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Short Communication

Predominance of A2063G mutant strains in the *Mycoplasma pneumoniae* epidemic in children: A clinical and epidemiological study in 2023 in Wuhan, China

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ABSTRACT

Objectives: The prevalence of respiratory infectious diseases has changed in the post-COVID-19 epidemic era, and *mycoplasma pneumoniae* (MP) infection in children has attracted wide attention.
Methods: Children hospitalized for pneumonia in Wuhan, China, in 2023 were enrolled. Respiratory secretions were obtained for the targeted next-generation sequencing (tNGS) including mutation of MP. Pulmonary inflammation was divided into bronchopneumonia and pulmonary consolidation/atelectasis according to lung computed tomography imaging.
Results: Of the 667 pediatric pneumonia, 478 were MP positive (72%). The positive rate of MP detected by tNGS increased from April, and MP had become the primary pathogen of pneumonia in children in 2023. The 23S rRNA mutations were all A2063G, accounting for 85% of detected MP. The clinical symptoms of the mutant and wild-type strains were similar, with half of them experiencing atelectasis and lung consolidation. Early bronchoscopic lavage combined with azithromycin in pediatric pulmonary consolidation was an effective therapy strategy, which could be an alternative selection to MP pneumonia treatment.
Conclusions: A2063G mutant strain MP was the primary pathogen of *mycoplasma pneumoniae* in children recently, which was often complicated by extra-pulmonary symptoms and complications.

Introduction

The sharp increase in *mycoplasma pneumoniae* (MP) infection among children in mainland China in 2023 has raised concerns about a traditional infectious disease epidemic [1]. However, it is still impossible to accurately judge the epidemic scale, the infected objectives, and the clinical outcome [2]. Until mid-2023, strong regional epidemics occurred in northern China, and *mycoplasma pneumoniae* pneumonia (MPP) in children attracted the attention of the World Health Organization [3].

Different from the past, the recent cluster infection of MP has been predominantly in school-aged children, with severe clinical symptoms and widespread resistance to macrolide antibiotics, which has caused significant obstacles in clinical treatment [4]. MP mutant infection is prevalent globally, especially in the Asia-Pacific region, with macrolide antibiotic resistance, which makes the infection difficult to control, along with a high prevalence of lethal complications and sequelae, such as pulmonary embolism and lung necrosis.

In this study, we investigated the distribution of mycoplasma mutant strains in children with pneumonia since April 2023 and found that MPP in central China was characterized by the prevalence of the A2063G mutant strain.

Methods

Study design

Patients and samples. Data were collected from April 2023 to December 2023 from hospitalized children with pneumonia, and nasopharyngeal swabs (NS) or bronchoalveolar lavage fluid (BALF)

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Table 1
General information on children pneumonia infections in 2023.

Indicators	Category	MP group (N = 478)	Non-MP group (N = 189)	*P*-value
Gender	Male, n (%)	261 (54.6%)	106 (56.08%)	0.679
	Female, n (%)	217 (45.4%)	83 (43.92%)	0.679
Age (y)	<1 n = 40 (6.45%)	9 (1.88%)	34 (17.99%)	<0.001
	1~3 n = 103 (15.44%)	37 (7.74%)	66 (34.92%)	<0.001
	4~6 n = 195 (29.24%)	150 (31.38%)	45 (23.81%)	<0.001
	≥7 n = 326 (48.88%)	282 (59%)	44 (23.28%)	<0.001
Prevalence (month)	1~6 n = 74 (11.09%)	24 (5.02%)	50 (26.46%)	<0.001
	7~12 n = 593 (88.91%)	454 (94.98%)	139 (73.54%)	<0.001
Symptoms	Duration of cough (d), m ± sd	8.41 ± 4.24	8.77 ± 6.99	0.514
	Fever duration (d), m ± sd	5.54 ± 2.92	3.68 ± 3.13	<0.001
	Digestive Symptoms, n (%)	6 (1.26%)	2 (1.06%)	1.000
	Rash, n (%)	13 (2.72%)	5 (2.65%)	0.948
	Pleural Effusion/Pulmonary Embolism, n (%)	12 (2.51%)	0 (0%)	0.06
	Length of hospitalization (d), m ± sd	6.02 ± 2.11	6.16 ± 1.76	0.427
Laboratory findings	White blood cell ($\times 10^9$/L), IQR	7.30 (5.83-8.97)	8.84 (6.07-11.98)	<0.001
	Neutrophil (%), IQR	61.95 (54.03-69.88)	51.20 (35.13-67.33)	<0.001
	lymphocyte (%), IQR	27.31 (19.83-34.10)	36.75 (21.68-51.43)	<0.001
	Elevated PCT, n (%)	24 (5.12%)	13 (6.95%)	0.365
	Elevated CRP, n (%)	312 (66.24%)	71 (37.97%)	<0.001
Samples	Throat swabs, n (%)	342 (71.55%)	186 (98.41%)	<0.001
	Bronchoalveolar Lavage Fluid, n (%)	136 (28.45%)	3 (1.59%)	<0.001
Diagnosis	Pneumonia, n (%)	237 (49.58%)	170 (89.95%)	<0.001
	Lung consolidation/Pulmonary Atelectasis, n (%)	241 (50.42%)	19 (10.05%)	<0.001

