International Journal of Contemporary Pediatrics
Joy Kallely SM et al. Int J Contemp Pediatr. 2026 Jun;13(6):885-889
https://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20261529

Original Research Article

Revisiting the usefulness of cold agglutinin test and direct Coombs test for bedside diagnosis of paediatric *Mycoplasma pneumoniae* pneumonia: a prospective observational study

Sneha Maria Joy Kallely*, Carol Sara Cherian, Jacob Abraham

Department of Pediatrics, Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India

Received: 21 February 2026
Revised: 18 March 2026
Accepted: 07 May 2026

***Correspondence:**
Dr. Sneha Maria Joy Kallely,
E-mail: drsnehamariajoykallely@gmail.com

ABSTRACT

Background: *Mycoplasma pneumoniae* is an important cause of community-acquired pneumonia in children. Access to specific serological assays is limited in many resource-constrained settings, necessitating evaluation of simple bedside diagnostic tests such as cold agglutinin test (CAT).
Methods: In this prospective observational study, children admitted with radiologically confirmed lobar pneumonia that showed no response to beta-lactams were evaluated with direct Coombs test (DCT) and CAT, and results were compared with *Mycoplasma pneumoniae* IgM serology as the reference standard. Clinical profile, inflammatory markers, treatment response and outcomes including need for paediatric intensive care and length of hospital stay were also recorded.
Results: Among IgM-positive children, normal total leucocyte counts were significantly more frequent than in IgM-negative cases, while erythrocyte sedimentation rate was commonly elevated, supporting an atypical pneumonia pattern. DCT showed low sensitivity and poor negative predictive value, whereas CAT demonstrated very high specificity and positive predictive value, making it a useful rule-in test but unsuitable as a stand-alone screening tool. CAT positivity was associated with a significantly longer duration of hospital stay, indicating more severe or protracted clinical course among children with demonstrable cold agglutinins.
Conclusions: *Mycoplasma pneumoniae* pneumonia in this cohort was characterized by normal total leucocyte counts with raised erythrocyte sedimentation rate, and CAT emerged as a highly specific rule-in test, though it could not replace confirmatory serology testing.

Keywords: *Mycoplasma pneumoniae*, Bedside cold agglutinin test, Direct coombs test, Paediatric pneumonia, Atypical pneumonia, Hospital stay

INTRODUCTION

Mycoplasma pneumoniae is a common cause of community-acquired pneumonia in school-aged children and adolescents, often presenting with an indolent course and extrapulmonary manifestations in nearly 50% cases. In many low- and middle-income settings, serological or molecular assays for definitive diagnosis remain costly, batched, or unavailable, leading to empirical therapy without microbiological confirmation. Simple bedside tests such as CAT have historically been used as surrogate marker of infection, but contemporary paediatric data, particularly from Indian settings, are limited.[1-4,6]

Accurate characterization of their diagnostic performance against a reference serological standard is essential to determine whether these tests can guide rational use of macrolides and other antimicrobials in routine practice.[3,4,6-8,11]

Bedside CAT was performed by combining blood with an equivalent amount of an anticoagulant in a tube. The tube was submerged in ice water (0 to 4°C) for 15-30 seconds. Tube is then retrieved from the ice and inspected for large clumps known as coarse agglutination, by observing the tube against a light source and gently rotating it. Upon heating/rewarming, the clumps should disperse and reappear upon cooling.[14]

The objective of the study was to evaluate the diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) of DCT and bedside CAT for detection of IgM-confirmed *Mycoplasma pneumoniae* infection in hospitalised children with radiologically confirmed lobar pneumonia. It also aimed to describe the clinical and laboratory profile (total leucocyte count, erythrocyte sedimentation rate, C-reactive protein, transaminitis) of children with IgM-confirmed *Mycoplasma pneumoniae* pneumonia and to assess the association between CAT positivity and hospital stay.

