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RESEARCH

Macrolide-resistant *Mycoplasma pneumoniae* is an independent risk factor for bronchial mucus plug formation in children

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Abstract

This study aimed to explore the characteristics of bronchial mucus plug formation and its risk factors. A total of 578 children who underwent bronchoalveolar lavage treatment between January 2022 and June 2024 at the Department of Pediatric, Women and Children's Hospital of Ganzhou were assessed in this study.The bronchoalveolar lavage fluid was tested by next-generation sequencing. The resistance genes of macrolides in *Mycoplasma pneumoniae* (MP) were detected by polymerase chain reaction. Data related to the basic information, clinical characteristics, and etiology were statistically analyzed. Logistic regression analysis was performed to identify the independent risk factors for bronchial mucus plug formation. There were 312 cases in the mucus plug group and 266 in the non-mucus plug group. The mucus plug group children were older, had longer hospital stays, exhibited a higher incidence of fever, experienced more complications, and had higher rates of hormone use when compared to those in the non-mucus plug group ($P < 0.05$). The detection rate of MP and macrolide resistance was significantly higher in the mucus plug group than in the non-mucus plug group ($P < 0.05$). According to the logistic regression analysis, macrolide-resistant MP (MRMP) acted as an independent risk factor for the formation of bronchial mucus plugs in children.

Conclusion: Bronchial mucus plugs were common in children with MP infection, often accompanied by fever and prolonged hospitalization. MRMP was thus identified as an independent risk factor for bronchial mucus plug formation.

What Is Known:
• *MP can lead to bronchial mucus plugs.*
What Is New:
• *MRMP is an independent risk factor for the formation of bronchial mucus plugs.*

Keywords Bronchoalveolar lavage · Children · Bronchial mucus plug · *Mycoplasma pneumoniae* · Macrolide-resistant

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Introduction

Bronchial mucus plugs are important endogenous foreign bodies in the respiratory tract that are formed due to bronchial mucosal bleeding, necrosis, inflammation, abnormal mucus secretion, and impaired mucus clearance, which lead to the accumulation of mucus in the bronchial lumen [1]. Mucus plugs often lead to bronchiectasis, pulmonary atelectasis, obstructive bronchitis, and even acute respiratory failure [2]. In some cases, the resulting condition can progress to life-threatening bronchial plasticity [3, 4]. The removal of mucus plugs is a necessary approach to promote recovery and reduce sequelae. In clinical practice, bronchoscopic lavage is often used for treatment. Bronchoscopy intervention can effectively clear the airways

and also conduct etiology tests. Presently, it is known that *Mycoplasma pneumoniae* (MP) and Adenovirus (ADV) are prone to the formation of bronchial mucus plugs [5, 6]. Mucus plug formation has been reported as one of the difficulties encountered in the treatment of MP pneumonia (MPP). Macrolide antibiotics are the first-line treatment for MPP. The main macrolide antibiotics include azithromycin, erythromycin, clarithromycin, and roxithromycin. However, the incidence of MRMP varies globally, with eastern Asia showing a greater degree of resistance [7]. Currently, the vast majority of MP isolates are resistant to macrolides in Europe and North America, wherein their prevalence is substantially lower compared to that in Asia [8]. However, there is limited information available on the relationship between MRMP and bronchial mucus plugs. Therefore, we retrospectively analyzed the medical history data of children treated with electronic bronchoscopy and alveolar lavage so as to explore the clinical features, etiology, and independent risk factors for mucus plug formation. The early detection of airway mucus plug formation is expected to guide physicians to remove airway mucus plug at the earliest possible, reduce the risk of complications such as atelectasis, and thereby promote the recovery of children.

