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Mycoplasma pneumoniae infection outbreak in Guangzhou, China after COVID-19 pandemic

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Abstract

Backgrounds *Mycoplasma pneumoniae* (*M. pneumoniae*) is a common pathogen causing respiratory diseases in children. This study aimed to characterize epidemiological and disease severity shifts of *M. pneumoniae*: infections in Guangzhou, China during and after the coronavirus disease 2019 (COVID-19) pandemic.

Methods Throat swab samples were obtained from 5405 hospitalized patients with symptoms of acute respiratory infections to detect *M. pneumoniae*. Differences in epidemiological and clinical characteristics of *M. pneumoniae*: infections were investigated during 2020–2022 and after COVID-19 pandemic (2023).

Results *M. pneumoniae* were detected in 849 (15.6%, 849/5405) patients. The highest annual positive rate was 29.4% (754/2570) in 2023, followed by 5.3% (72/1367) in 2022, 1.2% (12/1015) in 2021, and 2.0% (11/553) in 2020, with significantly increasing annual prevalence from 2020 to 2023. *M. pneumoniae* incidence peaked between July and December post-COVID-19 pandemic in 2023, with the highest monthly positive rate (56.4%, 165/293). Clinical characteristics and outcomes of patients with *M. pneumoniae* did not vary between periods during and after COVID-19 pandemic except that patients with *M. pneumoniae* post-COVID-19 pandemic were more likely to develop fever. Patients with severe *M. pneumoniae* pneumonia (SMPP) were more likely to develop respiratory complications, myocardial damage, and gastrointestinal dysfunction than those with non-SMPP. Patients with SMPP had lower lymphocytes, CD3$^+$ T cells, CD4$^+$ T cells, CD8$^+$ T cells, B cells, and higher IL-4, IL-6, IL-10 levels than those with non-SMPP. Bronchoalveolar lavage fluid specimens from infected patients were obtained to identify macrolide resistance mutations. Macrolide-resistant *M. pneumoniae* (MRMP) proportion in 2023 was 91.1% (215/236).

Conclusion Outbreaks of *M. pneumoniae*: occurred in Guangzhou, China in 2023 upon Non-pharmaceutical interventions easing. Despite the increasing incidence of *M. pneumoniae*, the disease severity remained similar during and after the COVID-19 pandemic.

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Li *et al. Virology Journal*   (2024) 21:183

Page 2 of 11

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*), which is the smallest self-living and cell-wall-less bacterium, commonly causes upper and lower respiratory tract infections, particularly in children and young adults [1–3]. *M. pneumoniae* infections contribute significantly to the disease burden in children, accounting for 10–30% of community-acquired pneumonia (CAP) cases [4, 5]. *M. pneumonia* infections may induce extrapulmonary complications, such as myocardial injury, abnormal liver function, kidney damage, encephalitis, gastrointestinal dysfunction, Stevens-Johnson syndrome, and arthritis [6–8].

During COVID-19 pandemic, the widespread adoption of non-pharmaceutical interventions (NPIs), such as mask wearing, hand hygiene, social distancing, travel restrictions, and school closure reduced the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and altered the prevalence patterns of other common respiratory pathogens [9]. Globally, *M. pneumonia* infections occur in regional outbreaks every three to seven years, each lasting for 1–2 years. A nationwide retrospective cohort study revealed that two epidemics of *M. pneumonia* (2011 and 2015) took place in South Korea [10]. Previous studies showed that there was an *M. pneumoniae* epidemic from 2015 to 2016 in Beijing [11]. Moreover, outbreaks of *M. pneumoniae* occurred in Southern Taiwan in 2019–2020, with the peak case numbers occurring between July 2019 and January 2020 [12]. Cheng et al. reported a *M. pneumoniae* outbreak in Beijing, China, in the summer of 2019, while *M. pneumoniae* activity notably reduced during the COVID-19 pandemic (2020–2021) [13]. Previous studies have shown significantly reduced *M. pneumoniae* positive rates in 2020 compared to 2012 and 2016 in Japan and Finland [14, 15]. Previous global *M. pneumoniae* surveillance studies revealed that the most recent *M. pneumoniae* epidemic occurred in late 2019-early 2020 simultaneously across multiple nations, predominantly in Europe and Asia; however, after the NPIs, *M. pneumoniae* positive rates dramatically dropped in 20 countries across Asia, Oceania, the Americas, and Europe during the COVID-19 pandemic in 2020–2022 [16, 17].

For most respiratory pathogens, a reduction was observed in the early phase of COVID-19 pandemic [18]. However, resurgence of respiratory pathogens was observed when NPIs eased [19]. Previous studies have reported a marked resurgence of respiratory syncytial virus(RSV) in England and Australia, which was associated with the relaxation of COVID-19 measures [20, 21].

Previous studies have reported an unusual resurgence of human metapneumovirus in South Korea and Western Australia after the COVID-19 pandemic [22, 23]. Global prospective surveillance data in previous study showed the re-emergence of *M. pneumoniae* in Europe and Asia in 2023 [24]. Changes in epidemiological, macrolide resistance, and clinical characteristics caused by *M. pneumoniae* infections when measures to restrict SARS-CoV-2 transmission were relaxed in Guangzhou, China remain unclear.

This study investigated the epidemiological, macrolide-resistant, and clinical characteristics of *M. pneumoniae* infections in two distinct periods, January 2020 to December 2022 and January 2023 to December 2023, encompassing the periods during and after the COVID-19 pandemic.

