Original Article

Analysis of *mycoplasma pneumoniae* infection among children with respiratory tract infections in hospital in Chengdu from 2014 to 2020

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Background: Respiratory tract infection (RTIs) is one of common diseases among the children. In recent years, the incidence of *mycoplasma pneumoniae* (*M. pneumoniae*) infection rate has been increasing, which cause respiratory tract infection. This study sought to analyze the epidemiological characteristics of *M. pneumoniae* hospitalized children with RTIs to provide a theoretical basis for clinical diagnosis and treatments in Chengdu, China.

Methods: The data of 22,882 cases of children who had been hospitalized for RTIs were collected. *M. pneumoniae* immunoglobulin M (IgM) antibody was detected using the indirect immunofluorescence method and passive agglutination method. The demographic features of patients, clinical diagnoses and laboratory data were also analyzed.

Results: A total of 4,213 children tested positive for *M. pneumoniae*. The total positive rate was 18.41% (18.30% for males and 22.72% for females). Female children had statistically significant higher positive rates than male children (χ^2=198.078, P<0.01). The positive rates of *M. pneumoniae* differed significantly among children of different ages (F=162.7532, P<0.01). The incidence rate of *M. pneumoniae* in 2017 and 2019 was significantly higher than the average (F=538.95, P<0.01). There were higher *M. pneumoniae* positive rates from April to May, and September to October (P<0.05) in 2016, 2017, 2018, and 2019. There was no correlation between *M. pneumoniae* infection and temperature and humidity (P>0.05). There was negative correlation with $PM_{2.5}$ (particulate matter in the air <2.5 μm) (R=−0.293, P<0.01) and PM_{10} (particulate matter in the air <10 μm). (R=−0.285, P<0.01). There were significant differences in the constituent ratios of cases of *M. pneumoniae* infection between in 2020 and other years (F=159.35, P<0.01). Bronchopneumonia accounted for the highest proportion of cases, followed by acute bronchitis and the exacerbation of asthma in 2020.

Conclusions: The epidemiological distribution of *M. pneumoniae* in children with RTIs in Chengdu was found to be related to gender, age, year and month; however, no relationship was found to temperature and humidity. There was a higher *M. pneumoniae* positive rate in children with bronchial pneumonia and asthma in cases. The prevention measures used to control Coronavirus Disease 2019 (COVID-19) also effectively controlled the *M. pneumoniae* infection rate.

Keywords: Respiratory tract infection (RTI); children; *mycoplasma pneumoniae* (*M. pneumoniae*); Coronavirus Disease 2019 (COVID-19)

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Introduction

Respiratory tract infections (RTIs) are one of the most common diseases among children. RTIs are caused by a board range of pathogens, including viruses, bacterial pathogens, and atypical bacterial pathogens. *Mycoplasma pneumoniae* (*M. pneumoniae*) is a common bacterial pathogen that causes lower RTIs in children and young adults. It is one of the smallest prokaryotic cell types of microorganisms that lack cell walls, is highly polymorphic, and can pass the bacteria filter and grow on inanimate objects. In recent years, the incidence of *M. pneumoniae* has been increasing (1-3). Different areas in different cities have different pathogen spectrums and epidemiological features (3,4). To date, the data available about the prevalence of *M. pneumoniae* infection in pediatric patients in Chengdu, Sichuan Province, China, including during the period in which Coronavirus Disease 2019 (COVID-19) spread in China, have been fragmentary. This study sought to investigate the effects of *M. pneumoniae* in hospitalized children between January 2014 and December 2020. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tp-21-139).

