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Serological and molecular detection of *Mycoplasma pneumoniae* in children with community-acquired lower respiratory tract infections☆

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ABSTRACT

This study was designed to evaluate the incidence of *Mycoplasma pneumoniae* infection in children with community-acquired lower respiratory tract infections (LRTIs). A total of 245 patients 6 months to 12 years of age were investigated for *M. pneumoniae* employing serological tests, polymerase chain reaction (PCR), nested PCR, and reverse transcription PCR (RT-PCR) on throat swab samples. Forty five (59.2%) children <5 years and 31 (40.7%) children ≥5 years age group were positive for *M. pneumoniae* infection, and this difference was statistically significant ($P \leq 0.01$).Clinical and radiological findings across *M. pneumoniae*–positive and –negative cases were comparable. Serology, PCR, nested PCR, and RT-PCR together detected *M. pneumoniae* infection in 76 (31%) patients. Sensitivity, specificity, and positive and negative predictive values of PCR were 16.18%, 95.48%, 57.89%, and 74.78%, respectively, and those of serology were 57.89%, 74.78%, 16.18%, and 95.48%, respectively. Serological and molecular detection in combination is useful for rapid and reliable diagnosis of *M. pneumoniae* infections in children with LRTIs.

1. Introduction

Lower respiratory tract infections (LRTIs) are considered a common cause for morbidity and mortality among children worldwide. The causative organisms of LRTIs are believed to be mainly respiratory viruses, *Streptococcus pneumoniae* (*S. pneumoniae*) and *Haemophilus influenzae* in young children and *S. pneumoniae* and *Mycoplasma pneumoniae* (*M. pneumoniae*) in older children (Kumar et al., 2011a, 2016). *M. pneumoniae* is widely recognized as a cause of respiratory tract infections in school-aged children and young adults (Pereyre et al., 2016). It infects both the upper and lower respiratory tracts, leading to tracheobronchitis, bronchitis, bronchiolitis, and community-acquired pneumonia (CAP) infections in 40% of cases (Kogoj et al., 2015; Waites and Talkington, 2004). *M. pneumoniae* can also cause extrapulmonary manifestations that affect almost every organ system (Ferwerda et al., 2001). Increase in CAP attributable to *M. pneumoniae* may occur many times during epidemics which occur at an interval of 4–7 years because of waning of herd immunity and introduction of new subtypes into the population (Kumar et al., 2018).

Correct and rapid detection of *M. pneumoniae* infection is critical to initiate appropriate therapy. However, confirmation of *M. pneumoniae* infection is clinically challenging since it is impossible to diagnose this disease merely based on clinical signs and symptoms; therefore, an optimal diagnostic tool is extremely important to allow for more precise diagnosis and appropriate treatment of patients with *M. pneumoniae* infection and reduce the misuse of antibiotics.

Mycoplasmas are not sensitive to β-lactam antibiotics, which are often used for empirical treatment of LRTIs (Dorigo-Zetsma et al., 1999); macrolides have long been regarded as the standard therapy for LRTIs caused by *M. pneumoniae.* During the last 10 years, macrolide resistance in *M. pneumoniae* has been reported worldwide, especially in Asian countries (Zhao et al., 2012). Culture of this pathogen is difficult to perform and time consuming. Serological methods, in particular enzyme-linked immunosorbent assays (ELISAs), are most widely used to diagnose *M. pneumoniae* infection (Meyer Sauteur et al., 2016). The golden diagnostic criteria of *M. pneumoniae* infection have been considered as the seroconversion or rising IgG titers, but the antibody results are susceptible to the children's age and immunity status especially facing to the difficulty in obtaining convalescent serum in a pediatric hospital (Wang et al., 2017). Nucleic acid amplification techniques (NAATs) have been increasingly recognized and implemented as the preferred method for identification of respiratory bacteria and viruses, including *M. pneumoniae*, in clinical specimens as a result of the high level of sensitivity and specificity and rapid turnaround time afforded by these methods (Diaz and Winchell, 2016). PCR is accepted as a rapid diagnostic test. PCR

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amplification from respiratory secretions, such as nasopharyngeal, oropharyngeal, or sputum samples, can provide more sensitive detection. PCR tests have been designed around the 16S rDNA, P1 adhesion protein, and the ATPase operon genes of *M. pneumoniae*. Sensitivity can be further increased by nested PCR, which involves reamplification of a PCR product with a different set of primers for the same target (Parrot et al., 2016). Since NAATs targeting DNA can detect both viable and nonviable organisms, detecting RNA by reverse transcriptase PCR (RT-PCR) or nucleic acid sequence–based amplification may be a useful method to identify productive *M. pneumoniae* infections.

