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Analysis of the characteristics of mixed infections with *Mycoplasma pneumoniae* in children

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353 hospitalized children diagnosed with *Mycoplasma pneumoniae* (MP) pneumonia were included in the study. They were divided into MP co-infection group and MP single infection group. 143 (40.5%) of the enrolled children had MP co-infections. The most common co-infecting pathogen was Rhinovirus (30.8%). Among the MP co-infections, 82 cases (57.3%) involved one pathogen, 44 cases (30.7%) involved two pathogens, 12 cases (8.4%) involved three pathogens, 4 cases (2.8%) involved four pathogens, and 1 case (0.7%) involved five pathogens. Significant differences were observed between the two groups in terms of severe MP pneumonia, macrolide resistance, bronchial mucus plug, and hormone use, with *P*-values of 0.039, 0.000, 0.000, and 0.035. The MP mixed virus or bacteria infection group was more likely to develop drug resistance compared to the mixed virus and bacteria group ($P = 0.007$ and $P = 0.046$). The MP mixed virus and bacteria group was more likely to develop severe pneumonia compared to the mixed virus or bacteria infection group ($P = 0.032$ and $P = 0.017$). In conclusion, MP was most commonly co-infected with Rhinovirus. Children with MP co-infections tend to exhibit higher rates of macrolide resistance, require more frequent use of hormones, and are more likely to develop severe pneumonia and bronchial mucus plug.

Keywords *Mycoplasma pneumoniae*, *Mycoplasma pneumoniae* pneumonia, Co-infection, Children

Mycoplasma pneumoniae pneumonia (MPP) is a significant public health issue affecting children worldwide, prompting extensive research in recent years[1]. Epidemics of *Mycoplasma pneumoniae* (MP) occur every 3–7 years, accounting for more than 40% of pediatric community-acquired pneumonia (CAP) cases during epidemic years[2]. Most studies have focused on refractory MP pneumonia (RMPP), macrolide resistant MP pneumonia (MRMPP), and severe MP pneumonia (SMPP). Few studies have investigated the influence of respiratory pathogens co-infection on the clinical course of MPP and their results have been inconclusive[3–5]. This study aims to explore the effects of respiratory pathogen co-infections on MPP in children.

Methods

Study patients and data collection

We retrospectively collected data from patients diagnosed with MPP who were admitted to the Women and Children's Hospital of Ganzhou and underwent bronchoalveolar lavage (BAL) therapy between May 2023 and July 2024. MPP was defined as patients presenting with clinical signs and symptoms of pneumonia underwent chest radiography, and MP infection was confirmed through laboratory tests[6]. SMPP was defined as MPP with any one of the follows[7]: (1) a poor general condition; (2) fastidium or dehydration; (3) disturbance of consciousness; (4) an increased respiratory rate (infants > 70 breaths/min and older children > 50 breaths/min); (5) dyspnea; (6) cyanosis; (7) extent of infiltration on chest X-ray ≥ 2/3 of one lung or multilobe involvement; (8) extra-pulmonary complications; (9) pleural effusion; (10) oxygen saturation in room air ≤ 92%. RMPP was defined as clinical and radiographic deterioration after at least seven days of macrolide antibiotic treatment[7]. MRMPP was defined as persistent fever ≥ 3 days after macrolide treatment, accompanied by the A2063G/2064G mutation[8]. Bronchial mucus plug was defined as endogenous foreign bodies in the respiratory tract. Due to the retrospective nature of the study, the Ethics and Research Council of the Women and Children's Hospital of Ganzhou waived the need for informed consent. All methods were performed in accordance with relevant guidelines and regulations, and all experimental protocols were approved by the Women and Children's Hospital of Ganzhou.