Methods

Subject information

The data of 22,882 in-patients suffering from RTIs, who had been admitted to Chengdu Women's and Children's Hospital, Chengdu, China, were used in this study. Diagnoses were made in accordance with the World Health Organization's criteria. Of the 22,882 patients, 13,600 children were male and 9,282 were female. Patients' ages ranged from 1 month to 17 years old. Patients were classified into the following age subgroups based on different physiological stages: infants (1 month–1 year old); toddlers (>1–3 years old); preschoolers (>3–6 years old); school-age children (7–17 years old). Venous blood was drawn from every patient. Serum samples were separated and tested for the *M. pneumoniae* immunoglobulin M (IgM) and the antibody. A patient was diagnosed with *M. pneumoniae i*nfection, if *M. pneumoniae* IgM was positive or the antibody ≥1:160 (5). The demographic features of patients, clinical diagnoses and laboratory data were analyzed. Data provided by the Chengdu Meteorological Service were examined to determine if there was an association between gender, age, month, year, $PM_{2.5}$ (particulate matter in the air <2.5 μm), PM_{10}(particulate matter in the air <10 μm), temperature, humidity and cases of *M. pneumoniae* infection. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China (NO.: B2021[8]). Individual consent for this retrospective study was waived.

Blood sampling

A sample of venous blood was drawn from each child. Each sample was centrifuged at 2,000 rpm for 10 min at 4 ℃. The serum was separated and stored at –20 ℃ until it was assayed with the *M. pneumoniae* IgM, and the antibody test was undertaken.

M. pneumoniae IgM test: Indirect immunity and the antibody positive titer test

Each slide had 1 well containing *M. pneumoniae* antigens. Serum samples were diluted 1:1 with phosphate buffered saline (PBS), and then treated with anti-human immunoglobulin G (IgG) sorbent. The sorbent-treated diluted serum was incubated for 90 min at 37 ℃ with the slide wells. Each slide was washed twice with PBS before the fluorescent secondary IgM antibody was added to the well and incubated at 37 ℃ for 30 minutes. Each slide was washed twice with PBS and the greenish-yellow fluorescent signal was detected with a fluorescence microscope (Zeiss, Oberkochen, Germany). IgM antibodies against *M. pneumoniae* in serum were detected using a passive agglutination kid (Fujirebio, Japan) in accordance with the manufacturer's instructions.

Statistical analysis

Categorical variables were presented as numbers with percentages and compared using a χ^2 test. A value of $P<0.05$ was considered statistically significant. The risk factors of *M. pneumoniae* infection were assessed using a binary logistic regression. Associations were quantified as odds ratios (ORs) and 95% confidence intervals (CIs). Analyses were carried out using the Statistical Package for the Social Sciences for

Table 1 The *M. pneumoniae* positive rates across different age groups

	Infants	Toddlers	Preschoolers	School-age children
Positive sample	405	2,708	753	347
Total sample	9,555	10,059	2,216	1,052
Positive percentage (%)	4.24	26.92	33.98	32.98

F=162.7532, P<0.01.

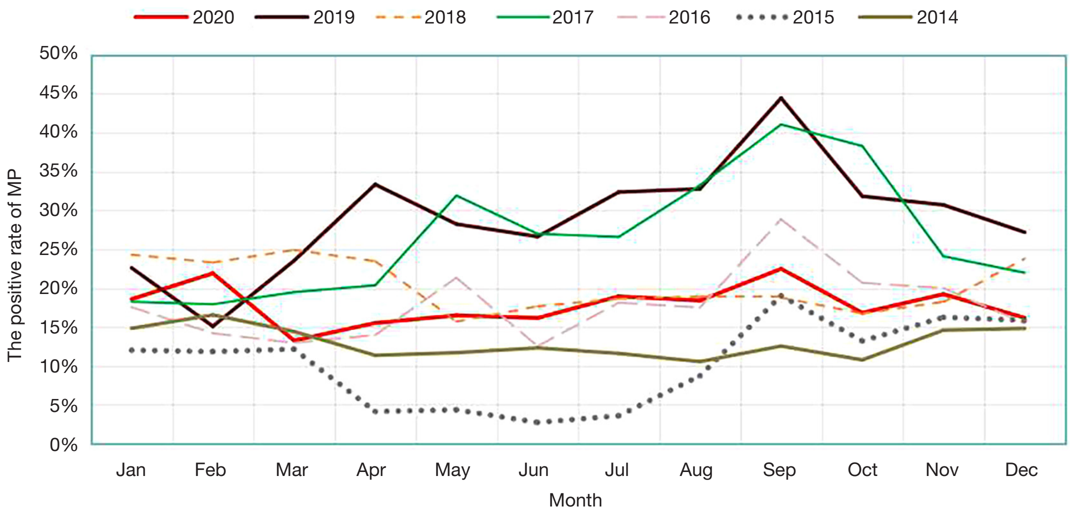

Figure 1 The seasonal incidence rates of *M. pneumoniae* from 2014 to 2020.

