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ORIGINAL RESEARCH

# Azithromycin Effectiveness in Children with Mutated *Mycoplasma Pneumoniae* Pneumonia

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**Objective:** *Mycoplasma pneumoniae* (MP) is highly resistant to macrolides in China. However, macrolides still exhibit clinical effectiveness in some macrolide-resistant patients. We tend to explore azithromycin effectiveness in *Mycoplasma pneumoniae* pneumonia (MPP) children with A2063/2064G mutation.

**Methods:** This retrospective observational cohort study was conducted at the Children's Hospital of the Chongqing Medical University. Children with macrolide-resistant mutations (A2063/2064G) diagnosed as MPP were retrospectively enrolled. Receiver operating characteristic (ROC) curves and logistic regression analysis were used to evaluate and identify independent risk factors for treatment failure (progress to refractory *Mycoplasma pneumoniae* pneumonia [RMPP]) in macrolide-unresponsive *Mycoplasma pneumoniae* pneumonia (MUMPP) children with the A2063/2064G mutation.

**Results:** One hundred fifty-five children were retrospectively enrolled. More than 20% (36/155, 23.23%) of patients experienced defervescence within 3 days of azithromycin treatment. RMPP was diagnosed in 54 patients (54/155, 34.84%) and the incidence of RMPP during hospitalization was 22.72 per 1000 person-days. Logistic regression analysis showed that lactate dehydrogenase (LDH) ≥ 399 (U/L) was an independent risk factor for RMPP (odds ratio [OR] 4.66, 95% confidence interval [CI] 1.31–17.10, P=0.017). During the year followed, RMPP patients had a significantly higher incidence of bronchiolitis obliterans and bronchiectasis than non-RMPP patients (16.67% vs 1.98%, P=0.001; 9.26% vs 0.00%, P=0.005, respectively).

**Conclusion:** Azithromycin was effective in children with MPP with the A2063/2064G mutation. For MUMPP children with A2063/2064G mutation, children with LDH ≥ 399 (U/L) had significant higher risk for progression to RMPP, and should consider to be treated with alternative antibiotics (eg tetracyclines, and fluoroquinolones).

**Keywords:** azithromycin effectiveness, A2063/2064G mutation, *mycoplasma pneumoniae* pneumonia, children

## Introduction

*Mycoplasma pneumoniae* (MP) is one of the most common pathogens that cause community-acquired pneumonia in children.[1] *Mycoplasma pneumoniae* pneumonia (MPP) is thought to be an acute, self-limiting infectious disease; however, it may sometimes cause severe short-term prognosis, such as refractory *Mycoplasma pneumoniae* pneumonia (RMPP), and long-term prognosis, such as bronchiolitis obliterans[2] and bronchiectasis.[3] Macrolides are first-line antibiotics for children with MPP. The incidence of MPP and macrolide-resistant MPP has increased over the past few years,[4] so do macrolide-resistant MPP.[5] One study showed that the detection rate of macrolide-resistant MP exceeded 90% in China.[6] Tetracyclines and fluoroquinolones are alternative antibiotics for macrolide-resistant MP infections, but their safety in children remains to be explored in children.[7] Therefore, it is important for clinicians to determine whether macrolides should be used in children with suspected macrolide-resistant MPP.

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RMPP is defined as clinical and radiological deterioration despite appropriate antibiotic therapy for at least 7 days,[8] is associated with high medical costs and poor long-term prognosis.[5] Therefore, RMPP is thought to be resistant to macrolides. However, macrolides remain effective in some patients with macrolide-resistant MPP or RMPP,[9,10] and macrolides combined with glucocorticoid therapy[11] or immunoglobulin therapy[12] may also be an option for RMPP. Considering the efficacy and safety of optional antibiotics against macrolide-resistant MP in children, early identification of RMPP may help adjust the therapeutic regimen in a timely manner and improve prognosis. Huang et al[13] suggested that C-reactive protein (CRP), lactate dehydrogenase (LDH), neutrophil proportion, D-dimer level, and long consolidation were predictors of RMPP. The A2063/2064G mutation accounts for > 90% of the macrolide-resistant MP infections.[4–6] Patients with persistent fever for ≥ 3 days after macrolide treatment are thought to be unresponsive to macrolides.[14] However, few studies have demonstrated the effectiveness of macrolides in children with MPP with the A2063/2064G mutation, and the indicators of treatment failure in macrolide-unresponsive *Mycoplasma pneumoniae* pneumonia (MUMPP) patients remain to be explored.

In this study, we aimed to evaluate the effectiveness of azithromycin in children with MPP with the A2063/2064G mutation and to explore the indicators of treatment failure (progression to RMPP) in children with MUMPP with the A2063/2064G mutation.

