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Mycoplasma pneumoniae Pneumonia and Co-Infection with Post-COVID-19: A Single Centre Analysis

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Abstract

Background: *Mycoplasma pneumoniae* (MP) is the primary causative agent of community-acquired pneumonia, which has increasingly become resistant to macrolides, complicating treatment regimens, especially with the co-infection factor. Its worldwide prevalence has fluctuated due to the influence of the COVID-19 pandemic. The study investigated co-infection patterns in children diagnosed with *Mycoplasma pneumoniae* pneumonia (MPP). **Methods:** From June 2022 to December 2023, we retrospectively analyzed the clinical data for hospitalized children with *Mycoplasma pneumoniae* pneumonia in Wuhan, China. We collected data on age, sex, clinical information, and pathogenic results. We also collected sputum or bronchoalveolar lavage fluid (BALF) samples to test respiratory pathogens and macrolide resistance using targeted microbial next-generation sequencing (tNGS). We analyzed the data using SPSS. **Results:** The study involved 417 patients diagnosed with MPP, of whom 86.33% had co-infections. Co-infections were notably linked to lobar pneumonia, prominent imaging shadows and higher macrolide resistance rate. Key bacterial pathogens were *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, rhinoviruses, and human adenoviruses (HADV). In MPP cases, *Candida albicans* was the fungal pathogen related to co-infections. The co-infection with HADV and human bocavirus 1 (HBoV1) correlated with prolonged fever, whereas *Bordetella pertussis* was linked to prolonged cough. In contrast, *Candida albicans* exhibited a weaker association with diffuse large-area infiltration on chest imaging, and its co-infection was less likely to result in severe disease. **Conclusion:** These results offer valuable insight into *Mycoplasma pneumoniae* pneumonia in children, highlighting the impact of co-infections on the disease's clinical outcomes.

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Keywords

Mycoplasma pneumoniae, Co-Infections, Pneumonia, Children, Post-COVID-19

1. Introduction

Mycoplasma pneumoniae (MP) is a significant respiratory pathogen that often contributes to community-acquired pneumonia (CAP), particularly in children. Although it typically leads to mild respiratory illness, it is commonly associated with co-infections involving other bacterial or viral pathogens that can complicate clinical outcomes by exacerbating symptoms, lengthening the duration of illness, and making treatment more challenging [1]. A study in Central China reveals a 30.62% prevalence of MP infection among pediatric patients diagnosed with acute respiratory infections. The study highlights notable fluctuations in infection rates, including a peak in 2018 and a resurgence in 2023. During the COVID-19 pandemic from 2019 to 2022, the infection rate was notably lower, reaching its lowest point in 2021. The data indicates seasonal variations, with the highest positive rate of MP infection occurring in autumn. Additionally, school-aged children exhibited the highest rate of infection. The study found a co-infection rate of 14.27%, with viral co-infections being more common, particularly among infants, who are more susceptible to such co-infections [2].

Co-infections complicate the clinical diagnosis of *Mycoplasma pneumoniae* pneumonia, as the symptoms often overlap with those of other respiratory pathogens. A major challenge in clinical practice is distinguishing between viral and MP infections, as their clinical presentations and radiographic findings are frequently indistinguishable. Furthermore, laboratory evaluations may not provide clear differentiation, often rendering them non-diagnostic, which can lead to delayed appropriate treatment [3]-[5]. Also, the reported case study highlights atypical CT scan findings in an adult patient who was co-infected with the Influenza B virus and *Mycoplasma pneumoniae*, resulting in severe symptoms and a delayed diagnosis [6]. Common co-pathogens include respiratory viruses such as influenza and adenovirus, alongside bacteria like *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* [7]. The study found that those with adenovirus co-infections experienced more severe symptoms, with 37.5% developing extrapulmonary complications [8].

Macrolide resistance is significantly high in MP infections, and studies suggest that patients with resistant strains face an increased risk of co-infections. According to a survey conducted in Korea, the co-infection rate with other respiratory pathogens was found to be 88.49% [9]. Research suggests that viral infections may worsen the primary MP infection or raise the likelihood of secondary bacterial infections. Patients with viral co-infections have been observed to display a greater frequency of fever, elevated white blood cell counts (WBCs), increased levels of C-

reactive protein (CRP) and procalcitonin (PCT), as well as a greater proportion of lactate dehydrogenase (LDH) levels exceeding 250 U/L [10].

As a significant urban center, Wuhan exhibits a high population density that can promote the rapid transmission of respiratory pathogens, including *Mycoplasma pneumoniae*. During epidemics, such as the influenza season, the incidence of co-infections in children may increase, making it vital to understand the dynamics of these diseases. Additionally, Wuhan also served as the focal point of the first COVID-19 epidemic, which raises particular concerns about co-infections involving SARS-CoV-2, *Mycoplasma pneumoniae*, and other bacterial pathogens. A study conducted in Wuhan revealed that co-infections of Influenza A with *Mycoplasma pneumoniae*, as well as *Bordetella pertussis* co-infected with rhinovirus, were among the most prevalent combinations of respiratory pathogens in hospitalized pediatric patients from December 2023 to April 2024 [11]. The main objective of this research was to examine the detection of MP and the co-infection rate in Wuhan following the COVID-19 pandemic. The aim was to identify any changes in the prevalence of common respiratory pathogens after restrictions were eased. Additionally, since respiratory epidemics can vary by region, the findings of this study would offer insights into the causes and trends of community-acquired pneumonia (CAP) in Wuhan.