METHODS

Study design and setting

This was a prospective observational study conducted in a tertiary-care paediatric centre (Pushpagiri Institute of Medical Sciences and Research Center, Thiruvalla) during a period of 4 months (December, 2024 to March, 2025). Infants and children between 1 month to 15 years of age who were admitted with radiologically confirmed lobar pneumonia with no response to beta-lactams were enrolled consecutively. Children were managed either in the paediatric ward or paediatric intensive care unit (PICU) at the discretion of the treating team.

Participants

Children with clinical features suggestive of lower respiratory tract infection with chest radiograph evidence of lobar pneumonia and not responding to beta-lactams were eligible for inclusion. Those with incomplete diagnostic work-up for *Mycoplasma* pneumonia, those who had chronic lung disease and significant immunosuppression and those who did not consent for study were excluded. All included children underwent testing for *Mycoplasma pneumoniae* IgM antibodies, DCT and CAT at admission, along with routine haematological and biochemical investigations.

Index tests and reference standard

The index tests evaluated were DCT and bedside CAT performed using standard procedures, and results were categorised as positive or negative as per predefined criteria.[1-4] Bedside CAT was performed by the principal investigator. *Mycoplasma pneumoniae* IgM serology served as the reference standard for defining infection status, with IgM-positive cases considered true positives and IgM-negative cases true negatives for diagnostic accuracy calculations. IgM *Mycoplasma* equivocal results were excluded because of uncertain classification near the assay cut-off. Principal investigator was unaware of the final serology results at the time of performing CAT, and serology was processed independent of bedside test results.

Data collection and variables

For each participant, demographic details (age, gender), DCT, CAT and *Mycoplasma* IgM results, total leucocyte count (TC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), site of care (PICU or ward), complications, transaminitis, radiographic findings, duration of hospital stay and treatment response were recorded in a structured proforma and entered into an electronic spreadsheet. Laboratory variables were categorized (e.g. TC as normal or high, ESR as normal or raised) according to institutional age-adjusted reference ranges.

Statistical analysis

Diagnostic accuracy indices of DCT and CAT (sensitivity, specificity, positive predictive value, negative predictive value) were calculated with 95% confidence intervals, using IgM serology as the reference standard. Categorical variables were compared between IgM-positive and IgM-negative groups using Chi-square or Fisher's exact test, while continuous variables such as duration of hospital stay were compared using independent-samples t tests, and p value less than 0.05 was considered statistically significant.

RESULTS

Study population

A total of 24 children with radiologically confirmed lobar pneumonia and complete *Mycoplasma pneumoniae* work-up were included in the analysis. The cohort comprised of 42% boys and 58% girls with a mean age of 7.6 years. Seventeen children were IgM-positive for *Mycoplasma pneumoniae* which formed the infected group. Also, there were 2 cases with equivocal titer of IgM *Mycoplasma*. PICU admission was required for 63% of children and 45% of them had associated complications like synpneumonic effusion and pulmonary thromboembolism.

Clinical, laboratory and radiographic profile

Among IgM-positive children, all had normal TLC, whereas IgM-negative children showed a mix of normal counts (40%) and leukocytosis (60%); this difference was statistically significant (Pearson χ^2=11.811, p=0.001; Fisher's exact test p=0.006), indicating a strong association between *Mycoplasma pneumoniae* infection and normal leukocyte profile. Raised ESR was observed

in 88.2% of IgM-positive cases, while 60% IgM-negative children had elevated ESR, and this difference did not reach statistical significance. CRP levels were frequently high in both groups, and transaminitis was documented in 29% of IgM-positive patients, often in association with more severe radiological or clinical disease. Among the *Mycoplasma* positive cases, 7 (41%) children had complications like synpneumonic effusion and 1 child had pleural effusion with pulmonary thromboembolism. Of all the cases, 48% showed left lower lobe involvement, followed by right upper lobe involvement (20%). Among the infected cases, 37% showed a left lower lobe predilection, followed by right upper lobe (25%).

