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Influence of COVID-19 pandemic on the epidemiology of *Mycoplasma pneumoniae* infections among hospitalized children in Henan, China

Jiayue Ma, Pengbo Guo, Shiyue Mei, Mingchao Li, Zhidan Yu, Yaodong Zhang, Adong Shen, Huiqing Sun [*], Lifeng Li [**]

Henan International Joint Laboratory of Children's Infectious Diseases, Department of Neonatology, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China

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ABSTRACT

Background: Increasing reports have indicated that non-pharmaceutical interventions to control the COVID-19 pandemic may also have an effect on the prevalence of other pathogens. *Mycoplasma pneumoniae* is an important atypical pathogen prevalent in children with high rates of macrolide resistance. The aim of this study was to investigate the epidemiological characteristics of *M. pneumoniae* infection in children before and during the COVID-19 pandemic.
Methods: In this study, *M. pneumoniae* detection results were extracted from Henan Children's Hospital from 2018 to 2021. The epidemiological characteristics of pediatric *M. pneumoniae* infection were analyzed.
Results: We found that the highest positive rate of *M. pneumoniae* infection was 11.00 % in 2018, 14.01 % in 2019, followed by 11.24 % in 2021 and 8.75 % in 2020 ($p < 0.001$). Most tested children had respiratory system manifestations, and pneumoniae was the most common diagnosis (53.23 %). An increase in the number of positive cases was observed with an increase in age, with a higher number of cases among children over 6 years old. No positive cases were identified among children aged 1–28 days. The decrease in the positive rate among children aged between1–6 years old in 2020 and 2021 was found to be statistically significant ($p < 0.001$). The pre-pandemic period demonstrated a higher incidence rate in the fall, whereas the summers and winters exhibited a significantly higher positive rate during the pandemic period ($p < 0.001$). Different regions in Henan also showed different epidemic patterns.
Conclusions: In summary, strict pandemic measures influenced the spread of *M. pneumoniae* to some extent and changed demographic characteristics, including age, season and regional distribution. Continuous monitoring is required for the control and prevention of related diseases.

1. Introduction

Since Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), was first

* Corresponding author.
** Corresponding author.
E-mail addresses: s_huiqing@sina.com (H. Sun), lsbks1017@126.com (L. Li).

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reported in December 2019, there have been high mortality rates worldwide [1]. Henan is a populous province in central China that is subject to high levels of migration. As of February 29, 2020, COVID-19 cases have been reported in all 18 provincially administered cities in Henan, including pediatric cases [2]. Researchers have found that non-pharmaceutical interventions (such as maintaining social distance, wearing masks, limiting crowd gathering, and restricting outdoor activities) for COVID-19 have not only limited the spread of SARS-CoV-2, but also changed the demographic characteristics of other pathogen infections such as influenza virus, respiratory and enteric viruses, *Acinetobacter baumannii* and *Streptococcus pneumoniae* [3–7].

Mycoplasma pneumoniae (*M. pneumoniae*) is one of the smallest prokaryotic organisms without a rigid cell wall, which attaches to host cells mainly via the attachment organelle, a 170 kDa protein called P1 [8]. *M. pneumoniae* spreads through droplet transmission, and has an incubation period of one to three weeks [8]. *M. pneumoniae* primarily infects the respiratory tract, and is a prevalent cause of community-acquired pneumonia (CAP) worldwide [9]. However, the spread of infection by autoimmune mechanisms can also result in complications in other systems [10]. The most common clinical symptoms are cough, expectoration, fever, myalgia and dyspnea [11], whereas asymptomatic infection is also possible due to recurrent infection, thus causing outbreaks in families [8]. *M. pneumoniae* epidemiological characteristics can vary by age, gender, geography and season, and be influenced by detection methods [12,13]. The similarity of *M. pneumoniae* transmission pathways with COVID-19 transmission pathways may indicate similar effects by the non-pharmaceutical interventions.

The treatment of *M. pneumoniae* infections in children typically involves the use of macrolides and other alternative antibiotics such as tetracyclines or fluoroquinolones [14].However, there is a significant prevalence of macrolide resistance in Asia, ranging from 90 to 100 % [15]. Our earlier study showed that the rate of macrolide-resistant *M. pneumoniae* reached 100 % by detecting mutations in the 23S rRNA gene [9]. There are differences in the resistance rates among different countries and regions, with previous studies indicating that even though *M. pneumoniae* infection is self-limiting disease, whereas severe cases of *M. pneumoniae* infection could lead to poor clinical outcomes in patients with serious complications [16].

In this study, we aimed to analyze the epidemiological characteristics of pediatric *M. pneumoniae* infection in Henan, China from 2018 to 2021 to explore the influence of the COVID-19 pandemic on *M. pneumoniae* prevalence features and to provide guidance for disease treatment and infection prevention.