Materials and methods

Study population

We retrospectively collected the data of patients who underwent electronic bronchoscopy and bronchoalveolar lavage (BAL) therapy from January 2022 to June 2024 and obtained their demographics, clinical data, and laboratory data. The BAL fluid was sent to a third-party testing agency (Guangzhou KingMed Center for Clinical Laboratory) for next-generation sequencing testing. The MP antibiotic-resistance gene testing was also conducted by the same third-party testing agency. Full-length sequencing of the 23S rRNA gene of MP was performed by polymerase chain reaction. Based on the findings under the microscope, the patients were assigned to the mucus plug group and the non-mucus plug group.

Procedure for BAL

All patients completed preoperative preparations. After anesthesia, the bronchoscope was entered into the airway from the glottis through the nasal cavity and throat. We observed the tracheal carina, each lobe, and segmental bronchus. Then, normal saline at 37℃ was used for irrigation in stages, using 0.5–1.0 mL/kg each time, and the inflammation or sputum supposition was brushed and rinsed according to the situation of the site. The douche solution was inhaled into a sterile container for inspection and the intraoperative situation of the child was closely monitored [9].

Statistical analyses

The data were analyzed by using the SPSS 20 software package. Continuous variables were reported as the mean ± standard deviation and were compared by Student's t-test or the nonparametric Mann–Whitney U-test. The categorical variables were presented as numbers and compared by the Chi-square test. $P < 0.05$ was considered to indicate statistical significance. Logistic regression analysis was performed to identify the independent risk factors for bronchial mucus plug formation in children.

Results

The demographic and clinical information

A total of 578 cases (male:female = 1.62:1, age range: 2–156 months, median age: 49 months) were enrolled, of which 312 were assigned to the mucus plug group and 266 to the non-mucus plug group. The pathogenic detection rate reached 95.6%, with 236 (40.8%) cases of single infection and 317 (54.8%) cases of mixed infection. In addition, 353 (61.1%) cases tested positive for MP, and 210 (36.3%) cases had a single MP infection. A total of 183 (31.7%) patients had point mutations in the MP 23S rRNA gene; these patients are referred to as A2063G mutations. The pathogen detection rate in the mucus plug group was the highest for MP, followed by that for *Streptococcus pneumoniae* and Rhinovirus (Fig. 1). There were 182 (31.5%) cases with complications, including hypoxemia, atelectasis, pleural effusion, bronchiectasis, emphysema, pneumothorax, and empyema.

Comparisons between the mucus plug group and the non-mucus plug group

The demographics, clinical characteristics, laboratory findings, and pathogens of children in the mucus plug group and the non-mucus plug group are summarized in Table 1. The mucus plug group exhibited no statistically significant differences when compared to the non-mucus plug group in terms of gender, cough, and wheezing ($P > 0.05$). However, children in the mucus plug group were older, had longer hospital stays, had a higher