# Methods

## Study Design and Population

This retrospective, observational cohort study was conducted at the Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorder. Hospitalized children with MPP associated with macrolide-resistant mutations (A2063/2064G) were enrolled between January 2019 and December 2022. All patients were followed for at least one year. The inclusion criteria were as follows: (i) hospitalized children and (ii) diagnosed with macrolide-resistant mutation (A2063/2064G) associated MPP. The exclusion criteria were as follows: (i) patients who experienced defervescence before admission, (ii) patients with incomplete clinical information, and (iii) patients with hematologic malignancies.

This study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University (approval number: 2021–179). The requirement for informed consent for this retrospective study was waived by the Ethics Committee of Children's Hospital of Chongqing Medical University. Neither human nor animal experiments were conducted in this study. All Methods were performed in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

## Data Collection and Definitions

We retrospectively obtained information including demographic data, underlying conditions, laboratory data (routine blood examination, liver function, and coagulation function), chest imaging data, antibiotic therapy during hospitalization, fever duration, cough duration, diagnosis, and prognosis. The diagnostic criteria for MPP were based on the Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2022[15] and the Chinese Guidelines for Diagnosis and Treatment of Mycoplasma Pneumoniae Pneumonia in Children (2023 Edition).[16] Macrolide-unresponsive *Mycoplasma pneumoniae* pneumonia (MUMPP) is defined as persistent fever ≥3 days after macrolide treatment.[17] RMPP is defined as clinical and radiological deterioration despite appropriate antibiotic therapy for at least seven days.[8] Treatment failure was defined as the progression to RMPP in children with MUMPP with the A/2063/2064G mutation. The children who experienced defervescence within 3 days of azithromycin treatment or those who did not progress to RMPP were thought to show effectiveness to azithromycin. Diagnoses of bronchiolitis obliterans[18] and bronchiectasis[19] are based on a combination of history, clinical symptoms, and physiological and radiological findings. Patients diagnosed with bronchiolitis obliterans or bronchiectasis within one year of discharge were considered to have a poor prognosis.

## Detection of Macrolide Resistance Gene

Bronchoalveolar lavage fluid (BALF) or nasopharyngeal aspirates (NPA) were collected for A2063G/A2064G gene mutation detection. The resistance genes were detected by a nested PCR-linked capillary electrophoresis and single-strand conformation polymorphism analysis (nPCR-CE-SSCP) based on a previous method.[20]

## Clinical Outcome

To evaluate the effectiveness of azithromycin in MPP children with A2063/2064G mutation.

## Statistical Analysis

Continuous variables were compared using the Mann–Whitney *U*-test or Student's *t* test and are presented as medians and interquartile ranges (IQRs). Categorical variables were compared using Pearson's $\chi^2$ test or Fisher's exact test, and are presented as numbers (n) and percentages (%). Receiver operating characteristic (ROC) curves were used to evaluate the candidate indicators of treatment failure (progression to RMPP). The area under the curve (AUC) and corresponding 95% confidence interval (CI) were calculated, and the prediction efficacy of indicators was classified into less predictive ($0.5 < AUC \leq 0.7$), moderately predictive ($0.7 < AUC \leq 0.9$) and highly predictive ($0.9 < AUC < 1$).[21,22] To evaluate the effectiveness of azithromycin, logistic regression analysis were applied to explore the risk factors of poor prognosis in MUMPP children with A2063/2064G mutation. Variables with *P*-level $\geq 0.10$ in univariate logistic analysis were further enrolled in multivariate logistic regression analysis. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were calculated. The differences were statistically significant (two-sided, *P*-value <0.05 (2-sided). All analyses were performed using the R software version 4.3.2.

# Results

## Study population

From January 2019 to December 2022, 332 children were eligible for inclusion in the study. A total of 177 children were excluded (128 patients experienced defervescence before admission, 47 patients had incomplete clinical information, and two patients had hematologic malignancy). Then, 155 children with MPP with the A2063/2064G mutation were enrolled in this study, and there were 119 children with A2063/2064G mutation diagnosed with MUMPP. Finally, those 119 MUMPP children were divided into Non-RMPP group and RMPP group. A flow chart of the study population is shown in Figure 1. Among MPP patients with the A2063/2064G mutation enrolled from 2019 to 2022, the proportion of RMPP decreased from 65.52% to 26.32%. The number of children with MPP with A2063/2064G mutations in different years is shown in Figure 2.