Windows (SPSS, version 11.0).

Results

The positive rates of M. pneumoniae infection across different genders

Of the total of 22,882 samples tested, 4,213 samples (18.41%) were positive for *M. pneumoniae* infection. Of these, 2,104 (18.30%) males were positive, and 2,109 (22.72%) females were positive (χ^2=198.078, P<0.01).

The positive rates of M. pneumoniae infection across different age groups

Overall, the positive percentages of *M. pneumoniae* infection were 4.24% in infants, 26.92% in toddlers, 33.98% in preschoolers, and 32.98% in school-age children. Notably, the positive percentage rate increased as the age of the patients increased (F=162.7532, P<0.01; see *Table 1*).

Annual incidence and seasonal incidence of M. pneumoniae infection

The positive rate of *M. pneumoniae* infection was significantly different among children of different ages (F=538.95, P<0.01). Notably, the incidence of *M. pneumoniae* infection in 2014 (13.14%), 2015 (10.47%), and 2020 (17.98%) was relatively low. However, the incidence of *M. pneumoniae* infection was significantly higher than the average rate in 2017 (26.59%) and 2019 (28.18%). Further, the incidence of *M. pneumoniae* infection was high from April to May, and September to October in 2016 (F=38.3378, P=0.0001), 2017 (F=110.8096, P<0.01), 2018 (F=21.6136, P=0.0275), and 2019 (F=35.6517, P=0.0002); however, no mensal preference was observed in 2014 (F=4.7018, P>0.05), 2015 (F=10.5878, P=0.4784), or 2020 (F=10.1404, P=0.5178; see *Figure 1*).

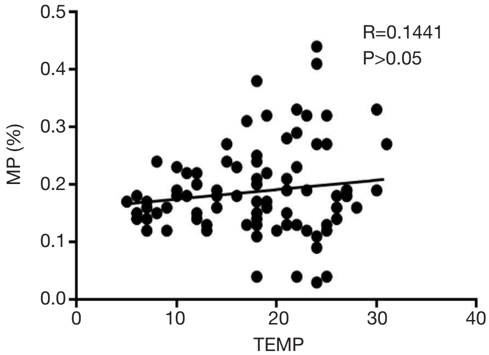

Figure 2 The correlation between a positive rate of *M. pneumoniae* infection and temperature.

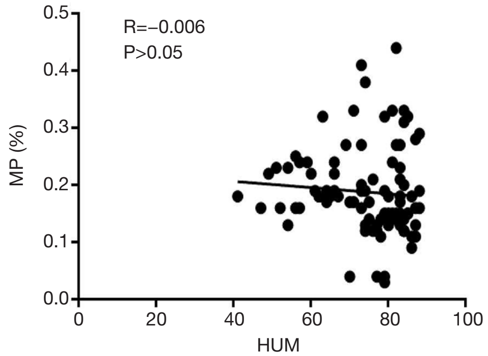

Figure 3 The correlation between a positive rate of *M. pneumoniae* infection and humidity.

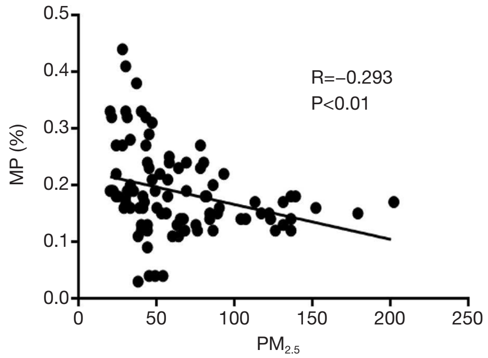

Figure 4 The correlation between the rate of *M. pneumoniae* infection and $PM_{2.5}$.

