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Epidemiology of pediatric Mycoplasma pneumoniae pneumonia and clinical outcomes by pathogen co-detection pattern: a two-center retrospective study

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Abstract

Background

Epidemiological patterns of pediatric *Mycoplasma pneumoniae* pneumonia changed during the implementation and adjustment of COVID-19-related non-pharmaceutical interventions. Multicenter evidence on pathogen co-detection and clinical outcomes remains limited. We aimed to characterize its epidemiology and assess these associations.

Methods

This two-center retrospective study included hospitalized children aged 0–16 years with community-acquired pneumonia (CAP) from January 1, 2022 to December 31, 2025. *M. pneumoniae* positivity among CAP admissions was compared by year, sex, age, season, and intervention stage. Children with *M. pneumoniae* pneumonia (MPP) were classified as *M. pneumoniae* alone, with bacteria, with viruses, or with both bacteria and viruses. Length of stay was analyzed using a Gamma generalized linear model; pulmonary consolidation and pleural effusion were analyzed using multivariable logistic regression. Models adjusted for center, sex, age, season, and intervention stage.

Results

Among 28,067 children with CAP, 8,328 had MPP (29.7%). Annual positivity was 19.7%, 38.3%, 38.2%, and 12.0% from 2022 through 2025 ($P < 0.001$). Compared with *Mycoplasma pneumoniae* alone, bacterial co-detection and bacterial-viral co-detection were associated with longer hospital stay, with adjusted mean ratios of 1.071 (95% confidence interval 1.047–1.094) and 1.064 (1.037–1.092), respectively (both $P < 0.001$); viral co-detection was not ($P = 0.173$). Center-by-pattern interactions were significant, and associations with imaging outcomes were less consistent.

Conclusions

Pediatric *Mycoplasma pneumoniae* infection showed marked temporal, seasonal, and demographic variation. Bacterial-containing co-detection patterns were associated with modestly longer hospitalization in overall models, but effect estimates varied between centers, supporting cautious interpretation of co-detection as a clinical marker.

Background

Mycoplasma pneumoniae is a common cause of community-acquired pneumonia (CAP) in children [1, 2], and it typically occurs in cycles of approximately 3–5 years and exhibits seasonal variations [3, 4]. During the coronavirus disease 2019 pandemic, non-pharmaceutical interventions (NPIs), including masking

and reduced social mixing, altered the transmission of multiple respiratory pathogens and were accompanied by a marked reduction in *M. pneumoniae* detection [5, 6]. After relaxation of pandemic-related restrictions, delayed re-emergence of *M. pneumoniae* and other respiratory pathogens was reported across Asia, Europe, the Americas, and Oceania, with changes in annual and seasonal patterns [4, 7–10].

Detection of respiratory bacteria or viruses in addition to *M. pneumoniae* is common among children with *M. pneumoniae* pneumonia (MPP) [11–13]. Previous studies suggest that different co-detection patterns may be associated with distinct clinical phenotypes and disease burden [14, 15]. However, some studies have analyzed all co-detections as a single category, and differences in diagnostic platforms or pathogen panels between centers can substantially influence the observed pathogen spectrum. In this setting, co-detection should not automatically be interpreted as simultaneous causative infection.

We therefore analyzed hospitalized children with CAP and MPP from two participating hospitals between January 1, 2022 and December 31, 2025. The objectives were to describe temporal, demographic, and seasonal patterns of *M. pneumoniae* positivity and to compare four pathogen detection patterns—*M. pneumoniae* alone, *M. pneumoniae* plus bacteria, *M. pneumoniae* plus viruses, and *M. pneumoniae* plus both bacteria and viruses—in relation to hospital length of stay and pulmonary imaging outcomes. We also assessed the robustness of the findings to alternative time adjustments and explored potential center heterogeneity.

Methods

Study design and participants

This was a two-center retrospective observational study of children aged 0–16 years hospitalized with community-acquired pneumonia (CAP) at Center A or Center B in Nantong, Jiangsu Province, China, between January 1, 2022 and December 31, 2025. All eligible CAP admissions were included in the epidemiological analysis. For these analyses, *M. pneumoniae* positivity was calculated as the number of eligible CAP admissions meeting the study laboratory definition of *M. pneumoniae* positivity divided by the total number of eligible CAP admissions in the corresponding stratum. Children meeting the study criteria for *M. pneumoniae* pneumonia (MPP) and with sufficient laboratory data to assign a pathogen co-detection pattern and ascertain the major clinical outcomes were included in the analyses of co-detection patterns and outcomes. Pathogen testing was performed as part of routine clinical care and was not fully standardized between the two centers; center-specific methods are described below. Data were extracted from the electronic medical record and laboratory information systems and independently checked by the study investigators.