2. Methods

2.1. Patients and clinical specimens

A total of 66, 819 children with *M. pneumoniae* detection records were retrospectively analyzed between January 2018 and December 2021 at Henan Children's Hospital, a tertiary care teaching hospital located in Henan, China. Clinical data and epidemiological characteristics of children were obtained from electronic medical records. Part of the data were used in a publication of a letter to the editor [17]. The majority of the included cases were clinically diagnosed with respiratory and infectious diseases including pneumoniae, bronchitis, upper respiratory infections, Flu and asthma. Some patients admitted with other systemic disorders with indicators of infection and *M. pneumoniae* detection records were also included. All enrolled children meet the following criteria: (1) children aged below 18 years old; (2) at least one respiratory symptom (cough, dyspnea, combined with body temperature $>37.5\ ^\circ$C); (3) *M. pneumoniae* test performed; (4) SARS-CoV-2 tested negative; (5) without congenital malignant tumor; (6) without congenital pulmonary airway obstruction; (7) exclude recurrent chronic respiratory infection patients [18]. As a general pediatric hospital in a populous province, over 95 % of the patients come from different cities of Henan; the overall composition of patients can represent the whole Henan province to a certain extent. These patients were divided into five age groups: under 28 days (0–28 d), 1–12 months (1–12 m), 1–3 years (1–3 y), 3–6 years (3–6 y) and over six years old (>6 y). The seasonal, monthly and geographical distribution of *M. pneumoniae* were analyzed.

This retrospective study was approved by the Ethics Committee of Henan Children's Hospital (2023-K-078). Informed patient consent was waived.

2.2. M. pneumoniae RNA detection

M. pneumoniae RNA detection was performed routinely in clinical diagnosis using the kit provided by Shanghai Rendu Biotechnology Co. LTD with the StepOneRealTime fluorescence quantitative PCR instrument produced by Applied Biosystems (ABI) [9]. Nucleic acids were extracted using a magnetic bead separation device from throat swab, bronchial alveolar lavage fluid (BALF) and sputum samples. Primers and probes targeted to 16s rRNA were used to detect *M. pneumoniae*. The detection limit of this assay was 1000 DNA copies/mL.

2.3. Statistical analysis

Statistical analysis of the data was performed using SPSS version 25.0 software (IBM Corp., USA). Categorical variables including age, gender, sample types and seasons were analyzed using the Chi-squared test. Continuous-variable comparisons between the years were performed using the Student's t-test. P value (two-tailed) < 0.05 was considered statistically significant.

3. Results

3.1. Overall detection information of *M. pneumoniae* infection in children before and during the COVID-19 pandemic

Samples from a total of 66, 819 children were analyzed from January 2018 to December 2021 in Henan Children's Hospital, including 15,494 cases in 2018, 23,138 cases in 2019, 13, 627 cases in 2020 and 14, 560 cases in 2021 (Table 1). Before the COVID-19 pandemic (2018 and 2019), 12.80 % of children had tested *M. pneumoniae* positive 4, 946/38, 632), whereas during the first and second year of pandemic (2020 and 2021), the positive rates were 8.75 % (1193/13,627) and 11.24 % (1636/14,560), respectively, which was significantly lower than pre-pandemic ($\chi^2 = 244.840$, $p < 0.001$). In Henan, 7, 775 positive cases, 4, 386 (56.41 %) were boys, and 3, 389 (43.59 %) were girls, resulting in a gender ratio of 1:1.29. We statistically analyzed the gender ratio of the *M. pneumoniae* positive patients enrolled from 2018 to 2021 in this study by comparing with that of the *M. pneumoniae* negative patients. We analyzed the data of boys and girls every year and found that only in 2019, the positive rate of boys was significantly higher than that of girls ($\chi^2 = 19.183$, $p < 0.001$), and the overall gender difference from 2018 to 2021 showed that there was no difference between boys and girls in *M. pneumoniae* infection ($\chi^2 = 6.681$, $p = 0.083$).

Our analysis revealed that throat swabs were the most commonly obtained samples followed by BALF and sputum. Results indicated a significant increase in the positive rate of throat swab in 2021 as compared to 2019 (82.15 % vs 58.22 %, $\chi^2 = 31.913$, $p <$ 0.001), whereas the positive rates of both BALF and sputum samples were found to be significantly lower ($\chi^2 = 14.474$, $p < 0.001$; $\chi^2 =$ 57.318, $p < 0.001$) (Table 1).