The association between M. pneumoniae infection and $PM_{2.5}$, PM_{10}, temperature, and humidity

There was no correlation between *M. pneumoniae* infection and temperature (R=0.1441, P>0.05) or humidity (R=–0.006,

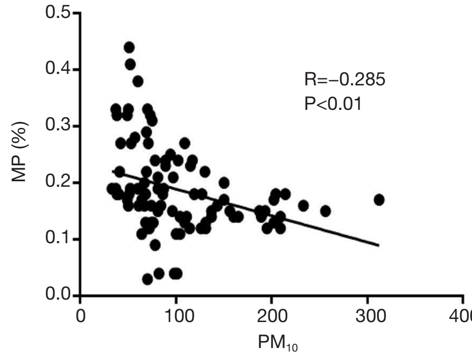

Figure 5 The correlation between the rate of *M. pneumoniae* infection and PM_{10}.

P>0.05; see *Figures 2,3*). However, there was a negative correlation between $PM_{2.5}$ (R=–0.293, P<0.01) and PM_{10} (R=–0.285, P<0.01) (see *Figures 4,5*).

The constituent ratios of cases of M. pneumoniae infection

There was no difference between the constituent ratios of cases of *M. pneumoniae* infection between 2014 and 2019 (F=32.34, P>0.05). Bronchopneumonia, a common respiratory disease of *M. pneumoniae* infection, accounted for the highest proportion of cases, followed by the exacerbation of asthma and severe pneumonia. However, there was a difference in the constituent ratio of cases of *M. pneumoniae* infection in 2020 compared to other years (F=159.35, P<0.01). The incidence of severe pneumonia decreased significantly in 2020, and bronchopneumonia accounted for the highest proportion of cases, followed by acute bronchitis and the exacerbation of asthma (see *Figure 6*).

Discussion

M. pneumoniae is a minimum microbe as bacteria and virus. It can cause pharyngitis, otitis, tracheobronchitis, or bronchopneumonia, and is transmitted by respiratory droplets. However, the positive rates of *M. pneumoniae* differ in different countries, different areas, across different ages, in different years and in different seasons. To provide more evidence for diagnoses and treatments, it is important to know the characteristics of *M. pneumoniae* in local areas.

The positive rate of *M. pneumoniae* was 18.41% for children in hospital for RTIs from 2014 to 2020 in Chengdu. This figure was lower than that of other areas in China (4). It was also lower than that of other countries,

