

Epidemiology, Co-infections, and Macrolide Resistance of *Mycoplasma pneumoniae* infection in Hangzhou, China: A Multicenter Study Analysis

Yi Wang

Hangzhou Center for Disease Control and Prevention

Longfang Jiang

Xiaoshan District Center for Disease Control and Prevention

Jing Wang

Hangzhou Center for Disease Control and Prevention

Xiaoxin Zhang

Hangzhou Center for Disease Control and Prevention

Yanyang Tao

Hangzhou Center for Disease Control and Prevention

Xinye Jin

Hangzhou Center for Disease Control and Prevention

Zhaokai He

Hangzhou Center for Disease Control and Prevention

Xiaojiang Duan

Hangzhou Center for Disease Control and Prevention

Liangliang Huo

huo11@hzcdc.com.cn

Hangzhou Center for Disease Control and Prevention

Research Article

Keywords: *Mycoplasma pneumoniae*, epidemiology, co-infection, macrolide resistance, multicenter study

Posted Date: August 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10076881/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Objectives

Mycoplasma pneumoniae (MP) is a leading cause of community-acquired pneumonia. Its epidemiology has shifted during and after the COVID-19 pandemic. Current evidence remains predominantly pediatric, creating a significant gap in systematic, all-age data—particularly among adults and hospitalized patients—which limits the development of tailored management strategies. This study employed a multicenter retrospective design to investigate hospitalized cases of MP infection across all age groups in the Hangzhou region of China.

Methods

Data were retrospectively obtained from inpatients at seven hospitals and four community health centers in Hangzhou, China. Patients of all age groups with acute respiratory tract infection and laboratory-confirmed MP infection were included. Epidemiological trends, age-specific incidence, co-infection pathogen spectra, and antimicrobial resistance-associated mutations patterns were analyzed.

Results

MP infection presented a “double-peak” pattern synchronous with acute respiratory infections, accounting for 5.2% of 99,730 included patients. Incidence remained < 5% in 2020–2021, increased rapidly, peaked at 12.3% in May 2024, and return to low levels. School-aged children most affected (48.6%); infections in older adults increased by five-fold. Most cases were single infections (83.5%), while 16.5% were co-infections, most commonly with adenovirus and *Chlamydia pneumoniae*, and the co-occurrence rate between parainfluenza virus and *Chlamydia pneumoniae* was as high as 73.8%. Macrolide resistance increased significantly, reaching 64.2% in 2023, primarily due to A2063G and A2064G mutations in the 23S rRNA gene.

Conclusion

The high burden in children, rising incidence in older adults, significant macrolide resistance, and distinct co-infection patterns highlight the need for enhanced, integrated surveillance and tailored stewardship strategies to manage MP infections.

1 Introduction

Mycoplasma pneumoniae (MP) is a major pathogen responsible for community-acquired pneumonia (CAP), accounting for 10% – 40% of CAP cases, rising up to over 70% during epidemic outbreaks[1, 2]. It exhibits cyclical epidemic patterns globally, typically with regional outbreaks occurring every 3 to 7 years.

However, substantial regional variations exist, with infection rates ranging from 9.5% to 66.5% [3]. The implementation of non-pharmaceutical interventions (NPIs) during the COVID-19 pandemic has markedly altered the global epidemiological patterns of infectious respiratory infectious diseases. Following the lifting of NPIs, an atypical and off-season resurgence of MP infections has been observed since the end of 2022 in multiple countries, including China, Denmark, and France—a phenomenon often attributed to the "immunity debt" effect. These trends suggest that the epidemiological characteristics of MP in the post-pandemic era may be undergoing profound restructuring and evolution [4–7].

As a major city in eastern China, Hangzhou has experienced a sharp increase rise in MP infections during this outbreak, providing a crucial observational window for studying the epidemiological patterns of MP during the post-pandemic era. Although China has comprehensively strengthened its respiratory pathogen surveillance since 2023, the existing systems remain inadequate for the complete elucidation of the epidemic profile. Moreover, studies on the epidemiological characteristics of MP infections in Hangzhou before and after the lifting of NPIs remain scarce. Another prominent issue is that approximately 90% of current MP studies focus exclusively on children and adolescents, leaving a significant gap in systematic research across all age groups, particularly in adults and the older populations [8]. These bias limits our understanding of the true infection rates, clinical manifestations, and transmission dynamics of MP in different age cohorts, thereby constraining the development of transmission chain models and the formulation of age-stratified prevention and control strategies.

Macrolides have long been the first-line treatment choice for MP infections. However, in recent years, the proportion of macrolide-resistant MP (MRMP) in China has been continuously rising, posing a significant challenge for clinical treatment [9]. Global data indicate a marked increase in macrolide resistance rates in MP, with particularly high levels observed in Asia. In China, the resistance rates range from 26.9% – 100% [10, 11]. However, systematic studies on macrolide resistance across all age groups remain insufficient. Continuous monitoring of MP epidemiology and mutation profiles is crucial for guiding clinical practice.

In addition, MP frequently co-occurs with bacterial pathogens, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*; or viral agents, including the influenza and respiratory syncytial viruses. Such co-infections are strongly associated with exacerbated disease severity in pediatric patients [12, 13]. However, existing studies are predominantly constrained by limited sample sizes, often including fewer than 1,000 patients. Additionally, most investigations have been restricted to specific pathogen combinations or a single clinical center, resulting in limited representativeness. Given these methodological constraints, re-evaluation of the diagnostic utility of serological testing for the accurate identification of MP is warranted.

Currently, there is a scarcity of systematic research on the detailed epidemiological characteristics of prevailing infections in China, including the precise temporal dynamics, shifts in age distribution, patterns of co-infection, and mutation trends of antimicrobial resistance genes in circulating strains. To address this gap, the present study systematically collected data on multi-pathogen co-infection

analysis; resistance mutation profiles of prevalent strains from on hospitalized MP infection cases from medical institutions in Hangzhou, China. This study aimed to provide a comprehensive elucidation of the epidemiological patterns; co-infection dynamics; and shifts in antimicrobial resistance of MP in the post-pandemic era from both epidemiological and etiological perspectives.

2 Materials and Methods

2.1 Study Population

This retrospective, multicenter observational study was conducted across seven hospitals and four community health centers in Hangzhou, China, from January 1, 2020, to December 31, 2024. Clinical data were extracted from inpatient databases, encompassing patients who met the following inclusion criteria: (1) diagnosis of acute respiratory tract infection (ARTI) across all age groups, including acute upper respiratory tract infections, influenza, pneumonia, and other acute lower respiratory tract infections; and (2) laboratory-confirmed MP infections, defined by a positive result in nucleic acid detection, antigen testing, or IgM antibody serology.

2.2 Data Collection

Demographic, clinical, and laboratory data were extracted from the electronic health records using standardized case report forms. The variables encompassed demographic characteristics (age, sex, educational level, and occupation), coinfection status (defined by the detection of pathogens such as MP, influenza virus, adenovirus, rhinovirus, SARS-CoV-2, human metapneumovirus, parainfluenza virus, bocavirus, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* species), and information on antimicrobial resistance mutation sites. For inpatients, samples including nasopharyngeal swabs of secretions and bronchoalveolar lavage fluid were analyzed for common macrolide resistance-associated point mutations in the V region of the 23S rRNA gene (specifically A2063G and A2064G) using targeted polymerase chain reaction assays. All the data were independently entered by two researchers and cross-verified to ensure accuracy and reliability.

2.3 Statistical Analysis

In descriptive analysis, categorical variables were summarized using frequencies and percentages. Normally distributed continuous variables were presented as the mean $\pm$ standard deviation. Non-normally distributed continuous variables were reported as medians and interquartile ranges. To explore the co-infection patterns, pairwise co-occurrence relationships among pathogens were analyzed using contingency tables and chi-square tests (or Fisher's exact test), and odds ratios (ORs) were calculated. All analyses were performed using R software (version 4.3.2). Statistical significance was set at $p < 0.05$.

