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ORIGINAL RESEARCH

Prevalence of Macrolide-Resistant *Mycoplasma pneumoniae* Infections After the COVID-19 Pandemic in Southern Italy, 2023–2025

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ABSTRACT

***Introduction*:** The COVID-19 pandemic and associated non-pharmaceutical interventions (NPIs) markedly reduced the circulation of respiratory pathogens. In late 2023, a resurgence of *Mycoplasma pneumoniae* (MP) infections, including macrolide-resistant strains (MRMP), was documented worldwide. This study aimed to determine MRMP prevalence and epidemiological characteristics in Southern Italy during the post-pandemic period.

***Methods*:** Between January 2023 and May 2025, 5362 respiratory specimens were tested for *M. pneumoniae* and other respiratory pathogens using multiplex real-time polymerase chain reaction (PCR). Macrolide resistance-associated mutations in the 23S rRNA gene were identified through PCR amplification and Sanger sequencing. Data were stratified by age group and clinical setting.

***Results*:** MP prevalence peaked at 15.8% in May 2025. Of 305 positive cases, the median age was 10 years, 52.1% were male, and 86.9% were hospitalized. Coinfections occurred in 23.3% of cases, particularly among children < 5 years. Macrolide resistance was detected in 7.5% of MP-positive samples, predominantly the A2063G mutation (96%), with the highest prevalence in patients aged 10–14 years (12.6%). No resistance was identified in ICU patients.

***Conclusion*:** The post-pandemic resurgence of *M. pneumoniae* in Southern Italy, coupled with sustained macrolide resistance, underscores the need for continuous molecular surveillance and targeted antimicrobial stewardship to optimize therapy and prevent further resistance spread.

Keywords: Epidemiology; *Mycoplasma pneumoniae*; Macrolide resistance; Prevalence; Southern Italy

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Key Summary Points

Why carry out this study?

In the post-COVID19 period a pronounced resurgence of *Mycoplasma pneumoniae* (MP) infections has been reported internationally, with limited epidemiological data from Italy.

This is the first post-pandemic (2023–2025) report on the prevalence of MP infections and associated macrolide resistance (MRMP) in Southern Italy, with detailed age-stratified analysis.

What was learned from this study?

Overall MRMP rate of 7.5%, peaking in preadolescents (12.6%, aged 10–14 years) and predominantly associated with the A2063G mutation.

Coinfections more frequent among younger patients, highlighting potential clinical complications and the need for thorough differential diagnosis.

Continued molecular surveillance, integration into routine diagnostics, and targeted antimicrobial stewardship are needed to limit the spread of MRMP and to optimize clinical management and antimicrobial stewardship.

INTRODUCTION

Mycoplasma pneumoniae (MP) is a leading cause of community-acquired respiratory tract infections, especially in children, adolescents, and young adults [1]. Globally, it accounts for a significant proportion of community-acquired pneumonia (CAP) cases and is characterized by cyclical epidemic peaks every 3–7 years [2]. Macrolides are the first-line treatment, particularly in pediatric populations, due to their efficacy and safety profile. However, the emergence of macrolide-resistant *M. pneumoniae* (MRMP) has become a substantial clinical and public health concern [2].

The COVID-19 pandemic and the widespread adoption of NPIs, including school closures, mask use, social distancing, and enhanced hygiene, disrupted the transmission dynamics of respiratory pathogens, resulting in a marked global decline in *M. pneumoniae* incidence, particularly in children [3]. Since late 2023, a pronounced resurgence of MP infections has been reported internationally, attributed to altered population immunity, behavioral changes, and the pathogen's long incubation period [4, 5].

In Italy, MRMP strains have been reported over the past decade, with prevalence rates varying according to geographic region and patient population. Pediatric outbreaks in Southern Italy have shown resistance rates up to 26% [6], whereas reported rates of adult CAP cases in the same region were around 7% [7]. Such variability highlights the importance of regional surveillance to inform empirical treatment and optimize clinical outcomes.

This study aimed to provide an updated assessment of MRMP prevalence in Southern Italy following the COVID-19 pandemic, describing molecular resistance patterns, and demographic distribution.

METHODS

Study Design and Population

We conducted a retrospective observational study including all respiratory specimens processed at the Molecular Epidemiology and Public Health Laboratory, AOUC Polyclinic of Bari, between January 2023 and May 2025. Eligible patients were both hospitalized and outpatient individuals with acute respiratory tract infection symptoms who underwent etiological testing. If multiple samples were received from the same patient during the same respiratory tract infection episode, only the first sample was included. Demographic data were retrieved from laboratory records.