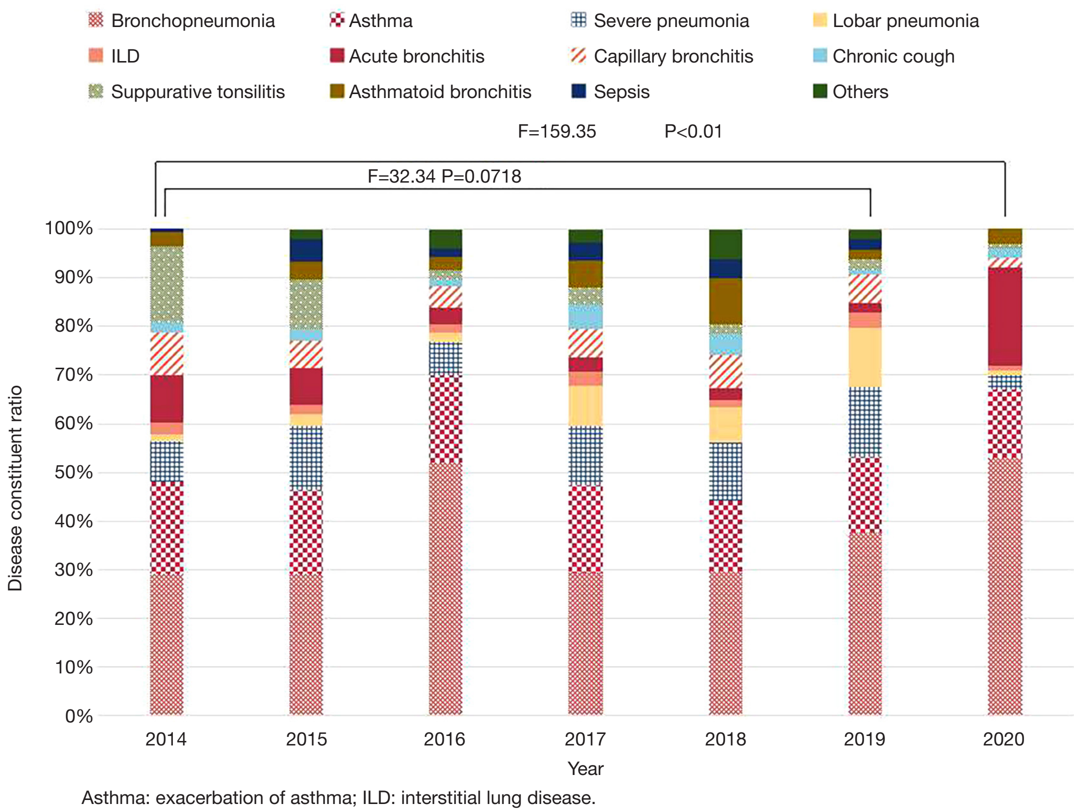

Figure 6 The constituent ratios of cases of *M. pneumoniae* infection.

such as Japan and Peru (6,7). However, it was higher than that in Russia where *M. pneumoniae* was detected to be about 15.9% (8). Thus, geographical factors appear to contribute to apparent differences in *M. pneumoniae* infection. However, it should be noted that the different results could have been affected by the various research samples and the different detection methods used.

The distribution of *M. pneumoniae* across sex differed across reports (6). The present study found that the positive percentage of *M. pneumoniae* in male children was lower than that in female children. Similar results have been reported in other areas in China (9,10). However, the reasons for such differences between the sexes remain unexplained. Thus, more studies need to be conducted to examine this issue in the future.

The data showed a significant association among age groups. Age is an important factor that can affect pathogen distribution. We found that preschoolers and school-age children had high incidence rates of *M. pneumoniae* infection of 33.98% and 32.98%, respectively. In China, children go to daycare when they are about 3 years old and to primary school when they are about 7 years old. Compared to the spacious housing conditions of infants and toddlers, the preschoolers and school-aged children lived or studied in crowded places because of the large populations, and *M. pneumoniae* spread easily among children (see *Table 1*; P<0.05). A study in Indian also reported the positive rate of *M. pneumoniae* infection was 59.5% in children (11). However, in older children, the immune response of organisms improves, and the pathogen can produce an effective response. Thus, *M. pneumoniae* infection plays an important role in this age group. Future research should seek to examine the learning and living environments of preschoolers and school-age children as conditions allow.

The positive percentage of *M. pneumoniae* was different in different years from 2014 to 2020. Epidemics of *M. pneumoniae* infection occur at intervals of 3 to 7 years (12). There was a general upward trend in volatility in the last 7 years of our study. It appears that in 2017 and in 2019 in Chengdu, there was a *M. pneumoniae* infection epidemic (the rates for these years were 26.59% and 28.18%, respectively). Similarly, that there was an outbreak of *M. pneumoniae* in

Finland in 2017 (13). Somewhat surprisingly, there was a lower incidence of *M. pneumoniae* infection in 2020 than other years, when COVID-19 spread in China. Further, there was no seasonal variation in the incidence of *M. pneumoniae* infection in 2020. The prevention and control of the COVID-19 epidemic began in January 2020. It appears that these prevention measures effectively controlled the infection rate of *M. pneumoniae*. *M. pneumoniae* infection can occur at any time of year, and were the most common from April to May and September to October in 2016, 2017, 2018, and 2019 in Chengdu (see *Table 1*; P<0.05); however, this was different in other areas (14,15). The present study demonstrated that there were seasonal variations in the incidence of *M. pneumoniae* infection. Interestingly, we also found that there was a relatively low rate of *M. pneumoniae* infection in winter vacation (February) and summer vacation (July to August) every year in Chengdu. It may be that the physical changes after vacations and crowded school environments play an important role in *M. pneumoniae* infection in children. It is important that doctors are aware of the character of sustainability and the aperiodic spreading of *M. pneumoniae* in local areas, and also regularly monitor *M. pneumoniae* to gain insights in how to avoid *M. pneumonia* infection at school.