Materials and methods

Study population

From 1 January 2020 to 31 December 2023, hospitalized children under 14 years of age who were diagnosed with acute respiratory tract infections (ARTIs) at the First Affiliated Hospital of Guangzhou Medical University were enrolled. Diagnosis of pediatric *M. pneumoniae* pneumonia (MPP) was performed by attending physicians according to the Guidelines for Diagnosis and Treatment of *M. pneumoniae* pneumonia in Children as follows: (i) fever, or acute respiratory symptoms (cough, tachypnea, difficulty breathing) or both; (ii) low breathing or dry, wet rales; (iii) chest film findings characterized by lung portal lymph node and lung gate shadow, bronchopneumonia, interstitial pneumonia, and large and high-density shadow; (iv) children with positive polymerase chain reaction (PCR) results or *M. pneumoniae* antibody titer≥1:160. SMPP was defined as MPP with one of the following: (i) disturbance of consciousness; (ii) hypoxemia, consisting of respiratory rate (RR)≥70/min (infant), RR≥50/min (over 1 year old), assisted breathing (groan, nasal fan, three concave sign), intermittent apnea, and oxygen saturation<92%; (iii) persistent high fever for more than 5 days; (iv) dehydration; (v) chest X-ray showed that multiple lobes were involved or complicated with pleural effusion; and (vi) serious extrapulmonary complications, according to the guideline for diagnosis and treatment of CAP in children (2019 version) [25].

Specimen detection

Throat swab samples were collected within 24 h after admission for *M. pneumoniae* screening following established clinical protocols. Real-time polymerase chain

reaction (RT-PCR) testing was performed to *M. pneumoniae* using a commercial kit (Daan Gene Co., Ltd., Guangzhou, China) based on the recommended protocol. Amplifications were performed using an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, USA).

Lymphocyte subset detection

Ethylene diamine tetra acetic acid anticoagulated whole blood samples (2 mL) were collected from patients with SMPP ($n=108$) and non-SMPP ($n=281$) within 24 h after admission. Briefly, $CD3^+T$ cells, $CD3^+CD4^+T$ cells, $CD3^+CD8^+T$ cells, $CD19^+B$ cells, and $CD16^+CD56^+NK$ cells were measured using multi-color flow cytometry with panels 1 and 2. Panel 1 included CD3-FITC, CD4-APC, CD8-PE, and CD45-PerCP. Panel 2 comprised CD3-FITC, CD19-APC, CD16-PE, CD56-PE and CD45-PerCP (Agilent Multitest). Cells were acquired on a NovoCyte D3000 flow cytometer (Agilent Technologies, Palo Alto, USA) following the manufacturer's instructions. All flow cytometry data were acquired and analyzed using Agilent NovoExpress software.

Cytokine assays

The serum samples from patients with SMPP ($n=142$) and non-SMPP ($n=323$) were collected within 24 h after admission. The serum levels of interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17 A, interferon-α (IFN-α), IFN-γ, and tumor necrosis factor-α (TNF-α) were detected according to the 12 cytokine detection kit manufacturer's instructions (Jiangxi Saiji Biotechnology Limited Company) on a MateCyte 2L6C flow cytometer (Challen Biotechnology Co., Ltd., Hunan, China). The FCAP Array™ v 3.0.1 software returned data as Median Fluorescence Intensity and concentration (pg/ml).

Data collections

Demographic information, age, sex, clinical symptoms and signs, extrapulmonary complications, treatment and length of hospital stay were collected retrospectively from patient records. Laboratory findings including white blood cell (WBC) counts, neutrophil counts, lymphocyte counts, erythrocyte counts, hemoglobin (Hb), platelet (PLT), K+, Na+, Cl-, total protein (TP), albumin (ALB), total biliary acid (TBA), gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), procalcitonin (PCT), C-reactive protein (CRP), prothrombin time (PT), Fibrinogen (FIB), Activated partial thromboplastin time (APPT), D-dimer, Ceruloplasmin, immunoglobulin (Ig)G, IgM, and IgA were conducted within 24 h after admission.

### Macrolide-resistant mutations of *M. pneumoniae*

The main indications of bronchoalveolar lavage fluid (BALF) were as follows: (i) diagnosis of infection: persistent infection symptoms despite empirical treatment, necessitating clarification of the etiology, suspected infections involving unusual pathogens, mixed pathogens, or drug-resistant bacteria, and severe pneumonia requiring mechanical ventilation; (ii) therapeutic removal of airways materials; (iii) diagnosis of noninfectious lung diseases; (iv) evaluation of alveolar inflammatory processes [26]. Among the 754 cases of *M. pneumoniae* infection in 2023, 236 BALF samples from infected patients were obtained and transferred by an uninterrupted cold-chain transportation to KingMed, Guangzhou, China for tNGS detection to identify pathogens and macrolide-resistance mutation. Nucleic acid extraction was performed using the MetaPure DNA & RNA Extraction Kit (KingCreate, Guangzhou, China), and library preparation was performed using the RP100™ Respiratory Pathogen Microorganisms Multiplex Testing Kit (KingCreate, Guangzhou, China). Sequencing was carried out on Illumina MiniSeq Platform using the MiniSeq Rapid Reagent Kit (Illumina, CA, USA) as previously described [27]. The sequencing data generated was analyzed using a customized bioinformatics workflow. Fastp v0.20.1 was used for adaptor trimming and low-quality filtering, and Bowtie2 v2.4.1 was utilized to map the reads of each sample against tNGS special authoritatively classified database in very-sensitive mode. If the best alignment of the reads dropped into one target amplicon region, the reads were counted. The count number of each target amplicon was summed and normalized to 100,000. The number of reads per 100,000 (RPhK) was calculated for species or genus level. and subsequently indicated as the target pathogen result (RPhK≥10). Macrolide-resistance mutations of *M. pneumoniae* including 23 S rRNA: A2063G, A2064G, A2067G, and A2617G, were calling using samtools mpileup [28]. The mutation rate was obtained by calculating the ratio of the depth of the mutation at the site to the total depth. The threshold for mutation calling was 10%.