3 Results

3.1 Epidemiological Characteristics of Hospitalized Patients with MP Infection

3.1.1 Temporal Distribution Trends of Hospitalized Patients with ARTIs and MP Infection

The epidemiological characteristics of MP infection-related hospitalizations in Hangzhou (Figure 1A) revealed that from 2020 to 2024, the number of hospitalized MP infection cases were 124, 160, 282, 1,811, and 2,828, respectively. Within the overall population, both ARTIs (blue bars) and MP infections (red bars) exhibited a simultaneous peak in the winter of 2023. Hospitalizations due to ARTIs reached a maximum of 4,015 cases in January 2024, while MP infection cases peaked at 425 cases during the same period, forming a distinct “bimodal pattern.” Furthermore, during the winter of 2023 (December to February of the following year), MP infection-related hospitalizations increased 39-fold compared with the same period in 2020 (1,110 vs. 28).

Age-stratified analysis (Figure 1B) indicated that children and adolescents aged 0–17 years bore the primary disease burden. School-aged children (6–17 years) were the most affected subgroup, accounting for 21.41% (2,532/11,828) of all ARTIs, followed by the 0–5 year group at 4.89% (1,138/23,292). Among adults, the proportion decreased with increasing age: the 18–59 years group accounted for 2.56% (730/28,527), while the older adult group (aged ≥ 60 years) had the lowest proportion at 2.23% (805/36,083). Although all age groups exhibited typical seasonal fluctuations (peaking in winter) from 2020 to 2023, an anomalous population-wide outbreak of MP infections occurred from late 2023 to early 2024. During this peak, the proportion of MP infections among ARTIs in school-aged children reached a maximum of 35.00% (84/240). Notably, the older adult group, which typically show a low incidence, exhibited a marked increase; from 2020 to 2022, the proportion of MP infections in this group remained below 1.5%; however, in the winter of 2023, it surged sharply to 8.18% (108/1,321), which is approximately a five-fold increase from baseline levels (Supplementary Figure 1). Although the absolute proportion of MP infections in older adults remained lower than that of children, this anomaly suggests potential changes in the epidemiology of MP infections among older adults. This abnormal pattern gradually returned to baseline levels after the summer of 2024.

The relative importance of MP infection among hospitalized ARTI patients displayed a clear three-phase evolutionary trend (Figure 1C); from 2020 to 2024, MP infection accounted for 5.22% (5,205/99,730) of all ARTI hospitalizations. Specifically, a low-fluctuation phase (proportion below 5%) was observed from 2020 to 2022, followed by an active fluctuation phase beginning in 2023; the proportion of MP infections first increased to an initial peak of 11.4% in mid-to-late 2023. After a brief decline, it surged again, reaching a historical peak of 12.3% in May 2024 and forming a second peak. Subsequently, a rapid decline phase ensued, with the proportion nearing pre-active phase levels by the end of 2024.

3.1.2 Age Distribution and Seasonal Trends

Figure 2A illustrates the epidemiological characteristics of hospitalized MP infection cases in Hangzhou, China, from 2020 to 2024. First, school-aged children (6–17 years) bore the highest disease burden, accounting for 48.64% (2,532/5,205) of total hospitalized MP infections, followed by the 0–5 years age group at 21.86% (1,138/5,205), the 18–59 years age group at 14.02% (730/5,205), and the ≥60 years age group at 15.47% (805/5,205). Regarding temporal trends (Figure 2A), the school-aged cohort exhibited a marked seasonal fluctuation, with peak incidence observed during the winter of 2023 (December to February of the following year); a 93.6-fold increase, compared to the same period in 2021 (468 vs. 5 cases). The older adult group (≥60 years) also showed a rapid upward trend, with a 41.14-fold increase in the winter of 2023, relative to the same period in 2021 (288 vs. 7 cases).

Age-specific analysis (Figure 2B) was performed using an enhanced point heat map that integrates age groups with monthly data and visualizes case density through both dot size and color intensity. This visualization confirmed the heightened susceptibility of the 6-17-year age group, which exhibited a prominent infection hotspot, characterized by large dots and dark brown coloring, in the fourth quarter of 2023. During this period, the average number of hospitalizations per month was 160 in the 6–17-year age group, whereas in all other age groups it remained <50 hospitalizations per month (medium-sized dots, bright yellow distribution).

The seasonal distribution and evolution of infection intensity (Figure 2C) quantitatively validated the winter epidemic effect: case numbers in all age groups peaked during winter, with significantly higher incidences observed, compared to other seasons, indicating that colder periods constitute a critical risk interval for MP infection. From 2020 to 2022, although the winter peak persisted, its intensity remained relatively moderate, and differences across age groups were not markedly pronounced. In contrast, the epidemic pattern in the winter of 2023 exhibited distinct characteristics of high intensity and broad spread.

3.1.3 Age Distribution and Seasonal Trends

Analysis of the sociodemographic characteristics of hospitalized patients with MP infection in Hangzhou, China, from 2020 to 2024 revealed that males accounted for 51.5% (2,680/5,205), while females accounted for 48.5% (2,525/5,205). The proportion of males was significantly higher than that of females ($\chi^2 = 4.62$, $p < 0.05$). Educational attainment exhibited a distinctly tiered distribution ($\chi^2 = 27,441$, $p < 0.001$). Patients with primary school education or below constituted the largest proportion at 77.8% (4,048 cases), followed by those with secondary education at 13.4% (696 cases). In contrast, higher education groups were underrepresented: vocational/technical school graduates accounted for only 2.0% (102 cases), bachelor's degree holders for 1.6% (83 cases), and postgraduates or higher for 0.2% (8 cases) (Figure 3A).

Occupational distribution showed a high degree of concentration, with statistically significant differences among groups ($\chi^2 = 15,326$, $p < 0.001$). Students constituted the largest patient group (46.9%, 2,443 cases), followed by non-kindergarten-attending children (21.9%, 1,141 cases), accounting for 70.0% of all hospitalized patients. The remaining occupational categories included other/unknown occupations (10.2%, 533 cases), industrial workers (4.5%, 233 cases), clerical staff (3.3%, 174 cases), agricultural workers (1.7%, 87 cases), commercial/service workers (1.5%, 77 cases), professionals/technical personnel (1.1%, 56 cases), and the unemployed (0.4%, 19 cases), all of which represented relatively small proportions (Figure 3B).

3.2 Co-infection Profile

This study further examined three aspects of co-infection among 5,205 hospitalized patients with MP infection: pathogen prevalence, types of co-infecting agents, and patterns of pathogen co-occurrence. The distribution of coinfection types across the study population (Figure 4A) revealed that 83.5% (4,344 cases) involved single infections, whereas 16.5% (861/5,205) exhibited mixed infections. Among the co-infections, infections with a single additional pathogen were the most common (12.2%, 635 cases), followed by dual-pathogen co-infections (2.3%, 118 cases), and triple or more pathogen co-infections (2.1%, 108 cases). As shown in Figure 4B, the most common co-infecting pathogens were adenovirus (4.3%, 224/5,205), *Chlamydia pneumoniae* (4.1%, 214/5,205), and influenza virus (3.7%, 190/5,205). Viral pathogens dominated the co-infection spectrum (three of the top four pathogens were viruses), whereas the detection rates of bacterial pathogens such as *Staphylococcus aureus* (0.5%, 25/5,205) and *Klebsiella pneumoniae* (0.2%, 11/5,205) were relatively low.