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Microbiological analyses

On the day of admission, 3 mL of venous blood was collected from patients, and the passive agglutination assay was used to detect MP antibody (MP Antibody Test Kit, Fujitsu Joint Stock Company). An MP antibody titer ≥ 1:160 was considered positive. BALF samples were collected using flexible fiberoptic bronchoscopy. Briefly, after the bronchoscope was inserted into the affected lung lobe, warm sterile saline was instilled and gently suctioned. A 1.5–2 mL aliquot of BALF from each patient was used for traditional bacterial culture within 30 min of collection[9]. Another 1.5–2 mL of BALF samples were sent to a third-party testing agency (Guangzhou KingMed Center for clinical laboratory) for metagenomic next generation sequencing (mNGS). Patients were included in the MP group only if both the agglutination assay and molecular detection of MP in BALF yielded positive results. The sensitivity of mNGS depends on sequencing depth and the abundance of pathogens[10]. False positives may arise from sample contamination or database alignment errors, while false negatives may result from low pathogen abundance or insufficient sequencing depth[11]. To minimize false positives, we implemented negative controls and optimized the bioinformatics analysis process (e.g., removing host sequences and filtering low-quality data). Results were interpreted in conjunction with clinical information. A bacterial infection was defined as the presence of bacteria detected in BALF culture and confirmed by mNGS. Colonization was ruled out based on clinical presentation, pathogen abundance, inflammatory markers, imaging findings, and repeated testing[12]. MP antibiotic resistance gene testing was also conducted by the third-party testing agency (Guangzhou KingMed Center for Clinical Laboratory). Full length sequencing of the 23S rRNA gene of MP was performed by polymerase chain reaction (PCR).

Statistical analyses

Data were analyzed using SPSS 20 software. Continuous variables were reported as median (range) and compared using Student's t-test or the Mann–Whitney U-test. Categorical variables were presented as numbers (percentages) and compared using the Chi-square test. A P-value < 0.05 was considered statistically significant.

Results

Demographic characteristics and pathogens of the study population

A total of 353 MPP patients who underwent BAL therapy were included. The male-to-female ratio was 1.34:1. Age distribution was as follows: < 1 year (31 cases, 8.8%), 1–3 years (66 cases, 18.7%), 3–6 years (113 cases, 32%), and 6–14 years (143 cases, 40.5%). There were 72 cases (20.4%) of RMPP, 86 cases (24.4%) of SMPP, and 183 cases (51.8%) of MRMPP. Among the patients, 210 (59.5%) had MP single infections, and 143 (40.5%) had co-infections. Co-infections included 111 cases (31.4%) of MP mixed with viruses and 73 cases (20.7%) of MP mixed with bacteria. The most common co-infecting pathogen was Rhinovirus (RV, 30.8%), followed by *Streptococcus pneumoniae* (SP, 27.3%) and *Haemophilus influenzae* (HI, 16.1%). Co-infections with Coronavirus (CoV), Adenovirus (ADV), Parainfluenza virus (PIV), Epstein-Barr virus (EBV), Human Bocavirus (HBoV), Influenza virus (IV), Respiratory syncytial virus (RSV), *Staphylococcus aureus* (SA), and *Bordetella pertussis* were also observed (Fig. 1). When patients were co-infected with more than two respiratory viruses, each pathogen was counted independently. Among MP co-infections, 82 cases (57.3%) involved one pathogen, 44 cases (30.7%) involved two pathogens, 12 cases (8.4%) involved three pathogens, 4 cases (2.8%) involved four pathogens, and 1 case (0.7%) involved five pathogens (Table 1).