Statistical analysis

All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Normally distributed data were presented as mean±standard deviation. Categorical variables were summarized as frequencies and proportions. An unpaired t-test was used to compare the normally distributed parameters of the two groups, and all tests were two-tailed. The Chi-square or Fisher's exact test was utilized for classified variables. Linear regression models with gamma values were used to assess changes in prevalence over the calendar year. $P<0.05$ was considered statistically significant.

Table 1 Prevalence of *M. pneumoniae* in Guangzhou in 2020–2023

	2020	2021	2022	2023	gamma value	*P*	Trend
Number of cases	553	1015	1267	2570	–	–	–
Positive cases	11(2.0%)	12(1.2%)	72(5.3%)	754(29.4%)	0.827	< 0.001	Increasing
MPP	6(54.5%)	11(91.7%)	59(81.9%)	563(74.7%)	–	–	–
SMPP	4(36.4%)	0	12(16.7%)	185(24.5%)	–	–	–
Macrolide resistance	–	–	–	215/236(91.1%)	–	–	–
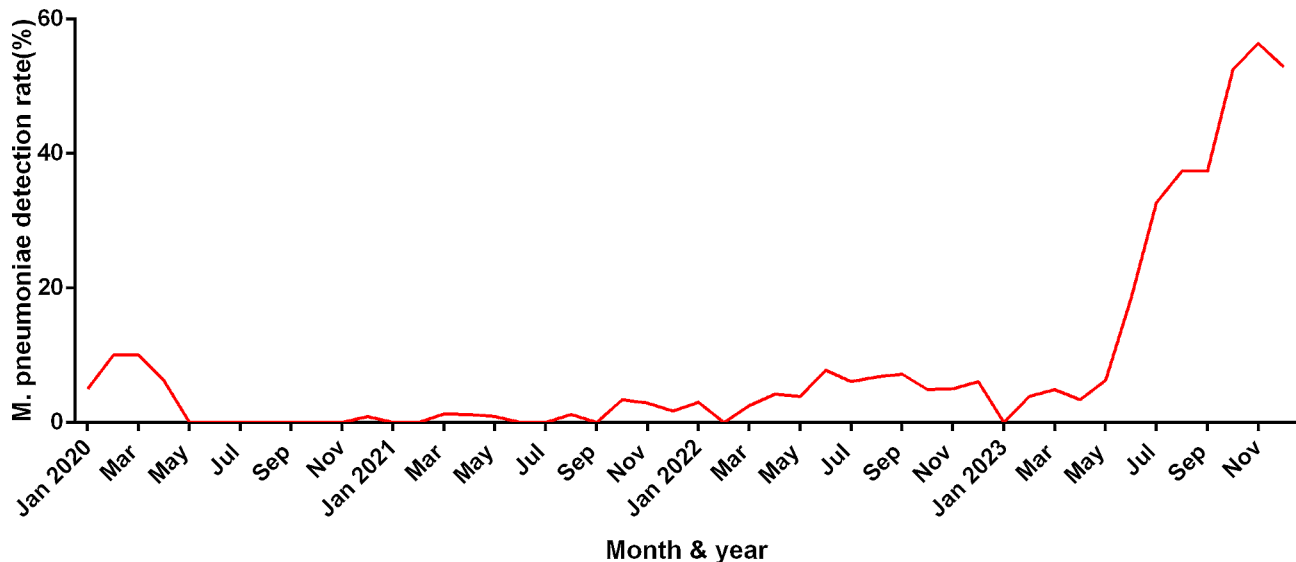
Fig. 1 The monthly distributions of *M. pneumoniae* in 2020–2023

Results

Study population and detection of *M. pneumoniae*

As shown in Table 1, from January 2020 to December 2023, 5405 hospitalized children with ARTI were enrolled, including 553 patients in 2020, 1015 patients in 2021, 1267 patients in 2022 (during the COVID-19 pandemic); and 2570 patients in 2023 (after the COVID-19 pandemic). A total of 849 (15.6%, 849/5405) cases were caused by *M. pneumoniae*. The highest annual positive rate was 29.4% (754/2570) in 2023, followed by 5.3% (72/1367) in 2022, 1.2% (12/1015) in 2021, and 2.0% (11/553) in 2020. The annual prevalence of *M. pneumoniae* showed an obvious increasing trend from 2020 to 2023 (*P* < 0.001).

Temporal distribution of *M. pneumoniae*

The monthly distributions of *M. pneumoniae* in 2020–2023 are illustrated in Fig. 1. During the COVID-19 pandemic, *M. pneumoniae* infections rarely occurred in 2020–2022, coinciding with the implementation of NPIs against COVID-19. Interestingly, *M. pneumoniae* notably increased since June and showed peaks between July and December after the COVID-19 pandemic in 2023, especially the highest monthly positive rate (56.4%, 165/293).

Macrolide-resistant *M. pneumoniae* (MRMP)

Among the 754 cases of *M. pneumoniae* infection in 2023, 236 BALF samples from patients infected with *M. pneumoniae* were obtained for tNGS detection to identify macrolide -resistance mutations. Of 236 clinical *M. pneumoniae* strains, 215 carried macrolide resistance mutations in the 23 S rRNA gene. All macrolide resistance mutations were A2063G transitions, and MRMP proportion was 91.1% (215/236) in 2023.

Clinical characteristics and laboratory findings of hospitalized patients infected with *M. pneumoniae* during and after the COVID-19 pandemic

We compared the clinical characteristics of hospitalized patients infected with *M. pneumoniae* during (2020 and 2022) and after the COVID-19 pandemic (2023) to investigate whether the clinical characteristics of *M. pneumoniae* infection changed during the post-pandemic period (Table 2). Patients with *M. pneumoniae* were older after the COVID-19 pandemic than during the COVID-19 pandemic (*P* < 0.001). A total of 754 patients with *M. pneumoniae* after the COVID-19 pandemic were categorized into four age groups, including 30 patients in age ≤ 1 years old group, 81 patients in age 1–3 years old group, 261 patients in 3–6 years old group, 382 patients in > 6 years old group, respectively.