More importantly, the pathogen co-occurrence heatmap (Figure 4C) revealed synergistic co-infection patterns among other respiratory pathogens in the context of MP infection. Analysis further demonstrated that in MP-positive patients, the parainfluenza virus and *Chlamydia pneumoniae* exhibited a pronounced symbiotic relationship, with a co-occurrence rate of 73.8% (OR [95% CI]: 160.4 [107.5–239.5]). Concurrently, *Staphylococcus aureus* and *Haemophilus influenzae* form a stable bacterial consortium, displaying a co-occurrence rate of 64.0% (OR [95% CI]: 74.3 [32.2–171.5]), with all p values < 0.05 . Furthermore, it is noteworthy that SARS-CoV-2, the human metapneumovirus, and bocavirus, demonstrated independent infection characteristics, as their co-occurrence rates with other pathogens were consistently less than 5%.

3.3 Analysis of antimicrobial resistance Mutations in Hospitalized Cases

From 2020 to 2024, 10,430 respiratory tract samples collected from hospitalized patients in Hangzhou, China were screened for MP antimicrobial resistance-associated gene mutations. In total, 1,553 positive cases were identified, with an overall mutation rate of 14.89% (1,553/10,430). Figure 5A illustrates the

age distribution of patients carrying these mutations, with a median age of 7 years. Analysis by mutation site (Figure 5B) revealed that the A2063G mutation accounted for 0.64% (10/1,553); A2063G or A2064G mutations accounted for 99.36% (1543/1,553). Temporal trend analysis (Figure 5C) demonstrated a notable increase in the mutation rate from a baseline level of 3.43% (35/1,019) in 2020, to 3.40% (34/999) in 2021, rising sharply to 9.09% (173/1,903) in 2022, peaking at 23.54% (898/3,814) in 2023, declining to 15.32% (413/2,695) in 2024, and remaining significantly elevated compared to earlier baseline levels.

Further analyses of antimicrobial-resistant gene mutations were conducted in 5,205 hospitalized patients with MP infection. Among these patients, 1,849 underwent testing, of which 942 tested positives for antimicrobial resistant gene mutations, yielding a mutation rate of 50.9%. Temporal trend analysis revealed that the mutation rate of antimicrobial-resistant genes in hospitalized MP infection cases increased significantly from a baseline level of 37.29% (22/59) in 2020 and 39.34% (24/61) in 2021, to 51.90% (123/237) in 2022, peaking at 64.17% (582/907) in 2023, and declining to 32.65% (191/585) in 2024. Despite this decline, the rate in 2024 remained notably higher than the earlier baseline levels.

4 Discussion

This study leveraged data from over 90,000 ARTI cases in Hangzhou, China, to conduct a comprehensive epidemiological analysis of MP. The extensive dataset facilitated a detailed examination of MP infection patterns and yielded valuable insights into the demographic characteristics across different age groups. The findings reveal a distinct three-phase evolutionary pattern in the relative significance of MP infection among hospitalized ARTI cases: an initial period of low-level fluctuation from 2020 to 2022, followed by a first surge peak in mid-to-late 2023 (11.4%), and subsequently a brief decline preceding a sharp resurgence that reached a historical peak of 12.3% in May 2024. Following subdued transmission during the COVID-19 pandemic, MP infections have demonstrated a remarkable rebound, characterized by a substantial increase in case numbers and fundamental shift in epidemic patterns. Importantly, these altered epidemiological features are not isolated phenomena; but align with global transitions in respiratory infectious disease dynamics, reflecting a new equilibrium in pathogen-host interactions in the post-pandemic landscape [14].

Consistent with prior research, this study demonstrates a substantial surge in MP infections from 2023 to 2024 from an epidemiological perspective [5]. Specifically, the number of hospitalized MP cases in the winter of 2023 increased 39-fold compared with the same period in 2020, with ARTI hospitalizations reaching 4,000 cases in January 2024. A key contributing factor is the “immunity debt” hypothesis: the strict NPIs implemented during the COVID-19 pandemic, while effective in curbing SARS-CoV-2 transmission, substantially reduced immune stimulation against common respiratory pathogens such as MP. This led to a marked accumulation of susceptible individuals [15–17]. As social activities gradually normalized, this accumulated susceptibility triggered the rapid spread of infections, a phenomenon also observed in Europe and North America [18–20]. Previous studies have indicated that MP infections

typically peak in summer or early autumn [21, 22]. However, our findings revealed a bimodal transmission pattern for both ARTI and MP hospitalizations during the winter of 2023, suggesting potential complex interactions between MP and other respiratory pathogens [23].

In terms of age distribution characteristics, our study confirms that school-aged children remain the demographic group at the highest risk of MP infection and notes a rising proportion of cases among the older population. The heightened susceptibility of school-aged children can be attributed to factors such as their congregate living environments, the developmental immaturity of their immune systems, and specific social behavioral patterns. As semi-enclosed and densely populated settings, schools provide optimal conditions for droplet transmission of MP. Furthermore, children's immune systems are not yet fully developed, resulting in relatively limited, specific immunological memory against MP. Collectively, these factors contribute to the high vulnerability of this population to MP infection [7]. Additionally, China relaxed its one-child policy in 2016, leading to an increase of approximately two million births in 2016–2017. This cohort of children now fall within the high-risk age group for respiratory infections. For the high-risk population of school-aged children, it is imperative to formulate targeted prevention and control measures such as strengthening school hygiene management, early case identification, and prompt isolation.

Although, the majority of existing research on MP has primarily focused on pediatric populations [24], an upward trend in infection rates among older individuals has been observed, reflecting the synergistic effects of multiple underlying mechanisms. First, antigenic variations and immune evasion by pathogens may be the contributing factors. The MP strain prevalent in 2023 potentially carries mutations in its key adhesion protein (P1 protein), thereby conferring partial immune escape capabilities [25]. Immunity established by prior infections offers protection against historical strains, which traditionally results in lower infection rates among adults and older individuals. However, the emerging variant may evade this preexisting immunity, leading to a marked increase in susceptibility within these demographic groups and contributing to a distinct infection wave, primarily involving adults. Second, enhanced clinical awareness regarding MP infections in the older age group and the wider adoption of advanced diagnostic techniques may also play a role. This finding holds significant implications and suggests the necessity of reassessing the age distribution characteristics of MP infections. Where appropriate, prevention and control measures should be extended to the entire population with intensified monitoring and interventions in high-risk settings such as nursing homes. In clinical practice, enhanced screening and surveillance of MP infections in older patients with CAP are particularly warranted.

Interestingly, in contrast to findings from South Korea, where co-infection was reported in 88.49% of cases, this study found that MP infection occurred predominantly as a single infection (83.5%), with a co-infection rate of only 16.5% [26]. A deeper analysis of coinfection patterns has demonstrated that viral pathogens dominate MP coinfections, with notably higher coinfection rates involving adenoviruses and *Chlamydia pneumoniae* [26, 27]. These findings are consistent with those of recent studies that underscore the prevalence and significance of respiratory pathogen co-infections [28, 29]. More importantly, co-occurrence analysis identified specific synergistic relationships among pathogens, such

as a strong symbiotic association between the parainfluenza virus and *Chlamydia pneumoniae*, providing critical insights for clinical diagnosis and treatment. Such synergy may arise through several mechanisms: viral cytopathic effects may compromise the integrity of the respiratory mucosa, facilitating adhesion and invasion by other pathogens; inflammation triggered by viral infections may alter the respiratory microenvironment and promote the proliferation of additional pathogens; and virus-induced immunomodulation may temporarily suppress host defense mechanisms, thereby impairing the clearance of co-infecting pathogens.

SARS-CoV-2 has an independent infection profile. It enters host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed only to a limited extent in respiratory epithelial cells. This mode of entry differs from that of common respiratory pathogens such as the influenza virus (which targets sialic acid receptors) and the parainfluenza virus (which binds to sialo glycoprotein receptors), thereby reducing the likelihood of coinfection of the same cell [30]. These findings hold significant implications for clinical practice. They suggest that during the diagnosis and treatment of respiratory infection syndromes, interactions among pathogens, particularly the potential for viral–bacterial/atypical pathogen co-infections, must be considered. When a specific viral infection is identified, clinicians should be aware of the possibility of concurrent infections, which would inform the formulation of more appropriate empirical anti-infective regimens. However, different pathogen combinations may yield diverse clinical effects; some may exacerbate disease severity, while others may modify clinical presentations. All of these factors warrant careful consideration in clinical decision-making.