2. Methods

2.1. Study Settings and Population

The study was carried out retrospectively in the Department of Pediatrics of Zhongnan Hospital of Wuhan University. From July 2022 to December 2023, we reviewed all pediatric patients aged from 1 month to 14 years who received a diagnosis of mycoplasma pneumonia (MP) infection and subsequently underwent testing for other respiratory pathogens. Cases were excluded if they involved active malignancies, severe congenital metabolic disorders, congenital immune deficiencies, or patients receiving immunosuppressive or immunomodulatory therapies (see Figure 1).

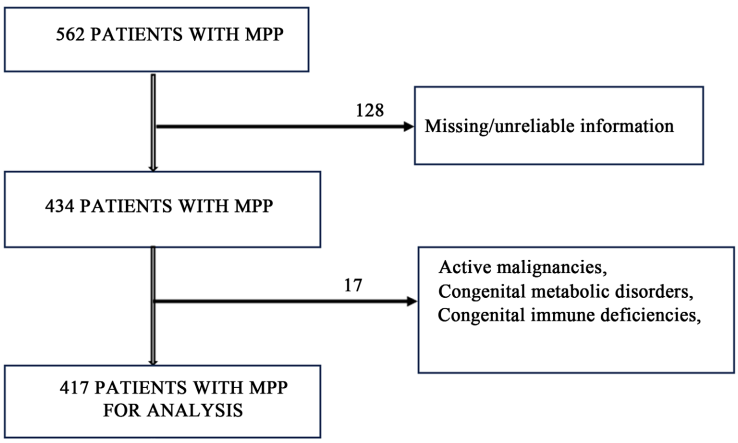

Figure 1. Patients' selection flowchart.

2.2. Data Collection

The data was obtained from departmental medical records containing patient data encompassing essential information such as admission details, clinical symptoms and signs, onset duration, laboratory results, and imaging results. Samples of bronchoalveolar fluid were obtained to test pathogens and assess macrolide resistance. The Ethical Committee of Zhongnan Hospital of Wuhan University for Clinical Research Projects authorized this study. As the research was conducted retrospectively, informed consent was waived.

2.3. Co-Infection and Macrolide Resistance Identification

MP was diagnosed via the MP serum-IgM antibody test (antibody titers ≥1:160), and to identify co-infections and macrolide resistance, the samples tested were sputum and Broncho alveolar lavage fluid (BALF). Collected samples were tested using targeted microbial next-generation sequencing (tNGS), performed by Guangzhou Jinyu Medical Laboratory Center Co. LTD. (King Med Diagnostics, Guangzhou, China). Concurrently, mutations were assessed in the 23S rRNA domain A2063G, A2064G, A2067G, and C2617G of MP [12]. MP primary infection was identified as the presence of MP with higher copies/mL (concentrations > 1.0×10^6 copies/mL) than any other detected pathogen. Co-infection was defined as MP infection identified with higher copies/mL alongside other pathogens (bacteria, viruses, and fungi), which were also selected for high copies/mL (concentration ranges >1.0×10^4 to >1.0×10^6 copies/mL).

2.4. Statistical Analysis

In our statistical analysis, we utilized the Statistical Package for Social Sciences (SPSS) Version 27 by IBM, Inc. Descriptive statistics were used to summarize numerical variables using mean and standard deviations. Frequencies and percentages were displayed as Categorical variables. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test, as appropriate. Independent t-tests or Mann-Whitney U tests were employed for continuous variables. A p-value of <0.05 is deemed statistically significant.

3. Results

Among 417 MP cases involved in the study, 360 (86.33%) cases had co-infections (see **Figure 2**), while 57 children (13.67%) did not have co-infections. There were 165 (84.18%) females and 195 (88.24%) males in the co-infection group. 91.59% of children aged 1 - 5 years and 84.21% of those older than 5 had co-infections. The co-infection group exhibited a maximum mean body temperature of 39.2˚C ± 0.86˚C. At the same time, the group without co-infection had a mean body temperature of 38.9˚C ± 0.96˚C. Cough was the main presented symptom, whereby 351 (86.45%) of children with co-infections had a cough. Among all these analyzed variables, there was no significant difference between the two groups of co-infections and those without co-infections (see **Table 1**).

Table 1. General information on children diagnosed with MPP.

	No co-infection	Co-infection	p-value
Variable	57 (13.67)	360 (86.33)	
Gender			0.229
Female	31 (15.82)	165 (84.18)	
Male	26 (11.76)	195 (88.24)	
Age			0.10
<1		6 (100.0)	
1 - 5	9 (8.41)	98 (91.59)	
>5	48 (15.79)	256 (84.21)	
Cough			0.659
No	2 (18.18)	9 (81.82)	
Yes	55 (13.55)	351 (86.45)	
Cough days			0.445
<7 days	38 (14.67)	221 (85.33)	
≥7 days	19 (12.03)	19 (87.97)	
Fever			0.201
No	1 (4.55)	21 (95.45)	
Yes	56 (14.32)	339 (85.82)	
Maximum body temperature	38.9˚C ± 0.96˚C	39.2˚C ± 0.86˚C	0.542
Fever days			0.326
No fever	1 (4.55)	21 (95.45)	
<7 days	38 (15.20)	212 (84.80)	
≥7 days	18 (12.41)	127 (87.59)	
Sputum			0.357
No	8 (18.18)	36 (81.82)	
Yes	49 (13.14)	324 (86.86)	
Convulsion			0.962
No	56 (13.36)	354 (86.34)	
Yes	1 (14.29)	6 (85.71)	
Vomit			0.102
No	54 (14.67)	314 (85.33)	
Yes	3 (6.12)	46 (93.88)	

Continued

Chest pain			0.183
No	54 (13.30)	352 (86.70)	
Yes	3 (27.27)	8 (72.73)	
Diarrhea			0.227
No	57 (13.97)	351 (86.03)	
Yes		9 (100.0)	
Rash			0.371
No	57 (13.83)	355 (86.17)	
Yes		5 (100.0)	
Wheeze			0.280
No	53 (13.28)	346 (86.72)	
Yes	4 (22.22)	14 (77.78)	
Rales			0.148
No	25 (11.36)	195 (88.64)	
Yes	32 (16.24)	165 (83.76)	

SD: Standard deviation.