Comparison between the MP single infection and MP co-infection group

No significant differences were observed between the MP co-infection and MP single infection groups in terms of gender, RMPP, fever, cough, wheezing, length of hospital stay, or complications ($P > 0.05$). However, patients in the MP single infection group were older than those in the MP co-infection group ($P = 0.007$). Significant differences were observed between the two groups in SMPP, macrolide resistance, bronchial mucus plug, and

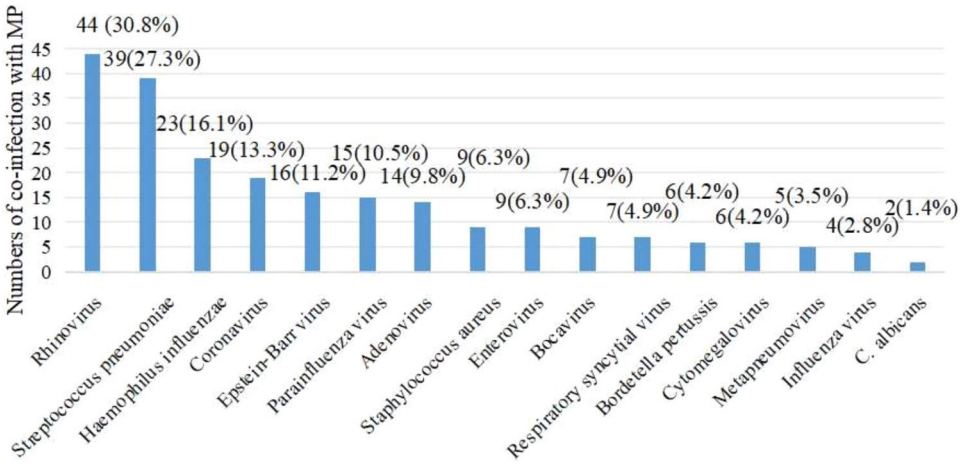

Fig. 1. Distribution of pathogens in MP co-infection.

Pathogens of MP co-infection	N (%)
One type	82 (57.3%)
MP + RV	22 (15.4%)
MP + SP	12 (8.4%)
MP + HI	9 (6.3%)
MP + ADV	6 (4.2%)
MP + EBV	7 (4.9%)
MP + PIV	4 (2.8%)
MP + IV	1 (0.7%)
MP + CoV	6 (4.2%)
MP + HBoV	2 (1.4%)
MP + *Bordetella pertussis*	4 (2.8%)
MP + SA	5 (3.5%)
MP + MPV	2 (1.4%)
MP + EV	2 (1.4%)
Two types	44 (30.7%)
MP + RV + SP	10 (7.0%)
MP + CoV + SP	4 (2.8%)
MP + RV + MPV	2 (1.4%)
MP + ADV + RV	2 (1.4%)
MP + HI + HBoV	2 (1.4%)
MP + SP + EBV	2 (1.4%)
MP + EBV + RSV	1 (0.7%)
MP + IV + RSV	1 (0.7%)
MP + EBV + SP	2 (1.4%)
MP + RSV + SA	1 (0.7%)
MP + HI + CMV	2 (1.4%)
MP + HI + PIV	2 (1.4%)
MP + *C. albicans* + IV	1 (0.7%)
MP + *Bordetella pertussis* + MPV	1 (0.7%)
MP + SP + HI	2 (1.4%)
MP + SP + PIV	3 (2.1%)
MP + CoV + *Bordetella pertussis*	1 (0.7%)
MP + RV + CoV	3 (2.1%)
MP + RV + HBoV	1 (0.7%)
MP + RSV + CMV	1 (0.7%)
Three types	12 (8.4%)
MP + RV + SP + *C. albicans*	1 (0.7%)
MP + SA + HI + EV	2 (1.4%)
MP + RSV + ADV + HBoV	1 (0.7%)
MP + CoV + PIV + EBV	2 (1.4%)
MP + ADV + HI + PIV	1 (0.7%)
MP + RV + ADV + EV	2 (1.4%)
MP + SP + SA + EV	1 (0.7%)
MP + SA + PIV + EV	1 (0.7%)
MP + RSV + CMV + IV	1 (0.7%)
Four types	4 (2.8%)
MP + SP + EV + CMV + CoV	1 (0.7%)
Continued	

Pathogens of MP co-infection	N (%)
MP + HI + EBV + PIV + CMV	1 (0.7%)
MP + HI + CoV + RSV + EV	1 (0.7%)
MP + HI + CoV + ADV + EBV	1 (0.7%)
Five types	1 (0.7%)
MP + RV + SP + HBoV + ADV + PIV	1 (0.7%)