3.2. Clinical manifestations of *M. pneumoniae* infection

Clinical manifestations of cases with a clear medical diagnosis or symptoms were analyzed. Respiratory system-associated symptoms accounted for 89.14 % (59, 562/66, 819) of cases with a significant difference in comparison to disorders affecting systems ($\chi^2 = 416.638$, $p < 0.001$), and most of these cased were diagnosed as pneumoniae caused by *M. pneumoniae*. The types of pneumonia included asthmatic pneumonia, lobar pneumonia, interstitial pneumonia, prolonged pneumonia and bronchopneumonia. Non-pneumoniae respiratory manifestations include respiratory tract infections, bronchitis, upper respiratory associated disease (such as rhinitis and tonsillitis), influenza, asthma and other disorders. *M. pneumoniae* was detected in 5.53 % of the children presenting with a fever and 5.48 % with cough, respectively (Table 2).

3.3. Age distributions of *M. pneumoniae* infection in children before and during the COVID-19 pandemic

The mean age of children positive for *M. pneumoniae* was 5.33 ± 2.65 years (ranging from 0.10 to 15.00 years). Prior to the pandemic, the mean age of children positive for *M. pneumoniae* was 5.24 ± 2.61 years (ranging from 0.10 to 15.00 years), and during the pandemic, the mean age was 5.47 ± 2.72 (ranging from 0.12 to 15.00 years), with a statically significant difference ($t = -3.609$, $p < 0.001$). Among the five age groups, there were no positive cases of *M. pneumoniae* was found in children between 0 and 28 days old from 2018 to 2021. The age distribution of *M. pneumoniae* is shown in Fig. 1. The positive rate and number of infections increased with age, with a peak in the >6-year-old age group, where the number of *M. pneumoniae* positive children was six times higher than that of children aged 1–12 months (522 and 3, 120) (Fig. 1A–B). In 2018, the number of children aged between 3 and 6 years old was prior to children over 6 years old. The high positive rate in the 1–3 y and 3-6 y age groups was statistically significant less than pandemic period ($\chi^2 = 82.390$, $p < 0.001$; $\chi^2 = 150.108$, $p < 0.001$), whereas in 2020, the detection rate of *M. pneumoniae* in children aged 1–12 m and >6 years (5.45 % vs 4.72 %; 44.17 % vs 39.80 %) ($\chi^2 = 6.495$; $p = 0.011$; $\chi^2 = 61.373$, $p < 0.001$) increased compared to 2019. The positive rate of >6 years showed climbing trend annually, the data during the pandemic were statistically higher than data before the pandemic ($\chi^2 = 22.740$, $p < 0.001$).

3.4. Seasonal and monthly distribution of *M. pneumoniae* infection in children before and during the COVID-19 pandemic

The seasonal distribution of *M. pneumoniae* positive cases from 2018 to 2021 was analyzed (Fig. 2A–C). Positive cases in most seasons decreased in 2020 and 2021, aside from winter in 2020 and summer in 2021 (Fig. 2A). The average positive rates in spring (March–May), summer (June–August), autumn (September–November) and winter (December–February) were 11.87 % (923/7, 775),

Table 1
Basic information of *M. pneumoniae* detection from 2018 to 2021.

Characteristic		2018 (n = 15,494)	2019 (n = 23,138)	2020 (n = 13,627)	2021 (n = 14,560)	χ^2	*P* value
M. pneumoniae positive specimens		1705 (11.00)	3241 (14.01)	1193 (8.75)	1636 (11.24)	244.840	<0.001
Gender	Male	972 (57.01)	1776 (54.80)	682 (57.17)	956 (58.44)	6.681	0.083
	Female	733 (42.99)	1465 (45.20)	511 (42.83)	680 (41.56)		
Sample types	Oropharyngeal swab	543 (31.85)	1887 (58.22)	788 (66.05)	1344 (82.15)	281.231	<0.001
	BALF[a]	544 (31.91)	1131 (34.90)	383 (32.10)	270 (16.50)	52.095	<0.001
	Sputum	617 (36.19)	223 (6.88)	22 (1.84)	22 (1.34)	101.717	<0.001

[a] BALF is short for Bronchoalveolar lavage fluid.

Table 2
Clinical manifestations of children with and without *M. pneumoniae* infection.

		Total (n = 66, 819)	Positive (n = 7, 775)	Negative (n = 59, 044)
Diagnosis	Respiratory and infectious diseases	59562	7457	52105
	Pneumoniae	35570	6351	29219
	Non-pneumoniae	23992	1106	22886
	Respiratory tract infection	8515	380	8135
	Bronchitis	8279	502	7777
	Upper respiratory tract associated diseases	5581	173	5408
	Flu	1688	39	1649
	Asthma	374	9	365
	Other systemic disorders with indicators of infection	7257	318	6939
Symptoms	Fever	2468	124	2344
	Sepsis	840	20	820
	Cough	383	21	362