The aim of this study was to analyze the role of *M. pneumoniae* in community-acquired LRTIs in children with use of serology, PCR, nested PCR, and RT-PCR.

2. Materials and methods

2.1. Study design

This was a prospective, single-center study designed to evaluate the incidence of *M. pneumoniae* infection in hospitalized children with community-acquired LRTIs. The study protocol was approved by the Ethical Committee of Maulana Azad Medical College and associated hospitals in New Delhi, India, and the work undertaken conformed to the provisions of the Declaration of Helsinky in 1995 (as revised in Edinburgh 2000).

Sample size was calculated using the formula $Z^2pq/L^2 = 4pq/L^2$.

Z = statistic for a level of confidence = 1.96.
n = sample size.
p = Prevalence used was 35.5%.
$q = 1 - p$.
L = allowable error = 20% of prevalence.
$n = 4 \times 0.35 \times 0.65 / (0.2 \times 0.35)^2$ = no. of samples required for study = 185.
No. of samples taken for study = 245.

2.2. Selection of cases

Two hundred forty-five children aged 6 months to 12 years with LRTIs admitted to the pediatric ward of Lok Nayak Hospital, Maulana Azad Medical College, New Delhi, India, were investigated for *M. pneumoniae* infection. Lower respiratory tract infection is defined as child with fever or cough with fast breathing/chest indrawing (fast breathing is present when respiratory rate is ≥60 breaths/min in a child of <2 months; ≥50 breaths/min in a child aged 2-12 months; ≥40 breaths/min in a child aged 12-60 months).

2.2.1. Inclusion criteria

Presence of cough and fever with chest indrawing of <30 days' duration, respiratory rate increase (with or without features of respiratory distress) on examination, and presence of signs of consolidation or bronchopneumonia with or without wheeze on auscultation.

2.2.2. Exclusion criteria

Hospital-acquired pneumonia, i.e., pneumonia that developed 72 h after hospitalization or within 7 days of discharge.

2.3. Enrollment and evaluation of patients

Written informed consent was taken from the parents or legal guardian of all children before being enrolled for this study and collecting clinical specimens in conformation with standard bioethical norms. Detailed history and clinical examination were performed on admission, and chest X-rays were conducted in all children, the details of which were noted in a predesigned pro forma.

2.4. Collection and transport of specimens

The laboratory samples taken at enrollment included blood specimens for detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against *M. pneumoniae* and a second blood sample obtained after 4–6 weeks of enrollment to assay for convalescent *M. pneumonia* IgG antibody titers. Throat swab samples were collected within 24 h of admission prior to receipt of antibiotics for detection of *M. pneumoniae* DNA by PCR, nested PCR, and RT-PCR analysis.

2.4.1. Blood

For serological tests 1–2 mL of blood was collected in a sterile centrifugation tube by venipuncture following all the usual sterile precautions and allowed to clot. Following clot retraction, the serum was separated by centrifugation and collected in a well-labeled Eppendorf tube. The acute phase sera were collected on the day of enrollment of the patients in the study. Convalescent phase sera were obtained after 4–6 weeks of enrollment. All sera were preserved at −20 °C until detected for antibodies to *M. pneumoniae*.

2.4.2. Throat swab

A sterile cotton swab was rubbed with rotation over 1 tonsillar area, then the arch of soft palate and uvula, the other tonsillar area, and finally the posterior pharyngeal wall. The head of the throat swab was broken into a vial containing 2 mL of pleuropneumonia-like organisms (PPLO) broth used as transport medium and transported to the laboratory after proper labeling of the vial. In the laboratory, the swabs were twirled thoroughly, expressed against the sides of the tube, and discarded. The samples were stored at −70 °C until PCR analysis could be done.