## Clinical Characteristics of 155 MPP Children with A2063/2064G Mutation

The median age was 6.17 (IQR 4.21–8.04) years, and 49.68% (77/155) of the patients were male. The median total fever duration was 10.00 (9.00–13.00) days and the median total cough duration was 16.00 (IQR 13.50–19.00) days. The median length of stay was 14.00 (IQR 11.32–17.59) days. There were 22.58% (35/155) patients with viral coinfection and 11.61% (18/155) with bacterial coinfection. More than three-fourths (123/155, 79.35%) of patients had lung consolidation, and approximately one-third (49/155, 31.61%) had pleural effusion. Approximately one-third of patients (47/155, 30.32%) were administered glucocorticoids or quinolones (44/155, 28.39%). There were 23 (14.84%, 23/155) patients experienced continuous positive airway pressure (CPAP). The incidence of MUMPP was 76.77% (119/155); in other words, more than 20% (36/155, 23.23%) of patients experienced defervescence within 3 days of azithromycin treatment. RMPP was diagnosed in 54 patients (34.84%, 54/155) and the incidence of RMPP during hospitalization was 22.72 per 1000 person-days. Eleven (7.10%, 11/155) patients had bronchiolitis obliterans and four (4/155, 2.58%) patients had bronchiectasis within one year after discharge. The details are presented in Table 1.