Diagnostic performance of DCT and CAT

DCT demonstrated low sensitivity for detecting IgM-positive *Mycoplasma pneumoniae* infection, with a substantial proportion of infected children testing negative, and it also showed limited negative predictive value, limiting its utility as a rule-out test. In contrast, CAT exhibited very high specificity and positive predictive value, both reaching 100%, such that a positive CAT result strongly indicated true *Mycoplasma pneumoniae* infection. However, CAT sensitivity was only 58.8%, indicating that almost half of the serologically confirmed cases would be missed if CAT were used as a stand-alone screening tool. Thus, a positive CAT result strongly supports *Mycoplasma pneumoniae* infection and can be used to rule in *Mycoplasma* infection with high confidence.

Treatment, outcomes and hospital stay

Among the *Mycoplasma* positive cases, 29% were macrolide resistant *Mycoplasma* pnaemoniae (MRMP), requiring escalation to doxycycline and 23% were refractory *Mycoplasma pnaeumonie* (RMPP) requiring steroid therapy.

Among the CAT-positive children, 45% had complications such as synpneumonic effusion or pleural effusion, and 63% required PICU admission. Also, CAT-positive children had a significantly longer mean duration of hospital stay than CAT-negative children (approximately 11.14±3.34 days vs. 7.46±2.44 days), with a mean difference of about 3.68 days and a statistically significant $p<0.05$. This finding suggests that the presence of cold agglutinins is associated with more severe or prolonged clinical course in paediatric *Mycoplasma pneumoniae* pneumonia.

Table 1: Blood counts in study group.

Variables	Total leucocyte counts		Pearson chi-square (p value)	Fisher's exact test (p value)
	Normal	Leucocytosis		
IgM positive (n=17)	17 (100%)	0	11.811 (0.001)	0.006
IgM negative (n=5)	2 (40%)	3 (60%)		

Table 2: Diagnostic accuracy of CAT for IgM *Mycoplasma pneumoniae* infection.

CAT result	IgM positive	IgM negative	Total
Positive	10	0	10
Negative	7	5	12
Total	17	5	22

Table 3: Diagnostic accuracy of DCT for IgM *Mycoplasma pneumoniae* infection.

DCT result	IgM positive	IgM negative	Total
Positive	7	1	8
Negative	10	4	14
Total	17	5	22

Table 4: Comparative diagnostic performance of CAT and DCT for IgM *Mycoplasma pneumoniae* infection.

Tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CAT	58.8%	100.0	100.0	41.7
DCT	41.2	80.0	87.5	28.6

Table 5: Association between CAT result and duration of hospital stay in children with pneumonia.

Variables	CAT positive, (n=10)	CAT negative, (n=12)	P value (t test)
Hospital stays (days) mean±SD	11.14±3.34	7.46±2.44	0.011

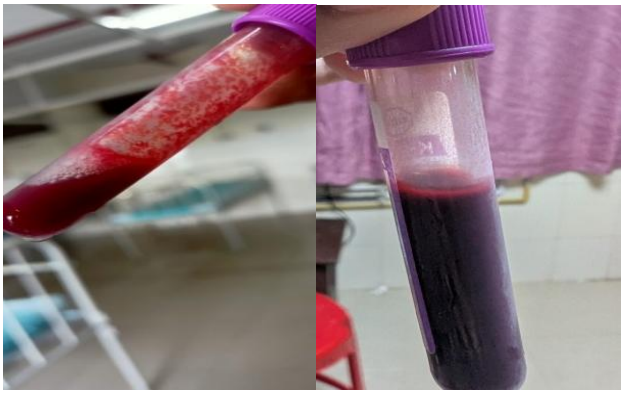

Figure 1: Positive CAT.