Eligibility criteria

CAP was defined according to the guideline for the management of community-acquired pneumonia in children [16]. The inclusion criteria for the CAP cohort were: (1) a diagnosis of pediatric CAP; (2) age 0–16 years; (3) hospitalization at either participating center during the study period; (4) complete demographic and admission information, including sex, age, and admission date; and (5) at least one interpretable M. pneumoniae test result obtained during hospitalization. For study purposes, the MPP cohort was retrospectively identified from the CAP cohort using prespecified criteria: a positive M. pneumoniae nucleic acid test or an M. pneumoniae-specific immunoglobulin M result at or above the contemporaneous assay-specific positive threshold, together with compatible clinical and imaging findings supporting a diagnosis of MPP [17, 18], and complete data on pathogen detection pattern and the principal outcomes.

Pathogen detection

Pathogen test results were extracted from the laboratory information systems of both centers. Laboratory *Mycoplasma pneumoniae* positivity was defined as a positive nucleic acid amplification test or an *M. pneumoniae*-specific immunoglobulin M (IgM) result meeting the assay-specific cutoff used at the respective center at the time of testing. The diagnosis of *M. pneumoniae* pneumonia additionally required compatible clinical and radiographic findings. Both centers used the iFlash 3000 chemiluminescence immunoassay platform (Shenzhen YHLO Biotech Co., Ltd., China) for quantitative detection of *M. pneumoniae*-specific IgM and Epstein–Barr virus-related IgM antibodies. Center A used a nucleic acid amplification platform to detect *M. pneumoniae* in nasopharyngeal swabs, whereas Center B used a real-time fluorescence PCR platform.

Testing strategies for non-M. pneumoniae pathogens differed between centers. At Center A, bacterial co-detection was based mainly on routine sputum culture, whereas viral co-detection was based predominantly on pathogen-specific IgM assays. At Center B, respiratory pathogens were identified mainly using multiplex PCR assays targeting respiratory viruses and bacterial pathogens. Additional pathogen results were extracted from laboratory reports obtained during hospitalization, and synonymous organism names were harmonized before classification. Because specimen types, assay principles, target panels, and testing strategies were not identical between centers, additional positive pathogen results were classified as pathogen co-detection and were not interpreted as evidence of concurrent active infection or causal involvement in the clinical presentation.

Definitions and outcomes

Age was categorized as infancy (< 1 year), toddler age (1 to < 3 years), preschool age (3 to < 6 years), and school age (≥ 6 years). Seasons were defined as spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). Based on the pandemic timeline and national policy changes, the study period was divided into an NPI implementation stage (January 1, 2022 to January 7, 2023) and a post-NPI-adjustment stage (January 8, 2023 to December 31, 2025). Major

clinical outcomes were hospital length of stay and pulmonary imaging findings, including pulmonary consolidation, pleural effusion, and atelectasis.

Children with MPP were classified into four mutually exclusive detection patterns according to pathogens detected in addition to *M. pneumoniae*: (1) *M. pneumoniae* alone; (2) *M. pneumoniae* plus at least one bacterium and no virus; (3) *M. pneumoniae* plus at least one virus and no bacterium; and (4) *M. pneumoniae* plus at least one bacterium and at least one virus.

Statistical analysis

Statistical analyses were performed using SPSS version 25.0. Categorical variables are presented as n (%) and were compared using the Pearson χ^2 test; Fisher exact testing was used when expected cell counts did not satisfy χ^2 assumptions. Overall annual differences in *M. pneumoniae* positivity were assessed using a 4×2 contingency-table χ^2 test, followed by Bonferroni-adjusted pairwise comparisons. Skewed continuous variables are presented as median (25th percentile, 75th percentile) and were compared across the four detection patterns using the Kruskal–Wallis H test.