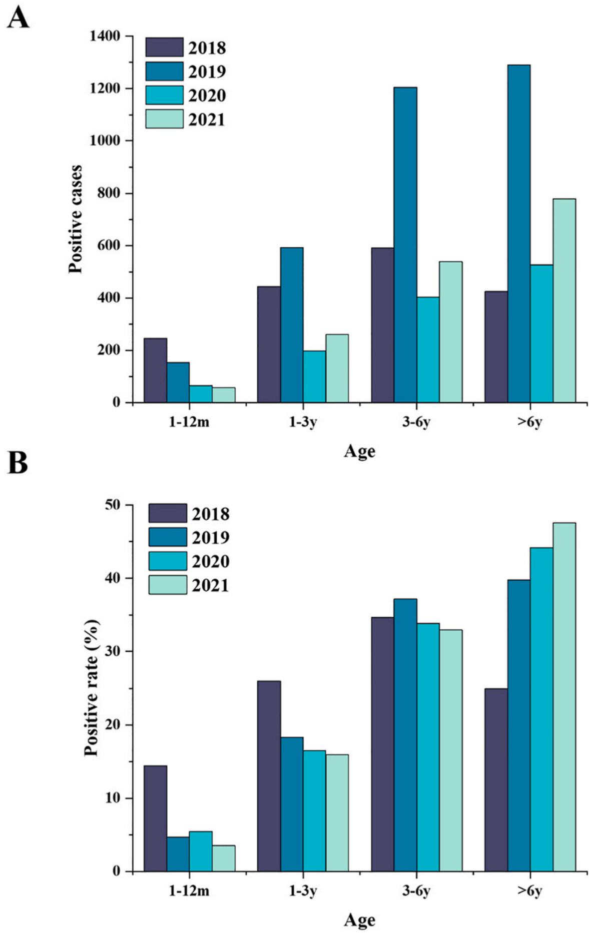

Fig. 1. Age distribution of *Mycoplasma pneumoniae*. Positive number (A) and positive rate (B) of *M. pneumoniae* in different age groups among children from 2018 to 2021.

23.41 % (1, 820/7, 775), 36.01 % (2, 800/7, 775), 28.71 % (2, 232/7, 775), respectively. Before the pandemic, *M. pneumoniae* detection rate peaked in the fall (43.63 %, 2, 158/4, 946), and there was a significant increase compared to other three seasons ($\chi^2 = 197.625, p < 0.001$; $\chi^2 = 136.113, p < 0.001$; $\chi^2 = 290.266, p < 0.001$). In 2020, the positive rate was highest in winter ($\chi^2 = 786.438, p < 0.001$), whereas the positive rate in summer of 2021 was significantly higher than winter ($\chi^2 = 367.972, p < 0.001$). We found that the *M. pneumoniae* positive rate in summer and winter before the outbreak was significantly lower compared to the outbreak period ($\chi^2 = 8.253, p = 0.004$; $\chi^2 = 6.462, p = 0.011$), whereas in fall, the positive rate in 2018 and 2019 was significantly higher than that in 2020 and 2021 ($\chi^2 = 414.101, p < 0.001$). The positive rate of *M. pneumoniae* infection during the pandemic did not show a similar

