniae* infections

Overall, *M. pneumoniae* was identified in 11.02% of all of the cases. All of the patients were divided into four groups: 0~12 months, 13~36 months, 37~60 months, and > 60 months. The prevalence of *M. pneumoniae* infections in each group was 5.99% (248/4137), 13.79% (285/2066), 17.11% (193/1128), and 20.94% (173/826), respectively (Figure 1).

From 2006 to 2010, the annual distribution of *M. pneumoniae* infections was 13.40%, 5.39%, 6.36%, 14.58%, and 13.99%, respectively. There was a significant difference between different years and seasons (Table 1). *M. pneumoniae* infections were detected throughout the year and mainly occurred from May to November of each year; this period included the autumn and summer seasons, with an epidemic month (June 2010) exhibiting a maximum infection prevalence of 25.97% (Figure 2).

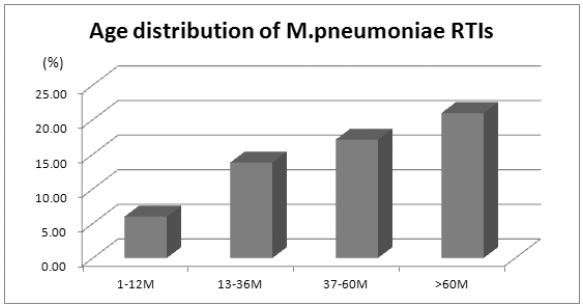

Figure 1: The detection rate of *M. pneumoniae* respiratory tract infections is 5.99% (248/4,137), 13.79% (285/2,066), 17.11% (193/1,128), 20.94% (173/826) in four age groups and it is increasing with the growing age (χ^2=248.35, P<0.0001).

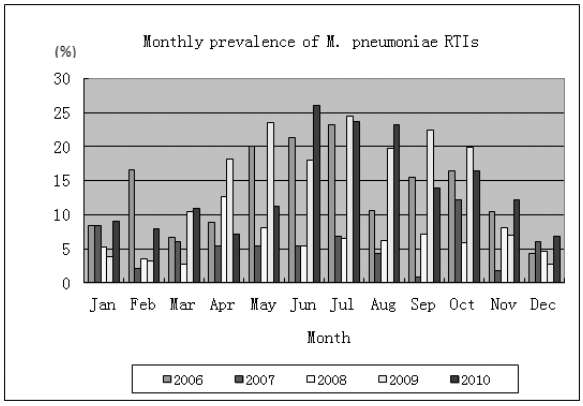

Figure 2: Monthly distribution of *M. pneumoniae* infection among children hospitalized with respiratory tract infections from January 2006 to December 2010. *M. pneumoniae* infection can be detected throughout the year and mainly occurs from May to November in each year, (autumn and summer respectively) with epidemic month (June 2010) up to 25.97%.

From 130 cases of *M. pneumoniae* co-infections that accounted for 14.5% (130/899) of *M. pneumoniae* infections cases, 33.1% (43/130) of the total cases of co-infections were RSV infections, and the remaining co-infection viruses were HBoV (16.9%), PIV-3 (13.8%), hMPV (13.1%), ADV (12.3%), IV-A (4.6%), PIV-1 (2.3%), and IV-B (0.8%). In addition, there were four cases of *M. pneumoniae* combined with two viral infections (Table 2).

Relationship between *M. pneumoniae* activity and meteorological factors

M. pneumoniae infections and mean temperature were positively correlated (r_s = 0.427, P = 0.0006); no correlation with other meteorological factors was observed (Table 3). Mean temperature was the only meteorological factor that was integrated into the formula by regression analysis (β=0.49, t=4.282, P<0.0001).

Table 1: The annual and seasonal distribution of M. *pneumoniae* respiratory tract infections. There was significant difference between the different years (χ^2 = 130.13, P <0.0001). Prevalence of 2007 and 2008 were significantly lower than year 2006, 2009, 2010 (P <0.0001). Prevalence of *M. pneumoniae* infections was different with different seasons (χ^2 = 93.59, P <0.0001). There were significant differences in seasons of 2006, 2009 and 2010 (both P <0.05) except for 2007, 2008.