2.5. Microbiological investigations

2.5.1. Serology

IgM and IgG antibodies against *M. pneumoniae* were performed by using commercially available ELISA-based kits (Calbiotech Inc., Austin Dr., Spring Valley, CA, USA). Tests were performed and results interpreted according to manufacturer's instructions. Antibody index of <0.9 or lower was recorded as negative, while a 4-fold or greater rise or standing antibody index of >1.1 or higher was regarded as positive for the presence of *M. pneumoniae* infection.

2.5.2. PCR for M. pneumoniae

2.5.2.1. DNA extraction. DNA was extracted using proteinase-K centrifugation method with minor modifications (Dorigo-Zetsma et al., 1999; Honda et al., 2000). PPLO broth (200 μL) inoculated with throat swab specimens was centrifuged at 12,000 revolutions per min (rpm) for 30 min. The pellets were suspended in 100 μL of proteinase-K buffer (50 mM KCl, 10 mM Tris-Cl, 2.5 mM $MgCl_2$, 0.5% Tween 20, 200 μg proteinase-K/ml, pH 8.3).The mixture was incubated for 2 h at 56 °C. Samples were boiled for 10 min and cooled, and the condensate representing partially purified DNA was used immediately or stored at −10 °C or lower for deployment in molecular analysis.

2.5.2.2. PCR amplification. A 277–base pair (bp) fragment of 16S rDNA gene of *M. pneumoniae* was selected for amplification with use of Mpn primer1 (sense) 5′-AAGGACCTGCAAGGGTTCGT-3′ and Mpn primer 2 (antisense) 5′-CTCTAGCCATTACCTGCTAA-3′ (Tjhie et al., 1994). Positive and negative controls were included in each run. Standard *M. pneumoniae* strain *Mycoplasma pneumoniae*-FH Lieu (ATCC 29085) was used as the positive control. The PCR was performed in a total volume of 25 μL containing 50 mM KCI, 10 mM Tris HCI (pH 8.3), 1.5 mM $MgCl_2$, 200 μM (each) deoxynucleoside triphosphates, 25 pmol of each primer, 2 U Taq DNA polymerase, and the 5 μL of sample DNA. Amplification was carried out for 40 cycles (PTC-0150 MiniCyclerTM, MJ Research, Inc., USA) consisting of initial denaturation for 4 min at 95 °C,

denaturation for 1 min at 95 °C, annealing for 1 min at 60 °C, extension for 1.5 min at 72 °C, and a final extension at 72 °C for 4 min. Amplified PCR products were electrophoresed on an ethidium bromide–stained agarose gel at 80 V for 1 h, along with a 100-bp DNA molecular weight marker. A band at 277 bp was taken to be positive PCR result.

2.5.3. Nested PCR for M. pneumoniae

In nested PCR, a 141-bp fragment of 16S rDNA was amplified employing *M. pneumoniae* inner primer 1 (antisense) 5′-CTCTAGCCATT ACCTGCTAA-3′ and inner primer 2 (GPO-1) 5′-ACTCCTACGGGAGG CAGCAGTA-3′ (Nour et al., 2005).

PCR product (4 μL) was transferred to 50-μL PCR reaction mixture for nested PCR employing 35 PCR cycles with denaturation for 1 min at 94 °C, annealing for 1 min at 56 °C, extension for 1 min at 72 °C, and a template delay step of 9 min at 72 °C. A band at 141 bp was taken to be positive nested PCR result.

2.5.4. RT-PCR

2.5.4.1. RNA extraction. RNA extraction was carried out by using the method described by Van Kuppeveld et al. (1992). Aliquots (200 μL) of PPLO broth containing throat swab specimens were centrifuged at 12,000 rpm for 30 min. The pellets were suspended in buffer (10 mM NaCl, 20 mM Tris HCl pH 8.0, 1 mM EDTA,1% sodium dodecyl sulfate, and 200 μg of proteinase-K per mL) and incubated for 2 h at 37 °C. Human placenta RNA inhibitor and 50–100 μL of diethyl pyrocarbonate–treated water were added to the RNA and stored at −20 °C.