Li *et al. Virology Journal*   (2024) 21:183

Page 5 of 11

Table 2 Clinical characteristics of patients with *M. pneumoniae* during (2020~2022) and after the COVID-19 pandemic (2023)

	2020~2022 (n=95)	2023 (n=754)	P value
Sex(male)	54(56.8%)	396(52.52%)	0.426
Age (months)	5.13±2.96	6.55±2.75	<0.001
≤1	14(14.7%)	30(4.0%)	<0.001
1~3	21(22.1%)	81(10.6%)	0.001
3~6	23(24.21%)	261(34.62%)	0.043
>6	37(38.95%)	382(50.66%)	0.031
Symptom			
Fever	77(81.05%)	690(91.51%)	0.001
Hyperpyrexia	38(40%)	345(45.76%)	0.288
Cough	92(96.8%)	736(97.61%)	0.916
Wheezing	23(24.2%)	45(5.97%)	<0.001
Shortness of breath	9(9.4%)	37(4.91%)	0.064
Rhinorrhea	23(24.2%)	212(28.12)	0.423
Pharyngodynia	50(52.6%)	465(61.67%)	0.089
Chest pain	1(1%)	12(1.59%)	1
Moist rales	57(60%)	487(64.59%)	0.380
Pleural effusion	3(3.1%)	29(3.85%)	0.963
Three concave sign	6(6.3%)	18(2.39%)	0.869
Hypoxemia	3(3.16%)	40(5.30%)	0.515
Headache	6(6.3%)	51(6.76%)	0.869
Myalgia	1(1%)	3(0.40%)	0.378
Erythra	0	4(0.53%)	1
Algor	4(4.2%)	44(5.84%)	0.518
Coma	0	1(0.13%)	1
Extrapulmonary manifestations			
Myocardial damage	2(2.11%)	26(3.45%)	0.700
Gastrointestinal dysfunction	8(8.42%)	34(4.51%)	0.160
Electrolyte disturbance	6(6.32%)	43(5.70%)	0.809
Liver dysfunction	2(2.11%)	3(0.40%)	0.099
Hypokalemia	4(4.21%)	38(5.04%)	0.920
Hyponatremia	2(2.11%)	8(1.06%)	0.701
Clinical outcome			
Severe pneumonia	16(16.84%)	185(24.54%)	0.096
MRMP	-	215/236(91.1%)	-
Therapy			
Bronchoscopy therapy	42(44.2%)	326(43.24%)	0.857
Nebulization	94(98.9%)	722(95.76%)	0.217
oxygen therapy	4(4.2%)	47(6.23%)	0.434
Antifungal therapy	1(1%)	1(0.13%)	0.211
Antibacterial therapy	95(100%)	744(98.67%)	0.532
Antiviral therapy	8(8.4%)	32(4.24%)	0.120

Most patients with *M. pneumoniae* after the COVID-19 pandemic were >3 years old (85.4%). The most common symptoms were cough, fever, and pharyngodynia. Clinical characteristics, extra-pulmonary manifestations and outcomes of *M. pneumoniae*-infected patients did not vary between the periods during and after the COVID-19 pandemic, except that patients with *M. pneumoniae* after the COVID-19 pandemic were more likely to have

fever ($P=0.001$). Patients infected with *M. pneumoniae* after the COVID-19 pandemic had a higher rate of co-infection with respiratory viruses than those during the COVID-19 pandemic ($P=0.03$) (Table 3).

The laboratory findings of hospitalized patients with *M. pneumoniae* between the periods during and after the COVID-19 pandemic are summarized in Table S1. Fibrinogen levels and neutrophil counts for these patients were higher after the COVID-19 pandemic than during the COVID-19 pandemic ($P<0.001$ and $P=0.007$, respectively). The lymphocyte counts in patients with *M. pneumoniae* after the COVID-19 pandemic were lower than those during the COVID-19 pandemic ($P<0.001$).

Clinical characteristics and laboratory findings of patients with non-SMPP and SMPP

Among the 849 cases of *M. pneumoniae* infection, 639 (75.3%) and 201 (23.7%) were diagnosed with non-SMPP and SMPP, respectively, and the clinical characteristics of patients diagnosed with non-SMPP and SMPP were compared (Table 4). No significant differences in sex or age were observed between the non-SMPP and SMPP groups ($P>0.05$). No significant differences in the rate of MRMP were observed between the non-SMPP and SMPP groups ($P>0.05$). Patients with SMPP were more likely to have fever, wheezing, shortness of breath, three concave signs, hypoxemia, and pleural effusion compared to those with non-SMPP ($P<0.05$). Patients with SMPP were more susceptible to myocardial damage, gastrointestinal dysfunction, and electrolyte disturbances than those with non-SMPP ($P<0.05$). Patients with SMPP received bronchoscopy therapy ($P<0.001$), and oxygen therapy ($P<0.001$) more often than those with non-SMPP. Patients with SMPP were more likely to have longer length of hospital stay, compared to those with non-SMPP ($P<0.001$). Patients infected with SMPP had a higher rate of coinfection with viruses and bacteria than those with non-SMPP ($P=0.007$ and $P=0.032$, respectively). Patients with SMPP were more likely to be coinfected with rhinovirus and *Streptococcus pneumoniae* than those with non-SMPP ($P=0.016$ and $P=0.009$, respectively) (Table 3).