Using *M. pneumoniae* alone as the reference category, pathogen detection pattern was the main exposure. Hospital length of stay was modeled using a generalized linear model with a Gamma distribution and log link, and results are reported as adjusted mean ratios (aMRs) with 95% confidence intervals (CIs). Pulmonary consolidation and pleural effusion were analyzed using multivariable logistic regression and are reported as adjusted odds ratios (aORs) with 95% CIs. The primary models prespecified adjustment for center, sex, age group, season, and NPI stage. Because NPI stage and calendar year were highly correlated, they were not entered simultaneously. Sensitivity analyses replaced NPI stage with calendar year as a categorical variable and separately restricted the analysis to the post-NPI-adjustment stage. Because detection-pattern distributions differed markedly between centers, exploratory models included a center-by-detection-pattern interaction; the overall interaction was assessed with a Wald χ^2 test, followed by center-stratified analyses. Atelectasis was uncommon and included a zero-event cell, so it was evaluated with exact testing and was not included in the main multivariable regression. All tests were two-sided, and $P < 0.05$ was considered statistically significant. No a priori sample-size calculation was performed because all eligible admissions during the prespecified study period were included.

Use of large language models

ChatGPT (OpenAI) was used during manuscript preparation to assist with English-language editing, manuscript formatting, and refinement of wording and organization. It was not used to generate or analyze study data, determine study results, or make clinical or statistical decisions. All AI-assisted changes were critically reviewed and verified by the authors, who take full responsibility for the content of the manuscript.

Results

Study population and center characteristics

A total of 28,067 children with CAP were included: 12,400 from Center A and 15,667 from Center B. The centers did not differ significantly in sex distribution ($P = 0.092$) or overall *M. pneumoniae* positivity ($P = 0.699$). Age distribution and seasonal composition differed between centers (both $P < 0.001$). Center A had a larger proportion of school-aged children, whereas Center B had larger proportions of infants and toddlers. Spring and summer accounted for larger proportions of CAP admissions at Center A, whereas autumn and winter accounted for larger proportions at Center B (Table 1).

Table 1
Characteristics of hospitalized children with CAP by study center

Variable	Center A CAP (n = 12400)	Center B CAP (n = 15667)	χ^2	*P*
Sex			2.833	0.092
Male	6805 (54.9)	8440 (53.9)		
Female	5595 (45.1)	7227 (46.1)		
Age group			1209.391	< 0.001
Infancy (< 1 y)	740 (6.0)	2221 (14.2)		
Toddler age (1 to < 3 y)	2617 (21.1)	3938 (25.1)		
Preschool age (3 to < 6 y)	4435 (35.8)	6288 (40.1)		
School age (≥ 6 y)	4608 (37.2)	3220 (20.6)		
Season			86.422	< 0.001
Spring (Mar–May)	2936 (23.7)	3457 (22.1)		
Summer (Jun–Aug)	3062 (24.7)	3287 (21.0)		
Autumn (Sep–Nov)	3375 (27.2)	4750 (30.3)		
Winter (Dec–Feb)	3027 (24.4)	4173 (26.6)		
M. pneumoniae status			0.149	0.699
Positive	3694 (29.8)	4634 (29.6)		
Negative	8706 (70.2)	11033 (70.4)		

Data are n (%) unless otherwise indicated; percentages are column percentages. CAP, community-acquired pneumonia; *M. pneumoniae*, *Mycoplasma pneumoniae*.

Temporal trends in M. pneumoniae positivity

Annual *M. pneumoniae* positivity among children with CAP was 19.7% in 2022, 38.3% in 2023, 38.2% in 2024, and 12.0% in 2025. The overall annual difference was significant (χ^2=1842.432, $P < 0.001$). After Bonferroni correction, positivity did not differ significantly between 2023 and 2024, whereas the other annual pairwise comparisons were statistically significant. Thus, positivity increased markedly from 2022, remained high during 2023–2024, and declined to 12.0% in 2025 (Table 2).

Table 2
M. pneumoniae positivity by demographic and temporal characteristics

Characteristic	CAP, n	*M. pneumoniae* positive, n	Positivity, %	χ^2	*P*
Calendar year				1842.432	< 0.001
2022	2974	586	19.7		
2023	8146	3122	38.3		
2024	9851	3768	38.2		
2025	7096	852	12.0		
Sex				133.500	< 0.001
Male	15245	4083	26.8		
Female	12822	4245	33.1		
Age group				1482.168	< 0.001
Infancy (< 1 y)	2961	287	9.7		
Toddler age (1 to < 3 y)	6555	1573	24.0		
Preschool age (3 to < 6 y)	10723	3002	28.0		
School age (≥ 6 y)	7828	3466	44.3		
Season				132.282	< 0.001
Spring	6393	1637	25.6		
Summer	6349	1984	31.2		
Autumn	8125	2725	33.5		
Winter	7200	1982	27.5		
NPI stage				158.211	< 0.001
Implementation stage	3076	612	19.9		
Post-adjustment stage	24991	7716	30.9		
Total	28067	8328	29.7		

CAP, community-acquired pneumonia; NPI, non-pharmaceutical intervention. Positivity was calculated as the number of *M. pneumoniae*-positive CAP admissions divided by the total CAP admissions in the corresponding stratum. Overall *P* values are from Pearson χ^2 tests. The annual overall comparison used a 4×2 contingency table.