Table 1. The number of pathogens in MP co-infections. MP, *Mycoplasma pneumoniae*; RV, Rhinovirus; SP, *Streptococcus pneumoniae*; HI, *Haemophilus influenzae*; ADV, Adenovirus; EBV, Epstein-Barr virus; PIV, Parainfluenza virus; IV, Influenza virus; CoV, Coronavirus; HBoV, Human Bocavirus; MPV, Metapneumovirus; SA, *Staphylococcus aureus*; RSV, Respiratory syncytial virus; CMV, Cytomegalovirus; EV, Enterovirus.

	MP single infection N = 210	MP co-infection N = 143	*P*
Sex (male)	119 (56.7%)	83 (58%)	0.80
Age (months)	64.4 (4–156)	54.7 (3–144)	0.007
Macrolide resistance	75 (35.7%)	108 (75.5%)	0.000
Bronchial mucus plug	125 (59.5%)	115 (80.4%)	0.000
SMPP	43 (20.5%)	43 (30.1%)	0.039
RMPP	37 (17.6%)	35 (24.5%)	0.12
Fever	188 (89.5%)	119 (83.2%)	0.084
Cough	209 (99.3%)	143 (100%)	0.41
Wheezing	38 (18.1%)	37 (25.9%)	0.079
Fever peak (℃)	39.2 (37.4–41)	39.3 (37.5–41.5)	0.57
Fever days	6.1 (1–32)	5.85 (1–19)	0.29
Length of hospital stay (days)	6.26 (1–14)	6.79 (1–16)	0.067
Complications	57 (27.1%)	49 (34.3%)	0.15
Hormone use	57 (27.1%)	54 (37.8%)	0.035
White blood cells	9.5 (2.67–27.7)	9.4 (2.13–30.4)	0.65
Percentage of neutrophils	59.1 (5.4–93.1)	55.7 (4–91)	0.62
C-reactive protein	13.6 (0.1–241)	14.4 (0.06–87)	0.58
Lactate dehydrogenase	309 (175–712)	320 (194–998)	0.31
Erythrocyte sedimentation rate	36 (1–122)	37 (1–99)	0.61
D-dimer	0.76 (0.02–8.1)	0.97 (0.06–14)	0.21

Table 2. Comparison of the baseline, clinical characteristics and laboratory findings between the MP single infection and MP co-infection groups. SMPP, Sever *Mycoplasma pneumoniae* pneumonia; RMPP, refractory *Mycoplasma pneumoniae* pneumonia; Complications, include intra-pulmonary and extra-pulmonary complications.

hormone use, with *P*-values of 0.039, 0.000, 0.000, and 0.035, respectively. No significant differences were found in laboratory findings between the two groups (Table 2).

Comparison between MP mixed virus and bacteria group

There were 70 cases (19.8%) of MP mixed with only viruses, 32 cases (9.1%) of MP mixed with only bacteria, and 41 cases (11.6%) of MP mixed with both viruses and bacteria. The MP mixed virus or bacteria infection group was more likely to develop drug resistance compared to the mixed virus and bacteria group ($P = 0.007$ and $P = 0.046$). The MP mixed virus and bacteria group was more likely to develop severe pneumonia compared to the mixed virus or bacteria infection group ($P = 0.032$ and $P = 0.017$). The MP mixed virus group had a longer hospital stay compared to the MP mixed bacteria group ($P = 0.009$). No significant differences were observed in gender, age, RMPP, bronchial mucus plug, fever, cough, wheezing, fever peak, fever duration, complications, or hormone use among the three groups. Additionally, no significant differences were found in laboratory findings among the three groups (Table 3).