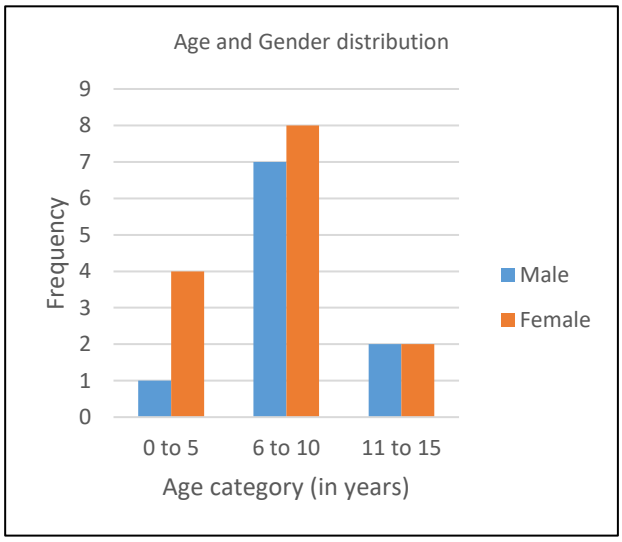

Figure 2: Demographic distribution of population.

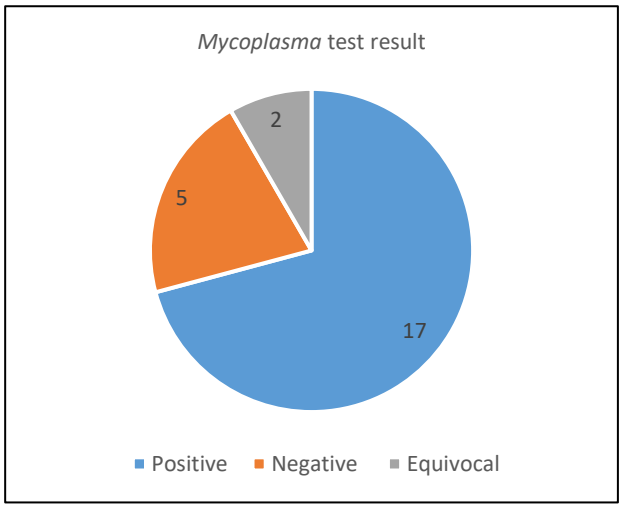

Figure 3: *Mycoplasma* test result.

DISCUSSION

This study provides contemporary paediatric data on the usefulness of two simple tests, DCT and CAT, for detecting *Mycoplasma pneumoniae* infection while using IgM serology as the reference standard. The predominance of normal TLC with elevated ESR among IgM-positive children is consistent with the recognized atypical pneumonia phenotype, and mirrors the findings of Cherian et al who reported that *Mycoplasma pneumoniae* accounted for 22.44% of pediatric respiratory infections and that normal leukocyte count with high ESR (>30 mm/hr) were significant features of *Mycoplasma pneumoniae*. These haematological features may help clinicians suspect *Mycoplasma pneumoniae* in appropriate clinical settings, particularly when access to serology or molecular assay is limited.[1-4,6,16]

CAT's very high specificity and positive predictive value support its role as a robust rule-in test, where a positive result can be used to confidently initiate or continue macrolide-based therapy for presumed *Mycoplasma pneumoniae* pneumonia. However, its modest sensitivity, coupled with the low sensitivity of DCT, indicates that neither test alone is adequate as a screening or rule-out tool.[1-4,6,13]

Cold antibody production is a well-recognised consequence of *Mycoplasma pneumoniae* infection, most commonly involving anti-I antibodies. These antibodies are detectable in approximately 50-60% of affected patients and may also occur in other conditions such as cytomegalovirus infection, *Klebsiella* infection, Epstein-Barr virus infection, lymphoid malignancies and various autoimmune diseases.[15]

The association between CAT positivity and longer hospital stay highlights a possible link between the immunohaematological response to *Mycoplasma pneumoniae* and disease severity or recovery time. While causality cannot be inferred, CAT positivity may identify a subgroup of children at higher risk of prolonged illness or complications, warranting closer monitoring and follow-up. Strengths of this study include the use of a serological reference standard and detailed clinical and laboratory characterization.