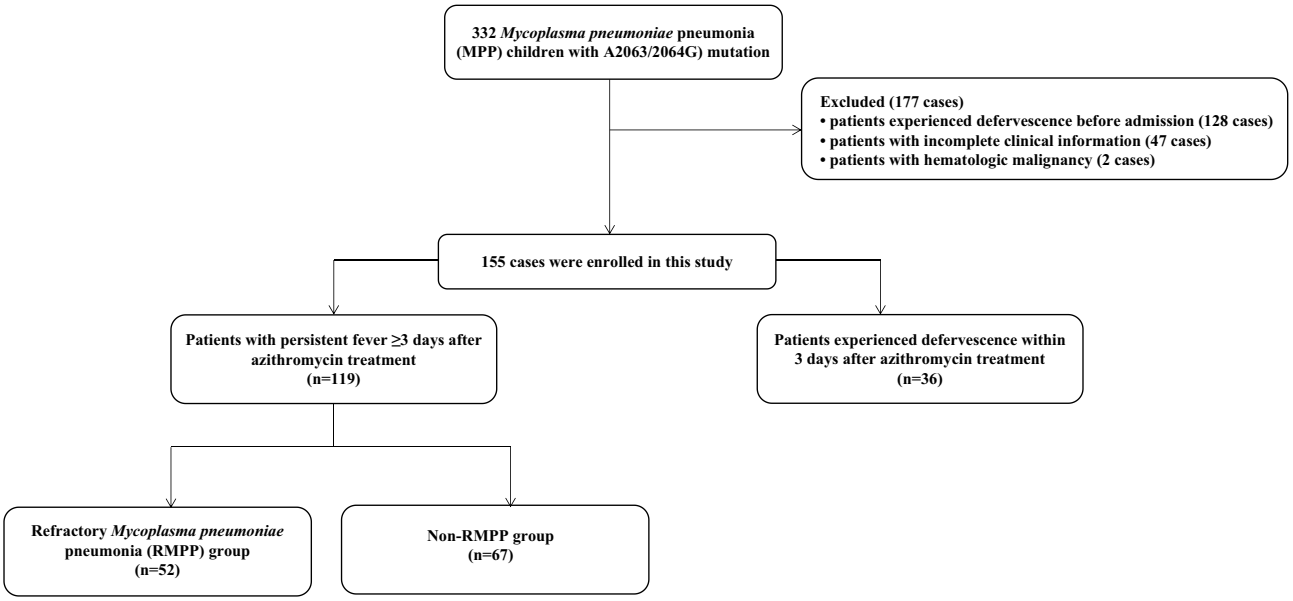


**Figure 1** The flow chart of the population.

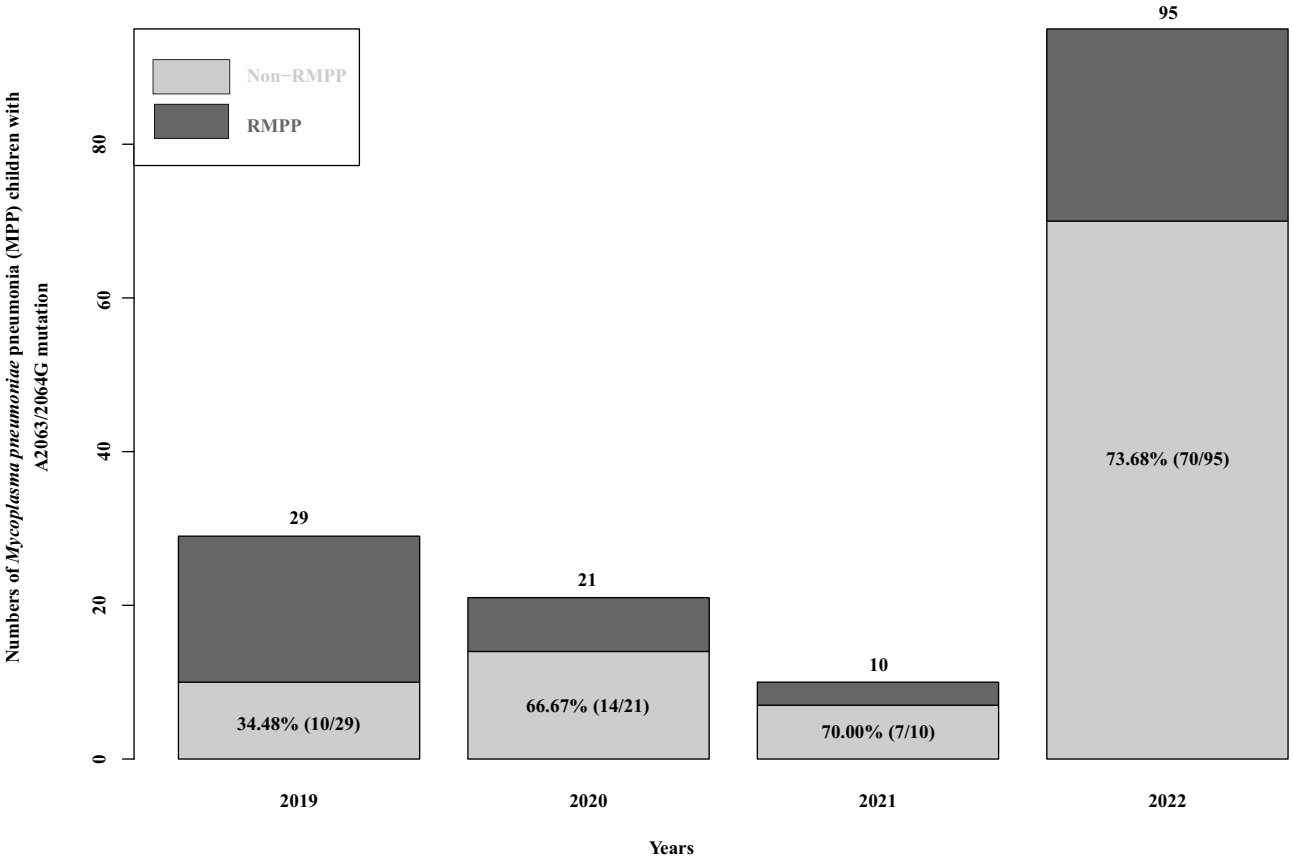


**Figure 2** Numbers of *Mycoplasma pneumoniae* pneumonia (MPP) children with A2063/2064G mutation in different years.

## Comparisons of Clinical Characteristics Between the Non-RMPP and RMPP Groups in 119 MUMPP Children with A2063/2064G Mutation

RMPP patients had a remarkably higher proportion of pleural effusions than non-RMPP patients (51.92% vs 25.37%, P=0.005). Compared to non-RMPP patients, RMPP patients had significantly lower albumin levels and significantly

**Table 1** Characteristics of 155 MPP Children with A2063/2064G Mutation

| Characteristics | Total (n=155) |
|---|---|
| Demographic data | |
| Age (years) (median, IQR) | 6.17 (4.21–8.04) |
| Male (n, %) | 77 (49.68%) |
| Underlying condition | |
| Viral coinfection (n, %) | 35 (22.58%) |
| Bacterial coinfection (n, %) | 18 (11.61%) |
| Laboratory data | |
| WBC (10^9/L) (median, IQR) | 6.81 (5.25–9.33) |
| CRP (mg/L) (median, IQR) | 22.22 (11.93–37.00) |
| PCT (ng/mL) (median, IQR) | 0.21 (0.10–0.53) |
| Fibrinogen (g/L) (median, IQR) | 4.52 (3.87–4.90) |
| D-dimer (ng/mL) (median, IQR) | 1.00 (0.47–2.17) |
| Albumin (g/L) (median, IQR) | 40.20 (35.60–43.30) |
| ALT (U/L) (median, IQR) | 18.00 (13.00–25.00) |
| AST (U/L) (median, IQR) | 36.00 (29.00–46.00) |
| LDH (U/L) (median, IQR) | 337.00 (283.00–465.65) |
| Chest imaging data | |
| Lung consolidation (n, %) | 123 (79.35%) |
| Pleural effusion (n, %) | 49 (31.61%) |
| With cephalosporins or penicillin | 77 (49.68%) |
| Requiring for CPAP (n, %) | 23 (14.84%) |
| Therapy adjustment during hospitalization | |
| Application of glucocorticoid (n, %) | 47 (30.32%) |
| Application of quinolones (n, %) | 44 (28.39%) |
| Application of immunoglobulin (n, %) | 28 (18.06%) |
| Fever duration before azithromycin therapy (days) (median, IQR) | 5.00 (4.00–7.00) |
| Total fever duration (days) (median, IQR) | 10.00 (9.00–13.00) |
| Cough duration before azithromycin therapy (days) (median, IQR) | 4.00 (3.00–6.50) |
| Total cough duration (days) (median, IQR) | 16.00 (13.50–19.00) |
| Length of stay (days) (median, IQR) | 14.00 (11.32–17.59) |
| MUMPP (n, %) | 119 (76.77%) |
| RMPP (n, %) | 54 (34.84%) |
| Poor prognosis within one year after discharge | 16 (10.32%) |
| Bronchiolitis obliterans (n, %) | 11 (7.10%) |
| Bronchiectasis (n, %) | 5 (3.23%) |