Discussion

Currently, there are limited studies on mixed infections involving MP. Previous studies have reported respiratory virus co-infection rates in children with MPP ranging from 27.3 to 56.1%[3,4,13]. This study found a respiratory virus co-infection rate of 31.4%, consistent with previous findings. Martin et al. reported that the most common co-infection combination was MP and PIV (15%), followed by MP and IV[14]. In this study, the most common

	[a]MP + Virus N = 70	[b]MP + Bacteria N = 32	[c]MP + Virus + Bacteria N = 41	*P*
Sex (male)	39 (55.7%)	16 (50%)	28 (68.3%)	$P^{ab} = 0.67$ $P^{ac} = 0.23$ $P^{bc} = 0.15$
Age (months)	51.8 (3–120)	61.8 (5–144)	54.1 (4–132)	$P^{ab} = 0.16$ $P^{ac} = 0.72$ $P^{bc} = 0.33$
Macrolide resistance	58 (82.9%)	26 (81.3%)	24 (58.5%)	$P^{ab} = 0.52$ $P^{ac} = 0.007$ $P^{bc} = 0.046$
Bronchial mucus plug	57 (81.4%)	27 (84.4%)	31 (75.6%)	$P^{ab} = 0.79$ $P^{ac} = 0.48$ $P^{bc} = 0.40$
SMPP	23 (32.9%)	4 (12.5%)	16 (39%)	$P^{ab} = 0.032$ $P^{ac} = 0.54$ $P^{bc} = 0.017$
RMPP	17 (24.3%)	8 (25%)	10 (24.4%)	$P^{ab} = 0.56$ $P^{ac} = 0.58$ $P^{bc} = 0.58$
Fever	59 (84.3%)	30 (93.8%)	30 (73.2%)	$P^{ab} = 0.22$ $P^{ac} = 0.22$ $P^{bc} = 0.031$
Cough	70 (100%)	32 (100%)	41 (100%)	-
Wheezing	19 (27.1%)	4 (12.5%)	14 (34.1%)	$P^{ab} = 0.13$ $P^{ac} = 0.52$ $P^{bc} = 0.054$
Fever peak (℃)	39.4 (38–41)	39.1 (38–42)	39.2 (38–41)	$P^{ab} = 0.044$ $P^{ac} = 0.061$ $P^{bc} = 0.76$
Fever days	6.3 (1–19)	5.6 (1–13)	5.2 (1–12)	$P^{ab} = 0.39$ $P^{ac} = 0.16$ $P^{bc} = 0.67$
Length of hospital stay (days)	7.4 (2–20)	6.0 (2–12)	6.5 (2–13)	$P^{ab} = 0.009$ $P^{ac} = 0.093$ $P^{bc} = 0.46$
Complications	25 (27.1%)	8 (34.3%)	16 (39%)	$P^{ab} = 0.36$ $P^{ac} = 0.84$ $P^{bc} = 0.22$
Hormone use	26 (37.1%)	16 (50%)	12 (29.3%)	$P^{ab} = 0.23$ $P^{ac} = 0.42$ $P^{bc} = 0.09$
White blood cells	9.7 (3–23)	8.3 (4–16)	9.9 (2–30)	$P^{ab} = 0.068$ $P^{ac} = 0.80$ $P^{bc} = 0.132$
Percentage of neutrophils	55.6 (16.1–83.7)	52.5 (4.8–79.4)	58.6 (4–91.1)	$P^{ab} = 0.43$ $P^{ac} = 0.36$ $P^{bc} = 0.17$
C-reactive protein	15.3 (0.1–87)	13.9 (0.06–51)	13.4 (0.6–61)	$P^{ab} = 0.65$ $P^{ac} = 0.56$ $P^{bc} = 0.88$
Lactate dehydrogenase	332.1 (202–618)	317.5 (210–998)	304.5 (194–996)	$P^{ab} = 0.59$ $P^{ac} = 0.20$ $P^{bc} = 0.641$
Erythrocyte sedimentation rate	33.6 (5–76)	40.5 (13–80)	40.9 (1–99)	$P^{ab} = 0.20$ $P^{ac} = 0.23$ $P^{bc} = 0.96$
D-dimer	1.2 (0.06–14.2)	1.0 (0.19–9.8)	0.6 (0.1–4.8)	$P^{ab} = 0.60$ $P^{ac} = 0.10$ $P^{bc} = 0.47$