Pathogen Detection

Respiratory specimens (nasopharyngeal swabs, bronchoalveolar lavage fluid, or tracheal

aspirates) were obtained from patients with a diagnosis of upper and lower respiratory tract infections. Specimens were collected using standardized procedures and promptly transported to the laboratory. Nucleic acids were extracted using the STARMag Universal Cartridge kit on the Nimbus IV platform (Seegene, Seoul, Korea). *M. pneumoniae* DNA and other respiratory pathogens, such as influenza virus types A and B, adenovirus, human parainfluenza virus types 1, 2, 3 and 4, rhinovirus, respiratory syncytial virus types A and B, metapneumovirus, human coronavirus 229E, NL63, and OC43, human bocavirus, enterovirus, and SARS-CoV-2. Bacteria including *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Legionella* spp. and *Legionella pneumophila*, *Chlamydia pneumoniae*, *Pneumocystis jirovecii*, *Bordetella pertussis*, and *Bordetella parapertussis* were detected using the Allplex™ Respiratory Panel Assays (Seegene, Seoul, Korea).

Macrolide Resistance Detection

All MP-positive samples were subjected to molecular analysis for macrolide resistance. Specifically, domain V of the 23S rRNA gene was amplified and sequenced to identify mutations associated with macrolide resistance (A2063G, A2064G). Polymerase chain reaction (PCR) amplification was performed using primers targeting the domain V region, followed by Sanger sequencing as previously described [7]. Sequences were analyzed using BioEdit and MEGA software, aligned with reference sequences to confirm point mutations.

Statistical Analysis

Positivity and resistance rates were calculated for the overall study period and stratified by age, clinical setting, and months of testing. Categorical variables were compared using chi-square or Fisher's exact tests. Statistical significance was set at $p < 0.05$. Analyses were performed using STATA v12 (StataCorp LLC, College Station, TX, USA).

Ethical Approval

Ethical approval for this retrospective study was waived because it was part of the legislated mandate of the Health Promotion and Public Health Department of the Apulia region, Italy (Prot. r_puglia/AOO_005/PROT/22/09/2022/0006271). All study procedures conformed to institutional and national ethical standards and the 2013 revision of the Declaration of Helsinki.

RESULTS

A total of 5362 respiratory specimens were tested, with 305 (5.7%) positive for MP. Monthly positivity rates increased sharply from negligible levels in early 2023 to a peak of 15.8% in May 2025 (Fig. 1).

The median patient age was 9 years (interquartile range 6–12, range 0–78 years), and 52.1% were male. The characteristics of MP-positive patients by age group are shown in Table 1. Most cases (86.9%) were hospitalized, and 2.0% required ICU admission. Hospitalization rates were similar across age groups, though ICU admissions were more frequent in older patients (>14 years).

Coinfections with other respiratory pathogens (*Haemophilus influenzae*, *Chlamydia pneumoniae*, *Streptococcus pneumoniae*, bocavirus, enterovirus, influenza virus B, parainfluenza virus 3, and rhinovirus) occurred in 23.3% of MP-positive cases, 84% of which were hospitalized. No other pathogens were detected. The proportion of coinfections varied significantly among age groups ($p < 0.001$), with the highest rate observed in children under 5 years (44.1%).

Macrolide resistance was identified in 23 cases (7.5%). No MRMP was detected in 2023, 12 (52.2%) in 2024, and 11 (47.8%) in the first 5 months of 2025. The distribution of MRMP cases did not increase in parallel with the increasing number of MP isolates. The prevalence of macrolide resistance varied by age group: 12.6% in children aged 10–14 years, 7.0% in 5–9 years, 4.4% in children older than 14 years, and 3.4% in the under 5 years group (Fig. 2). Among the MRMP strains, 22 (95.7%)