Year	Total positive cases n (%)	Spring (Mar-May) n (%)	Summer (Jun-Aug) n (%)	Autumn (Sep-Nov) n (%)	Winter (Dec-Feb) n (%)	P value
2006	**214(13.40)**	**43(11.0)**	**76(18.1)**	**57(13.87)**	**38(10.13)**	**0.0038**
2007	**82(5.39)**	**20(5.70)**	**21(5.43)**	**19(4.64)**	**22(5.90)**	**0.8735**
2008	**94(6.36)**	**32(7.84)**	**21(6.05)**	**24(6.84)**	**17(4.57)**	**0.2962**
2009	**266(14.58)**	**72(16.55)**	**104(20.68)**	**77(16.38)**	**13(3.12)**	**<0.0001**
2010	**243(13.99)**	**44(9.59)**	**110(24.34)**	**52(14.25)**	**37(8.03)**	**<0.0001**
Total	**899(11.02)**	**211(10.32)**	**332(15.74)**	**229(11.42)**	**127(6.35)**	**<0.0001**

Table 2: Distribution of co-infected viruses in children with M. *pneumoniae* infection.

Viruses + *M. pneumonia*	**n (%)**
Respiratory syncytial virus + *M. pneumoniae*	43 (33.1)
Human bocavirus + *M. pneumonia*	22 (16.9)
Parainfluenza virus 1 + *M. pneumonia*	18 (13.8)
Human metapneumovirus + *M. pneumoniae*	17 (13.1)
Adenovirus + *M. pneumonia*	16 (12.3)
Influenza virus A + *M. pneumonia*	6 (4.6)
Parainfluenza virus 1+ *M. pneumonia*	3 (2.3)
Influenza virus B + *M. pneumonia*	1 (0.8)
Parainfluenza virus 2 + *M. pneumonia*	0 (0)
Respiratory syncytial virus + Influenza virus A + *M. pneumoniae*	2 (1.5)
Parainfluenza virus 3 + Human bocavirus + *M. pneumoniae*	2 (1.5)
Total	130 (100)

Table 3: Relationship between M. *pneumoniae* respiratory tract infections and meteorological factors.

Meteorological factors	Mean±SD	Correlation coefficient r_s	P value *
mean temperature (ºC)	16.7±8.7	0.42795	0.0006
relative humidity (%)	68.9±4.8	0.116	0.3742
total rainfall monthly (mm)	85.7±57.7	0.148	0.258
total sunshine monthly (h)	142.2±51.9	0.1717	0.1894
mean wind velocity (m/s)	1.9±0.4	0.2269	0.0812

* $p < 0.05$ was considered statistically significant.

SD: standard deviation.

Table 4: Comparisons of clinical characteristics and laboratory findings between single *M. pneumoniae* infection and co-infection with viruses.

Clinical characteristics and laboratory findings	Single infection	Co-infection	P value *
Mean age (month, 95%CI)	42.9 (40.2-45.5)	34.6 (28.6-40.7)	0.017
Wheeze n (%)	188 (24.4)	47 (36.2)	0.006
Shortness of breath n (%)	50 (6.5)	19 (14.6)	0.0014
Bronchiolitis n (%)	94 (12.2)	30 (23.1)	0.001
Severe pneumonia n (%)	7 (0.9)	5 (3.8)	0.02
Neutrophils (%, 95%CI)	52.9 (51.6-54.2)	47.4 (44.1-50.8)	0.002
CRP (mg/L, 95%CI)	12.4 (10.9-13.8)	11.0 (6.5-15.4)	0.04
PLT (×10^9/L, 95%CI)	317 (310-325)	336 (317-355)	0.06

* $p < 0.05$ was considered statistically significant. Enumeration data in brackets counted as a percentage; measurement data in brackets show as 95% confidence interval. CRP: C-reactive protein, PLT: platelets.

Demographic data, clinical characteristics and laboratory findings of single *M. pneumoniae* infections and co-infections with the most important viruses

The mean age of children with *M. pneumoniae* infections was higher than those with *M. pneumoniae* co-infections. *M. pneumoniae* co-infections with wheezing and shortness of breath symptoms were more common than single *M. pneumoniae* infections. No other significant clinical characteristics were common to both single *M. pneumoniae* infections and co-infections, including cough, running nose, fever, dyspnea, feeding difficulties, cyanosis, and length of hospital stay. The proportion of *M. pneumoniae* co-infections in children with bronchiolitis and severe pneumonia was significantly higher than that in children with single *M. pneumoniae* infections. There were no significant differences in the incidence of URI, bronchitis, and pneumonia between these two groups. Percentages of neutrophils and CRP in children with single *M. pneumoniae* infections were higher than those in children with co-infections, while platelet levels were lower in single *M. pneumoniae* infections than those in co-infections (Table 4).