Monthly MPP case counts and MP positivity remained relatively low in 2022. A sustained increase began in mid-2023, peaking in October 2023. Both remained elevated through much of 2024 and declined markedly in 2025 (Fig. 1).

Demographic, seasonal, and NPI-stage patterns

Among the 28,067 children with CAP, 8,328 had MPP, corresponding to an overall *M. pneumoniae* positivity of 29.7%. Positivity was lower in boys than in girls (26.8% vs 33.1%; χ^2=133.500, $P < 0.001$). Positivity differed by age group (χ^2=1482.168, $P < 0.001$), increasing from 9.7% in infants to 24.0% in toddlers, 28.0% in preschool-aged children, and 44.3% in school-aged children. Seasonal differences were also significant (χ^2=132.282, $P < 0.001$), with the highest positivity in autumn (33.5%) and the lowest in spring (25.6%). Positivity was 30.9% during the post-NPI-adjustment stage compared with 19.9% during the NPI implementation stage (χ^2=158.211, $P < 0.001$) (Table 2).

Baseline characteristics by pathogen detection pattern

Among 8,328 children with MPP, 2,653 had *M. pneumoniae* alone, 2,062 had *M. pneumoniae* plus bacteria, 2,589 had *M. pneumoniae* plus viruses, and 1,024 had *M. pneumoniae* plus both bacteria and viruses. Sex distribution did not differ significantly across the four groups (χ^2=7.702, $P = 0.053$), whereas age, season, center, and NPI-stage distributions differed significantly (all $P < 0.001$). The viral co-detection group had the highest proportion of school-aged children (51.6%). The bacterial co-detection group was predominantly from Center B (97.0%), whereas the viral co-detection group was predominantly from Center A (89.0%). Bacterial and bacterial-viral co-detection was uncommon during the NPI implementation stage (Table 3).

Table 3
Baseline characteristics of children with MPP by pathogen detection pattern

Variable	*M. pneumoniae* alone (n = 2653)	+ bacteria (n = 2062)	+ viruses (n = 2589)	+ bacteria and viruses (n = 1024)	χ^2	*P*
Sex					7.702	0.053
Male	1319 (49.7)	1042 (50.5)	1212 (46.8)	510 (49.8)		
Female	1334 (50.3)	1020 (49.5)	1377 (53.2)	514 (50.2)		
Age group					302.893	< 0.001
Infancy	85 (3.2)	103 (5.0)	36 (1.4)	63 (6.2)		
Toddler age	617 (23.3)	383 (18.6)	329 (12.7)	244 (23.8)		
Preschool age	1071 (40.4)	683 (33.1)	887 (34.3)	361 (35.3)		
School age	880 (33.2)	893 (43.3)	1337 (51.6)	356 (34.8)		
Season					124.354	< 0.001
Spring	534 (20.1)	415 (20.1)	519 (20.0)	169 (16.5)		
Summer	739 (27.9)	377 (18.3)	688 (26.6)	180 (17.6)		
Autumn	805 (30.3)	764 (37.1)	795 (30.7)	361 (35.3)		
Winter	575 (21.7)	506 (24.5)	587 (22.7)	314 (30.7)		
Center					3994.814	< 0.001
Center A	1222 (46.1)	62 (3.0)	2303 (89.0)	107 (10.4)		
Center B	1431 (53.9)	2000 (97.0)	286 (11.0)	917 (89.6)		
NPI stage					577.599	< 0.001

Data are n (%); percentages are column percentages. MPP, *Mycoplasma pneumoniae* pneumonia; NPI, non-pharmaceutical intervention. Detection groups are mutually exclusive.