Limitations of this study include the relatively small sample size, single-centre design and the approximated nature of combined sensitivity estimates. Future research should focus on larger multicentric cohorts, integration of polymerase chain reaction assays where available, to refine the role of DCT and CAT in paediatric *Mycoplasma pneumoniae* pneumonia.[6-8,12]

CONCLUSION

Mycoplasma pneumoniae pneumonia in this cohort was marked by normal total leucocyte counts with frequently elevated erythrocyte sedimentation rate, consistent with an atypical inflammatory pattern. The bedside CAT showed excellent specificity and positive predictive value, supporting its role as a rule-in test, whereas the DCT test demonstrated poor standalone screening performance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee IEC Clearance No: PIMSRC/E1/388A/385/2026

REFERENCES

1. Cheng JH, Huang YC, Lin TY, Chang LY, Wong KS, Huang LM. A rapid cold agglutinin test in *Mycoplasma pneumoniae* infection in children. Acta Paediatr Scand. 1990;79(8-9):958-62.
2. Kim CH, Shin SM, Lee KY, Jung MH, Bahn JW. Diagnostic significance of cold agglutinin and anti-*Mycoplasma* antibody titers in pediatric pneumonia. Yeungnam Univ J Med. 1989;6(2):319-27.
3. Ozkaya S, Findik S, Atici AG, Yildiz F. Cold agglutination test and *Mycoplasma pneumoniae*: a retrospective study. Turk Thorac J. 2019;20(4):275-80.
4. Lin LJ, Chiu NC, Lee KS, Chiu YY, Chi H, Huang FY, et al. Diagnostic value of serological studies for *Mycoplasma pneumoniae* infection in pediatric community-acquired pneumonia. Asian Pac J Allergy Immunol. 2018;36(2):79-86.
5. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev. 2004;17(4):697-728.
6. Kumar S, Saigal SR, Sethi GR. *Mycoplasma pneumoniae*: a significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. Indian J Med Res. 2018;147(6):667-76.
7. Zhao F, Liu G, Wu J, Cao B, Tao X, He L, et al. Surveillance of macrolide-resistant *Mycoplasma pneumoniae* in Beijing, China, from 2008 to 2012. Antimicrob Agents Chemother. 2013;57(3):1521-3.
8. Chavan C, Patankar R, Gadgil A, Shankar D, Janani N, Prabu D. Detection of macrolide-resistant *Mycoplasma pneumoniae* in children with lower respiratory tract infection. J Pure Appl Microbiol. 2023;17(1):338-44.
9. Jindal SK. Community-acquired pneumonia in children: epidemiology and etiology. Indian J Pediatr. 2010;77(4):441-4.
10. Gaillard EA, Kantar A, Launes C. Community-acquired pneumonia in children-clinical features and diagnosis. Paediatr Respir Rev. 2016;20:60-7.
11. Bradley JS, Byington CL, Shah SS. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis. 2011;53(7):e25-76.
12. Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The clinical characteristics and predictors of refractory *Mycoplasma pneumoniae* pneumonia in children. PLoS One. 2016;11(5):e0156465.
13. Narita M. Pathogenesis of extrapulmonary manifestations of *Mycoplasma pneumoniae* infection with special reference to pneumonia. J Infect Chemother. 2010;16(3):162-9.
14. Salaria M, Singh M. Reply. Indian Pediatr. 2002;39:1061-1062. Available from: https://www.indianpediatrics.net/nov2002/nov-1061-1062.htm. Accessed on 06 February 2026.
15. Surendar K, Malay S. *Mycoplasma pneumonia*: Clinical features and Management. Lung India. 2010;27(2):75-85.
16. Cherian CS, Nair TS, Sushama Bai S, Oommen S. Prevalence and clinical profile of *Mycoplasma pneumonia* respiratory infection in children: a hospital-based study. Int J Contemporary Pediat. 2016;3:612-5.

Cite this article as: Joy Kallely SM, Cherian CS, Abraham J. Revisiting the usefulness of cold agglutinin test and direct Coombs test for bedside diagnosis of paediatric Mycoplasma pneumoniae pneumonia: a prospective observational study. Int J Contemp Pediatr 2026;13:885-9.