3.1. Pathogens Associated with Co-Infections in Children Diagnosed with MPP

Upon analysis, we identified various pathogens recognized as co-infecting organisms. See the detailed information in Figure 1.

3.1.1. Bacteria Pathogens

Streptococcus pneumoniae, Haemophilus influenzae, and *Staphylococcus aureus* are the leading bacterial pathogens associated with co-infections in children with MPP. Other bacterial co-infections, including *Klebsiella pneumoniae* and *E. coli,* were much less frequent.

3.1.2. Virus Pathogens

Rhinoviruses and the Human adenovirus group are the most frequently identified viral pathogens associated with co-infections in children with MPP. Other viral pathogens, such as respiratory syncytial virus (RSV) and influenza viruses, were present at much lower frequencies.

3.1.3. Fungal Pathogens

While fungal co-infections were uncommon overall, *Candida albicans* was notably the most frequent fungal pathogen associated with co-infections in MPP cases.

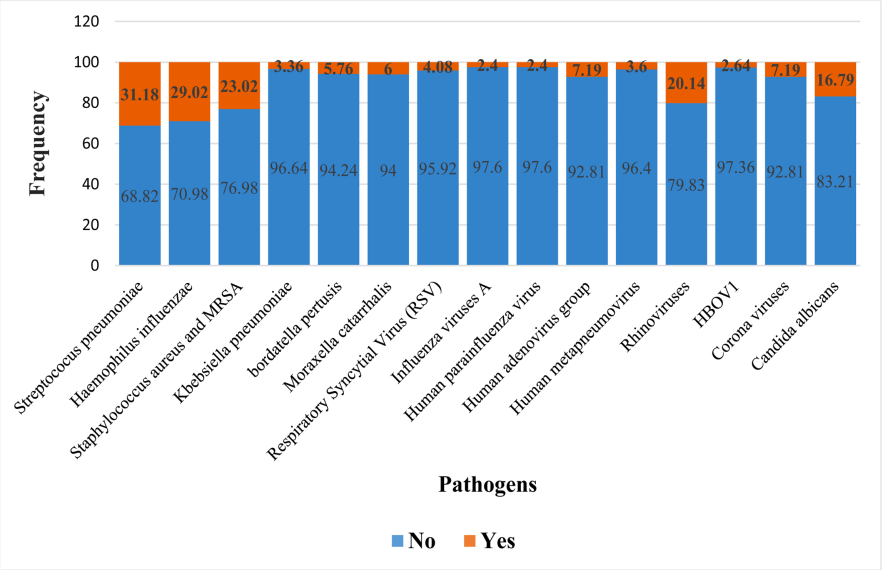

Figure 2. Pathogens specifically associated with co-infections in children with MPP.

3.2. Comparison of Outcomes for Children with MPP, with and without Co-Infections

Children with co-infections experienced more extended hospital stays (p = 0.0318) and had macrolide resistance (p = 0.0038). This resistance was linked to mutations in the 23S rRNA gene, all of which were A2063G. Co-infections were also significantly associated with lobar pneumonia (p = 0.0457) and extensive shadows on imaging (p = 0.0001). No significant differences were noted in severity, bilateral lung infections, pleural effusion, atelectasis, or fibrous lesions between children with and without co-infections (see Table 2).

Table 2. Comparison of the outcomes of children with MPP with and without co-infections.

	Co-infection				
Outcomes	**No**	**Yes**	**Mean diff**	**95% CI**	**p-value**
	57 (13.67)	360 (86.33)			
Length of hospital stay			2.714628	2.67 - 2.78	**0.0318***
<4 days		6 (100.0)			
4 - 7 days	9 (8.41)	98 (91.59)			
>7 days	48 (15.79)	256 (84.21)			
Severity			0.4028777	0.36 - 0.45	**0.2418**
Non severe	30 (12.05)	219 (87.95)			
Severe	27 (16.07)	141 (83.93)			

Continued

Macrolide resistance			1.872902	1.84 - 1.91	**0.0038***
RMP	43 (11.81)	321 (88.19)			
MP	14 (26.42)	39 (73.58)			
Lobar			0.5803357	0.53 - 0.63	**0.0457***
No	17 (9.71)	158 (90.29)			
Yes	40 (16.53)	202 (83.47)			
Large shadow			0.0791367	0.10 - 0.32	**0.0001***
No	45 (11.72)	339 (88.28)			
Yes	12 (36.36)	21 (63.64)			

*: $p < 0.05$.

3.3. Correlation between Fever Days and Pathogens

The correlation analysis between fever duration (categorized as <7 days and ≥7 days) and specific pathogens demonstrates varies associations. The analysis reveals that most pathogens show no significant correlation with fever duration ($p > 0.05$). However, notable correlations are observed for *Bordetella pertussis* was likely to be associated with shorter fever duration (<7 days), while Human adenovirus ($p = 0.0216$), and HBOV1 ($p = 0.0119$) were more likely associated with longer fever duration (≥7 days). Detailed results are shown in Table 3.

Table 3. Correlation between fever days (<7 days vs. ≥7 days) and pathogens.