In the present study, we examined whether there was an association between *M. pneumoniae* infection and $PM_{2.5}$ (particulate matter in the air <2.5 μm) and PM_{10} (particulate matter in the air <10 μm), temperature and humidity in Chengdu. Some cohort studies have identified consistent associations between ambient $PM_{2.5}$ and cardiorespiratory morbidity (16). Our study showed there was a negative correlation between the positive rate of *M. pneumoniae* infection and $PM_{2.5}$ and PM_{10}. Notably, there was serious air pollution in Chengdu in 2014 and 2015. This has been improved after some progress was made in environmental protection in 2020. Air pollution was found to increase the number RTI cases; however, it is not known whether the pathogeny of such cases was *M. pneumoniae*. The positive rate of *M. pneumoniae* infection was relatively low in 2014 and 2015. No association was found between *M. pneumoniae* infection and the average temperature and humidity of the climate, and it was different from other opinions (17). The temperature and humidity of the climate may affect the growth of *M. pneumoniae*; however, this study only analyzed the infection of *M. pneumoniae*, and did not examine the carriage of *M. pneumoniae*. It should be noted that high numbers of healthy children carry *M. pneumoniae* (18).

Of the children in-patients who were infected with *M. pneumoniae* between 2014 and 2019, cases of bronchopneumonia were the most common, followed by the acute exacerbation of asthma, and severe pneumonia. Some have conjectured that *M. pneumoniae* infection plays a key role in community-acquired pneumonia in children (19,20). However, other studies have shown that children with acute bronchitis have the highest positive rate of *M. pneumoniae* (21). These different results may have arisen as the data in this study was collected from in-patient children only and not outpatient children. Previous studies have shown that *M. pneumoniae* may play a role in the onset of asthma in predisposed children, and could trigger recurrent wheezing (22). The present study also confirmed that *M. pneumoniae* infection is frequently associated with exacerbations of asthma in children (23). It may be that children with *M. pneumoniae* infection with exacerbations of asthma have undergone empirical treatments before the exact pathogen had been identified. *M. pneumoniae* pneumonia is typically mild and self-limiting; however, severe *M. pneumoniae* pneumonia can cause severe pulmonary complications (e.g., obliterative bronchitis, bronchiectasis, and necrotizing pneumonia), and has increased in China (24). In the present study, we also found that cases of severe pneumonia are common in children in-patients who were infected with *M. pneumoniae*. Further, Lobar pneumonia increased in 2017, 2018, and 2019. However, the rate of severe pneumonia was significantly reduced in 2020. COIVD-19 may have weakened the virulence of *M. pneumoniae*. Future research should seek to confirm this in the future. However, in epidemic times, we should be aware that patients with *M. pneumonia* may be at risk of severe lung disease.

In conclusion, this retrospective analysis of *M. pneumoniae* in Chengdu from 2014 to 2020 revealed characteristics about *M. pneumoniae* in the local area. These results can help doctors to make clinical diagnoses, determine treatments, and prevent RTIs. By comparing the different characteristics of *M. pneumoniae* in 2020 to those of other years, we found that prevention strategies, such as health education, washing hands, and wearing masks, can control the epidemic of *M. pneumoniae*. This study had several limitations. First, it did not analyze the characteristics of outpatients with *M. pneumoniae* infection. Second, the tests conducted did not analyze patients with single *M. pneumoniae* infection or co-infections with other pathogenies.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/tp-21-139

Data Sharing Statement: Available at http://dx.doi.org/10.21037/tp-21-139

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tp-21-139). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China (NO.: B2021[8]). Individual consent for this retrospective study was waived.

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