**Abbreviations**: CPAP, Continuous positive airway pressure; MPP, *Mycoplasma pneumoniae* pneumonia; MUMPP, Macrolides-unresponsive *Mycoplasma pneumoniae* pneumonia; RMPP, Refractory *Mycoplasma pneumoniae* pneumonia.

higher C-reactive protein (CRP), procalcitonin (PCT), D-dimer, alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels. (P <0.05). Significantly more RMPP patients were administered cephalosporins or penicillin and required CPAP than non-RMPP patients (69.23% vs 38.81%, P=0.002; 28.85% vs 4.48%, P=0.001, respectively). Remarkably, more patients in the RMPP group were administered glucocorticoids and quinolones than in the ono-RMPP group (55.77% vs 20.90%, P<0.001; 53.85% vs 13.43%, P<0.001, respectively). Furthermore, the RMPP patients had remarkably longer cough duration (18.00 days (IQR 16.00–22.00 days) vs 15.00 days (IQR 13.00–17.00 days), P<0.001) or fever duration (13.00 days (IQR 11.00–15.00 days) vs 10.00 days (IQR 9.00–11.50 days), P<0.001) than those non-RMPP patients. The data showed that all patients with bronchiolitis obliterans or bronchiectasis were in the RMPP group. The demographic data, proportion of bacterial coinfection, proportion of viral coinfection, proportion of lung consolidation, white blood cell (WBC) count, fibrinogen level, usage of immunoglobulin,

fever duration or cough duration before anti-mycoplasma therapy, and length of hospital stay were not significantly different between the two groups. The details are presented in Table 2.

## Risk Factors of Treatment Failure (Progress to RMPP) in 119 MUMPP Children with A2063/2064G Mutation

To explore the optimum predictive values of candidate risk factors of treatment failure (progression to RMPP) in MPP children with the A2063/2064G mutation, ROC curves were constructed, and the cut-off values with maximum sensitivities and specificities were determined (Table 3). ROC curve analyses showed that the cut-off points for CRP, PCT, D-dimer, albumin level, ALT, AST, and LDH were 28.98 mg/L, 0.46 ng/mL, 1.32 ng/mL, 39.30 g/L, 21.30 U/L, 53, and 399 U/L, respectively. All candidate risk factors showed low or moderate predictive efficacy. The details are presented in Table 3.

**Table 2** Clinical Characteristics Comparisons of 119 MUMPP Children with A2063/2064G Mutation in Non-RMPP Group and RMPP Group