Pathogens	Symptom—Fever days		p-value
	<7 days	≥7 days	
	n = 250	n = 145	
	Mean (95% of CI)	Mean (95% of CI)	
Streptococcus pneumoniae	1.38 (1.32 - 1.43)	1.35 (1.26 - 1.44)	**0.6286**
Haemophilus influenzae	1.38 (1.32 - 1.44)	1.33 (1.24 - 1.42)	**0.334**
Staphylococcus aureus and MRSA	1.37 (1.31 - 1.42)	1.37 (1.26 - 1.48)	**0.9454**
Klebsiella pneumoniae	1.37 (1.32 - 1.42)	1.31 (1.02 - 1.59)	**0.6524**
Bordetella pertussis	1.38 (1.33 - 1.43)	1.14 (0.98 - 1.31)	**0.0285***
Moraxella catarrhalis	1.38 (1.33 - 1.43)	1.22 (1.04 - 1.39)	**0.1255**
Respiratory syncytial virus (RSV)	1.37 (1.32 - 1.42)	1.31 (1.06 - 1.57)	**0.6448**
Influenza viruses A	1.37 (1.32 - 1.41)	1.40 (1.03 - 1.77)	**0.8274**
Human para influenza virus	1.36 (1.31 - 1.41)	1.60 (1.23 - 1.97)	**0.1223**

Continued

Human adenovirus group	1.35 (1.30 - 1.40)	1.58 (1.37 - 1.78)	**0.0216***
Human metapneumovirus	1.37 (1.32 - 1.42)	1.23 (0.97 - 1.50)	**0.301**
Rhinoviruses	1.37 (1.32 - 1.43)	1.35 (1.24 - 1.45)	**0.6695**
HBOV1	1.36 (1.31 - 1.40)	1.73 (1.41 - 2.04)	**0.0119***
Corona viruses	1.37 (1.32 - 1.42)	1.31 (1.12 - 1.49)	**0.5169**
Candida albicans	1.36 (1.31 - 1.41)	1.40 (1.27 - 1.51)	**0.621**

HBOV1: Human bocavirus1, CI: Confidence interval, *: p <0.05.

3.4. Correlation between Cough Days and Pathogens

There were no significant correlations between most pathogens and cough duration (<7 days vs. ≥7 days). However, *Bordetella pertussis* exhibits a statistically significant correlation (p = 0.0335), suggesting it may be associated with prolonged cough duration (see Table 4).

Table 4. Correlation between cough days (<7 days vs. ≥7 days) and pathogens.

Pathogens	Symptom—Cough days		p-value
	<7 days	≥7 days	
	n = 259	n = 158	
	Mean (95% of CI)	Mean (95% of CI)	
Streptococcus pneumoniae	1.37 (1.31 - 1.43)	1.40 (1.31 - 1.49)	**0.551**
Haemophilus influenza	1.37 (1.32 - 1.43)	1.39 (1.31 - 1.48)	**0.6329**
Staphylococcus aureus and MRSA	1.39 (1.33 - 1.44)	1.35 (1.25 - 1.46)	**0.5814**
Klebsiella pneumoniae	1.38 (1.33 - 1.43)	1.29 (1.02 - 1.56)	**0.4659**
Bordetella pertussis	1.37 (1.32 - 1.41)	1.58 (1.37 - 1.80)	**0.0335***
Moraxella catarrhalis	1.39 (1.34 - 1.43)	1.28 (1.09 - 1.47)	**0.2942**
Respiratory syncytial virus (RSV)	1.38 (1.33 - 1.43)	1.41 (1.15 - 1.67)	**0.7761**
Influenza viruses A	1.37 (1.33 - 1.42)	1.60 (1.23 - 1.97)	**0.1453**
Human par influenza virus	1.38 (1.33 - 1.42)	1.50 (1.12 - 1.88)	**0.4255**
Human adenovirus group	1.37 (1.32 - 1.42)	1.50 (1.31 - 1.69)	**0.1565**
Human metapneumovirus	1.38 (1.33 - 1.43)	1.40 (1.12 - 1.68)	**0.8642**
Rhinoviruses	1.38 (1.33 - 1.44)	1.36 (1.25 - 1.46)	**0.6465**
HBOV1	1.38 (1.33 - 1.43)	1.36 (1.02 - 1.70)	**0.916**
Corona viruses	1.38 (1.33 - 1.43)	1.37 (1.18 - 1.55)	**0.8864**
Candida albicans	1.37 (1.32 - 1.42)	1.41 (1.29 - 1.53)	**0.5046**

HBOV1: Human bocavirus1, CI: Confidence interval, *: p <0.05.

3.5. Correlation between Large Shadow and Pathogens

Candida albicans was found to be less likely to be associated with the presence of a large shadow (diffuse large area of infiltration, p = 0.0071), while most pathogens had no statistically significant associations (see Table 5).

Table 5. Correlation between large shadow and pathogens.

Pathogens	Complication—Large shadow		p-value
	No	Yes	
	n = 384	n = 33	
	Mean (95% of CI)	Mean (95% of CI)	
Streptococcus pneumoniae	0.08 (0.05 - 0.11)	0.08 (0.04 - 0.13)	**0.7809**
Haemophilus influenza	0.08 (0.04 - 0.11)	0.07 (0.03 - 0.12)	**0.8186**
Staphylococcus aureus and MRSA	0.08 (0.05 - 0.11)	0.06 (0.01 - 0.11)	**0.4381**
Bordetella pertussis	0.08 (0.05 - 0.11)	0.04 (0.04 - 0.13)	**0.4848**
Moraxella catarrhalis	0.08 (0.05 - 0.11)	0.08 (0.03 - 0.19)	**0.9869**
Respiratory syncytial virus (RSV)	0.08 (0.05 - 0.11)	0.06 (0.07 - 0.18)	**0.7521**
Influenza viruses A	0.07 (0.05 - 0.10)	0.10 (0.12 - 0.33)	**0.8052**
Human adenovirus group	0.08 (0.05 - 0.11)	0.07 (0.03 - 0.16)	**0.7934**
Human metapneumovirus	0.79 (0.05 - 0.11)	0.66 (0.07 - 0.21)	**0.8558**
Rhinoviruses	0.08 (0.08 - 0.11)	0.07 (0.02 - 0.13)	**0.7703**
HBOV1	0.07 (0.05 - 0.10)	0.09 (0.11 - 0.29)	**0.8838**
Corona viruses	0.08 (0.05 - 0.11)	0.03 (0.03 - 0.10)	**0.3359**
Candida albicans	0.09 (0.06 - 0.13)	0.04 (0.04 - 0.12)	**0.0071***

HBOV1: Human bocavirus1, CI: Confidence interval, *: p <0.05.