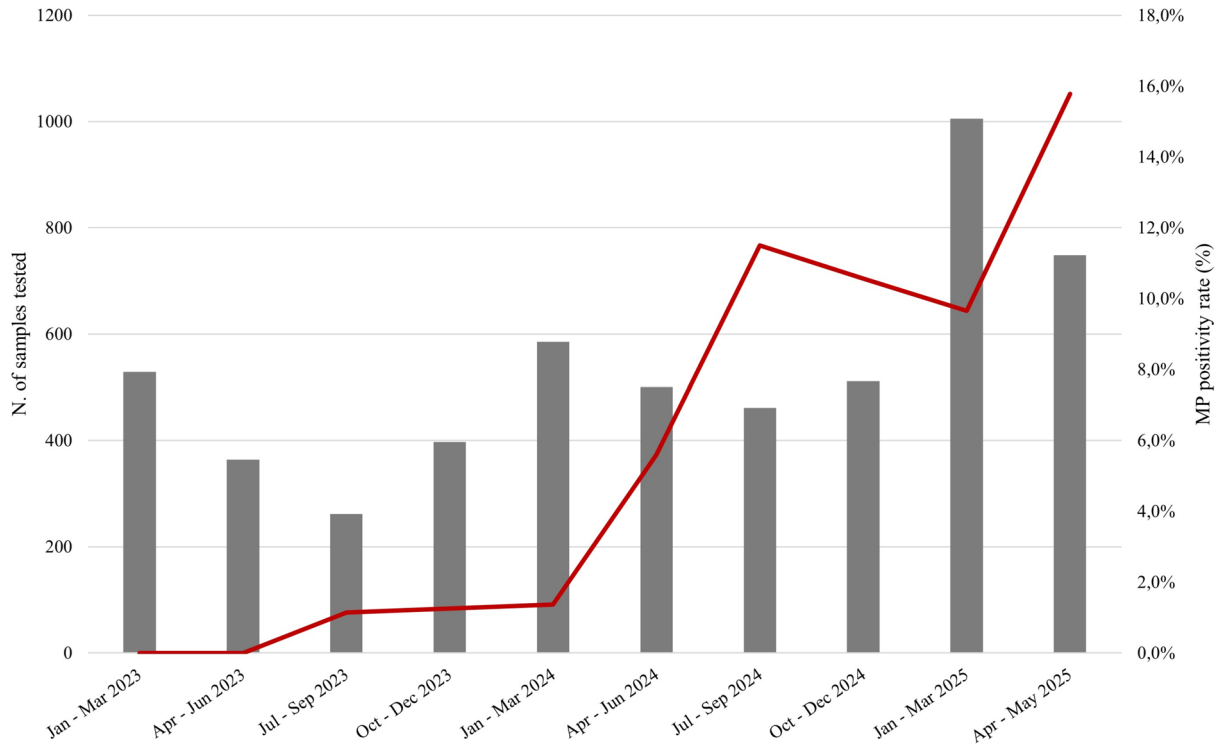

Fig. 1 Temporal trend in the total number of tests and positivity rates for *Mycoplasma pneumoniae* (MP) (January 2023–May 2025)

harbored the A2063G mutation whereas only 1 (4.3%) showed the A2064G mutation.

When analyzed by clinical setting, MRMP cases were identified in both hospitalized and outpatient populations, with 69.6% and 30.4% of resistant strains, respectively. No MRMP strains were detected among ICU patients.

DISCUSSION

This study provides an updated and comprehensive analysis of MRMP infections in Southern Italy during the post-COVID-19 era, highlighting substantial epidemiological changes. Our surveillance detected a marked resurgence of MP infections, with positivity rates in respiratory specimens rising from negligible levels in early 2023 to an average monthly positivity rate approaching 16% by May 2025. This upsurge, which emerged in late 2023, coincided temporally with the relaxation of COVID-19-related non-pharmaceutical interventions (NPIs), such as social distancing and universal masking. Similar rebound patterns in MP circulation have been reported in multiple European and Asian countries following NPI withdrawal [4, 5, 8, 9]. During the summer of 2025, persistent circulation of MP was recorded, although a decrease in the number of cases was observed after May 2025. Although there has been a clear resurgence of MP infections in the post-pandemic period, neither in our study nor in other countries around the world has any seasonality been observed in the occurrence of *M. pneumoniae* infection [4]. Further studies using data from a longer observation period are needed to explore seasonality of MP infections in the post-COVID19 era.