Discussion

Thanks to the improved diagnostic methods and the increased attention clinicians pay to the importance of the pathogen in recent years, the detection rate for *M. pneumoniae* infections among 8,157 children with community-acquired RTIs reached 11.2% in our study. Numerous epidemiological studies have shown that *M. pneumoniae* infection rates vary between 1.3% and 50% and that the infection rates during epidemic periods can be more than 50%[7]. In our study, the infection rate reached 25.97% during the epidemic month of June 2010. Differences between studies may be attributed to community outbreaks during the study periods and differences in the applied methods and the rigidity of diagnostic criteria. Differences may also result from the diversity of climate and geography in the study areas, as well as human activities. In the present study, the combination of serology and PCR was used for diagnosis. This combination is highly sensitive, achieving 95% to 100% detection[2,8], while the sensitivity of PCR alone is 75% and that of μ-capture ELISA is 66.7%[2]. This study shows that the rate of *M. pneumoniae* infections in Suzhou from 2007-2008 was significantly lower than in 2006, 2009, and 2010, indicating that 2007 and 2008 were non-epidemic years. Rasmussen et al[9] used PCR to monitor the *M. pneumoniae* infection situation and found that the epidemic year of *M. pneumoniae* in Denmark was 2004-2005, which was followed by a low positivity rate of approximately 3% from 2007-2009. The estimated national incidence of PCR-diagnosed *M. pneumoniae* infections in 2010 rose from 0.4 per 100,000 in week 34 to 3 per 100,000 in week 41, which indicated the possibility of a new epidemic. Nevertheless, in different regions, epidemics occur in different years.

This study also shows that *M. pneumoniae* was epidemic in the summer, followed by the spring and autumn seasons. Thus, from May to November of each year, the positivity rate of *M. pneumoniae* infections was high; this result similar to that of a study performed in Italy[10]. Unlike endemic disease, which may not demonstrate marked seasonal occurrences, outbreaks of *M. pneumoniae* infections in countries with temperate climates are generally thought to tend to occur in the summer or the early fall when the occurrence of other respiratory pathogens is usually lower[1]. Therefore, *M. pneumoniae* activity and climate are closely related. Denmark, Iceland and other regions have reported that *M. pneumoniae* is more common in the autumn and winter seasons, with an especially high rate in the winter[11,12]. 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. This study used combined PCR and ELISA techniques to detect *M. pneumoniae* and monitored the monthly temperature, monthly humidity, total rainfall, total sunshine and monthly wind velocity to establish the relationship between *M. pneumoniae* infections and climate. The present study has shown that in Suzhou, *M. pneumoniae* infections are positively correlated with temperature, especially in the summer when the temperature and rates of *M. pneumoniae* infections are higher. Thus, higher temperatures are associated with stronger *M. pneumoniae* activity. However, the prevalence of the respiratory viruses, RSV and IV-A, is inversely correlated with temperature[13]. Japanese researchers Onozuka et al[5] analyzed 13,056 cases of *M. pneumoniae* pneumonia in children in Fukuoka City for nine years. The study showed that when the weekly temperature rose by 1 ºC, then the incidence of *M. pneumoniae* pneumonia in children increased by 16.9% (95% CI 11.3% ~ 22.8%), 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% (95% CI 2.7% ~ 5.5%). Another study conducted by German researchers[14] reported that in the city of Mainz, *M. pneumoniae* infection was inversely associated with temperature (r=-0.19, P=0.022) but positively correlated with relative humidity (r=0.30, P <0.001); these findings are not in agreement with those of our present study. We presume that different climates may account for the different results among these studies. 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: over 68.9 ± 4.7 (%), with the minimal coefficient of variation of only 6.9%. 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. Mainz has an oceanic climate with moderate variations in temperature and significant variations in humidity. Thus, humidity exerts different influences on epidemics of *M. pneumoniae* in-

fections in Mainz. With high temperature and humidity, pathogenic microorganisms can form large aerosols[15]. Aerosol formation allows the *M. pneumoniae* microbe to survive longer, which increases the chances of infection and, thereby, leads to *M. pneumoniae* infection epidemics. Therefore, a clear understanding of the relationship between regional climate and *M. pneumoniae* infection and the establishment of *M. pneumoniae* infection and climate monitoring systems will help clinicians understand the factors underlying *M. pneumoniae* epidemics and provide better clinical diagnoses and treatments.