Variable	*M. pneumoniae* alone (n = 2653)	+ bacteria (n = 2062)	+ viruses (n = 2589)	+ bacteria and viruses (n = 1024)	χ^2	*P*
Implementation stage	447 (16.8)	11 (0.5)	152 (5.9)	2 (0.2)		
Post-adjustment stage	2206 (83.2)	2051 (99.5)	2437 (94.1)	1022 (99.8)		

Data are n (%); percentages are column percentages. MPP, *Mycoplasma pneumoniae* pneumonia; NPI, non-pharmaceutical intervention. Detection groups are mutually exclusive.

Unadjusted clinical outcomes

In unadjusted comparisons, hospital length of stay and several pulmonary imaging outcomes differed across detection patterns. Median length of stay was 7 days in the bacterial and bacterial-viral co-detection groups and 6 days in the *M. pneumoniae*-alone and viral co-detection groups (H = 118.806, $P < 0.001$). Pulmonary consolidation occurred in 9.2%, 6.8%, 15.4%, and 4.9% of the four groups, respectively (χ^2=137.938, $P < 0.001$), with the highest proportion in the viral co-detection group. Pleural effusion occurred in 1.2%, 1.9%, 1.3%, and 0.4%, respectively (χ^2=13.023, $P = 0.005$). Atelectasis was uncommon overall but differed across groups in exact testing ($P = 0.014$) (Table 4).

Table 4
Clinical outcomes of children with MPP by pathogen detection pattern

Clinical outcome	*M. pneumoniae* alone (n = 2653)	+ bacteria (n = 2062)	+ viruses (n = 2589)	+ bacteria and viruses (n = 1024)	Statistic	*P*
Length of stay, d, median (P25, P75)	6 (5, 8)	7 (6, 8)	6 (5, 7)	7 (6, 8)	H = 118.806	< 0.001
Pulmonary consolidation, n (%)	243 (9.2)	141 (6.8)	398 (15.4)	50 (4.9)	χ^2=137.938	< 0.001
Pleural effusion, n (%)	33 (1.2)	40 (1.9)	33 (1.3)	4 (0.4)	χ^2=13.023	0.005
Atelectasis, n (%)	6 (0.2)	4 (0.2)	15 (0.6)	0 (0.0)	Exact test	0.014

Length of stay is presented as median (P25, P75) and was compared using the Kruskal–Wallis H test. Percentages for imaging outcomes are calculated within each detection pattern. Pulmonary consolidation and pleural effusion were compared using Pearson χ^2 tests; atelectasis was assessed using the Fisher–Freeman–Halton exact test. MPP, *Mycoplasma pneumoniae* pneumonia.

Adjusted associations with clinical outcomes

After adjustment for center, sex, age group, season, and NPI stage, bacterial co-detection and bacterial-viral co-detection were associated with longer hospital stay than *M. pneumoniae* alone, with aMRs of 1.071 (95% CI 1.047–1.094; $P < 0.001$) and 1.064 (95% CI 1.037–1.092; $P < 0.001$), respectively. Viral co-detection was not associated with hospital length of stay (aMR 0.986, 95% CI 0.966–1.006; $P = 0.173$). In the primary model, bacterial co-detection was associated with pulmonary consolidation (aOR 1.325, 95% CI 1.007–1.742; $P = 0.044$). None of the detection patterns was significantly associated with pleural effusion in the primary model (Table 5).

Table 5
Adjusted associations between pathogen detection patterns and clinical outcomes in MPP

Outcome	**Detection pattern**	**Adjusted estimate (95% CI)**	***P***
Length of stay	*M. pneumoniae* alone	1.000 (reference)	—
	+ bacteria	aMR 1.071 (1.047–1.094)	< 0.001
	+ viruses	aMR 0.986 (0.966–1.006)	0.173
	+ bacteria and viruses	aMR 1.064 (1.037–1.092)	< 0.001
Pulmonary consolidation	*M. pneumoniae* alone	1.000 (reference)	—
	+ bacteria	aOR 1.325 (1.007–1.742)	0.044
	+ viruses	aOR 0.929 (0.772–1.118)	0.434
	+ bacteria and viruses	aOR 0.818 (0.582–1.149)	0.247
Pleural effusion	*M. pneumoniae* alone	1.000 (reference)	—
	+ bacteria	aOR 1.675 (0.968–2.897)	0.065
	+ viruses	aOR 1.333 (0.743–2.391)	0.335
	+ bacteria and viruses	aOR 0.371 (0.127–1.088)	0.071

M. pneumoniae alone is the reference category. Models were adjusted for center, sex, age group, season, and NPI stage. Length of stay was modeled using a Gamma generalized linear model with a log link and is expressed as an adjusted mean ratio (aMR); pulmonary consolidation and pleural effusion were modeled using multivariable logistic regression and are expressed as adjusted odds ratios (aORs). CI, confidence interval; NPI, non-pharmaceutical intervention; MPP, *Mycoplasma pneumoniae* pneumonia.