Laboratory findings of patients with non-SMPP and SMPP were analyzed (Table S2). Patients with SMPP had significantly higher neutrophil and lower lymphocyte counts than those with non-SMPP. CRP, PCT, D-dimer, AST, CK, and LDH levels were significantly higher in patients with SMPP than those with non-SMPP. Hemoglobin, TP, ALB, Na+, and Cl- levels were significantly lower in patients with SMPP than those with non-SMPP. As shown in Fig. 2, CD3+ T cells, CD4+ T cells CD8+ T cells, and B cells were significantly lower in patients with SMPP than those with non-SMPP. As shown in Fig. 3, there were no statistically significant differences in the

Table 3 Co-pathogen infection of patients with *M. pneumoniae*

Respiratory pathogens	2020～2022 (n=95)	2023 (n=754)	P value	non-SMPP (n=639)	SMPP (n=201)	P value
Virus	**7(7.4%)**	**119(15.8%)**	**0.030**	**84(13.2%)**	**42(20.9%)**	**0.007**
Infuenza virus A	0	14(1.9%)	0.362	14(2.2%)	2(1.0%)	0.432
Infuenza virus B	0	10(1.3%)	0.532	7(1.1%)	3(1.5%)	0.936
Respiratory syncytial virus	1(1.1%)	22(2.9%)	0.472	14(2.2%)	9(4.5%)	0.083
Adenovirus	1(1.1%)	20(2.7%)	0.551	16(2.5%)	5(2.5%)	0.99
Rhinovirus	1(1.1%)	34(4.5%)	0.186	20(3.1%)	14(7.0%)	0.016
Parainfuenza virus	1(1.1%)	15(2.0%)	0.816	15(2.3%)	1(0.5%)	0.168
Metapneumovirus	0	4(0.5%)	1	4(0.6%)	0	0.578
cytomegalovirus	1(1.1%)	4(0.5%)	0.448	2(0.3%)	3(1.5%)	0.171
Epstein-Barr virus	0	3(0.4%)	1	1(0.2%)	2(1.0%)	0.144
Herpesvirus hominis	0	2(0.3%)	1	0	2(1.0%)	0.057
Human bocavirus	1(1.1%)	6(0.8%)	0.566	6(0.9%)	1(0.5%)	0.876
Enterovirus	0	1(0.1%)	1	0	2(1.0%)	0.057
Coronavirus	1(1.1%)	2(0.3%)	0.300	1(0.2%)	2(1.0%)	0.144
Bacteria	**9(9.5%)**	**82(10.9%)**	**0.677**	**61(9.5%)**	**30(14.9%)**	**0.032**
Streptococcus pneumoniae	4(4.2%)	41(5.4%)	0.615	27(4.2%)	18(9.0%)	0.009
Haemophilus influenzae	2(2.1%)	26(3.4%)	0.700	22(3.4%)	6(3.0%)	0.752
Klebsiella pneumoniae	0	1(0.1%)	0.034	1(0.2%)	0	1
Staphylococcus aureus	0	19(2.5%)	0.231	13(2.0%)	6(3.0%)	0.604
Pseudomonas aeruginosa	1(1.1%)	1(0.1%)	0.211	1(0.2%)	1(0.5%)	1
Moraxella catarrhalis	0	5(0.7%)	1	3(0.5%)	2(1.0%)	0.750
Bordetella pertussis	0	4(0.5%)	1	3(0.5%)	1(0.5%)	1
Legionella pneumophila	0	4(0.5%)	1	2(0.3%)	2(1.0%)	0.243
Stenotrophomonas maltophilia	0	2(0.3%)	1	1(0.2%)	1(0.5%)	0.422
Enterococcus faecium	1(1.1%)	0	0.112	1(0.2%)	0	1
Staphylococcus equorum	1(1.1%)	0	0.112	1(0.2%)	0	1
Fungus	**0**	**1(0.1%)**	**1**	**1(0.2%)**	**0**	**1**
Chlamydia	**0**	**5(0.7%)**	**1**	**4(0.6%)**	**1(0.5%)**	**1**

serum levels of IL-1β, IL-2, IL-5, IL-8, IL-12P70, TNF-α, IFN-α, IFN-γ and IL-17 A between patients with non-SMPP and SMPP. Serum levels of IL-4, IL-6, and IL-10 were significantly higher in patients with SMPP than those with non-SMPP.

Discussion

In this study, we systematically compared the epidemiology, and clinical characteristics of *M. pneumoniae* among hospitalized children in Guangzhou, China, during and after the COVID-19 pandemic. Our study revealed a notable reduction in *M. pneumoniae* activity during the COVID-19 pandemic (2020–2022), coinciding with the implementation of NPIs. These findings are in line with several reports [13, 29–31], suggesting that COVID-19 restrictive measures had effectively reduced *M. pneumoniae* transmission. We further observed that outbreaks of *M. pneumoniae* occurred in Guangzhou, China in 2023, following the relaxation of measures to restrict SARS-CoV-2 transmission. Recently, Gong et al. reported that the increase in incidence of respiratory diseases among children in Beijing between September and November 2023 coincided with an increased positivity

rate of common respiratory pathogens, predominantly *M. pneumoniae* [32]. Additionally, since late October 2023, an epidemic of *M. pneumoniae* has occurred in Denmark [33]. As for explanations, the extraordinary absence of *M. pneumoniae* during the COVID-19 pandemic (2020–2022) probably resulted in young children without natural immunity to *M. pneumoniae*, thereby causing *M. pneumoniae* infection outbreaks upon NPIs easing. Additionally, we found that there was an *M. pneumoniae* epidemic in Guangzhou, China in 2019 (Figure S1). Therefore, the *M. pneumoniae* outbreaks in 2023 might be a return of the cyclical *M. pneumoniae* outbreaks witnessed prior to the COVID-19 pandemic, since *M. pneumoniae* epidemics typically occur every three to seven years. We found that *M. pneumoniae* revealed peaks between July and December in Southern China after the COVID-19 pandemic in 2023, which was largely consistent with other reports [34].