Regarding antimicrobial resistance, this study revealed that the positivity rate for antimicrobial resistant gene mutations among hospitalized patients was 14.89%. Among patients with MP infections, the rate of resistance mutations reached 50.9%. The positivity rate for such mutations rose sharply from a baseline level of 37.29% in 2020 to a peak of 64.17% in 2023 before declining moderately yet remaining elevated in 2024. This trend likely reflects the complex interplay between multiple contributing factors. Antibiotic-selective pressure is the principal driver of the development of resistance. During periods of high MP infection incidence, the extensive use of macrolide antibiotics may have facilitated the selection and dissemination of resistant strains [10, 31]. Competition among different genotypes of MP strains may also influence the dynamic changes in resistance rates [32]. When a balance is achieved between the transmission fitness of resistant strains and their resistance levels, relative stability or oscillations in resistance rates may emerge. Furthermore, shifts in population immunity may indirectly shape the distribution of resistance by modulating the transmission dynamics of bacterial strains.

MRMP were first isolated from Japanese patients in 2000. Subsequently, clinically resistant strains have been reported successively across Asia, Europe, and North America, with regional variations in resistance rates. In China, the rate of MRMP is notably high, exceeding 90% in some areas; and it is more prevalent among children than adults, complicating clinical management [33]. As the primary mechanism underlying macrolide resistance, the molecular characteristics and distribution patterns of 23S rRNA gene mutations are important for clinical diagnosis, treatment decisions, and antimicrobial resistance surveillance [34, 35]. The predominant mutation at position A2063G and A2064G, identified in

this study, aligns with findings from most regions worldwide, confirming its critical role in the development of resistance [32]. However, the variation in mutation rates across different loci suggests the potential existence of region-specific resistance mechanisms. This finding underscores the importance of personalized treatment strategies and the need to guide clinical practice based on local antimicrobial resistance surveillance data.

The limitations of this study include potential information bias due to its retrospective design and the possible underrepresentation of mild cases, as the analysis focused on hospitalized patients. In addition, inter-institutional variations in pathogen detection methods may have influenced the results. However, these limitations do not compromise the overall reliability of the study's main conclusions. Future research should aim to validate the current findings through prospective study designs, inclusion of outpatient populations, and implementation of standardized laboratory testing protocols, while further investigating the pathogenic mechanisms, immune response profiles, and optimization of treatment strategies for MP infection.

In conclusion, this study provides a comprehensive overview of the evolving epidemiological characteristics of MP infection in the post-pandemic era; and critical evidence for understanding the contemporary epidemiological patterns of MP infection. These findings indicated that MP emerged as a significant pathogen causing ARTIs during the winter of 2023, with the infection burden predominantly affecting school-aged children, and an increasing proportion of cases observed among older adults. Although co-infections remained uncommon, specific synergistic relationships, particularly with the parainfluenza virus and *Chlamydia pneumoniae*, were identified. Of particular concern was the persistent rise in macrolide resistance, which poses substantial challenges for first-line clinical treatment. These findings underscore the necessity of establishing an integrated surveillance network that monitors pathogen prevalence, resistance trends, and co-infection patterns. Furthermore, tailored prevention and control strategies should be developed based on age-specific disease burden profiles to enhance preparedness and response capabilities for MP infection.

Declarations

Acknowledgments

We would like to extend our sincere gratitude to the "Xiaoshan Health Data Platform" platform, the Xiaoshan District Center for Disease Control and Prevention in Hangzhou, Hangzhou GuoPo Medical Technology Co., Ltd., and all participating medical institutions for their valuable contributions to data collection and management.

Funding

This research was supported by Zhejiang Science and Technology Plan for Disease Prevention and Control (Project No.2025JK040) and Hangzhou Agricultural and Social Development Research Guidance Project (Project No.20241029Y078).

Author information

Authors and Affiliations

Infectious Disease Control Department, Hangzhou Center for Disease Control and Prevention (Hangzhou Health Supervision Institute), Hangzhou, China

Yi Wang, Jing Wang, Xiaoxin Zhang, Yanyang Tao, Xinye Jin, Zhaokai He, Xiaojiang Duan, Liangliang Huo

Xiaoshan District Center for Disease Control and Prevention (Xiaoshan District Health Supervision Institute), Hangzhou, China

Longfang Jiang

Corresponding author

Correspondence to [Liangliang Huo](#).

Author Contributions

Y.W.: study conceptualization, formal analysis, visualization, and writing—original draft, project administration. L.J. and J.W.: study conceptualization, methodology. X.Z. and Y.T.: writing—review and editing. X.J. and Z.H.: visualization and investigation. X.D.: validation, and writing—review and editing. L.H.: validation, and writing—review and editing.

Data Availability

The datasets generated and/or analysed during the current study are available in Figshare with the identifier <https://doi.org/10.6084/m9.figshare.32906600>.

Ethics approval

The research protocol was approved by the Ethics Committee of the Hangzhou Center for Disease Control and Prevention (Hangzhou Health Supervision Institute) (Approval No. MR-33-25-015753) and adhered to the ethical principles outlined in the Declaration of Helsinki. Given that this study does not involve any commercial interests and only uses data that has been fully anonymized, there is no potential risk to the participants. Therefore, in accordance with applicable ethical guidelines, the ethics committee has approved the exemption from obtaining informed consent.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos).

Competing interests

The authors declare no competing interests.

References

1. Meyer Sauter PM. Childhood community-acquired pneumonia. *Eur J Pediatr*. 2024;183:1129–36.
2. Meyer Sauter PM, Krautter S, Ambroggio L, Seiler M, Paioni P, Rely C, Capaul R, Kellenberger C, Haas T, Gysin C, et al. Improved Diagnostics Help to Identify Clinical Features and Biomarkers That Predict *Mycoplasma pneumoniae* Community-acquired Pneumonia in Children. *Clin Infect Dis*. 2020;71:1645–54.
3. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. *Mycoplasma pneumoniae* from the Respiratory Tract and Beyond. *Clin Microbiol Rev*. 2017;30:747–809.
4. Yue Y, Wu D, Zeng Q, Li Y, Yang C, Lv X, Wang L. Changes in children respiratory infections pre and post COVID-19 pandemic. *Front Cell Infect Microbiol*. 2025;15:1549497.
5. Sun Y, Li P, Jin R, Liang Y, Yuan J, Lu Z, Liang J, Zhang Y, Ren H, Zhang Y, et al. Characterizing the epidemiology of *Mycoplasma pneumoniae* infections in China in 2022–2024: a nationwide cross-sectional study of over 1.6 million cases. *Emerg Microbes Infect*. 2025;14:2482703.
6. Urbieto AD, Barbeito Castiñeiras G, Rivero Calle I, Pardo Seco J, Rodríguez Tenreiro C, Suárez Camacho R, Pérez Del Molino Bernal ML, Martínón Torres F: *Mycoplasma pneumoniae* at the rise not only in China: rapid increase of *Mycoplasma pneumoniae* cases also in Spain. *Emerg Microbes Infect*. 2024;13:2332680.
7. Wang F, Cheng Q, Duo H, Wang J, Yang J, Jing S, Li J, Zhou X, Shang Y, Chen N, et al. Childhood *Mycoplasma pneumoniae*: epidemiology and manifestation in Northeast and Inner Mongolia, China. *Microbiol Spectr*. 2024;12:e0009724.
8. Meyer Sauter PM, Beeton ML, Uldum SA, Bossuyt N, Vermeulen M, Loens K, Pereyre S, Bébéar C, Keše D, Day J, et al. *Mycoplasma pneumoniae* detections before and during the COVID-19 pandemic: results of a global survey, 2017 to 2021. *Euro Surveill*. 2022;27:2100746.
9. Xie P, Zhang Y, Qin Y, Fang Y, Yang N, Bai Y, Zhi S, Niu W, Wang F, Yuan X. Macrolide resistance in *Mycoplasma pneumoniae* in adult patients. *Front Cell Infect Microbiol*. 2025;15:1496521.
10. Chih-Cheng L, Hsueh CC, Hsu CK, Tsai YW, Hsueh PR. Disease burden and macrolide resistance of *Mycoplasma pneumoniae* infection in adults in the Asia-Pacific region. *Int J Antimicrob Agents*. 2024;64:107205.
11. Wang YS, Zhou YL, Bai GN, Li SX, Xu D, Chen LN, Chen X, Dong XY, Fu HM, Fu Z, et al. Expert consensus on the diagnosis and treatment of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. *World J Pediatr*. 2024;20:901–14.
12. Yu A, Ran L, Sun X, Feng T. Significance of respiratory virus coinfection in children with *Mycoplasma pneumoniae* pneumonia. *BMC Pulm Med*. 2024;24:585.
13. Li F, Zhang Y, Shi P, Cao L, Su L, Fu P, Abuduxikuer K, Wang L, Wang Y, Lu R, et al. *Mycoplasma pneumoniae* and Adenovirus Coinfection Cause Pediatric Severe Community-Acquired Pneumonia.