When calendar year replaced NPI stage, the associations of bacterial and bacterial-viral co-detection with longer hospital stay remained significant (both $P < 0.001$), whereas the association between bacterial co-detection and pulmonary consolidation was no longer significant ($P = 0.314$). Restriction to the post-NPI-adjustment stage yielded similar findings for hospital length of stay, while none of the detection patterns was significantly associated with pulmonary consolidation. Detailed sensitivity estimates are presented in Supplementary Table S1.

Because detection patterns were strongly imbalanced by center, we evaluated interaction between center and detection pattern. The overall interaction was significant in both the NPI-stage-adjusted model (Wald χ^2=9.526, df = 3, P for interaction = 0.023) and the year-adjusted model (Wald χ^2=11.051, df = 3, P for interaction = 0.011). In center-stratified analyses, viral co-detection was associated with a slightly shorter stay, whereas bacterial-viral co-detection was associated with a longer stay at Center A. At Center B, bacterial co-detection was consistently associated with a longer stay, while the association for bacterial-viral co-detection was sensitive to time adjustment. The complete center-stratified estimates are provided in Supplementary Table S2.

Discussion

In this two-center retrospective study, overall *M. pneumoniae* positivity among hospitalized CAP admissions was 29.7% from 2022 through 2025. Positivity varied substantially over time, increasing from 19.7% in 2022 to approximately 38% in 2023–2024 before declining to 12.0% in 2025. *M. pneumoniae* positivity was also higher among girls, school-aged children, and in autumn. Among children with MPP, 68.1% had at least one additional bacterial or viral pathogen detected. In the primary adjusted analysis, bacterial co-detection and bacterial-viral co-detection were associated with modestly longer hospital stays than *M. pneumoniae* alone. However, center-by-pattern interactions were significant, and associations with pulmonary imaging outcomes were not stable across alternative time-adjustment strategies. These findings support interpretation of the overall estimates as average associations rather than uniform effects across centers.

The overall *M. pneumoniae* positivity in the present CAP cohort was close to the 26.4% reported in a pediatric study from Suzhou, suggesting that a substantial burden of *M. pneumoniae* infection has been observed across parts of eastern China [19]. The marked rise after the NPI implementation period is also consistent with reports from Suzhou and Shanghai showing increased *M. pneumoniae* activity in 2023 [9, 10]. Together with international observations of delayed re-emergence after pandemic restrictions [7, 8], our temporal findings are compatible with a post-pandemic resurgence. Nevertheless, the comparison of the NPI implementation and post-NPI-adjustment stages is inherently time-dependent. The higher positivity observed after policy adjustment should therefore not be interpreted as evidence that the policy change itself caused the resurgence.

Age and season showed clear associations with *M. pneumoniae* positivity. Positivity increased across age groups and was highest in school-aged children, consistent with previous epidemiological studies in China and the United States [19–21]. Autumn had the highest positivity in our cohort, whereas spring had the lowest. Multicenter studies from Beijing and South Korea have also reported prominent autumn or autumn-winter activity [22, 23], while surveillance from Suzhou suggested a shift from a summer peak before the pandemic to an autumn peak after the pandemic [9]. These differences indicate that *M. pneumoniae* seasonality may vary with geography, epidemic cycle, population mixing, and contemporaneous circulation of other respiratory pathogens. The distinct age and seasonal composition

of the two participating centers further reinforces the need to account for center-level case mix in multicenter analyses.

Pathogen co-detection was frequent in the MPP cohort. Viral co-detection was the largest co-detection category, and the overall proportion with any additional pathogen detected was 68.1%. Previous pediatric studies have likewise reported frequent detection of other respiratory pathogens in MPP [13, 24]. Importantly, the distribution of detection patterns was highly center-dependent: 97.0% of bacterial co-detections occurred in Center B, whereas 89.0% of viral co-detections occurred in Center A. This imbalance likely reflects, at least in part, differences in patient populations, laboratory platforms, testing indications, and pathogen panels. For that reason, we used the term co-detection rather than assuming true mixed causative infection, adjusted for center a priori, and examined interaction and center-stratified estimates. This distinction is clinically relevant because detection of a respiratory organism may reflect current infection, colonization, prolonged shedding, or evidence of a recent infection rather than simultaneous causation [25].