We further compared the clinical characteristics and laboratory findings of hospitalized patients infected with *M. pneumoniae* during (2020 and 2022) and after the COVID-19 pandemic (2023). Our results suggest that patients with *M. pneumoniae* after the COVID-19

Table 4 Clinical characteristics of patients with non-severe *M. pneumoniae* pneumonia (non-SMPP) and severe *M. pneumoniae* pneumonia (SMPP)

	non-SMPP (n = 639)	SMPP (n = 201)	P value
Sex(male)	341(53.4%)	105(52.2%)	0.780
Age (months)	6.31 ± 2.74	6.61 ± 2.99	0.188
≤1	33(5.2%)	11(5.5%)	0.864
1~3	77(12.2%)	23(11.4%)	0.811
3~6	217(33.96%)	65(32.34%)	0.671
>6	312(48.83%)	102(50.75%)	0.635
Clinical characteristic			
Fever	554(86.70%)	191(95.02%)	0.001
Hyperpyrexia	271(42.41%)	101(50.25%)	0.051
Cough	623 (97.5%)	198(98.5%)	0.569
Wheezing	44(6.9%)	23(11.4%)	0.037
Shortness of breath	23(3.6%)	23(11.4%)	< 0.001
Rhinorrhea	182(28.5%)	51(25.3%)	0.391
Pharyngodynia	392(61.3%)	123(61.1%)	0.969
Chest pain	7(1.1%)	6(2.9%)	0.117
Moist rales	403(63.07%)	141(70.1%)	0.067
Pleural effusion	10(1.6%)	21(10.4%)	< 0.001
Three concave sign	12(1.9%)	12(5.9%)	0.002
Hypoxemia	3(0.47%)	40(19.90%)	< 0.001
Headache	43(6.7%)	14(6.9%)	0.908
Myalgia	3(0.5%)	1(0.4%)	1
Erythra	4(0.3%)	0	0.578
Algor	39(6.1%)	9(4.4%)	0.386
Coma	0	1(0.4%)	0.239
Extrapulmonary manifestations			
Myocardial damage	16(2.50%)	12(5.97%)	0.017
Gastrointestinal dysfunction	26(4.07%)	16(7.96%)	0.027
Electrolyte disturbance	28(4.38%)	21(10.45%)	0.001
Liver dysfunction	2(0.31%)	3(1.49%)	0.171
Hypokalemia	24(3.76%)	18(8.96%)	0.003
Hyponatremia	4(0.63%)	6(2.99%)	0.021
Clinical outcome			
Length of hospital stay (d)	6.96 ± 2.81	9.05 ± 3.70	< 0.001
MPMP	125/138(90.6%)	90/98(91.8%)	0.738
Therapy			
Bronchoscopy therapy	234(36.6%)	133(66.1%)	< 0.001
Nebulization	620(97.0%)	190(94.5%)	0.096
Oxygen therapy	12(1.9%)	39(19.40%)	< 0.001
Antifungal therapy	1(0.2%)	1(0.4%)	0.422
Antibacterial therapy	632(98.9%)	200(99.5%)	0.730
Antiviral therapy	34(5.3%)	6(2.9%)	0.175

pandemic (2023) had a higher rate of coinfection with respiratory viruses than those during the COVID-19 pandemic. Previous studies revealed that resurgence of respiratory pathogens was observed after NPIs easing, such as influenza A, influenza B, RSV, and human metapneumovirus [19]. Our data revealed that patients with *M. pneumoniae* were older in 2023 than those in 2020–2022. Maison et al. presented a shift in the average age of influenza A and RSV infection to older children upon NPIs easing in Germany [35]. One possible explanation is the accumulation of a susceptible population and subsequent decline in population immunity due to NPIs implemented during the COVID-19 pandemic. Meanwhile, with the lifting of NPIs, school-aged children attended school, leading to a widespread trend. Hence, some researchers have speculated whether a large outbreak and more severe pulmonary and extrapulmonary symptoms will be caused by *M. pneumoniae* infection [17]. In our study, no significant differences were found in the clinical characteristics and outcomes of patients with *M. pneumoniae* during and after the COVID-19 pandemic, except that patients with *M. pneumoniae* after the COVID-19 pandemic were more likely to have fever.

In recent years, severe *M. pneumoniae* pneumonia had been reported worldwide, resulting in a significant disease burden on children. In our study, 201 (23.7%, 201/849) cases were diagnosed with SMPP. We found that patients with SMPP were more likely to have fever, wheezing, shortness of breath, three concave sign, hypoxemia, and pleural effusion than those with non-SMPP. Our results also indicated that patients with SMPP were more likely to show myocardial damage and gastrointestinal dysfunction, consistent with previous reports [36, 37].