Microbiol Spectr. 2022;10:e0002622.

14. Wu Q, Pan X, Han D, Ma Z, Zhang H. New Insights into the Epidemiological Characteristics of *Mycoplasma pneumoniae* Infection before and after the COVID-19 Pandemic. *Microorganisms* 2024, 12.
15. Xu L, Wang P, Wang Y, Liu B, Xu X, Yang Q, Gao C, Sun H, Xu Y, Xu Q, et al. Epidemiological, clinical, and genotypic characteristics of pediatric *Mycoplasma pneumoniae* infections: an 8-year survey in Suzhou, China in the pre- and post-COVID-19 eras. *Front Microbiol.* 2024;15:1483152.
16. Jiang TT, Sun L, Wang TY, Qi H, Tang H, Wang YC, Han Q, Shi XQ, Bi J, Jiao WW, Shen AD. The clinical significance of macrolide resistance in pediatric *Mycoplasma pneumoniae* infection during COVID-19 pandemic. *Front Cell Infect Microbiol.* 2023;13:1181402.
17. Chen J, Zhang J, Lu Z, Chen Y, Huang S, Li H, Lin S, Yu J, Zeng X, Ji C, et al. *Mycoplasma pneumoniae* among Chinese Outpatient Children with Mild Respiratory Tract Infections during the Coronavirus Disease 2019 Pandemic. *Microbiol Spectr.* 2022;10:e0155021.
18. Leber AL, Embry T, Everhart K, Taveras J, Oyeniran SJ, Wang H. Macrolide-Resistant *Mycoplasma pneumoniae* Infections among Children after COVID-19 Pandemic, Ohio, USA. *Emerg Infect Dis.* 2025;31:555–8.
19. Waldeck F, Kramer TS, Boutin S, Matten J, Kramer J, Rupp J. Re-emergence of *Mycoplasma pneumoniae* before and after COVID-19 pandemic in Germany. *BMC Infect Dis.* 2025;25:318.
20. Edouard S, Boughammoura H, Colson P, La Scola B, Fournier PE, Fenollar F. Large-Scale Outbreak of *Mycoplasma pneumoniae* Infection, Marseille, France, 2023–2024. *Emerg Infect Dis.* 2024;30:1481–4.
21. Zhang L, Lai M, Ai T, Liao H, Huang Y, Zhang Y, Liu Y, Wang L, Hu J. Analysis of mycoplasma pneumoniae infection among children with respiratory tract infections in hospital in Chengdu from 2014 to 2020. *Transl Pediatr.* 2021;10:990–7.
22. Qu J, Yang C, Bao F, Chen S, Gu L, Cao B. Epidemiological characterization of respiratory tract infections caused by *Mycoplasma pneumoniae* during epidemic and post-epidemic periods in North China, from 2011 to 2016. *BMC Infect Dis.* 2018;18:335.
23. Wang X, Li M, Luo M, Luo Q, Kang L, Xie H, Wang Y, Yu X, Li A, Dong M, et al. *Mycoplasma pneumoniae* triggers pneumonia epidemic in autumn and winter in Beijing: a multicentre, population-based epidemiological study between 2015 and 2020. *Emerg Microbes Infect.* 2022;11:1508–17.
24. Diaz MH, Hersh AL, Olson J, Shah SS, Hall M, Edens C. *Mycoplasma pneumoniae* Infections in Hospitalized Children - United States, 2018–2024. *MMWR Morb Mortal Wkly Rep.* 2025;74:394–400.
25. Chen Y, Jia X, Gao Y, Ren X, Du B, Zhao H, Feng Y, Xue G, Cui J, Gan L, et al. Increased macrolide resistance rate of *Mycoplasma pneumoniae* correlated with epidemic in Beijing, China in 2023. *Front Microbiol.* 2024;15:1449511.
26. Shin S, Koo S, Yang YJ, Lim HJ. Characteristics of the *Mycoplasma pneumoniae* Epidemic from 2019 to 2020 in Korea: Macrolide Resistance and Co-Infection Trends. *Antibiot (Basel)* 2023, 12.

27. Zhu X, Liu P, Yu H, Wang L, Zhong H, Xu M, Lu L, Jia R, Su L, Cao L, et al. An outbreak of *Mycoplasma pneumoniae* in children after the COVID-19 pandemic, Shanghai, China, 2023. *Front Microbiol.* 2024;15:1427702.
28. Oliva A, Siccardi G, Migliarini A, Cancelli F, Carnevalini M, D'Andria M, Attilia I, Danese VC, Cecchetti V, Romiti R, et al. Co-infection of SARS-CoV-2 with *Chlamydia* or *Mycoplasma pneumoniae*: a case series and review of the literature. *Infection.* 2020;48:871–7.
29. De Francesco MA, Poiesi C, Gargiulo F, Bonfanti C, Pollara P, Fiorentini S, Caccuri F, Carta V, Mangeri L, Pellizzeri S, et al. Co-infection of *chlamydia pneumoniae* and *mycoplasma pneumoniae* with SARS-CoV-2 is associated with more severe features. *J Infect.* 2021;82:e4–7.
30. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell.* 2020;181:271–e280278.
31. Wang G, Wu P, Tang R, Zhang W. Global prevalence of resistance to macrolides in *Mycoplasma pneumoniae*: a systematic review and meta-analysis. *J Antimicrob Chemother.* 2022;77:2353–63.
32. Rothstein TE, Cunningham SA, Rieke RA, Mainella JM, Mutchler MM, Patel R. Macrolide Resistance in *Mycoplasma pneumoniae*, Midwestern United States, 2014 to 2021. *Antimicrob Agents Chemother.* 2022;66:e0243221.
33. Jiang Y, Dou H, Xu B, Xu B, Zhou W, Wang H, Ge L, Hu Y, Han X, Qin X, et al. Macrolide resistance of *Mycoplasma pneumoniae* in several regions of China from 2013 to 2019. *Epidemiol Infect.* 2024;152:e75.
34. Nguyen DD, Ho NT, Dover LG, Vo AHM, Ly HTT, Doan PM, Nguyen HT, Luu NTT, Pham AN, Tran HTT. Novel Variant and Known Mutation in 23S rRNA Gene of *Mycoplasma pneumoniae*, Northern Vietnam, 2023. *Emerg Infect Dis.* 2024;30:1034–6.
35. Wang N, Xu X, Xiao L, Liu Y. Novel mechanisms of macrolide resistance revealed by in vitro selection and genome analysis in *Mycoplasma pneumoniae*. *Front Cell Infect Microbiol.* 2023;13:1186017.