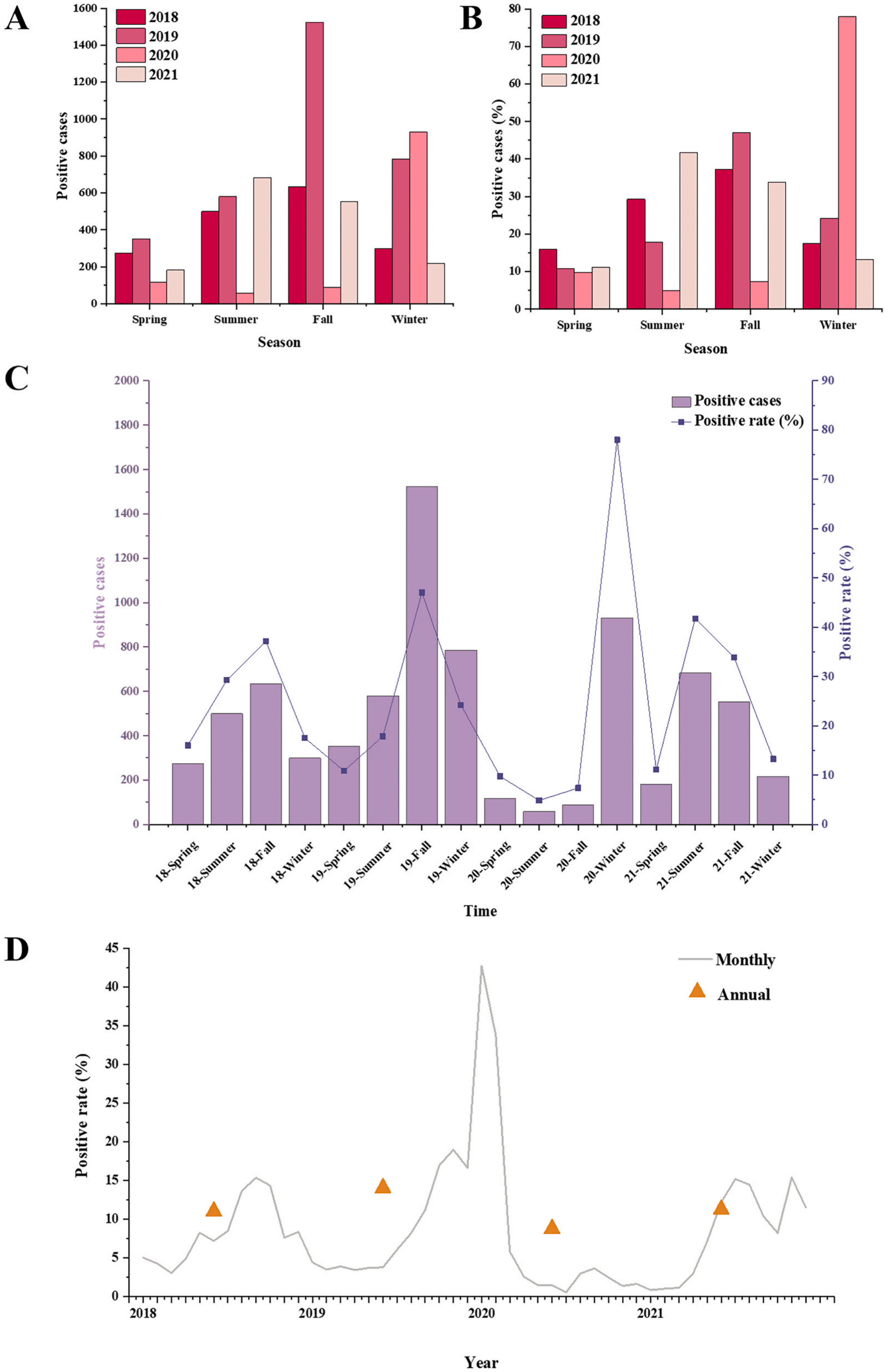

Fig. 2. Seasonally and monthly distribution of *Mycoplasma pneumoniae* infection among children from 2018 to 2021. *M. pneumoniae* positive number (A) and positive rate (B) in different seasons. General trend of *M. pneumoniae* positive rate from 2018 to 2021 in different seasons (C) and different months (D).

trend to 2018 and 2019 either, which corresponds with the results of the seasonal analysis (Fig. 2D).

3.5. Geographical distribution of *M. pneumoniae* infection in children before and during the COVID-19 pandemic

Geographical distribution of *M. pneumoniae* infections among 61, 647 children with definite geographical information were analyzed, with 34, 600 infections occurring prior to the COVID-19 pandemic and 27, 047 infections occurring during the pandemic, another 5172 cases were not included in the geographical statistics due to the ambiguity of electronic medical records (Fig. 3A–B).