Molecular characterization revealed a macrolide resistance prevalence of 7.5%, predominantly linked to the A2063G point mutation within domain V of the 23S rRNA gene, in agreement with global surveillance reports [10, 11]. Resistance was predominantly observed in

Table 1 Characteristics of patients positive for *Mycoplasma pneumoniae* (MP) and positive for macrolide-resistant *Mycoplasma pneumoniae* (MRMP) by age group, Southern Italy (January 2023–May 2025)

	Age groups (years)										*p* value
	N	(%; 95% CI)	< 5		5–9		10–14		> 14		
			N	(%; 95% CI)	*N*	(%; 95% CI)	*N*	(%; 95% CI)	*N*	(%; 95% CI)	
MP positive											
Total	305	(100)	59	(19.3; 14.9–23.8)	114	(37.4; 31.9–42.8)	87	(28.5; 23.5–33.6)	45	(14.8; 10.8–18.7)	
Gender											
Male	159	(52.1; 46.5–57.7)	29	(49.2; 36.4–61.9)	66	(57.9; 48.8–67)	42	(48.3; 37.8–58.8)	22	(48.9; 34.3–63.5)	0.48
Female	146	(47.9; 42.3–53.5)	30	(50.8; 38.1–63.6)	48	(42.1; 33–51.2)	45	(51.7; 41.2–62.2)	23	(51.1; 36.5–65.7)	
Setting											
Outpatient	40	(13.1; 9.3–16.9)	12	(20.3; 10.1–30.6)	13	(11.4; 5.6–17.2)	10	(11.5; 4.8–18.2)	5	(11.1; 1.9–20.3)	0.34
Inpatient	265	(86.9; 83.1–90.7)	47	(79.7; 69.4–89.9)	101	(88.6; 82.8–94.4)	77	(88.5; 81.8–95.2)	40	(88.9; 79.7–98.1)	
ICU	6	(2.0; 0.4–3.5)	0	(0.0)	0	(0.0)	2	(2.3; 0.0–5.4)	4	(8.9; 0.6–17.2)	NA
Coinfection	71	(23.3; 18.5–28)	26	(44.1; 31.4–56.7)	33	(28.9; 20.6–37.3)	7	(8.0; 2.3–13.8)	5	(11.1; 1.9–20.3)	< 0.001
MRMP											
Total	23		2		8		11		2		
Gender											
Male	13		1		6		5		1		NA
Female	10		1		2		6		1		

Table 1 continued

	Age groups (years)								*p* value
	N	(%; 95% CI)	< 5		5–9		10–14		> 14		
			N	(%; 95% CI)	*N*	(%; 95% CI)	*N*	(%; 95% CI)	*N*	(%; 95% CI)	
Setting											
Inpatient	16		2		5		7		2		NA
Outpatient	7		0		3		4		0		
ICU	0		0		0		0		0		
Coinfection											
Yes	5		1		3		1		0		NA
No	18		1		5		10		2		
Mutation											
A2063G	22		2		8		10		2		NA
A2064G	1		0		0		1		0		

ICU intensive care unit, *CI* confidence interval, *NA* not applicable

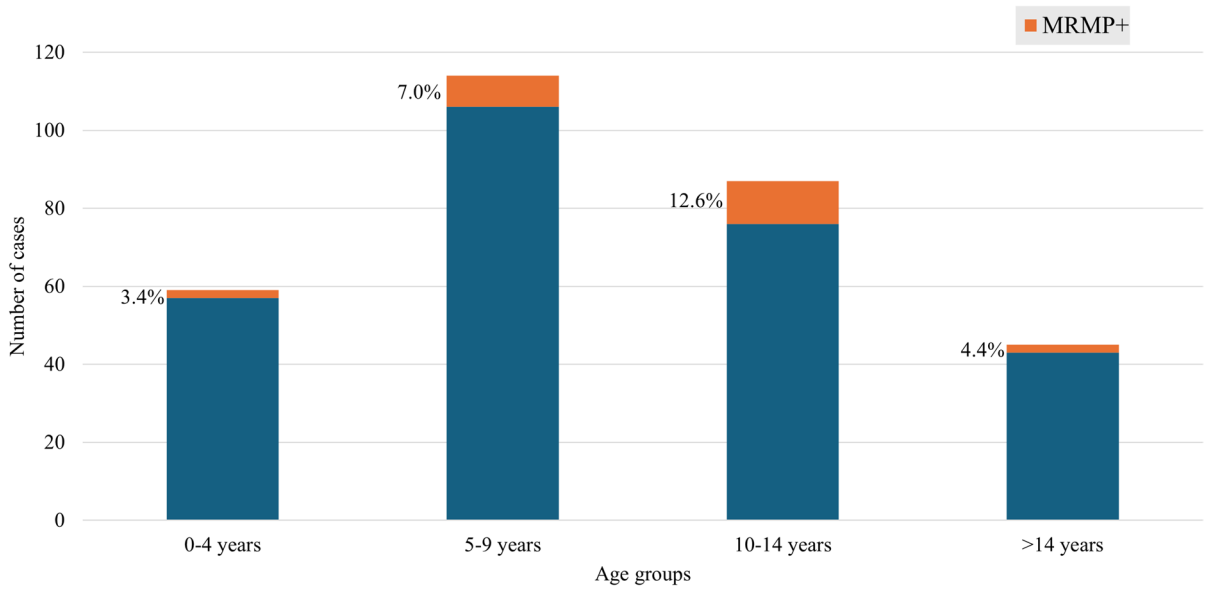

Fig. 2 Distribution of MP and MRMP positive cases by age groups (January 2023–May 2025). *Mycoplasma pneumoniae* (MP), macrolide-resistant strains (MRMP)