Figures

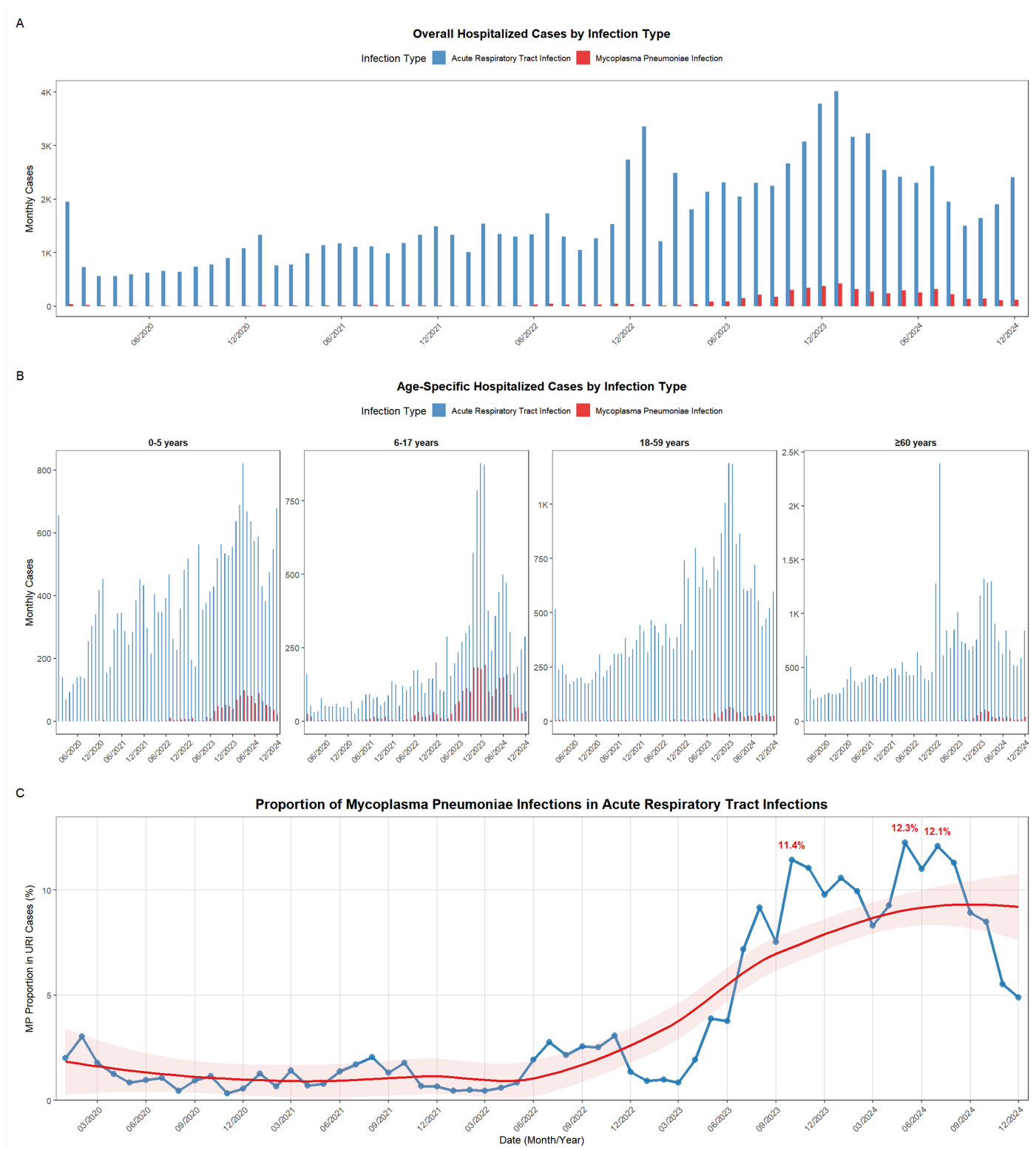


Figure 1

Temporal Distribution of Hospitalized Patients with ARTI Infections and MP Infections.

Figure 1A Distribution of infection types among hospitalized cases across all age groups. Blue bars represent the number of hospitalizations for acute upper respiratory tract infections, while red bars indicate the number of hospitalizations for MP infections. **Figure 1B** Age-stratified distribution patterns of MP and

acute upper respiratory tract infections. Blue bars denote hospitalization counts for acute upper respiratory tract infections, and red bars denote those for MP infections. **Figure 1C** Temporal trend in the proportion of MP infections among acute respiratory infections. The blue line represents the monthly percentage of MP infection cases relative to total acute respiratory infections, and the red confidence band illustrates the 95% confidence interval of the predicted trend based on LOESS smoothing.

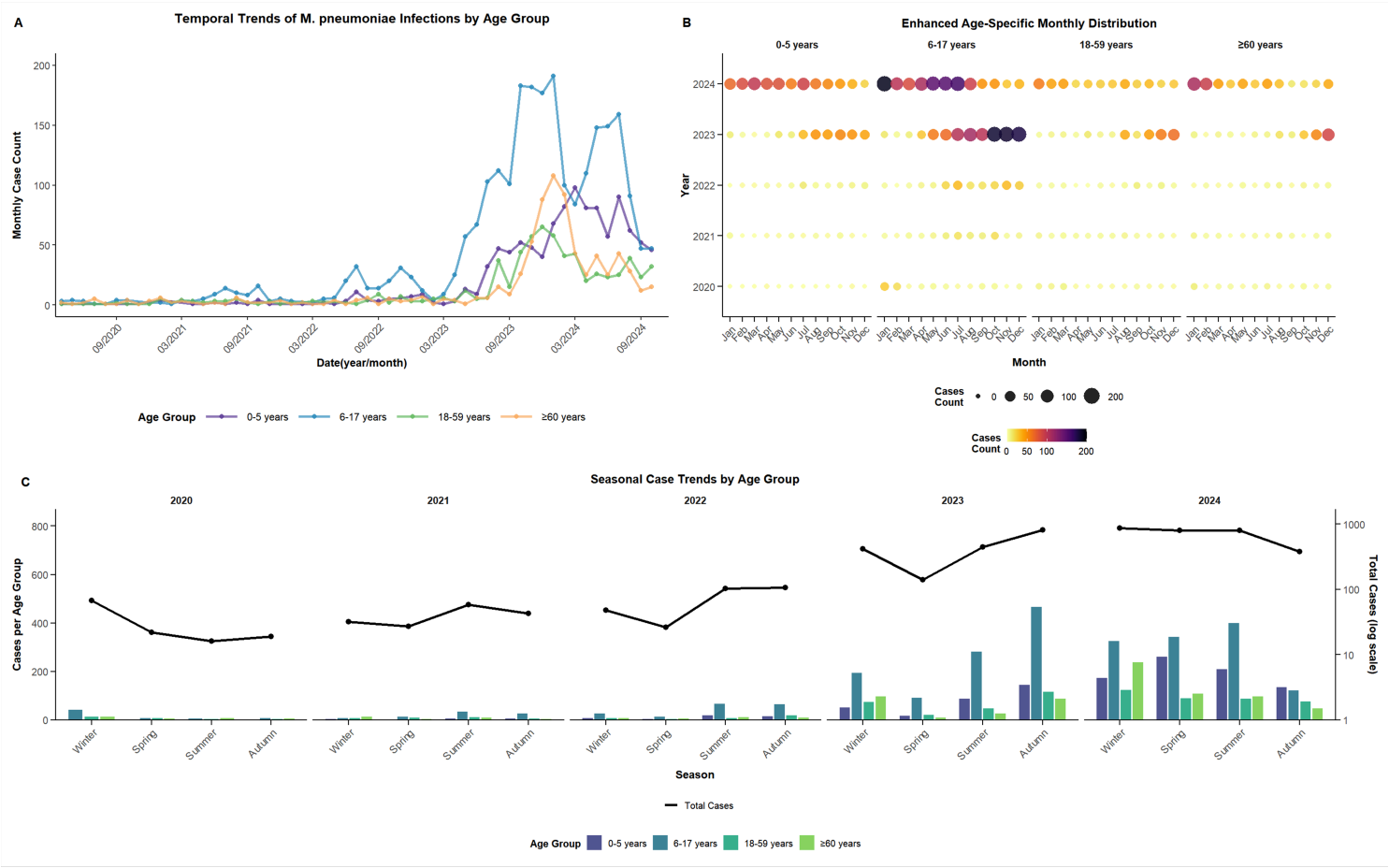


Figure 2

Temporal Distribution of MP Infection Hospitalizations by Age.