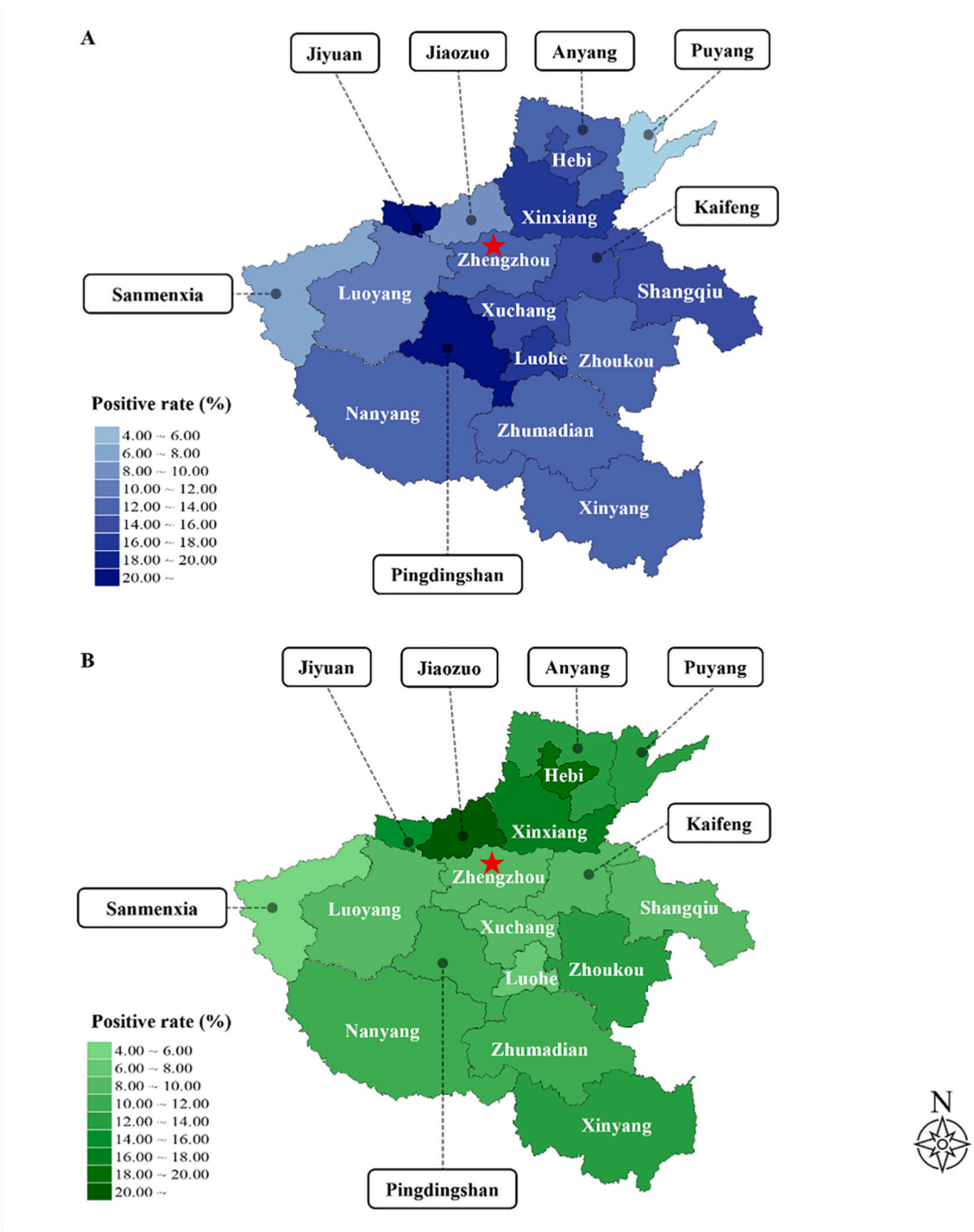

Fig. 3. Comparison between different cities in Henan province according to *Mycoplasma pneumoniae* positive rate during 2018–2021. (A) *M. pneumoniae* positive rate of Henan in 2018 and 2019 pre-pandemic; (B) *M. pneumoniae* positive rate map of Henan in 2020 and 2021 during pandemic period.

98.33 % of them were in Henan Province, distributed across all eighteen cities in Henan. Zhengzhou, the capital city of the province of Henan, recorded the highest number of cases, which was consistent with its population density. The detection rate was both higher than the provincial overall detection rate before and after the pandemic (13.70 % vs 12.80 %, 10.06 % vs 10.04 %) in Zhengzhou, and had dramatically decreased in pandemic ($\chi^2 = 96.871$, $p < 0.001$). According to Fig. 3, nine cities exhibited positive rates *M. pneumoniae* infection above 14.00 % in 2018 and 2019, compared to the positive rates in four cities in 2020 and 2021. The top five *M. pneumoniae* positive cities were Jiyuan, Pingdingshan, Luohe, Xinxiang and Hebi, respectively in 2019. Jiaozuo, Hebi, Xinxiang, Jiyuan and Anyang recorded the highest positive rate in 2020 and 2021. The *M. pneumoniae* positive rate in Anyang, Hebi, Jiaozuo, Puyang and Xinyang showed an increasing proneness of infection, while there was a significantly high in the infection of Jiaozuo and Puyang ($\chi^2 = 27.676, p < 0.001$; $\chi^2 = 7.986, p = 0.005$). Of the additional thirteen cities, six cities had statistically significant declining detection rates, consisting of Zhenghou, Pingdingshan, Xuchang, Shangqiu, Kaifeng, Luohe and Zhumadian had significant differences ($\chi^2 = 96.871, p < 0.001$; $\chi^2 = 24.000, p < 0.001$; $\chi^2 = 13.317, p < 0.001$; $\chi^2 = 9.833, p = 0.002$; Kaifeng $\chi^2 = 7.885, p = 0.005$; $\chi^2 = 7.256, p = 0.007$; $\chi^2 = 2.875, p = 0.090$).

4. Discussion

Since the outbreak of COVID-19, the government has taken various actions to control the pandemic. Mitigating measures used for children included the adoption of online classes, the restriction of outdoor sports, and the expansion of vaccination coverage to the adolescent demographic. Without non-pharmaceutical interventions, the number of COVID-19 cases would have increased 67 times in the first two months of the outbreak (until 29 February 2020) in China [19]. During the pandemic (2020–2021), our region suffered two large-scale lockdowns, one was from January to May 2020, the other was from July to October 2021. Residents were asked to keep indoors, and the schools and day care facilities suspended offline teaching, in order to reduce transmission of the virus among patients of all ages. These interventions may also affect the transmission of other respiratory tract infections.