3.6. Correlation between Severity and Pathogens

Candida albicans demonstrated a statistically significant correlation (p = 0.0139), suggesting that it may be associated with a less severe disease presentation. However, Other pathogens, including *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae,* and others, did not show a significant relationship with disease severity (p > 0.05), as detailed in Table 6.

3.7. Correlation between Length of Hospital Stay and Pathogens

Most pathogens were found to have no significant association with hospital stay duration (p > 0.05). However, Coronaviruses demonstrated a likelihood of shorter hospital stay duration (p = 0.027) (see Table 7).

Table 6. Correlation between severity and pathogens.

Pathogens	Severity		p-value
	Non severe	Severe	
	n = 249	n = 168	
	Mean (95% of CI)	Mean (95% of CI)	
Streptococcus pneumoniae	0.41 (0.36 - 0.47)	0.38 (0.29 - 0.46)	**0.4683**
Haemophilus influenzae	0.39 (0.34 - 0.45)	0.41 (0.32 - 0.50)	**0.7836**
Staphylococcus aureus and MRSA	0.40 (0.35 - 0.45)	0.41 (0.30 - 0.51)	**0.8519**
Klebsiella pneumoniae	0.39 (0.35 - 0.44)	0.50 (0.20 - 0.80)	**0.4523**
Bordetella pertussis	0.40 (0.35 - 0.45)	0.42 (0.20 - 0.63)	**0.8875**
Moraxella catarrhalis	0.41 (0.36 - 0.45)	0.36 (0.16 - 0.56)	**0.6531**
Respiratory syncytial virus (RSV)	0.40 (0.35 - 0.44)	0.47 (0.21 - 0.74)	**0.5622**
Influenza viruses A	0.40 (0.35 - 0.45)	0.50 (0.12 - 0.88)	**0.5273**
Human par influenza virus	0.40 (0.36 - 0.45)	0.40 (0.03 - 0.77)	**0.9851**
Human adenovirus group	0.40 (0.35 - 0.45)	0.43 (0.25 - 0.62)	**0.7248**
Human metapneumovirus	0.41 (0.36 - 0.45)	0.33 (0.06 - 0.60)	**0.577**
Rhinoviruses	0.39 (0.33 - 0.44)	0.46 (0.365 - 0.57)	**0.2**
HBOV1	0.41 (0.36 - 0.45)	0.27 (0.04 - 0.59)	**0.3736**
Corona viruses	0.41 (0.36 - 0.46)	0.27 (0.09 - 0.43)	**0.1149**
Candida albicans	0.43 (0.38 - 0.48)	0.27 (0.16 - 0.38)	**0.0139***

HBOV1: Human bocavirus1, CI: Confidence interval, *: p <0.05.

Table 7. Correlation between length of hospital stay and pathogens.

Pathogens	Length of hospital stay		p-value
	<7 days	≥7 days	
	n = 303	n = 114	
	Mean (95% of CI)	Mean (95% of CI)	
Streptococcus pneumoniae	1.28 (1.23 - 1.33)	1.25 (1.17 - 1.32)	**0.548**
Haemophilus influenzae	1.28 (1.22 - 1.33)	1.26 (1.18 - 1.34)	**0.6157**
Staphylococcus aureus and MRSA	1.25 (1.21 - 1.31)	1.33 (1.23 - 1.43)	**0.1948**
Klebsiella pneumoniae	1.28 (1.23 - 1.32)	1.21 (0.97 - 1.46)	**0.6148**
Bordetella pertussis	1.27 (1.23 - 1.32)	1.29 (1.09 - 1.49)	**0.8365**
Moraxella catarrhalis	1.28 (1.24 - 1.33)	1.16 (1.01 - 1.31)	**0.1904**

Continued

Respiratory syncytial virus (RSV)	1.28 (1.23 - 1.31)	1.24 (1.01 - 1.46)	**0.7198**
Influenza viruses A	1.27 (1.23 - 1.31)	1.40 (1.03 - 1.77)	**0.3644**
Human par influenza virus	1.27 (1.23 - 1.32)	1.30 (0.95 - 1365)	**0.8488**
Human adenovirus group	1.28 (1.24 - 1.33)	1.17 (1.03 - 1.31)	**0.1742**
Human metapneumovirus	1.27 (1.23 - 1.32)	1.27 (1.01 - 1.52)	**0.9528**
Rhinoviruses	1.28 (1.23 - 1.32)	1.26 (1.17 - 1.36)	**0.7923**
HBOV1	1.28 (1.23 - 1.32)	1.18 (0.91 - 1.45)	**0.491**
Corona viruses	1.29 (1.24 - 1.33)	1.10 (0.99 - 1.21)	**0.027***
Candida albicans	1.27 (1.22 - 1.32)	1.29 (1.17 - 1.39)	**0.8002**

CI: Confidence interval, *: $p < 0.05$.