Figure 2A Temporal trend of MP infections stratified by age group. The purple, blue, cyan, and orange lines represent the infection trends for the 0-5 years, 6-17 years, 18-59 years, and ≥60 years age groups, respectively. **Figure 2B** Age-specific annual and monthly distribution visualized as an enhanced heat map. Dot size (small dot [2 mm]: ≤10 cases; medium dot [5 mm]: 50 cases; large dot [8 mm]: ≥200 cases) and color intensity (bright yellow: low case count, ≤10; dark brown: high case count, ≥200) reflect case density. **Figure 2C** Seasonal distribution and logarithmic trend of infections across age groups. In the left coordinate system, a Viridis-colored bar chart quantifies quarterly cases distributions for the four age groups (0-5, 6-17, 18-59, and ≥60 years), with bar height proportional to case number. In the right coordinate system, the black line—after log10 transformation—displays the trend in total quarterly case counts.

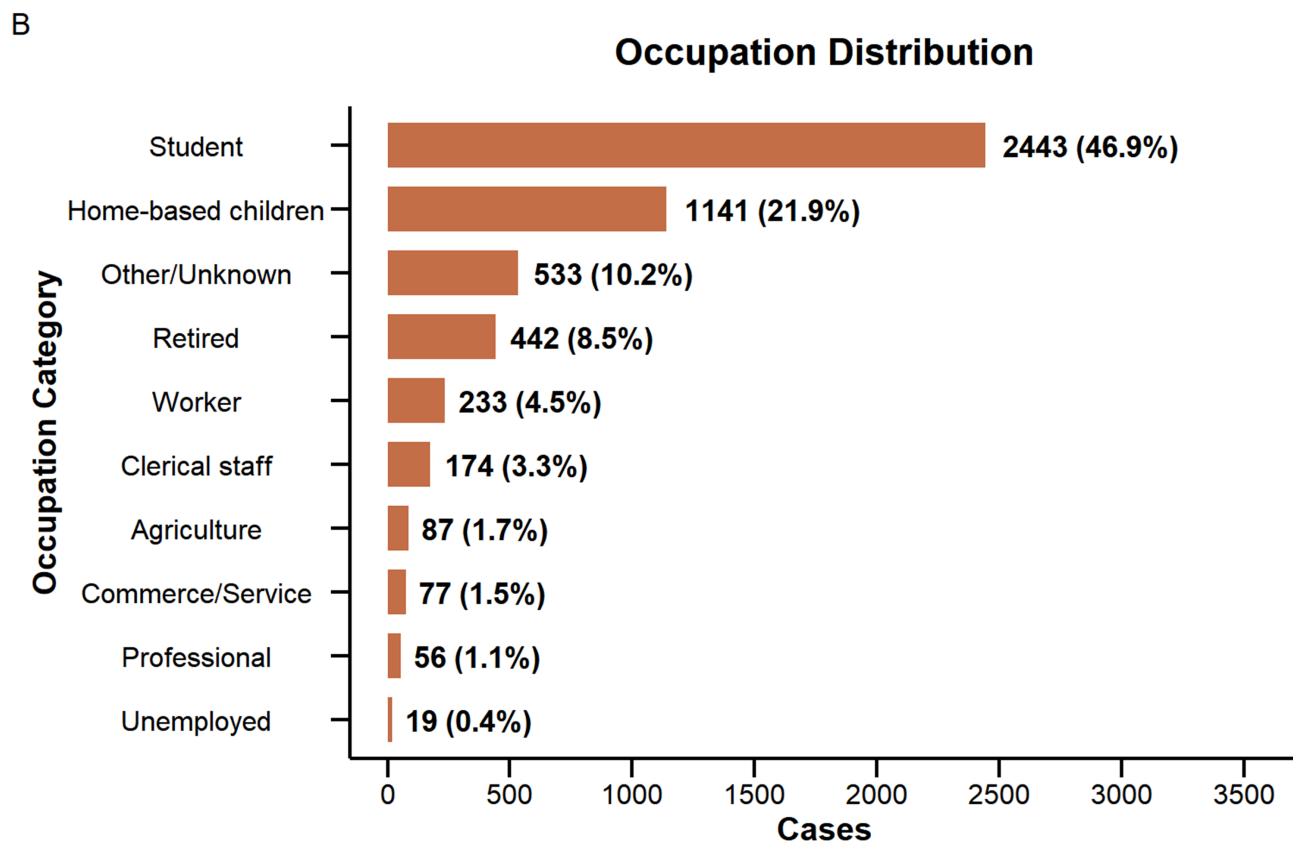
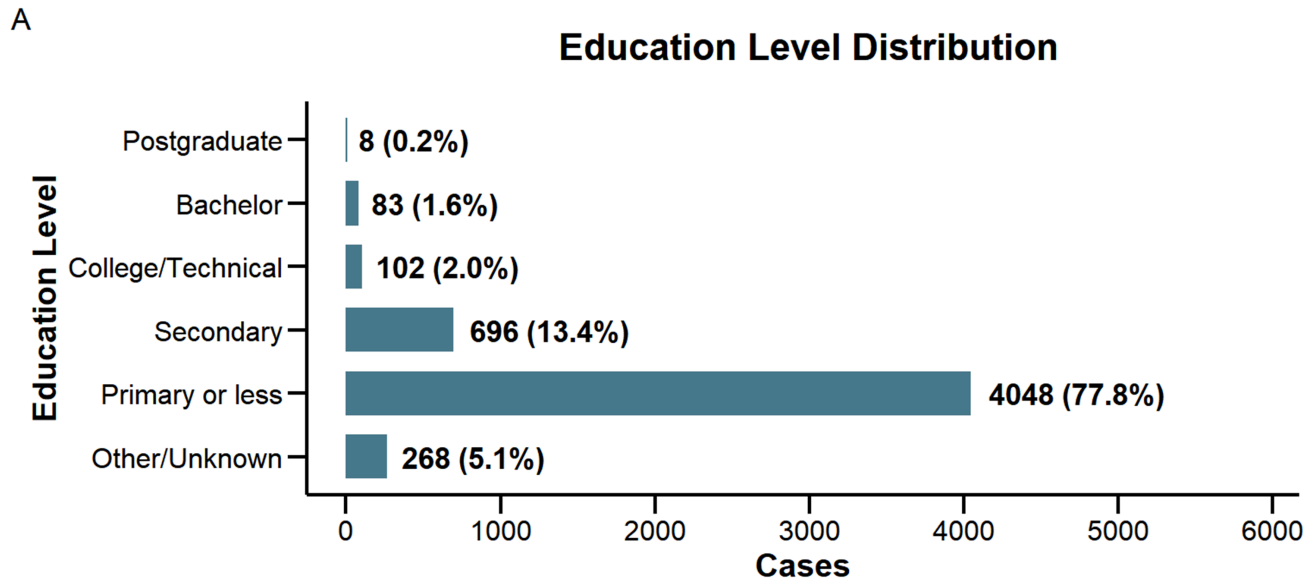


Figure 3

Demographic Distribution of Hospitalized Patients with MP Infection.