The most consistent outcome association involved hospital length of stay. After covariate adjustment, bacterial co-detection and bacterial-viral co-detection were associated with a 5%–7% longer mean hospital stay relative to *M. pneumoniae* alone, and the direction of these associations was generally preserved when calendar year replaced NPI stage and when analyses were restricted to the post-NPI-adjustment period. These findings are broadly compatible with reports that selected co-detection patterns may be associated with greater clinical burden [15, 26]. However, the effect sizes were modest, and significant center-by-pattern interaction indicated heterogeneity. At Center A, bacterial-viral co-detection showed the more consistent association with longer stay, whereas at Center B bacterial co-detection was more consistently associated with longer stay. Hospital length of stay is influenced not only by disease severity but also by admission thresholds, discharge criteria, local practice patterns, and resource use. Accordingly, the aMRs observed here should be interpreted as adjusted associations in this study population rather than direct measures of pathogen-specific biological severity.

Associations with pulmonary imaging outcomes were less robust. The bacterial co-detection group had higher adjusted odds of pulmonary consolidation only in the primary model; the association was attenuated and no longer significant when calendar year replaced NPI stage or when the analysis was restricted to the post-NPI-adjustment period. Previous studies indicate that imaging findings in MPP are influenced by age, inflammatory response, and overall disease severity, and reports regarding the effect of co-detected pathogens on radiologic abnormalities have been inconsistent [26–30]. Pleural effusion and atelectasis were also uncommon in the present cohort, limiting statistical precision. The apparent lower odds of pleural effusion for bacterial-viral co-detection in sensitivity models should therefore be interpreted cautiously, particularly given the small number of events and the strong center imbalance in detection patterns.

This study has several limitations. First, it was retrospective, and pathogen testing was performed as part of routine clinical care. Testing indications and coverage may therefore have varied over time and

between centers, which could have introduced ascertainment bias into comparisons of *M. pneumoniae* positivity. In addition, MPP classification could be supported by a single positive *M. pneumoniae*-specific IgM result together with compatible clinical and radiographic findings; because IgM may persist after recent infection, some diagnostic misclassification cannot be excluded. Second, the two centers did not use identical non-*M. pneumoniae* pathogen-testing platforms or panels. The marked center imbalance in several detection patterns creates potential detection bias and residual confounding even after center adjustment, interaction testing, and stratified analyses. Third, hospital length of stay may be affected by center-specific admission and discharge practices. Fourth, co-detection does not establish concurrent causation; some detected pathogens may represent colonization, persistent carriage, or recent infection. Fifth, some detection-pattern categories were uncommon during the NPI implementation stage, and pleural effusion and atelectasis were rare, reducing the stability of several effect estimates. Finally, both centers were located in the same city, which may limit generalizability. Prospective multicenter studies using standardized pathogen panels and harmonized testing strategies are needed to confirm whether specific co-detection patterns provide reproducible prognostic information across settings.

Conclusions

Pediatric *M. pneumoniae* infection showed marked annual, seasonal, and demographic variation from 2022 through 2025. In overall adjusted models, bacterial co-detection and bacterial-viral co-detection were associated with modestly longer hospitalization among children with MPP, but center-stratified results indicated heterogeneity. Associations with pulmonary imaging outcomes were less consistent. These findings support cautious interpretation of pathogen co-detection and emphasize the importance of center-level testing differences in multicenter MPP research.

Abbreviations

aMR
adjusted mean ratio
aOR
adjusted odds ratio
CAP
community-acquired pneumonia
CI
confidence interval
COVID-19
coronavirus disease 2019
IgM
immunoglobulin M
MP
Mycoplasma pneumoniae
MPP

Mycoplasma pneumoniae pneumonia
NPI
non-pharmaceutical intervention
PCR
polymerase chain reaction
SPSS
Statistical Package for the Social Sciences.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Nantong Maternity and Child Health Hospital (also known as Nantong University Affiliated Maternal and Child Health Hospital; approval No. Y2026019) and the Ethics Committee of the Second Affiliated Hospital of Nantong University (also known as Nantong First People's Hospital; approval No. 2026-KT272-01). The study was conducted in accordance with the Declaration of Helsinki. Owing to the retrospective design of the study and the use of de-identified medical records, the requirement for informed consent from the participants and/or their legal guardians was waived by both ethics committees.