Patients with SMPP were more likely to be coinfected with rhinovirus and *Streptococcus pneumoniae* than those with non-SMPP in this study, which was largely consistent with other reports [34]. Innate and adaptive immune responses play critical roles in the defense against pathogen infection and disease progression. In this study, *M. pneumoniae* infections resulted in a broad reduction in immune cells, including lymphocytes, CD3$^+$ T cells, CD4$^+$ T cells, CD8$^+$ T cells and B cells in patients with SMPP. Our results suggest that decreases in lymphocyte, CD3$^+$ T cell, CD4$^+$ T cell, CD8$^+$ T cell and B cell counts were correlated with disease severity in patients with *M. pneumoniae*. Previous studies revealed that CD4$^+$ effector memory T cells form the major population of the pathogen-specific IFN-γ response in children with MPP and that the presence of these cells in peripheral blood correlates with pulmonary disease severity [38]. Similar to bacterial or viral infection, *M. pneumoniae* infection can also stimulate a variety of immunoactive cells to produce hyperactive cytokine-mediated inflammatory response, leading to body damage. Previous reports revealed that compared to healthy controls, serum levels of PCT, IL-6, IL-8, TNF-α, and IL-10 were significantly higher in patients with *M. pneumoniae* [39]. We further found that the serum levels of IL-4, IL-6, and IL-10 were higher in patients with SMPP than those with non-SMPP. Deng et al. reported that patients with SMPP
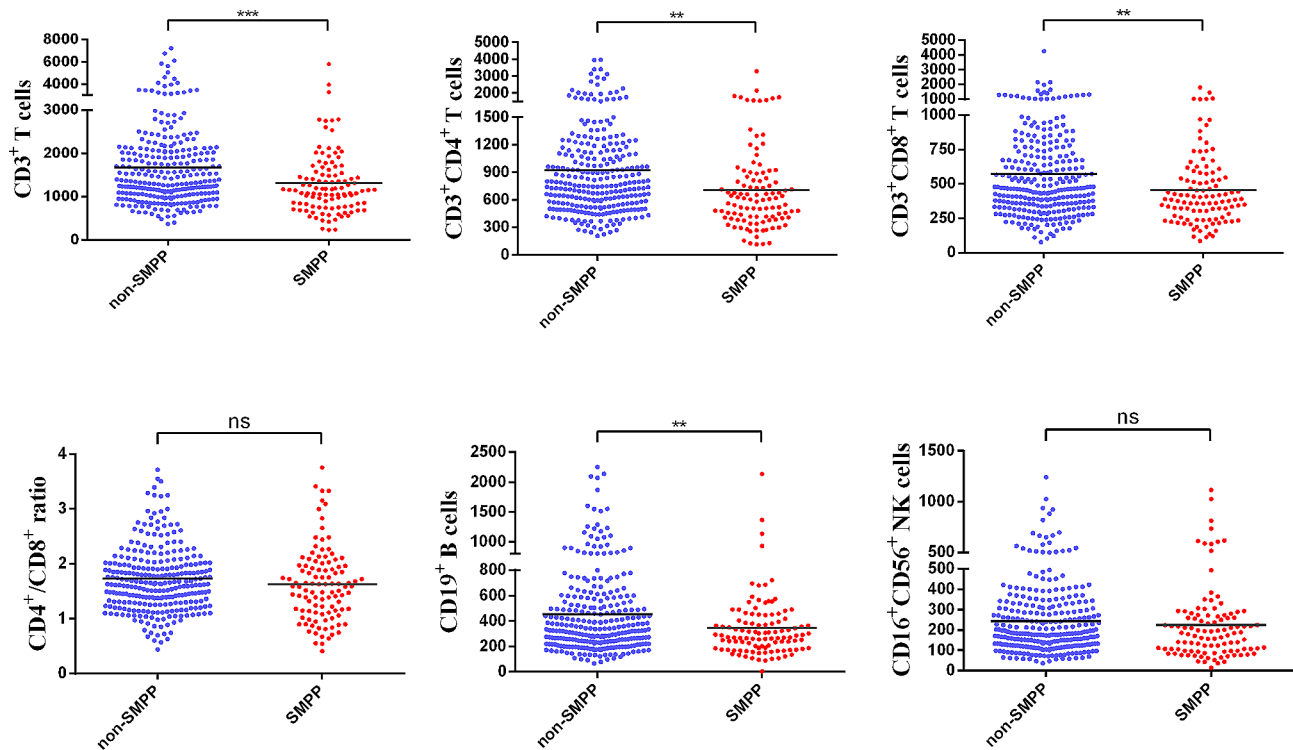
Li *et al. Virology Journal* (2024) 21:183

Page 8 of 11

Fig. 2 Comparison of peripheral lymphocyte subsets between patients with SMPP ($n=108$) and patients with non-SMPP ($n=281$).*, $P<0.05$; **, $P<0.01$;***, $P<0.001$; ns, not significant

had significantly higher levels of TNF-α, IL-6, and IL-10 in their BALF than those with non-SMPP [40]. Collectively, these findings imply that elevated levels of IL-6 and IL-10 are indicative of the severity of inflammation and pneumonia.

Macrolides are first-line agents for treating *M. pneumoniae* infections. Understanding the prevalence of MRMP is crucial due to limited number of alternative therapies available to children. The global prevalence of MRMP infection, especially in Asian regions, is increasing rapidly. We found a high rate of MRMP (91.1%, 215/236) among children with *M. pneumoniae* in Guangzhou, China in 2023. No significant differences in the rate of MRMP were observed between the non-SMPP and SMPP groups in this study, which was largely consistent with other reports [41]. In total, 36 (63%, 36/57) strains were classified as MRMP with mutations in A2063G in Shenzhen, China in 2010–2011 [42]. A multicenter population-based epidemiological study revealed that the total MRMP proportion in patients with ARTI from 35 sentinel hospitals in Beijing from 2018 to 2020 was 84.7% (402/474), with 74.4% in 2018, 90.6% in 2019, and 93.9% in 2020 [43]. Wu et al. reported that the total proportion of MRMP in Taiwan from 2017 to 2019 was 38.4% (53/138), with 10.6% in 2017, 47.5% in 2018, and 62.5% in 2019 [44]. In 2015, the rate of MRMP was 87.2% (82/94) in children with *M. pneumoniae* pneumonia in Korea

[45]. These findings revealed that the increase in MRMP in many regions is due to the selective antibiotic pressure on children during periods of extensive macrolide use. Therefore, continuous monitoring of occurrence and antimicrobial susceptibility of MRMP is necessitated to control its spread and establish strategies for treating second-line antibiotic resistant *M. pneumoniae* infections.

This study has several limitations. First, this was a single-center retrospective study, not a multicenter retrospective study. Second, the study population included hospitalized pediatric patients with *M. pneumoniae* and did not include outpatients. Third, *M. pneumoniae* strains were exclusively screened for gene mutations associated with macrolide resistance; however, a phenotypic assessment of drug resistance was not conducted. Fourth, some hospitalized patients with *M. pneumoniae* were coinfected with other bacteria or viruses.