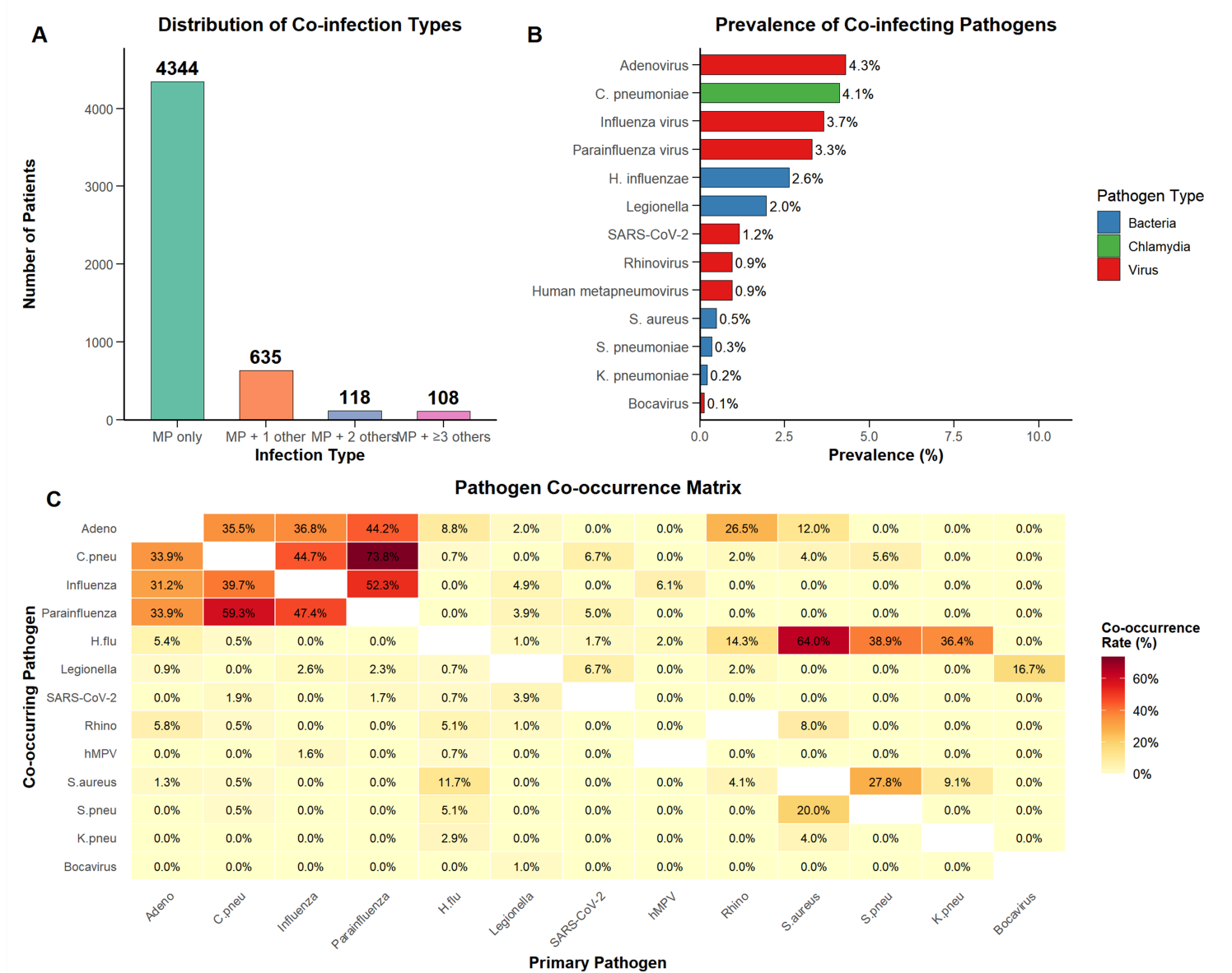


Figure 4

Co-infection Patterns in Hospitalized Patients with MP Infection.

Figure 4A Population distribution of co-infection patterns, illustrating the number of patients across four infection categories: MP infection alone (MP only), MP co-infected with one additional pathogen, MP co-infected with two additional pathogens, and MP co-infected with three or more additional pathogens. **Figure 4B** Prevalence distribution of co-infecting pathogens, displaying the distribution of 13 pathogens in descending order of positive rate. The positive rate for each pathogen is calculated as (number of patients positive for the pathogen / total number of hospitalized patients) $\times$ 100%, with viral pathogens highlighted in red and bacterial pathogens in blue. **Figure 4C** Heatmap depicting pathogen co-occurrence relationships, where the x-axis represents the primary pathogens and the y-axis denotes co-occurring pathogens. The color gradient from yellow to red in the cells indicates an increasing co-occurrence rate, calculated as (number of patients infected with both A and B / number of patients infected with A) $\times$ 100%.

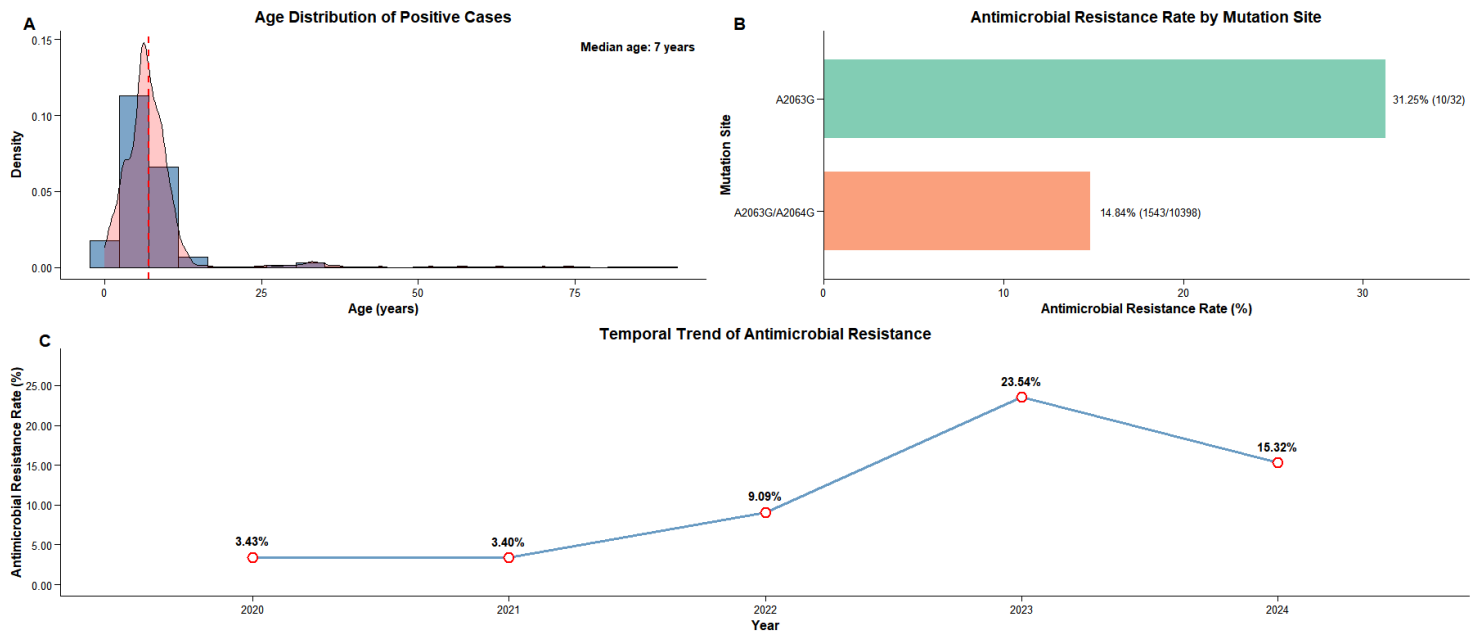


Figure 5

Analysis of antimicrobial resistance in Inpatients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMartials.docx](#)