Consent for publication

Not applicable. No individual-level identifiable data are presented in this manuscript.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available because they contain potentially sensitive clinical information. De-identified data may be available from the corresponding author on reasonable request, subject to applicable institutional and ethical requirements.

Competing interests

The authors declare that they have no competing interests.

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Clinical trial number

Clinical trial number: not applicable.

Authors' contributions

JL contributed to study design, data curation, statistical analysis, preparation of figures and tables, and drafting of the manuscript. XZ contributed to study design, quality control, interpretation of the results, study supervision, and critical revision of the manuscript. JZ contributed to data validation, review of imaging outcomes, interpretation of the results, manuscript revision, and funding acquisition. CX contributed to data acquisition, data verification, and manuscript revision. All authors read and approved the final manuscript.

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Figures

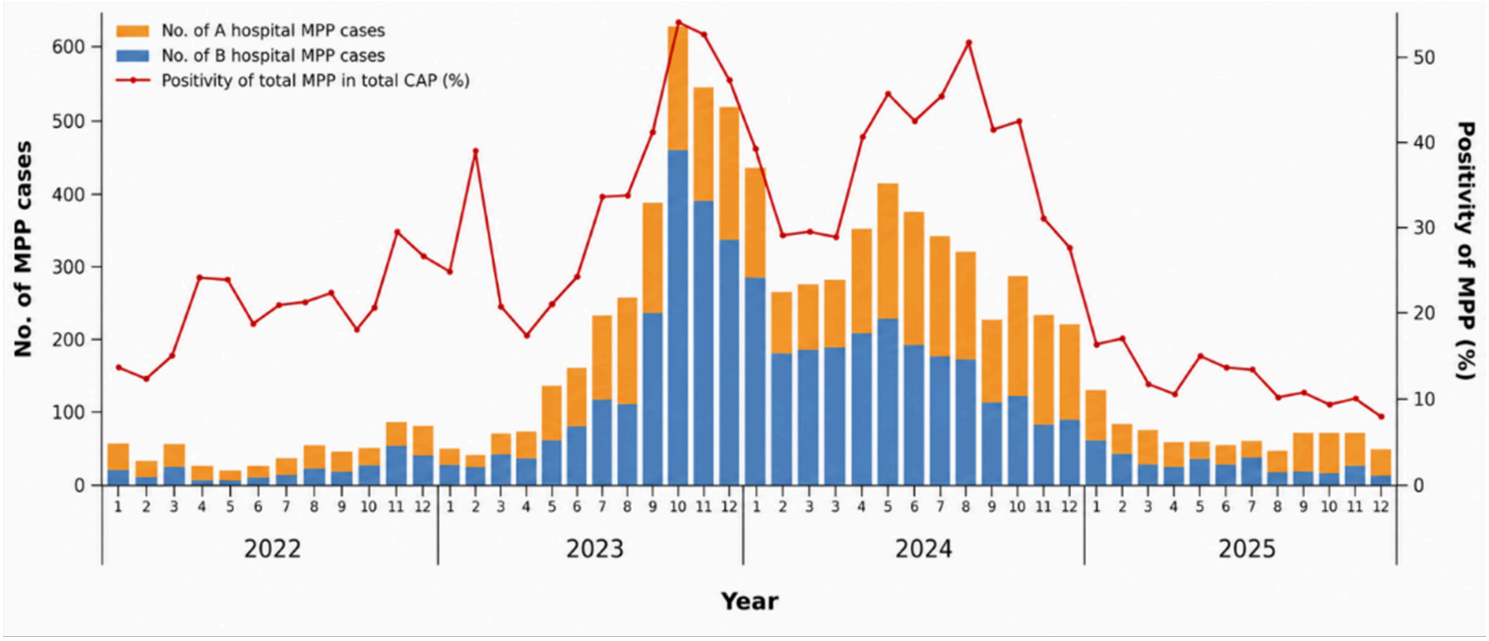


Figure 1

Monthly MPP case counts and MP positivity at two centers, 2022–2025. Stacked bars show monthly numbers of MPP cases from Center A and Center B; the line shows the proportion of MPP among all hospitalized CAP cases. MPP, *Mycoplasma pneumoniae* pneumonia; MP, *Mycoplasma pneumoniae*; CAP, community-acquired pneumonia.

Supplementary Files

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- SupplementaryTables.docx