In conclusion, this study revealed that the COVID-19 restrictive measures had effectively reduced the transmission of *M. pneumoniae* during the COVID-19 pandemic (2020–2022). There were outbreaks of *M. pneumoniae* in Guangzhou, China in 2023, following the easing of NPIs. The rate of MRMP was 91.1% (215/236) among children with *M. pneumoniae* in Guangzhou, China in 2023. Clinical characteristics and outcomes of patients with *M. pneumoniae* did not vary during and after the COVID-19 pandemic. Patients with SMPP were more likely to
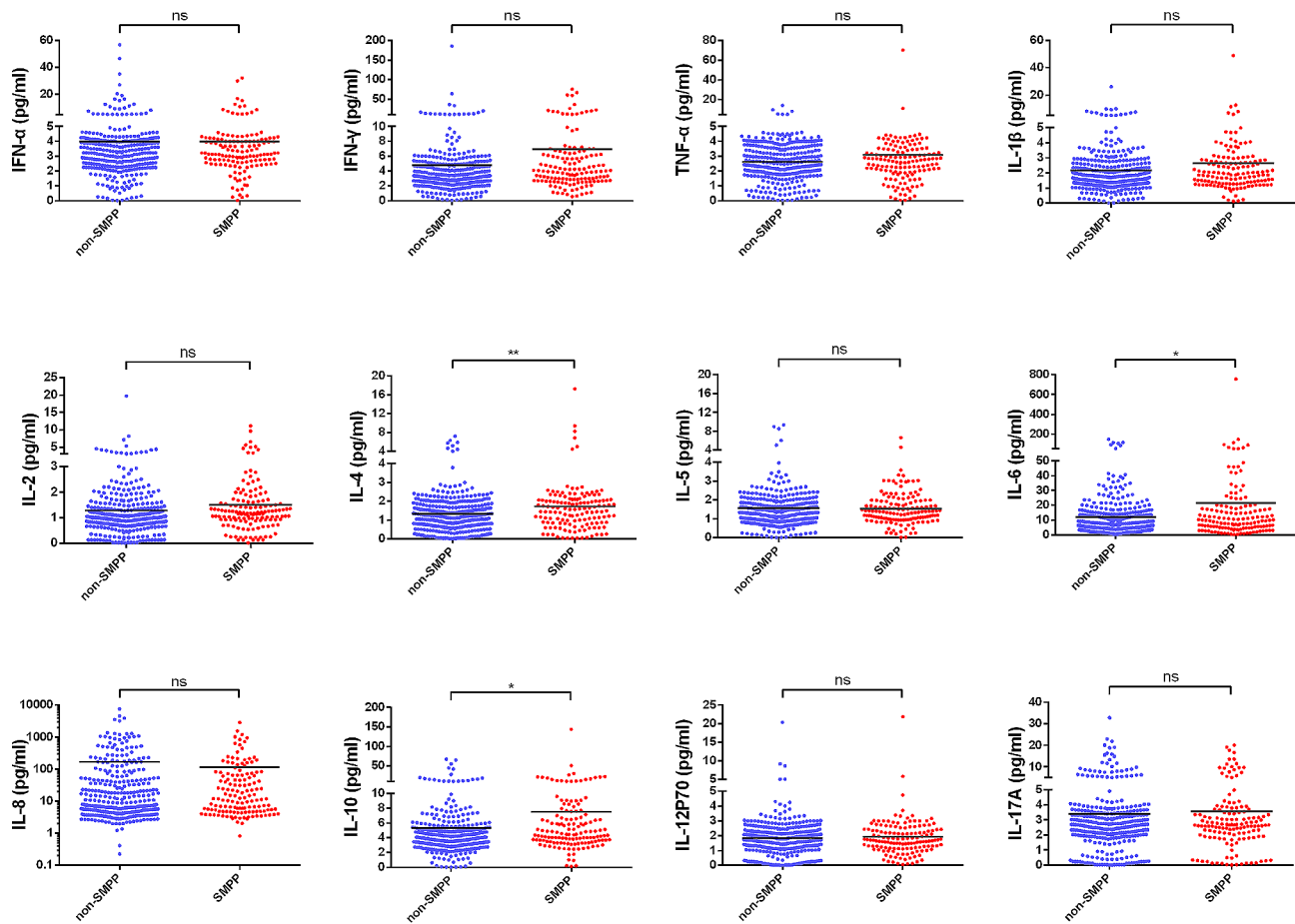
Fig. 3 Comparison of serum cytokine levels between patients with SMPP ($n=142$) and patients with non-SMPP ($n=323$).*, $P<0.05$; **, $P<0.01$; ns, not significant

develop respiratory complications, myocardial damage, electrolyte disturbance and gastrointestinal dysfunction than those with non-SMPP. This study comprehensively analyzed the changes in *M. pneumoniae* during and after the COVID-19 pandemic in terms of incidence, temporal distribution, prevalence of macrolide resistance, and clinical features, providing invaluable data for monitoring, infection prevention, control, and treatment of *M. pneumoniae* in the post-pandemic.

Abbreviations

ARTIs	Acute respiratory tract infections
CAP	Community-acquired pneumoniae
COVID-19	Coronavirus disease 2019
M. pneumoniae	Mycoplasma pneumoniae
MPP	*M. pneumoniae* pneumonia
MRMP	Macrolide-resistant Mycoplasma pneumoniae
NPIs	Non-pharmaceutical interventions
RSV	Respiratory syncytial virus
SMPP	Severe *M. pneumoniae* pneumonia
tNGS	Targeted next-generation sequencing

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions
Y.L., M.-Z.W., and Y.L. contributed equally to this manuscript. Q.-G. C., Y. L., and Y.-P.L. contributed to conceptualization, writing of the original draft, review and editing, and resources. Q.-G.C., Y.L., M.-Z.W., Y.L., Y.-H.Y., W.-Y.G., Y.-Z.D., T.W., C.-W.T., C.L., and F.-F.L. contributed to methodology, investigation, formal analysis, and data curation. Q.-G. C. contributed to project administration. All authors have reviewed and approved the final manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

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