M. pneumoniae is a common pathogen, which can cause upper respiratory tract infection, lower respiratory tract infection, and extrapulmonary clinical symptoms, and the most common infection is CAP. According to statistics, 10 %–40 % of pneumonia cases in school-age children and adolescents can be attributed to *M. pneumoniae* [9]. According to a national surveillance in China from 2009 to 2019, the all-age incidence of *M. pneumoniae* was 18.6 %, which ranked the third most common pathogen among children and the most prevalent among school-age children [20]. Scholars have reported a reduction of *M. pneumoniae* transmission influenced by COVID-19 non-pharmaceutical interventions [21,22]. Both SARS-CoV-2 and *M. pneumoniae* spread via the respiratory tract. Hence, it is important to analyze the prevalent features of *M. pneumoniae* infection in children before and during the COVID-19 pandemic.

In this study, *M. pneumoniae* positive rate of Henan in 2018 and 2019 was 12.80 %. Study of *M. pneumoniae* epidemiology from 2014 to 2019 across Luoyang, a city in Henan, indicated that *M. pneumoniae* detection rate was 45.6 % [23]. Higher rate compared to this study could due to the inclusion criteria were narrower than in this study and the regional difference. By comparing the annual data, we found that the number of children tested for *M. pneumoniae* fell by an average of 13.52 % per year. The positive rate of *M. pneumoniae* before the pandemic was 1.28 times that after the pandemic. The total number of children tested and the positive rate showed a declining trend. This was consistent with previous studies. Cai et al. [24] found that the positive rate of *M. pneumoniae* decreased from 32.9 % in 2019 to 21.5 % in 2020 in Zhejiang, China. According to epidemiological statistics of *M. pneumoniae* from 2017 to 2020 in Chengdu, China [25], the total number of confirmed *M. pneumoniae* pediatric cases significantly decreased in the second quarter of 2020. What is noteworthy is that after the end of social quarantine restrictions, other pathogens resurged in the community and respiratory syncytial virus resumed the normal epidemic cycle [26], whereas the *M. pneumoniae* positive rate was still lower than pre-pandemic rates [21,27]. The possible reason for the lower positive rate is the slower generation time (6 h) and incubation period (1–3 weeks) of *M. pneumoniae*, which may require a longer time interval to re-establishment in the population after non-drug interventions are discontinued [27]. Otherwise, changes of *M. pneumoniae* subtypes could also be one of the possible factors leading to dynamic changes of *M. pneumoniae* epidemic. Our previous study indicated that *M. pneumoniae* adhesion protein P1 could shift from type 1 to type 2, which was related to different clinical manifestations and macrolide resistance phenotype [28]. Therefore, the different prevalent subtypes of *M. pneumoniae* may cause the epidemiological changes.

Since usual examinations are unable to distinguish *M. pneumoniae* infections from other manifestations, clinicians do not have a reliable way to affirm *M. pneumoniae* infection without diagnostic tests [11]. Empiric treatment could accompany inappropriate antibiotic use, which might induce higher antibiotic resistance and worsen the prognosis. Hence, it is important to summarize the clinical symptoms of *M. pneumoniae* infection in children to assist with diagnosis. Fever, cough and shortness of breath were considered as a triad of typical symptoms [29]. Wang et al. [30] reported that among children with CAP, the presence of chest pain and crepitations were associated with *M. pneumoniae* infection. The former was found to significantly increase the likelihood of infection by more than twofold. Otherwise, a combination of prodromal fever and respiratory symptoms >6 days; pathological findings on pulmonary auscultation and biomarker procalcitonin <0.25 μg/L and elevated C-reactive protein levels could improve clinical diagnostic performance [31,32]. Even though *M. pneumoniae* infection was associated with various systems, in our study, most of the cases were pneumonia. Upper respiratory tract associated diseases were relatively rare, which was consistent with previous studies [29]. Fever and cough were commonly observed among the pediatric population with *M. pneumoniae* infection. In some cases, children presenting with sepsis as the primary complaint also tested positive for *M. pneumoniae*, suggesting the possibility of co-infection with other pathogens or the occurrence of *M. pneumoniae* or antibiotic resistance.