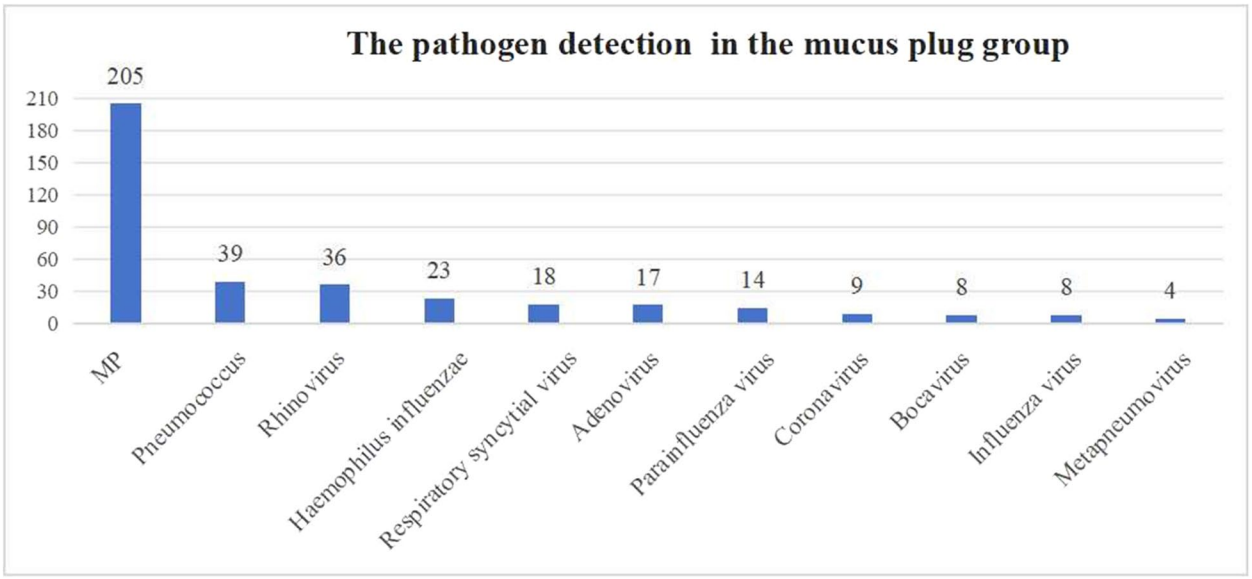

Fig. 1 The pathogen detection in the mucus plug group

Table 1 Comparisons between the mucus plug group and the non-mucus plug group

	The mucus plug group (n = 312)	The non-mucus plug group (n = 266)	*P*
Sex (male/female)	183/129	174/92	0.103
Age (months)	54.49 ± 33.342	46.98 ± 36.644	0.01
Fever	269	188	0.000
Wheezing	84	78	0.577
Cough	310	263	0.666
Use of hormones	169	39	0.000
Hospital stays	6.87 ± 2.767	6.07 ± 3.197	0.001
Complications	110	72	0.039
Severe pneumonia	88	57	0.068
White blood cells	9.39 ± 4.923	10.66 ± 5.039	0.002
Percentage of neutrophils	56.12 ± 16.806	51.41 ± 18.901	0.002
Percentage of Lymphocyte	34.17 ± 15.759	38.4 ± 18.182	0.003
C-reactive protein	12.83 ± 16.627	12.85 ± 24.942	0.989
Lactate dehydrogenase	322.41 ± 108.846	291.93 ± 88.513	0.000
Erythrocyte sedimentation rate	34.64 ± 21.987	32.96 ± 22.287	0.501
D-dimer	0.84 ± 1.349	0.66 ± 1.050	0.111
MP single infection	125	85	0.000
MRMP	169	14	0.000
Co-infection	171	146	0.817

incidence of fever, had more numbers of complications, and used more hormones when compared to those in the non-mucus plug group ($P < 0.05$). The detection rate of MP and the resistance rate of macrolides were significantly higher in the mucus plug group than in the non-mucus plug group, with statistically significant differences ($P < 0.05$).

Logistic regression analysis of bronchial mucus plug formation

A binary logistic regression analysis was performed using the variables showing statistically significant differences (Table 1) as independent variables and the presence of bronchial mucus plugs as a dependent variable. Logistic

Table 2 Logistic regression analysis of bronchial mucus plug formation

	B	*S.E*	*Wald*X^2	*P*	*OR*	95%*CI*
MP resistance	2.846	0.355	64.408	0.000	17.226	8.596–34.522
MP single infection	−0.118	0.334	0.125	0.724	0.889	0.462–1.711
Age	0.002	0.004	0.143	0.706	1.002	0.993–1.010
Fever	−0.930	0.421	4.874	0.027	0.395	0.173–0.901
WBC	0.038	0.028	1.909	0.167	1.039	0.984–1.097
LDH	−0.001	0.001	0.687	0.407	0.999	0.996–1.002

regression analysis indicated that the MRMP acted as an independent risk factor for the formation of bronchial mucus plugs in children (Table 2).