Previous studies showed that *M. pneumoniae* infection mainly occurs in children over 5 years of age. With the recent improvement of detection methods, many studies found that *M. pneumoniae* infections are relatively more common in children under age 5[16,17]. The present study shows that *M. pneumoniae* infections are also common in hospitalized patients less than 5 years old, with a positivity rate of 9.9% (726/7331). This finding is consistent with that of a study performed in France[18],which reported that the number of young children who attend daycare centers on a regular basis is greater than that in previous years and demonstrated that RTIs can be spread by young children sharing respiratory secretions.

M. pneumoniae co-infection with viruses is common in children with RTIs. Bezerra et al reported that 19.3% of the total *M. pneumoniae* infections in children less than 5 years old presented with two or more pathogens[19], which is consistent with the results of our study but lower than the 26.5% (9/34) observed in a similar study conducted in Greece[20] that also detected *Chlamydia pneumoniae* in children with RTIs. Whether the combination of two pathogens causes more severe clinical illnesses remains unverified, according to previous study[21]. However, this study found the following: the mean age of children with co-infections is younger than the age of those with single *M. pneumoniae* infections; their clinical symptoms are relatively more severe; and they are more prone to wheezing and shortness of breath. As a result, children with co-infections are at risk of developing severe pneumonia. The children with co-infections were also observed to have relatively more severe clinical symptoms and are more prone to suffer wheezing and shortness of breath, which also puts them at risk for developing severe pneumonia. RSV, the common causal factor of severe pneumonia in young and premature infants[22], is the most frequent virus combined with *M. pneumoniae* that contributes to a high risk of developing severe pneumonia. In the present study, 9.3% of the total RSV and *M. pneumoniae* co-infection cases were diagnosed with severe pneumonia.

This study shows that the total number of white blood cells in children with *M. pneumoniae* infections alone did not increase. Additionally, neutrophils were the dominant cellular infiltrate observed, accounting for 52.9%, and CRP was increased. These findings are consistent with the findings of Esposito et al (N% = 66 ± 17%, CRP = 53 ± 83 mg/L)[21]. In the co-infection group, neutrophil percentages and CRP were lower than in the single *M. pneumoniae* infection group, suggesting that the viruses that co-infected with *M. pneumoniae* may play an important role in pathopoiesis.

The five-year monitoring of *M. pneumoniae* infections in Suzhou indicated that *M. pneumoniae* is a common respiratory tract pathogen in children with RTIs. *M. pneumoniae* can be detected throughout the year and has a unique seasonality. Co-infection with viruses is common in children with RTIs. In addition, meteorological factors, especially the temperature, play an important role in epidemics of *M. pneumoniae* infections. Understanding the pathogen's epidemic characteristics and clinical features can facilitate the early detection of possible respiratory diseases and the early prevention and suitable treatment of RTIs in children.

Acknowledgement
Yan YD is co-first author due to equal contribution to this manuscript.

Conflict of interest
Authors have no conflict of interest.