Table 3. Comparison of the baseline, clinical characteristics and laboratory findings between the MP mixed virus and bacteria group. SMPP, Sever *Mycoplasma pneumoniae* pneumonia; RMPP, refractory *Mycoplasma pneumoniae* pneumonia; Complications, include intra-pulmonary and extra-pulmonary complications. [a]Only MP and Virus. [b]Only MP and Bacteria. [c]MP, Virus and Bacteria coinfection.

co-infecting pathogen was RV, followed by SP, HI, CoV, EBV, PIV, and ADV. Yun et al. reported that the most commonly co-detected bacteria were SP, SA, *and Escherichia coli*[15]. To date, there have been no reports of MP being frequently co-infected with RV. This discrepancy may be due to differences in study populations and specimen types. Most literature focuses on specific subgroups, such as patients with RMPP or SMPP. Zhang et al. reported that 27.0% of RMPP patients had co-infections with other pathogens, including SP, HI, SA, HBoV, RV, and RSV[16]. There was no consensus on the impact of co-infection on disease severity, which might depend on the specific pathogens involved[17]. However, this study found that MP co-infection was more likely to cause

severe pneumonia. Co-infections with ADV have been associated with more severe clinical manifestations[18]. Some case studies have reported that patients co-infected with MP and EBV suffered more severe symptoms[19]. In this study, children with MP co-infection tended to exhibit higher rates of macrolide resistance. Co-infections may indirectly increase the risk of antibiotic resistance through mechanisms such as increased antibiotic selection pressure, promotion of gene transfer, or interference with immune clearance. Research suggested that co-infected patients have higher rates of macrolide resistance gene mutations (e.g., A2063G) in Japan and China[20,21]. Some studies suggested that drug resistance is primarily related to host variability and prior antibiotic misuse[22]. However, large-scale prospective studies directly linking co-infections to antibiotic resistance are lacking and require further investigation. Yan et al. found that co-infections with other respiratory pathogens were an independent risk factor for the development of resistance genes[23]. This study also found that MP co-infections were associated with a higher likelihood of bronchial mucus plug, which has rarely been reported.

Several studies have shown that the rate of MP mixed bacteria infection is relatively low. In Chen et al. reported that 10.9% (22/201) of RMPP patients had bacterial co-infections[24]. Jin et al. confirmed that bacterial co-infections in pediatric RMPP were rare[9]. This study also found a low rate of MP mixed with bacterial infections. In terms of co-infection with bacteria, MP was found to compete for nutrients to eliminate other bacteria[25] and activate the host inflammatory response[26], resulting in limited bacterial diversity in MPP[27]. In contrast, viral infections can damage the lung epithelium and suppress the immune response, promoting bacterial growth and leading to secondary bacterial infections[28], which may increase bacterial diversity.

This study has several limitations. It was a retrospective study with a small sample size, which might introduce selection bias. Future research should involve prospective studies with larger sample sizes. In conclusion, MP was most commonly co-infected with RV. Children with MP co-infections tend to exhibit higher rates of macrolide resistance, more severe pneumonia, and a higher likelihood of bronchial mucus plug.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions
Li Yuan wrote the main manuscript text and Lili Zhou prepared Figs. 1 and table 1–3. Diao Mingyue helped to analyzed and interpreted the data. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
Due to the retrospective nature of the study, the Ethics and Research Council of Women and Children's Hospital of Ganzhou waived the need of obtaining informed consent. All methods were performed in accordance with the relevant guidelines and regulations. All experimental protocols were approved by Women and Children's Hospital of Ganzhou.

Additional information
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