CRP, C-reactive protein; IQR, interquartile range; m ± sd, mean ± standard deviation; MP, *mycoplasma pneumoniae*; PCT, procalcitonin.

were sampled for targeted microbial next-generation sequencing (tNGS). The children's clinical data were obtained from Zhongnan Hospital of Wuhan University, China. Only children hospitalized for a lower respiratory tract infection were included. Patients with known bronchopulmonary dysplasia, congenital heart disease, immunodeficiency or suspected nosocomial infections, and ventilator-associated pneumonia were excluded.

tNGS detection. The tNGS sequencing was commissioned by Guangzhou Jinyu Medical Laboratory Center Co., LTD. (King Med Diagnostics, Guangzhou, China). Sequencing targeted the highly conserved regions of 153 respiratory pathogens, used super-multiplex polymerase chain reaction combined with NGS technology for gene sequencing, and read the detection results of pathogens. Those 153 regions include common respiratory pathogens such as viruses, bacteria, mycoplasma, mycobacteria, and fungi. This protocol simultaneously examined mutations in the 23S rRNA domain A2063G, A2064G, A2067G, and C2617G of MP [5].

Results

General information

Of 667 children with pneumonia, MPP accounted for the highest prevalence with 478 cases (71.66%). Other commonly identified pathogens included *streptococcus pneumoniae, haemophilus influenzae, rhinovirus, staphylococcus aureus, coronavirus, adenovirus, parainfluenza virus*, and *respiratory syncytial virus* (Supplementary Figure 1). The positive rate of MP increased rapidly since May 2023, reaching 55.56% in July, and soaring to a high of 90% in November. MPP cases were predominantly observed in children aged four and above, with an increasing detection rate as age increased (Table 1). Among different sample types, the positive rate of BALF was 97.84%, significantly higher than that of NS ($P < 0.001$). Compared with other pathogens causing pneumonia in the same period, children with MPP had more extended fever and elevated CRP ($P < 0.001$). The incidence of lung consolidation associated with MPP reached 50.42%, accounting for 93.01% of all cases.

Clinical characteristics of A2063G mutant infection

Among MP, 404 (84.52%) showed A2063G mutations in the 23S rRNA domain. The clinical characteristics of the mutant strain MPP are shown in Table 2. Compared with the wild-type strain, the A2063G mutant pneumonia showed no difference in the time of fever, cough, hospital stay, white blood cell count, percentage of neutrophil, and the incidence of lung consolidation (Supplementary Figure 2A,2B).

None of the children progressed to require ventilator support, and there were no death cases. Two A2063G mutant pneumonia cases developed recurrent fever, chest tightness, and dyspnea after bronchoscopic alveolar lavage. These symptoms were confirmed to be pulmonary embolism by high-resolution computed tomography (CT) and pulmonary angiography. The cases resolved after two weeks of anticoagulation and thrombolytic therapy (Supplementary Figures 2C and 2D).

Effect of anti-MP therapy

The effectiveness of macrocyclic lipid antibiotics was assessed in light of the prevalent resistance of A2063G mutations to 14-membered ring macrolides (Supplementary Table 1). The doxycycline treatment group was superior to azithromycin only in shortening hospital stays ($P < 0.05$), while there was no significant difference in controlling fever and shortening cough duration. Combined with fibril-bronchoalveolar lavage therapy, azithromycin and doxycycline demonstrated similar therapeutic effects.

Co-detection of mycoplasma and other microorganisms

Analysis of co-detection showed that tNGS generally detected multiple pathogens in the same sample, including pathogenic and suspected colonizing bacteria (Supplementary Figure 3). The sequenced results showed that the most common co-detection with MP were *streptococcus pneumoniae, haemophilus influenzae, rhinovirus, staphylococcus aureus, adenoviruses, respiratory syncytial virus, metapneumoviruses, parainfluenza virus, coronavirus*. Two patients with pulmonary embolism, mycoplasma A2063G mutant,

Table 2
Clinical characteristics of drug-resistant strains of children *Mycoplasma pneumoniae* pneumonia.