4. Discussion

The study involved 417 patients diagnosed with MP, of whom 86.33% had co-infections. Specifically, 88.24% of males and 84.18% of females were affected, while 91.59% of children aged 1 to 5 and 84.21% of those older than 5 had co-infections. No significant differences were found in symptom presentation or disease severity between co-infected and non-co-infected patients; however, co-infected children had longer hospital stays and higher macrolide resistance. Co-infections were notably linked to lobar pneumonia and prominent imaging shadows. Key bacterial pathogens were *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*; Rhinoviruses and Human adenoviruses were also present. In MPP cases, *Candida albicans* was the frequent fungal pathogen associated with co-infections. Furthermore, the co-infection with HADV and HBOv1 was associated with prolonged fever, with *Bordetella pertussis* correlated with fewer fever days (<7 days) but with prolonged cough, while *Candida albicans* was found to be less associated with a large shadow on chest imaging (diffuse large area of infiltration) and its co-infection was less likely to cause severe disease.

Similar to other studies, we observed that the co-infection rate was higher in younger children compared to older ones. This must have been attributed to their weaker immune systems, which make them more susceptible to other respiratory pathogens [13] [14]. In this study, we observed that MP patients coinfected with other pathogens had shown an extended hospital stay. This can be thought to be due to prolonged symptoms and presentations of severe symptoms, which necessitate prolonged treatment. Although our study did not assess factors contributing to the extended hospital stay, a prior study indicated that a longer hospital stay was linked to increased febrile days following the start of antibiotic treatment. This association was also noted with factors such as a history of previous respiratory disease admissions, having a sibling, more vigorous immune response in individuals with co-infections, as was highlighted by specific laboratory measurements,

including WBC, CRP, AST, and ALT levels, and presence of macrolide drug resistance also contributed to prolonged hospitalization [8] [15] [16].

The imaging results for MP patients have been explained differently; in our study, lobar pneumonia and large shadows (diffuse large area of infiltration) on imaging were more prominent in patients with co-infections. This might have resulted from the presence of coinfecting pathogens. As studied previously, co-infections, particularly with *Streptococcus pneumoniae* or viral agents like adenovirus, can provoke an intensified immune reaction, and macrolide resistance can result in delayed disease resolution, leading to significant extensive lung consolidation and infiltrates [1] [8]. Although in our study, when we assessed the individual coinfecting pathogens, *Candida albicans* was found to be less associated with a large shadow, while other pathogens demonstrated insignificant correlation. Considering the severity of imaging results in patients with co-infections, healthcare providers should maintain a high level of awareness for potential co-pathogens and contemplate more extensive medication coverage when appropriate.

Similarly to other studies, the most identified coinfecting organisms were *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus* in terms of bacteria, which tally with the previous analysis of almost similar sample size but their methods of diagnosis were bacteria culture differing from ours of nucleic acid test through Targeted next-generation sequencing technology (tNGS), and their study population involved children diagnosed with refractory *Mycoplasma pneumoniae* pneumonia (RMPP) suggesting that there is a need to study more about the persistent interaction between these mentioned pathogens [7]. Also, the study from Vietnam had similar findings [17]. The coinfecting viruses included Rhinoviruses and the Human adenovirus (HADV). These pathogens have long been mentioned in previous studies, and their interactions with MP have even been studied. The presence of co-infection with HADV in this study was associated with prolonged fever, indicating that its co-infection is associated with the extended time needed for the pathogen to clear from the body and worsen the host's immune response, resulting in prolonged periods of inflammation [18]. In a multicenter study conducted in Korea, rhinovirus was found to be the highest coinfecting pathogen among viruses, and it was demonstrated in patients with macrolide-sensitive MP (MSMP) [19]. In contrast to our study, another recent study found Respiratory syncytial virus (RSV) as the most coinfecting virus, although this study involved both children and adults [10]. *Candida albicans* was notably the most frequent fungal pathogen associated with co-infections in MPP cases, although its presence in the study was associated with less severe disease, suggesting that the co-infection with Candida does not play a role in the severity of MPP. The frequent occurrence of co-infections in patients with MPP underscores the importance of comprehensive diagnostic testing for accurate identification and effective management of the disease. Viral and bacterial co-infections can complicate the clinical presentation and progression of MPP, making reliance on a single test insufficient and potentially leading to misdiagnosis or delayed treatment. The comprehensive diagnostic

strategy allows clinicians to distinguish between primary MP infections and co-infections, facilitating targeted treatment decisions and appropriate antibiotic or antiviral therapies, ultimately improving patient outcomes. Early and precise diagnosis enables timely intervention and helps prevent complications and reduces the unnecessary use of broad-spectrum antibiotics, which can contribute to antimicrobial resistance [20] [21].

The current study found that the rate of macrolide resistance in individuals with co-infections was significantly higher, approximately 88.19%, compared to the 56.07% reported in a study conducted before the COVID-19 pandemic [8]. Notably, macrolide resistance has been particularly elevated in Asian countries, especially in the Asia-Pacific. In China, resistance rates in MP range from 26.9% to as high as 100% [22].

In discussing the severity of the illness, our study did not demonstrate statistical significance about the severity outcome of the disease between the two groups. Several studies have reported that co-infection is the risk factor for disease severity, explaining that severe disease presentation was more noticed in individuals coinfected with *Streptococcus pneumoniae* and those with viral co-infection [1] [14]. However, our study correlated the co-infection with *Candida albicans* with non-severe disease presentations. But some studies align with our findings that there was no statistically significant difference in disease severity between the two groups despite the higher co-infection rate. The observed variability in this scenario may imply that Macrolide resistance, driven by particular genetic modifications, could significantly impact the severity and presentation of the disease, independent of co-existing infections. In addition, the coinfecting pathogens associated with *Mycoplasma pneumoniae* (MP) might be less virulent, leading to less severe disease manifestations, even in cases of co-infection [23]-[25]. Clinicians should continue closely monitoring these patients even if this study did not uncover a significant correlation between co-infection and disease severity. Even when there isn't a direct correlation between co-infections and MPP severity, they can nevertheless cause unforeseen complications and a longer recovery time, which can complicate the clinical course of the illness. Thus, a watchful, proactive strategy is necessary in clinical practice to guarantee prompt treatments and avoid negative consequences.