2.5.4.2. Complementary DNA (cDNA) synthesis and amplification of the cDNA product. A 277-bp fragment of 16S rDNA of *M. pneumoniae* was selected for amplification (Tjhie et al., 1994) using primers-primer 1 (sense) 5′-AAGGACCTGCAAGGGTTCGT-3′ and primer 2 (antisense) 5′-CTCTAGCCATTACCTGCTAA-3′. Synthesis of cDNA and amplification of rRNA were performed as described by Cornelissen et al. (1990). The rRNA was transcribed in cDNA in 20 μL of reaction mixture containing 75 mM KCl, 50 mM Tris HCl (pH 8.3), 6 mM $MgCl_2$, 10 mM DTT, 0.2 mM each deoxynucleoside triphosphate, 50 pmol of antisense primer, and 20 U of RNase inhibitor, denatured total RNA. The reaction was started with 5 U avian myeloblastosis virus reverse transcriptase and incubated for 30 min at 42 °C, and then the RT was inactivated at 95 °C for 5 min. The reverse transcription reaction product was used directly in amplification. Amplification was performed in 50-μL reaction volume containing 50 mM KCl, 10 mM Tris HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.01% gelatin, 200 μM (each) deoxynucleoside triphosphates, 25 pmol of each primer, 1 U of Taq DNA polymerase, and the cDNA product. Amplification was carried out with 40 cycles consisting of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and elongation at 72 °C for 2 min. Positive and negative controls were also run along with the samples. A band of 277 bp was taken to be positive RT-PCR result.

2.6. Statistical analysis

Data analysis was performed using the statistical software Epi info version 3.5.3, CDC, Atlanta, GA, USA. The chi-square test and the Fisher exact test were used for testing the difference of proportion between the qualitative variables. A P value of <0.05 was considered statistically significant.

3. Results

A total of 245 children with LRTIs were included in this study.

3.1. Demographic profile

In the present study, 190 (77.5%) children were of age < 5 years, while 55 (22.5%) were ≥5 years with mean age (mean ± SD) of 35.59 ± 37.15 months. The association of *M. pneumoniae* infection was statistically significant across ≥5-year age group ($P < 0.001$; Table 1). *M. pneumoniae* infection was positive in 44 (29.5%) male and 32 (33.3%) female children, and the difference in prevalence of *M. pneumoniae* infection across male and female groups was statistically insignificant ($P = 0.53$; Table 1).

3.2. Clinical and radiological findings

Crepitations, rhonchi, and tachypnea with subcostal retraction were not found to have any statistically significant association with *M. pneumoniae* infection. Documentation of bronchopneumonia, interstitial infiltrates, hyperinflation, consolidation, consolidation and pleural effusion, hyperinflation + infiltrates, and normal chest X-rays were numerically comparable and differences were statistically insignificant in *M. pneumoniae*–positive and –negative categories (Table 2).

3.3. Microbiological profile

Serological evidence of *M. pneumoniae* infection was observed in 68 (27.7%) patients: specific IgM antibodies in 25 (36.7) patients, specific IgG antibodies in 16 (23.5%), specific IgM and IgG antibodies in 20 (29.4%) patients, and 4-fold rise in IgG antibodies alone in 7 (10.2%) patients in convalescent phase sera which were available only in 103 (42%) out of 245 patients.

M. pneumoniae PCR was positive in 16 (6.5%) patients:9 (56.2%) serologically proven and 7 (43.7%) serologically unproven *M. pneumoniae* infection. Nested PCR was positive in 19 (7.7%) patients: 11 (57.8%) cases were serologically proven and 8 (42.1%) serologically unproven. RT-PCR was positive in 15(6.1%) cases: 9 (60%) were serologically proven and 6 (40%) serologically unproven. PCR, nested PCR, and RT-PCR were positive for *M. pneumoniae* infection in 19 (7.7%) patients. Serology, PCR, nested PCR, and RT-PCR together detected *M. pneumoniae* infection in 76 (31.0%) patients. When serology was considered as the diagnostic standard, sensitivity, specificity, and positive and negative predictive values of PCR were 16.18%, 95.48%, 57.89%, and 74.78%, respectively; in contrast, when PCR was considered as the diagnostic standard, sensitivity, specificity, and positive and negative predictive values of serology were 57.89%, 74.78%,16.18%, and 95.48%, respectively (Tables 3 and 4).