Three different types of samples, including oropharyngeal swab, BALF and sputum were collected to detect MP-RNA. Previous findings indicate that oropharyngeal swab and sputum specimens were more effective than nasopharyngeal swab specimen in detecting *M. pneumoniae* [33,34]. The collection of BALF specimens is more challenging, and it is typically used for severe

M. pneumoniae pneumonia cases. Oropharyngeal swab specimens increased during pandemic period, while the positive rate of BALF and sputum specimens decreased. This might result from two reasons: first, the collection method for oropharyngeal swab specimens was improved, similar to that used for SARS-CoV-2 nucleic acid collection, and second, measures taken to respond to the COVID-19 pandemic likely reduced total cases of *M. pneumoniae* pneumonia, leading to a decrease in the collection of BALF specimens. Clinically, BAL was conducted only in cases with severe pneumonia during the pandemic. Therefore, the overall rate of *M. pneumoniae* infections could be influenced.

According to the age distribution of *M. pneumoniae* infections, incidences of *M. pneumoniae* occurred in children over 6 years old. This corresponds with the findings of a report that summarized data from 2017 to 2019, which pointed out that the highest incidence rate of *M. pneumoniae* pneumonia occurred among children between 7 and 16 years old [35]. Reports in India [36], America [11] and some European countries [37] showed similar results. Children of school age or older are more prone to infect *M. pneumoniae* by droplet transmission compared to infants and preschoolers. They get the highest infection might be due to increased exercise and exposure to the external world as compared to the infants. Meanwhile, before the age of five is a critical period for the development of the immune system, with the loss of mother-derived immune mediators, which may be another important factor for the high incidence of *M. pneumoniae* infection in children at this age [38]. Nevertheless, Li et al. [20] analyzed *M. pneumoniae* infection data from 2009 to 2019 and found that there was an obvious descending turning point for *M. pneumoniae* cases at 7 years old. This turning point dropped with increasing age, which is contrary to our findings. These contrary results could be due to differences in collection times, geographical locations, testing methods and COVID-19 impact. Furthermore, our data showed that the positive rates among children aged of 1–6 years old during pandemic were significantly lower than those in the pre-pandemic era, suggesting that interventions to control the spread of SARS-CoV-2 may have had an impact on limiting the dissemination of *M. pneumoniae*. Studies have reported a decline in the incidence of *M. pneumoniae* outbreaks in primary schools [39] and military academies [40] because of COVID-19 prevention measures. Our analysis of gender differences of *M. pneumoniae* infections revealed no disparity between infected boys and girls, which correspond with previous findings [25,41,42]. Previous studies have reported differences in the seasonal distribution of *M. pneumoniae* infections, with higher incidences during the fall and winter compared to spring and summer [41,43]. However, seasonal tendencies can vary annually [44]. Our data revealed that the incidence of *M. pneumoniae* infections were higher in fall compared to other seasons before the pandemic, whereas cases during the summer and winter were more prevalent after the pandemic. In a monthly analysis, the highest positive rate was observed in January 2020, at the onset of the pandemic. Two peaks in the incidence of *M. pneumoniae* infections were noted in November and July 2021, with the former being a common time for *M. pneumoniae* infections and the latter being potentially influenced by temperature as seen in a previous report that showed a positive correlation between increased temperature and *M. pneumoniae* infections [45]. Overall, the positive rate during the pandemic period was lower compared to the pre-pandemic period, and the seasonal distribution of *M. pneumoniae* infections has changed, suggesting an impact induced by COVID-19 restriction measures.

5. Conclusion

In conclusion, this study demonstrated that the overall incidence of *M. pneumoniae* infections has decreased after the COVID-19 outbreak, especially in children aged 1–6 years old. This decrease may be influenced by the measures taken to control the spread of SARS-CoV-2. The susceptible seasons for *M. pneumoniae* infection have changed from the fall to summer and winter because of the pandemic. Our study provides important complementary information on the molecular epidemiology of *M. pneumoniae* infection in children in Henan, China. Further continuous surveillance and multi-center studies with long-term longitudinal data are needed to gain a more comprehensive understanding of the epidemiology of *M. pneumoniae* to enhance the prevention and treatment of related diseases.

Ethical approval

This retrospective study was approved by the Ethics Committee of Henan Children's Hospital (2023-K-078). Informed patient consent was waived.

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Data availability statement

As a retrospective analysis, the data are not uploaded to a public database. Meanwhile, key data could be obtained from the chart in the article. The raw datasets used in the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Jiayue Ma: Writing – original draft, Methodology, Conceptualization. **Pengbo Guo:** Resources, Investigation, Formal analysis.

Shiyue Mei: Methodology, Formal analysis. **Mingchao Li:** Writing – review & editing, Methodology. **Zhidan Yu:** Writing – review & editing, Data curation. **Yaodong Zhang:** Writing – review & editing, Supervision. **Adong Shen:** Writing – review & editing, Supervision. **Huiqing Sun:** Writing – review & editing, Supervision, Formal analysis. **Lifeng Li:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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