children, suggesting possible age-related differences in antimicrobial exposure, host immunity, or transmission dynamics, although this finding should be interpreted with caution because of the small number of MRMP positive cases. Further data are needed to better investigate the predictive and risk factors for resistance. Detection of MRMP in both inpatients and outpatients underscores its circulation in both community and hospital settings. Our prevalence estimates are consistent with previous Italian adult cohort data (about 7%) [7], but remain markedly lower than the 26% reported during earlier pediatric outbreaks in the same region [6]. This relative stability contrasts with reports from East Asia, where MRMP rates have risen considerably, complicating empirical treatment strategies [10, 12].

Internationally, our findings align with reports of increased MP infections and the emergence of MRMP during the post-pandemic period, including outbreaks in the USA [13], Denmark [14], and Germany [15]. Notably, the prevalence in our Southern Italian cohort appears higher than in these countries, where reported rates range from below 2% to 2.61%. This discrepancy may reflect variations in antimicrobial prescribing practices, differences in surveillance intensity, or distinct strain introduction and transmission dynamics. Furthermore, the prevalence of macrolide resistance in MP found in this study seems to differ significantly from that estimated for Italy (approximately 3%) in a recent report [11], given that no samples from Italian patients were tested for macrolide resistance. This suggests that more standardized and geographically focused investigations should be conducted in order to obtain more accurate data.

Clinically, the persistence of MRMP strains poses significant therapeutic challenges, given that macrolides remain the first-line empirical treatment for MP, particularly in pediatric populations owing to their safety and efficacy profiles. The presence of resistance increases the risk of treatment failure, prolonged symptomatology, and additional healthcare utilization. Additionally, our data also demonstrated a high coinfection rate (23.3%), with the greatest burden in children under 5 years of age. These coinfections, often involving viral or bacterial respiratory pathogens, can exacerbate disease severity, complicate diagnosis, and guide inappropriate antimicrobial use [2]. Most patients with coinfections were hospitalized. Unfortunately, no

information is available on the antibiotic treatment received by patients, as this information was not evident from the laboratory records and therefore it was not possible to draw conclusions on the possible use of antibiotics as a driver of resistance development.

The drivers of this resurgence and sustained resistance are likely multifactorial. Contributing factors may include the temporary suppression of transmission during NPIs, shifts in healthcare-seeking behavior, changes in antibiotic prescribing patterns, and selective pressures favoring resistant clones [3, 4, 16, 17]. Further investigations are warranted to elucidate these dynamics and inform targeted public health interventions.

Strengths of this study include the integration of molecular diagnostics with sequencing-based resistance detection, enhancing the accuracy of MRMP prevalence estimates. Limitations include its retrospective single-center design, the small sample size especially in the MRMP-positive group, and limited availability of clinical and therapeutical data. These may limit generalizability and precluded a deeper analysis of disease severity and treatment outcomes. Nevertheless, our findings provide valuable epidemiological data for post-COVID-19 MRMP surveillance in southern Europe.

CONCLUSIONS

The resurgence of *M. pneumoniae* infections in Southern Italy, coupled with sustained macrolide resistance, highlights the necessity for continued molecular surveillance, integration into routine diagnostics, and targeted antimicrobial stewardship. Further multicenter prospective studies are essential to track resistance trends, guide treatment strategies, and mitigate public health impact.

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Author Contributions. Maria Chironna conceived this study and designed the analysis. Marisa Accogli, Alfredo Marziani and Valentina Annachiara Orlando obtained the data. Francesca Centrone, Raffaella Melilli and Davide Sacco, cleared up the datasets; Francesca Centrone, Raffaella Melilli, Anna Sallustio, Daniele Casulli, Vittoria Scolamacchia, Nicola Netti and Raffaella Melilli performed the data analyses. Anna Sallustio, Francesca Centrone, and Raffaella Melilli interpreted the results of the data analyses. Francesca Centrone and Maria Chironna drafted for the manuscript; all authors read and approved the final manuscript.