4. Discussion

M. pneumoniae is an important cause of respiratory tract infections, and its etiological diagnosis is a constantly challenging issue due to the lack of a rapid, sensitive, and specific diagnostic gold standard. Our study shows higher and significant prevalence of *M. pneumoniae* in children ≥5 years of age in agreement with the previous studies (Waites and Talkington, 2004; Waris et al., 1998) but in contrast to Kumar et al. (2011b) which reported higher positivity (41%) in <1-year-old children with LRTIs. Kumar et al. (2010) reported a case of persistent pneumonia due to *M. pneumoniae* in a 3-week-old neonate. This notion was corroborated in the recent study on community-acquired pneumonia, where *M. pneumoniae* was detected significantly more frequently in children ≥5 years of age (19%) than in younger children (3%) (Jain et al., 2015).

Table 1
Association of *Mycoplasma pneumoniae* with age and sex in 245 children with community-acquired lower respiratory tract infections.

Characteristic		MP positive ($n = 76$) n (%)	MP negative ($n = 169$) n (%)	Total ($n = 245$) n (%)	P value
Age	<5 years	45 (59.2%)	145 (85.7%)	190 (77.5%)	<0.001
	≥5 years	31 (40.7%)	24 (14.2%)	55 (22.4%)	
Sex	Male	44 (29.5%)	105 (70.4%)	149 (60.8%)	0.53
	Female	32 (33.3%)	64 (66.6%)	96 (39.2%)	

MP = *Mycoplasma pneumoniae*.

Table 2
Association of *Mycoplasma pneumoniae* with clinical and radiological profile in the study group.

Clinical findings	MP positive ($n = 76$) n (%)	MP negative ($n = 169$) n (%)	Total ($n = 245$) n (%)	P	Odds ratio (95% CI)
Crepitations	36 (47.3%)	76 (44.9%)	112 (45.7%)	0.72	1.10 (0.64–1.89)
Rhonchi	8 (10.50%)	30 (17.7%)	38 (15.5%)	0.14	0.54 (0.23–1.25)
Tachypnea with subcostal retraction	57 (75%)	116 (68.6%)	173 (70.6)	0.31	1.37 (0.74–2.52)
Radiological findings					
Bronchopneumonia	4 (5.2%)	7 (4.1%)	11 (4.48%)	0.49	2.0 (0.27–14.69)
Interstitial infiltrates	31 (40.7%)	62 (36.6)	93 (37.9%)	0.50	1.75 (0.34–8.92)
Hyperinflation	10 (13.1%)	29 (17.1%)	39 (15.9%)	0.83	1.20 (0.21–6.79)
Consolidation	11 (14.4%)	23 (13.6%)	34 (13.8%)	0.55	1.67 (0.29–9.42)
Consolidation + pleural effusion	5 (6.5%)	6 (3.5%)	11 (4.48%)	0.28	2.91 (0.40–20.8)
Hyperinflation + infiltrates	5 (6.5%)	15 (8.8%)	20 (8.16%)	0.87	1.16 (0.18–7.56)
WNL	8 (10.5%)	20 (11.8%)	28 (11.2%)	0.71	1.40 (0.23–8.24)
X-ray findings unavailable	2 (2.6%)	7 (4.1%)	9 (3.6%)	0.91	1

M.P. = *Mycoplasma pneumoniae*; WNL = within normal limits.

The percentage of *M. pneumoniae*–positive patients was slightly higher in female than male children, but no significant association between sex of the patient and incidence of *M. pneumoniae* infection could be found ($P = 0.53$). Our study is in agreement with Vervloet et al. (2010) who reported higher *M. pneumoniae* infection in females than males but in contrast to previous findings by Ferwerda et al. (2001) and Medjo et al. (2014).