| Characteristics | Non-RMPP (n=67) | RMPP (n=52) | P value |
|---|---|---|---|
| Demographic data | | | |
| Age (years) (median, IQR) | 6.67 (4.50–8.21) | 5.96 (4.25–7.67) | 0.565 |
| Male (n, %) | 35 (52.24%) | 23 (44.23%) | 0.495 |
| Coinfections | | | |
| Viral coinfection (n, %) | 13 (19.40%) | 13 (25.00%) | 0.611 |
| Bacterial coinfection (n, %) | 6 (8.96%) | 8 (15.38%) | 0.428 |
| Laboratory data | | | |
| WBC (10^9/L) (median, IQR) | 7.02 (4.93–9.67) | 6.55 (5.09–9.20) | 0.787 |
| CRP (mg/L) (median, IQR) | 17.91 (0.78–30.30) | 29.33 (13.71–52.44) | 0.014* |
| PCT (ng/mL) (median, IQR) | 0.18 (0.10–0.41) | 0.41 (0.14–0.76) | 0.006* |
| Fibrinogen (g/L) (median, IQR) | 4.53 (3.99–4.92) | 4.53 (3.85–4.79) | 0.493 |
| D-dimer (ng/mL) (median, IQR) | 0.60 (0.43–1.06) | 2.36 (1.06–4.41) | <0.001* |
| Albumin (g/L) (median, IQR) | 41.50 (39.15–43.80) | 35.85 (32.70–40.70) | <0.001* |
| ALT (U/L) (median, IQR) | 16.00 (13.00–21.00) | 22.45 (15.50–42.25) | <0.001* |
| AST (U/L) (median, IQR) | 34.00 (28.00–43.50) | 43.85 (33.75–61.50) | 0.001* |
| LDH (U/L) (median, IQR) | 310.00 (271.00–364.00) | 496.80 (348.75–686.50) | 0.004* |
| Chest imaging data | | | |
| Lung consolidation (n, %) | 52 (77.61%) | 47 (90.38%) | 0.109 |
| Pleural effusion (n, %) | 17 (25.37%) | 27 (51.92%) | 0.005* |
| With cephalosporins or penicillin | 26 (38.81%) | 36 (69.23%) | 0.002* |
| Requiring for CPAP (n, %) | 3 (4.48%) | 15 (28.85%) | 0.001* |
| Therapy adjustment during hospitalization | | | |
| Application of glucocorticoid (n, %) | 14 (20.90%) | 29 (55.77%) | <0.001* |
| Application of quinolones (n, %) | 9 (13.43%) | 28 (53.85%) | <0.001* |
| Application of immunoglobulin (n, %) | 11 (16.42%) | 14 (26.92%) | 0.243 |
| Fever duration before azithromycin therapy (days) (median, IQR) | 5.00 (3.00–6.00) | 5.00 (3.00–7.00) | 1.000 |
| Total fever duration (days) (median, IQR) | 10.00 (9.00–11.50) | 13.0 (11.00–15.00) | <0.001* |
| Cough duration before azithromycin therapy (days) (median, IQR) | 4.00 (2.00–5.00) | 4.00 (3.00–7.00) | 0.498 |
| Total cough duration (days) (median, IQR) | 15.00 (13.00–17.00) | 18.00 (16.00–22.00) | <0.001* |
| Length of stay (days) (median, IQR) | 13.75 (11.07–17.25) | 13.92 (11.38–17.50) | 0.949 |
| Poor prognosis within one year after discharge | | | |
| Bronchiolitis obliterans (n, %) | 0 (0.00%) | 8 (15.38%) | 0.001* |
| Bronchiectasis (n, %) | 0 (0.00%) | 5 (9.62%) | 0.014* |

**Note:** *with statistical significance, P <0.05.

**Abbreviations**: CPAP, Continuous positive airway pressure; MUMPP, Macrolides-unresponsive *Mycoplasma pneumoniae* pneumonia; RMPP, Refractory *Mycoplasma pneumoniae* pneumonia.

**Table 3** Predictive Values of Candidate Risk Factors of Treatment Failure (RMPP) in 119 MUMPP Children with A2063/2064G Mutation

| Risk Factors | Cutoff value | Sensitivity | Specificity | AUC (95% CI) | P-value |
|---|---|---|---|---|---|
| CRP (mg/L) | 28.98 | 51.92% | 73.13% | 0.63 (0.53–0.73) | 0.007* |
| PCT (ng/mL) | 0.46 | 50.00% | 77.61% | 0.65 (0.55–0.75) | 0.007* |
| D-dimer (ng/mL) | 1.32 | 71.15% | 80.60% | 0.79 (0.71–0.88) | <0.001* |
| Albumin (g/L) | 39.30 | 71.15% | 74.63% | 0.74 (0.64–0.83) | <0.001* |
| ALT (U/L) | 21.30 | 55.77% | 79.10% | 0.70 (0.60–0.79) | 0.002* |
| AST (U/L) | 53.00 | 34.62% | 92.54% | 0.66 (0.55–0.76) | <0.001* |
| LDH (U/L) | 399.00 | 67.31% | 82.09% | 0.78 (0.69–0.87) | 0.033* |
| Pleural effusion | - | 51.92% | 74.63% | 0.63 (0.55–0.71) | 0.002* |
| Requiring for CPAP | - | 28.85% | 95.52% | 0.62 (0.55–0.69) | <0.001* |
| With cephalosporins or penicillin | - | 69.23% | 61.12% | 0.65 (0.57–0.74) | <0.001* |

**Note:** *with statistical significance, P <0.05.
**Abbreviations**: CPAP, Continuous positive airway pressure; MUMPP, Macrolides-unresponsive *Mycoplasma pneumoniae* pneumonia; RMPP, Refractory *Mycoplasma pneumoniae* pneumonia.