		A2063G group (n = 404)	Non-A2063G group (n = 74)	*P*-value
Samples	Bronchoalveolar Lavage Fluid, n (%)	106 (77.94%)	30 (22.06%)	0.012
	Nasopharyngeal swabs, n (%)	298 (87.13%)	44 (12.87%)	0.012
Gender	Male, n (%)	220 (54.46%)	41 (55.41%)	0.88
	Female, n (%)	184 (45.54%)	33 (44.59%)	0.88
	Age (y), m ± sd	6.96 ± 2.55	6.59 ± 3.20	0.844
	Duration of cough (d), m ± sd	8.51 ± 4.33	7.88 ± 3.70	0.241
	Fever duration (d), m ± sd	5.57 ± 3.02	5.36 ± 2.33	0.500
	Length of hospitalization (d), m ± sd	5.96 ± 2.13	6.35 ± 2.02	0.156
	White blood cell ($\times 10^9$/L), IQR	7.33 (6.00-9.02)	7.03 (5.50-8.53)	0.251
	Neutrophil (%), IQR	62.5 (54.70-70.80)	59.70 (48.85-69.10)	0.108
	lymphocyte (%), IQR	26.9 (19.65-33.55)	29.80 (20.55-37.55)	0.076
	Elevated PCT, n (%)	20 (5.05%)	4 (5.56%)	1.000
	Elevated CRP, n (%)	265 (66.75%)	47 (63.89%)	0.589
	Digestive Symptoms, n (%)	6 (1.49%)	0 (0.00%)	0.626
	Rash, n (%)	11 (2.72%)	2 (2.70%)	1.000
	Pleural Effusion, n (%)	9 (2.23%)	1 (1.35%)	0.966
	Pulmonary Embolism, n (%)	2 (0.5%)	0 (0.00%)	1.000
	Lung consolidation/Pulmonary Atelectasis, n (%)	206 (49.72%)	35 (49.28%)	0.559

CRP, C-reactive protein; IQR, interquartile range; m ± sd, mean ± standard deviation; PCT, procalcitonin.

acinetobacter baumannii, and *adenovirus* were detected. Note that in this group of tNGS, we found that *B. pertussis* accounted for 5.2%, indicating that this uncommon bacterium is not rare in this epidemic.

Discussion

This study showed that in 2023, MP outbreaks in children in central China caused severe lower respiratory tract infections. MPP accounted for a substantial proportion of childhood pneumonia cases, with approximately half of them exhibiting lung consolidation. Despite the MP epidemic strains being resistant to macrolides due to mutations, there were no significant differences observed in clinical symptoms, lung consolidation, or clinical outcomes compared to wild-type strains.

The unexpected widespread prevalence of MP and the significant occurrence of lung consolidation in pediatric patients from the latter half of 2023 caught clinicians by surprise [6]. Our study strongly indicates that recent MP infection was associated with more severe pulmonary pathology [7]. The leading susceptible group was school-aged children, who exhibited a higher susceptibility than under three. Throughout the prevalence of MP, the identification rate of other prevalent respiratory pathogens experienced a rapid decline, particularly in the case of *respiratory syncytial virus* and *rhinovirus*. In contrast, the detection rate of *influenza virus, coronavirus*, and *adenovirus* remained consistently low. The predominant bacteria identified in the detection of the MP epidemic are *streptococcus pneumoniae* and *haemophilus influenzae*. This observation implies that respiratory pathogens commonly associated with infections may also exhibit mixed or colonization infections during the MP epidemic, potentially influenced by the sampling method and the susceptibility detection techniques employed. Notably, *B. pertussis* was concurrently identified in this cohort of children, comprising approximately 5.2% of cases, therefore, the importance of vaccination against pertussis should be emphasized.

The occurrence of lung consolidation due to MPP has prompted clinical scrutiny regarding the potential challenges in clinical treatment and the emergence of severe complications resulting from pathogen mutation. Our findings indicate that the A2063G mutant strain was the predominant pathogen responsible for childhood pneumonia, however, the pulmonary CT imaging changes were not distinguishable from those caused by wild strains. Nevertheless, it is vital to acknowledge the potential risk of pleural effusion and pulmonary embolism.

It has been shown that A2063G mutations in the 23S rRNA sequence confer resistance to 14-membered ring macrolides [8]. Additionally, these mutations may also result in resistance to 15-membered ring macrolides, like azithromycin [9]. The previous studies showed that early treatment with tetracycline and quinolone antibiotics significantly benefited those macrolide-resistant MP patients [10]. However, in this study, azithromycin and doxycycline exhibited comparable efficacy in managing cough and fever symptoms, reducing the duration of hospitalization or complications in patients with MPP. This could be correlated to the earlier bronchoscopic intervention in those in childhood with lung consolidation in this group of pediatric pneumonia.

This study has several limitations. There is a lack of data on early simultaneous tNGS as a control group and the study does not include complete test data throughout the entire year. Additionally, the data obtained only from hospitalized children with severe disease and obvious lung lesions may not fully represent the overall scenario of respiratory infection during the same period.

In summary, the outbreak of A2063 mutant MP in 2023 primarily affected school-age children, who were at a high risk of developing pulmonary consolidation and pulmonary embolism. The combination of azithromycin and early bronchoalveolar lavage proved effective in alleviating the clinical symptoms.

Declaration of competing interest

The authors have no competing interest to declare.

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Ethical approval

This study was approved by the Medical Ethics Committee of Zhongnan Hospital of Wuhan University (pre-grant 2023172K).

Author contributions

Drs. Dekyi, Yujia Xiao, and Xia Wang conducted the data collection, data analysis, data interpretation, writing, made the Figures and literature review, Drs. Yuxin Wang, Shuwen Feng, Lihong Liao, Youping Deng, and Shouyi Wang participated in data analysis, and

Dr. Dongchi Zhao and Junwen Zheng made the study design data, interpretation, and writing. All authors read and approved the final report and have no conflicting issues with the contributions listed herein.

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107074.

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