References

1. Waites KB. New concepts of *Mycoplasma pneumoniae* infections in children. Pediatr Pulmonol. 2003; 36: 267-278.
2. Souliou E, Almasri M, Papa A, Theodoridou A, Diza E. Laboratory diagnosis of *Mycoplasma pneumoniae* respiratory tract infections in children. Euro J Clin Microb Infect Dis. 2007; 26: 513-515.
3. Dorigo-Zetsma JW, Zaat SA, Wertheim-van Dillen PM, Spanjaard L, Rijntjes J, van Waveren G, et al. Comparison of PCR, culture, and serological tests for diagnosis of *Mycoplasma pneumoniae* respiratory tract infection in children. J Clin Microbiol. 1999; 37: 14-17.
4. Zhao F, Cao B, He LH, Yin YD, Tao XX, Song SF, et al. Evaluation of a new real-time PCR assay for detection of Mycoplasma pneumoniae in clinical specimens. Biomed Environ Sci. 2012; 25: 77-81.
5. Onozuka D, Hashizume M, Hagihara A. Impact of weather factors on *Mycoplasma pneumoniae* pneumonia. Thorax. 2009; 64: 507-511.
6. Hardegger D, Nadal D, Bossart W, Altwegg M, Dutly F. Rapid detection of *Mycoplasma pneumoniae* in clinical samples by real-time PCR. J Microbiol Methods. 2000; 41: 45-51.
7. Liu FC, Chen PY, Huang F, Tsai CR, Lee CY, Wang LC. Rapid diagnosis of *Mycoplasma pneumoniae* infection in children by polymerase chain reaction. J Microbiol Immunol Infect. 2007; 40: 507-512.
8. Waris ME, Toikka P, Saarinen T, Nikkari S, Meurman O, Vainionpää R, et al. Diagnosis of Mycoplasma pneumoniae pneumonia in children. J Med Microbiol. 1998; 36: 3155-3159.
9. Rasmussen JN, Voldstedlund M, Andersen RL, Ellermann-Eriksen S, Jensen TG, Johansen HK, et al. Increased incidence of *Mycoplasma pneumoniae* infections detected by laboratory-based surveillance in Denmark in 2010. Euro Surveill. 2010; 15: 19708.
10. Defilippi A, Silvestri M, Tacchella A, Giacchino R, Melioli G, Di Marco E, et al. Epidemiology and clinical features of Mycoplasma pneumoniae infection in children. Respir Med. 2008; 102: 1762-1768.
11. Lind K, Bentzon MW. Changes in the epidemiological pattern of *Mycoplasma pneumoniae* infections in Denmark. A 30 years survey. Epidemiol Infect.1998; 101: 377-386.
12. Hauksdottir GS, Löve A, Sigurdardottir V, Jonsson T. Seasonal

variation of *Mycoplasma* infections in Iceland and Israel: different associations with meteorological variables. Eur Respir J. 1997; 10: 2432-2433.
13. Tang JW, Lai FY, Wong F, Hon KL. Incidence of common respiratory viral infections related to climate factors in hospitalized children in Hong Kong. Epidemiol Infect. 2010; 138: 226-235.
14. du Prel JB, Puppe W, Gröndahl B, Knuf M, Weigl JA, Schaaff F, et al. Are meteorological parameters associated with acute respiratory tract infections? Clin Infect Dis. 2009; 49: 861-868.
15. Yusuf S, Piedimonte G, Auais A, Demmler G, Krishnan S, Van Caeseele P, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. Epidemiol Infect. 2007; 135: 1077-1090.
16. Bosnak M, Dikici B, Bosnak V, Dogru O, Ozkan I, Ceylan A, et al. Prevalence of *Mycoplasma pneumoniae* in children in Diyarbakir, the south-east of Turkey. Pediatr Int. 2002; 44: 510–512.
17. Othman N, Isaacs D, Kesson A. *Mycoplasma pneumoniae* infections in Australian children. J Paediatr Child Health. 2005; 41: 671–676.
18. Layani-Milon MP, Gras I, Valette M, Luciani J, Stagnara J, Aymard M, et al. Incidence of upper respiratory tract *Mycoplasma pneumoniae* infections among outpatients in Rhône-Alpes, France, during five successive winter periods. J Clin Microbiol. 1999; 37: 1721–1726.
19. Bezerra PG, Britto MC, Correia JB, Duarte Mdo C, Fonceca AM, Rose K, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. PLoS One. 2011; 6: e18928.
20. Almasri M, Diza E, Papa A, Eboriadou M, Souliou E. *Mycoplasma pneumoniae* respiratory tract infections among Greek children. Hippokratia. 2011; 15: 147-152.
21. Esposito S, Blasi F, Bellini F, Allegra L, Principi N. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections in children with pneumonia. Eur Respir J. 2001; 17: 241-245.
22. Berkley JA, Munywoki P, Ngama M, Kazungu S, Abwao J, Bett A, et al. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA. 2010; 303: 2051-2057.