**Table 4** Logistic Regression Analysis for Risk Factors of Treatment Failure (RMPP) in 119 MUMPP Children with A2063/2064G Mutation

| Variables | Univariate Analysis | | | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | P | OR | 95% CI | P |
| LDH ≥ 399 (U/L) | 9.79 | 4.61–21.76 | <0.001* | 4.66 | 1.31–17.10 | 0.017* |
| D-dimer ≥ 1.32 (ng/mL) | 9.34 | 4.46–20.52 | <0.001* | | | |
| Albumin ≤ 39.30 (g/L) | 5.99 | 2.93–12.63 | <0.001* | | | |
| Requiring for CPAP | 5.65 | 2.23–15.74 | <0.001* | | | |
| ALT ≥ 21.30 (U/L) | 4.17 | 2.06–8.62 | <0.001* | | | |
| Pleural effusion | 4.10 | 2.02–8.53 | <0.001* | | | |
| Neutrophil proportion ≥ 68.76 (%) | 3.80 | 1.91-7-74 | <0.001* | | | |
| With cephalosporins or penicillin | 3.78 | 1.89–7.83 | <0.001* | | | |
| CRP ≥ 28.98 (mg/L) | 3.65 | 1.70–8.05 | 0.001* | | | |
| PCT ≥ 0.46 (ng/mL) | 3.56 | 1.76–7.53 | <0.001* | | | |
| AST ≥ 53.00 (U/L) | 3.21 | 1.60–6.55 | 0.001* | | | |

**Note:** *with statistical significance, P <0.05.
**Abbreviations**: MUMPP, Macrolides-unresponsive *Mycoplasma pneumoniae* pneumonia; RMPP, Refractory *Mycoplasma pneumoniae* pneumonia.

In univariate logistic analysis, CRP ≥28.98 mg/L, PCT ≥0.46 ng/mL, D-dimer ≥1.32 ng/mL, albumin level ≤39.30 g/L, ALT ≥21.30 U/L, AST ≥53 U/L, LDH ≥399 U/L, pleural effusion requiring CPAP, and administration of cephalosporins or penicillin were associated with an increased risk of RMPP. In multivariate analysis, LDH ≥399 U/L was an independent risk factor for RMPP in MUMPP children with the A2063/2064G mutation (odds ratio [OR]4.66, 95% confidence interval [CI] 1.31–17.10, P =0.017). Details are presented in Table 4.

## Discussion

MP with gene mutations in 23S rRNA has been reported worldwide since 2000, and the A2063/2064G mutation accounts for more than 90% of macrolide-resistant MP infections.[4–6] The macrolide resistance rate in MP exceeds 90% in China.[6] In our study, we found that the incidence of RMPP showed a decreasing trend (from 65.52% to 26.32%) in MPP patients with the A2063/2064G mutation. A possible explanation for this is as follows. Azithromycin combined with immunoglobulin or glucocorticoids has been shown to have a better clinical therapeutic effect than azithromycin alone.[8,12] Approximately one-third of the patients (30.32%, 47/155) in our study received glucocorticoid therapy, and approximately one-fifth (18.06%, 28/155) received immunoglobulin therapy for patients with persistent fever ≥3 days after

macrolide treatment. We assumed that timely immunoglobulin or glucocorticoid therapy might help patients with MUMPP avoid progressing to RMPP.

For patients with MUMPP, second-line antibiotics (such as tetracyclines and fluoroquinolones) may be required.[17] However, the use of tetracyclines and fluoroquinolones is restricted in children because of their unknown safety in this population.[7] Lee et al[23] found that macrolides are clinically effective in some patients with macrolide-resistant MP, although with a relatively low effectiveness. Zuckerman et al[24] reported a possible association between a decrease in MP DNA load and defervescence within 48 h of macrolide treatment.[24] However, Kawai et al[25] found that some macrolide-resistant patients showed defervescence, whereas there was no decrease in the MP DNA load after 48 h of macrolide treatment. Inappropriate excessive immune responses play a key role in MP infection,[17] and the immunomodulatory effects of macrolides[26] may explain defervescence after macrolide treatment in patients with macrolide-resistant MP. In this study, we also found more than twenty percent (36/155, 23.23%) of patients showed defervescence within three days of azithromycin treatment. It was assumed that in vitro susceptibility testing of MP may not completely represent clinical effectiveness. In other words, for some children with MPP and the A2063/2064G mutation, azithromycin remains a safe and effective option in the first three-day course of treatment. In patients with MUMPP, second-line antibiotics (eg tetracyclines, and fluoroquinolones),[17] glucocorticoids[11] and immunoglobulin therapies[12] should be further evaluated. WBC, PCT, CRP, LDH, and D-dimer levels have been reported to be predictive factors for RMPP.[17,27] In this study, we found that LDH ≥399 U/L was an independent risk factor for treatment failure (progression to RMPP) in MUMPP patients with the A2063/2064G mutation (OR 4.66, 95% CI 1.31–17.10, P =0.017).