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Data Availability. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest. The authors Francesca Centrone, Marisa Accogli, Raffaella Melilli, Alfredo Marziani, Valentina A Orlando, Daniele Casulli, Vittoria Scolamacchia, Nicola Netti, Davide Sacco, Anna Sallustio, and Maria Chironna have no conflicts of interest to declare.

Ethical Approval. Ethical approval for this retrospective study was waived because it was part of the legislated mandate of the Health Promotion and Public Health Department of the Apulia region, Italy (Prot. r_puglia/AOO_005/PROT/22/09/2022/0006271). All study procedures conformed to institutional and national ethical standards and the 2013 revision of the Declaration of Helsinki.

REFERENCES

1. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev. 2004;17(4):697–728.

2. Kim K, Jung S, Kim M, Park S, Yang HJ, Lee E. Global trends in the proportion of macrolide-resistant *Mycoplasma pneumoniae* infections: a systematic review and meta-analysis. JAMA Netw Open. 2022;5(7):e2220949.

3. Xu L, Wang P, Wang Y, 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.

4. ESGMAC MAPS Study Group. Global spatiotemporal dynamics of *Mycoplasma pneumoniae* re-emergence after COVID-19 pandemic restrictions: an epidemiological and transmission modelling study. Lancet Microbe. 2025;6(4):101019.

5. Novazzi F, Arcari G, Perniciaro S, et al. Ongoing post-pandemic peak of *Mycoplasma pneumoniae* cases in July 2024: a single-center experience in north-west Italy. IJID Reg. 2024;14:100554.

6. Chironna M, Sallustio A, Esposito S, et al. Emergence of macrolide-resistant strains during an outbreak of *Mycoplasma pneumoniae* infections in children. J Antimicrob Chemother. 2011;66(4):734–7.

7. Loconsole D, De Robertis AL, Mallamaci R, et al. First description of macrolide-resistant *Mycoplasma pneumoniae* in adults with community-acquired pneumonia in Italy. BioMed Res Int. 2019;2019:7168949.

8. Xing FF, Chiu KHY, Chao-Wen D, et al. Post-COVID-19 pandemic rebound of macrolide-resistant *Mycoplasma pneumoniae* infection: a descriptive study. Antibiotics. 2024;13(3):262.

9. Sun Y, Li P, Jin R, 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(1):2482703.

10. Miyashita N, Ogata M, Fukuda N, Yamura A, Ito T. Macrolide-resistant *Mycoplasma pneumoniae* infection prevalence increases again in Osaka. Respir Investig. 2025;63(4):517–20.

11. Meyer Sauteur PM, European Society of Clinical Microbiology and Infectious Diseases Study Group for *Mycoplasma* and *Chlamydia* Infections (ESGMAC) *Mycoplasma pneumoniae* Surveillance (MAPS) study group. *Mycoplasma pneumoniae*: re-emergence and beyond. Lancet Microbe. 2025;16:101191.

12. Jia X, Chen Y, Gao Y, et al. Increased *in vitro* antimicrobial resistance of *Mycoplasma pneumoniae* isolates obtained from children in Beijing, China, in 2023. Front Cell Infect Microbiol. 2024;14:1478087.

13. Leber AL, Embry T, Everhart K, et al. Macrolide-resistant *Mycoplasma pneumoniae* infections among children after COVID-19 pandemic, Ohio, USA. Emerg Infect Dis. 2025;31(3):555–8.

14. Nordholm AC, Søborg B, Jokelainen P, et al. *Mycoplasma pneumoniae* epidemic in Denmark, October to December, 2023. Euro Surveill. 2024;29(2):2300707.

15. Dumke R. The high-incidence period of *Mycoplasma pneumoniae* infections 2023/2024 in southeast Germany was associated with a low level of macrolide resistance. Infection. 2024;52(6):2525–7.

16. Ma J, Guo P, Mei S, et al. Influence of COVID-19 pandemic on the epidemiology of *Mycoplasma pneumoniae* infections among hospitalized children in Henan, China. Heliyon. 2023;9(11):e22213.

17. Qi J, Li H, Li H, et al. Epidemiological changes of *Mycoplasma pneumoniae* among children before, during, and post the COVID-19 pandemic in Henan, China, from 2017 to 2024. Microbiol Spectr. 2025;13(7):e0312124.