5. Limitations

The study was constrained by its retrospective design, as the data were collected for clinical use rather than specifically for research purposes, which may result in incomplete information or potential biases. Also, our study did not assess treatment and treatment response among these sample groups. Moreover, the study population may not fully represent the larger target demographic; it is limited to those who had access to healthcare and were admitted during a specific period. Since the research was conducted at a single institution, the results may not be easily applicable to all pediatric patients suffering from *Mycoplasma pneumoniae* pneumonia

and coexisting infections. Nevertheless, despite these constraints, our study offers important insights and a general understanding of this patient group, laying the groundwork for future investigations in this field.

6. Conclusion

This study significantly associated co-infections with lobar pneumonia and prominent imaging shadows. Key bacterial pathogens identified included *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*, alongside the presence of rhinoviruses and human adenoviruses. *Candida albicans* was the most frequently identified fungal pathogen coinfecting with MP. The co-infection with HADV and HBOV1 was associated with prolonged fever, *Bordetella pertussis* was associated with prolonged cough, while *Candida albicans* was found to be less associated with a large shadow on chest imaging and its co-infection was less likely to cause severe disease. Our study offers an overview of the trend regarding *Mycoplasma pneumoniae* pneumonia with co-infections, particularly following the COVID-19 era. We call for extensive, multicenter studies to examine the trends of individual pathogen co-infections and their impact on disease progression.

Ethical Approval and Consent to Participate

This study was authorized by the Ethics Committee of Zhongnan Hospital of Wuhan University for Clinical Research Projects, which followed the Helsinki rules and norms for medical research on children. As the research was conducted retrospectively, informed consent was waived.

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Authors' Contributions

ANK and AAM created and wrote the manuscript. BDS interpreted the data and reviewed it. YPD supervised the whole process. All authors reviewed and approved the final version submitted.

Conflicts of Interest

The authors affirm that this research was carried out free of any commercial or financial relationships that might be viewed as a conflict of interest.

References

[1] Chiu, C., Chen, C., Wong, K., Tsai, M., Chiu, C. and Huang, Y. (2015) Impact of Bacterial and Viral Coinfection on Mycoplasmal Pneumonia in Childhood Community-Acquired Pneumonia. *Journal of Microbiology, Immunology and Infection*, **48**, 51-56. https://doi.org/10.1016/j.jmii.2013.06.006

[2] Mao, J., Niu, Z., Liu, M., *et al*. (2024) The Epidemiological Characteristics of *Mycoplasma pneumoniae* Infection and Coinfection among Children in Central China from 2018 to 2023. https://doi.org/10.21203/rs.3.rs-4617945/v1

[3] Lin, X., Xu, E., Zhang, T., Zhu, Q., Liu, Y. and Tian, Q. (2025) Cytokine-Based Nomogram for Discriminating Viral Pneumonia from *Mycoplasma pneumoniae* Pneumonia in Children. *Diagnostic Microbiology and Infectious Disease*, **111**, Article ID: 116611. https://doi.org/10.1016/j.diagmicrobio.2024.116611

[4] Skevaki, C.L., Tsialta, P., Trochoutsou, A.I., Logotheti, I., Makrinioti, H., Taka, S., *et al*. (2015) Associations between Viral and Bacterial Potential Pathogens in the Nasopharynx of Children with and without Respiratory Symptoms. *Pediatric Infectious Disease Journal*, **34**, 1296-1301. https://doi.org/10.1097/inf.0000000000000872

[5] Tomsa, N., Melit, L.E., Popescu, T. and Marginean, C.O. (2024) *Mycoplasma pneumoniae* Pneumonia—Challenges Related to Diagnosis and Treatment in Children. *Romanian Journal of Infectious Diseases*, **27**, 46-50. https://doi.org/10.37897/rjid.2024.1.12

[6] Shinozaki, T., Sasahara, K., Iwami, E., Kuroda, A., Matsuzaki, T., Nakajima, T., *et al*. (2018) A Case of Influenza B and *Mycoplasma pneumoniae* Coinfection in an Adult. *Case Reports in Infectious Diseases*, **2018**, Article ID: 3529358. https://doi.org/10.1155/2018/3529358

[7] Zhang, X., Chen, Z., Gu, W., Ji, W., Wang, Y., Hao, C., *et al*. (2018) Viral and Bacterial Co-Infection in Hospitalised Children with Refractory *Mycoplasma pneumoniae* Pneumonia. *Epidemiology and Infection*, **146**, 1384-1388. https://doi.org/10.1017/s0950268818000778

[8] Zhou, Y., Wang, J., Chen, W., Shen, N., Tao, Y., Zhao, R., *et al*. (2020) Impact of Viral Coinfection and Macrolide-Resistant Mycoplasma Infection in Children with Refractory *Mycoplasma pneumoniae* Pneumonia. *BMC Infectious Diseases*, **20**, Article No. 633. https://doi.org/10.1186/s12879-020-05356-1

[9] Shin, S., Koo, S., Yang, Y. and Lim, H. (2023) Characteristics of the *Mycoplasma pneumoniae* Epidemic from 2019 to 2020 in Korea: Macrolide Resistance and Co-Infection Trends. *Antibiotics*, **12**, Article 1623. https://doi.org/10.3390/antibiotics12111623