Discussion

Currently, reports on the etiology of bronchial mucus plug formation are limited. We found that the most common pathogen associated with bronchial mucus plug formation was MP, followed by *S. pneumoniae* and Rhinovirus. Moreover, there was not much difference between single infection and mixed infection cases in terms of the formation of mucus plugs. However, patients with MP single infection, fever, complications, rising levels of white blood cells (WBC), and lactate dehydrogenase (LDH) were prone to mucus plug formation. Some studies have identified the age, duration of fever, C-reactive protein (CRP) levels, and LDH as risk factors for airway mucus plug formation in children with refractory MPP (RMPP) [2, 5]. In this study, the mutation of the A2063G gene site of the 23S rRNA resistance gene was identified, which concurs with a past report [10]. Most of the MRMP isolated in Japan carry the A2063G mutation in the domain V of the 23S rRNA gene, which confers strong resistance to 14- and 15-membered macrolides and lincosamides [11]. Presently, the isolation rate of MRMP from pediatric patients is estimated at 50–90% in Japan. In Taiwan, the macrolide resistance rate was 12% in 2011, 20% between 2015 and 2016, and 50% between 2017 and 2018, respectively [12]. In this study, the rate of MRMP was 31.7%, which was lower than that in Japan. This difference may be related to the difference in the sample size or the prevalence characteristics of MP across regions.

MRMP was isolated more frequently from adolescent and pediatric patients than from adults, which is likely related to the frequent use of macrolides for treatments of mycoplasmal infections at younger ages. MRMP served as an independent risk factor for mucus plug formation. This aspect was partially consistent with the literature findings. The literature focused on RMPP, which was prone to the formation of bronchial mucus plugs. However, the present subjects were not limited to RMPP.

This study results showed that patients in the mucus plug group were relatively older, which may be related to the susceptible population for MP [13, 14]. In the physiological regulation of normal growth and development in children, the age range of 4–7 years refers to a period wherein the immune system develops rapidly. As the child grows older, the immune response triggered after infection by pathogens becomes more intense [15]. This aspect may be related to the older age of the children in the mucus plug group in this study.

The mechanism of mucus plug formation is complex, involving multiple factors that disrupt airway mucus secretion and ultimately lead to mucus plug formation [16]. After MP infection, direct damage occurs to airway epithelial cells, resulting in an increase in cell shedding within the airways [17]. Moreover, various mediators cause hemolysis and damage to mucosal and post-capillary endothelial cells, which affect the microcirculation in the infected area and alter the physical and chemical properties of the airway secretions [18]. Ultimately, under the influence of various immune-mediated inflammatory factors, immune imbalance occurs, triggering a vicious cycle in the local infection, which, in turn, exacerbates the condition and results in the formation of mucus plugs. In recent years, it has been found that the formation of airway mucus plugs is closely related to changes in the airway microbiome and the disruption of the local airway immune function, which involves the occurrence of several abnormal changes in inflammatory cytokines [19].

This study has certain limitations. First, as a retrospective study with a relatively small sample size, it may have been subject to selection bias. Future research should therefore focus on prospective studies with larger cohorts to validate these findings. Second, the study included a relatively high number of cases of MP infection because of the MP epidemic period, which limited the analysis of bronchial mucus plug formation caused by other pathogens.

In conclusion, bronchial mucus plugs were found to be more common in children with MP infection and were often accompanied by fever and prolonged hospitalization requirements. Although co-infection did not increase the risk, MRMP was identified as a significant factor in mucus

plug formation, thereby highlighting the need for early detection and intervention in such cases.

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Authors' contributions Xie chunlian and Zhou lili wrote the main manuscript text, Zhang libin helped to analyzed data and prepared Figs. 1 and Xiao yichun prepared Table 1–2. All authors reviewed the manuscript.

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

Declarations

Ethics approval The study protocol was approved by the Ethics and Research Council of Women and Children's Hospital of Ganzhou (2021–203). The data were collected from the patients anonymously. This study complied with the Declaration of Helsinki.

Competing interests The authors declare no competing interests.

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