According to Guidelines for Diagnosis and Treatment of *Mycoplasma Pneumoniae* Pneumonia in Children (China, 2023 Edition),[16] tetracyclines or fluoroquinolones may be recommended for MUMPP patients with A2063/2064G mutation, however, the specific opportunity for therapy adjustment was unclear. Our study found that for MUMPP patients with A2063/2064G mutation, those with LDH ≥399 U/L should consider to be treated with alternative antibiotics (eg tetracyclines, and fluoroquinolones).

Children aged ≥ 5 years have a more developed immune system and are more likely to develop RMPP than younger children.[17] The median age was 6.17 (IQR 4.21–8.04) years and 54 (54/155, 34.84%) children with macrolide-resistant mutation (A2063/2064G) associated MPP had RMPP in our study. The incidence of RMPP during hospitalization was 22.72 per 1000 person-days. Among the MUMPP patients with the A2063/2064G mutation, RMPP patients had a significantly higher proportion of bronchiolitis obliterans and bronchiectasis (15.38% vs 0.00%, P=0.001; 9.62% vs 0.00%, P=0.014, respectively). Chiu et al[28] suggested that coinfection may influence the prognosis of MP infection, and Zhang et al[29] found that coinfection was positively correlated with severity in children with RMPP. However, we found no significant difference in coinfections between the RMPP and non-RMPP patients. It was assumed that coinfections might not play a key role in the occurrence of bronchiolitis obliterans or bronchiectasis. Although the mechanism of RMPP remains to be explored, an excessive host immune response may play a key role in disease progression.[9] Immune evasion may result in uncontrolled proliferation of MP, and inflammatory injury may induce host tissue damage.[30] We found that RMPP patients had significantly higher CRP and LDH levels than non-RMPP patients. CRP and LDH levels may be positively associated with the inflammatory responses in the body.[13] Excessive and overactive immune response in RMPP may lead to severe sequelae.[9] Furthermore, for patients with RMPP, it has been reported that bacterial coinfection was rare and the non-anti-MP antibiotics showed little effectiveness in early treatment.[31] Our Results showed that 18 (11.61%) patients had bacterial coinfection. However, more than half (62/155, 52.10%) of the patients were administered cephalosporins or penicillin, indicating that non-anti-MP antibiotics need to be decreased in children with MPP.

This study has some limitations. First, this was a retrospective, single-center study with a small sample size, and a prospective, multicenter study is expected to strengthen our results. Second, some patients with incomplete clinical information were excluded, and this may also induce potential biases in our study. Third, azithromycin showed good effectiveness to more than twenty percent of MPP children with the A2063/2064G mutation in our study, but the mechanisms require further exploration. Lastly, more than two-thirds of the patients in our study showed persistent fever ≥3 days after macrolide treatment; however, the safety of second-line antibiotics (such as tetracyclines and fluoroquinolones) remains to be further explored.

## Conclusion

In this study, we found that azithromycin was effective in children with MPP with the A2063/2064G mutation. For MUMPP children with A2063/2064G mutation, children with LDH ≥ 399 (U/L) had significant higher risk for progression to RMPP, and should consider to be treated with alternative antibiotics (eg tetracyclines, and fluoroquinolones).

## Abbreviations

ALT, Alanine transaminase; AST, Aspartate aminotransferase; AUC, Area under the curve; CI, Confidence interval; CPAP, Continuous positive airway pressure; CRP, C-reactive protein; IQR, Inter-quartile range; LDH, Lactate dehydrogenase; MP, *Mycoplasma pneumoniae*; MPP, *Mycoplasma pneumoniae* pneumonia; MUMPP, Macrolides-unresponsive *Mycoplasma pneumoniae* pneumonia; OR, Odds ratio; PCT, Procalcitonin; RMPP, Refractory *Mycoplasma pneumoniae* pneumonia; ROC, Receiver operating characteristic; WBC, White blood cell.

## Data Sharing Statement

Data from this study can be obtained from the corresponding author with rational request.

## Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University. (Approval number: 2021-179). The requirement for informed consent for this retrospective study was waived by the Ethics Committee of Children's Hospital of Chongqing Medical University. Neither human nor animal experiments were conducted in this study. All methods were performed in accordance with relevant guidelines and regulations.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

Jie Cheng and Ya Liu are co-first authors for this study. The authors report no competing interests in this study.

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