[10] Wu, X., Chen, R., Mo, D., Wang, Y., Xia, Y. and He, M. (2024) Analysis of *Mycoplasma pneumoniae* Pneumonia and Viral Coinfection in Southern Guangzhou after the COVID-19 Pandemic. *Journal of Thoracic Disease*, **16**, 6789-6798. https://doi.org/10.21037/jtd-24-729

[11] Han, Y., Wang, D., Wang, Q., Liu, Y., Yan, M., Ren, F., *et al*. (2025) Seasonal Shifts in Respiratory Pathogen Co-Infections and the Associated Differential Induction of Cytokines in Children. *Cytokine*, **186**, Article ID: 156847. https://doi.org/10.1016/j.cyto.2024.156847

[12] Dekyi,, Xiao, Y., Wang, X., Feng, S., Wang, Y., Liao, L., *et al*. (2024) Predominance of A2063G Mutant Strains in the *Mycoplasma pneumoniae* Epidemic in Children: A Clinical and Epidemiological Study in 2023 in Wuhan, China. *International Journal of Infectious Diseases*, **145**, Article ID: 107074. https://doi.org/10.1016/j.ijid.2024.107074

[13] Chen, Q., Lin, L., Zhang, N. and Yang, Y. (2024) Adenovirus and *Mycoplasma pneumoniae* Co-Infection as a Risk Factor for Severe Community-Acquired Pneumonia in Children. *Frontiers in Pediatrics*, **12**, Article 1337786. https://doi.org/10.3389/fped.2024.1337786

[14] Choo, S., Lee, Y.Y. and Lee, E. (2022) Clinical Significance of Respiratory Virus Coinfection in Children with *Mycoplasma pneumoniae* Pneumonia. *BMC Pulmonary Medicine*, **22**, Article No. 212. https://doi.org/10.1186/s12890-022-02005-y

[15] Sung, M., Roh, E.J., Lee, E.S., Lee, J.Y., Kim, H., Ahn, Y., *et al*. (2022) Assessment of Variables Associated with Prolonged Admission Duration in Children with *Mycoplasma pneumoniae* Pneumonia. *The Clinical Respiratory Journal*, **16**, 756-767. https://doi.org/10.1111/crj.13549

[16] Xu, Y., Li, S., Liu, J., Zhou, J., Jin, F., Chen, X., *et al*. (2020) Impact of Epstein-Barr Virus Coinfection in *Mycoplasma pneumoniae* Pneumonia. *Medicine*, **99**, e19792. https://doi.org/10.1097/md.0000000000019792

[17] Tran Quang, K., Tran Do, H., Pham Hung, V., Nguyen Vu, T., Tran Xuan, B., Larsson, M., *et al*. (2021) Study on the Co-Infection of Children with Severe Community-Acquired Pneumonia. *Pediatrics International*, **64**, e14853. https://doi.org/10.1111/ped.14853

[18] Gao, J., Xu, L., Xu, B., Xie, Z. and Shen, K. (2020) Human Adenovirus Coinfection Aggravates the Severity of *Mycoplasma pneumoniae* Pneumonia in Children. *BMC Infectious Diseases*, **20**, Article No. 420. https://doi.org/10.1186/s12879-020-05152-x

[19] Sung, M., Choi, H.J., Lee, M.H., *et al*. (2022) Regional and Annual Patterns in Respiratory Virus Co-Infection Etiologies and Antibiotic Prescriptions for Pediatric *Mycoplasma pneumoniae* Pneumonia. *European Review for Medical and Pharmacological Sciences*, **26**, 5844-5856. https://doi.org/10.26355/eurrev_202208_29524

[20] del Rosal, T., Bote-Gascón, P., Falces-Romero, I., Sainz, T., Baquero-Artigao, F., Rodríguez-Molino, P., *et al*. (2024) Multiplex PCR and Antibiotic Use in Children with Community-Acquired Pneumonia. *Children*, **11**, Article 245. https://doi.org/10.3390/children11020245

[21] Ly, V.K., Pham, V.H., Ly, X.V. and Pham, P.M. (2024) Bacterial and Viral Co-Infections in Community-Acquired Pneumonia in Adults: A Prospective Study of Multiple Hospital Centers. *MedPharmRes*, **8**, 153-161. https://doi.org/10.32895/ump.mpr.8.3.17

[22] Lai, C.C., Hsueh, C., Hsu, C., Tsai, Y. and Hsueh, P. (2024) Disease Burden and Macrolide Resistance of *Mycoplasma pneumoniae* Infection in Adults in the Asia-Pacific Region. *International Journal of Antimicrobial Agents*, **64**, Article ID: 107205. https://doi.org/10.1016/j.ijantimicag.2024.107205

[23] Pereyre, S., Goret, J. and Bébéar, C. (2016) *Mycoplasma pneumoniae*: Current Knowledge on Macrolide Resistance and Treatment. *Frontiers in Microbiology*, **7**, Article 974. https://doi.org/10.3389/fmicb.2016.00974

[24] Guo, Q., Li, L., Wang, C., Huang, Y., Ma, F., Cong, S., *et al*. (2022) Comprehensive Virome Analysis of the Viral Spectrum in Paediatric Patients Diagnosed with *Mycoplasma pneumoniae* Pneumonia. *Virology Journal*, **19**, Article No. 181. https://doi.org/10.1186/s12985-022-01914-y

[25] Chironna, M., Sallustio, A., Esposito, S., Perulli, M., Chinellato, I., Di Bari, C., *et al*. (2011) Emergence of Macrolide-Resistant Strains during an Outbreak of *Mycoplasma pneumoniae* Infections in Children. *Journal of Antimicrobial Chemotherapy*, **66**, 734-737. https://doi.org/10.1093/jac/dkr003