In our study, none of the clinical and radiological findings were significantly associated with *M. pneumoniae* infection, enabling to predict *M. pneumoniae* infection based on clinical findings, which can emphasize that clinical and laboratory features alone cannot predict the etiology of community-acquired pneumonia in children and are not useful in therapeutic decision making, in accordance with study by Principi et al. (2001). None of the radiological findings were significantly associated with *M. pneumoniae* infection. In *M. pneumoniae* LRTIs, radiologic changes are reported as nonspecific where *M. pneumoniae*–positive and –negative cases were not possible to differentiate on the basis of radiological picture of chest (Kumar et al., 2011b).

Serological evidence of acute infection was observed in 68 (34%) children similar to study by Kumar et al. (2011b) who reported 34% *M. pneumoniae* positivity by serology in children with community-acquired LRTIs. Liu et al. (2007) reported serological positivity of 30% in children with respiratory infections. Ayyez et al. (2014) found seropositivity of 33.07% by IgM ELISA in 42 out of 127 patients with respiratory infections. Sensitivity, specificity, and positive and negative predictive values of serology were 57.89%, 74.78%, 16.18%, and 95.48%, respectively, by using *M. pneumoniae* PCR as the "diagnostic standard," in line with earlier report where sensitivity ranged from 35% to 77% and specificity from 49% to 100% (Beersma et al., 2005).

Nucleic acid amplification techniques targeted towards DNA or RNA have progressively been explored for identification of pathogens including *M. pneumoniae* in infectious respiratory diseases. Determination and comparison between laboratory diagnostic methods of *M. pneumoniae* infection for LRTIs in larger clinical data base have been only scantily reported (Wang et al., 2017). Few of the currently available NAATs have been extensively validated against culture. The sensitivity of NAATs is almost always superior to that of traditional procedures, and they are more and more considered as the "new gold standard" (Loens et al., 2016). PCR approaches have been the most valuable method for rapid, sensitive, and specific diagnosis of *M. pneumoniae* infection. *M. pneumoniae* PCR was positive in 19 (7.7%) children in accordance with Maheshwari et al. (2011) who reported 16S rDNA PCR positivity of 6.7% for *M. pneumoniae* in children with community-acquired LRTIs employing throat swabs. Nested PCR positivity (7.7%) was in accordance with Nour et al. (2005) and Abele-Horn et al. (1998) who reported 8.69% and 8% positivity by nested PCR, respectively. PCR (6.6%) and the RT-PCR (6.1%) had almost identical positivity, in accordance with previous report where RT-PCR was performed on substantial part of samples negative by direct PCR and no additional positive samples were found by RT-PCR in this group (Tjhie et al., 1994). Positive PCR results in serologically negative cases may be due to an inadequate immune response, early successful antibiotic treatment, the collection of specimens before specific antibody synthesis could occur, a false-positive PCR result, the detection of carrier state not manifesting systemic antibody response, or false-negative serology in spite of active infection (Kumar et al., 2011b).

Negative PCR results in serologically proven infections may be due to inhibitors or other technical problems with the assay, its gene target, and antibiotics administration (Atkinson et al., 2008).

When serology was considered as the diagnostic standard, sensitivity, specificity, and positive and negative predictive values of PCR were 16.18%, 95.48%, 57.89%, and 74.78%, respectively. This heterogeneity between studies regarding sensitivity of PCR method could be due to different PCR types, different gene targets, or different sample types and time point for sampling.

The low concordance between serology and PCR in our study is in accordance with Menendez et al. (1999) who reported that the PCR assay in throat swab samples for the diagnosis of *M. pneumoniae* has a lower sensitivity than serologic methods and that 11.4% patients were positive by serology and only 3 (1.6%) were positive by PCR. Ayyez et al. (2014) found that 42 (33.07%) samples were positive for *M. pneumoniae* IgM, whereas 19/127 (14.96%) cases were positive by RT-PCR.

Limitation of our study was the time point of the acute phase serum because specific IgM emerges within 1 week after initial infection and about 2 weeks before IgG. Although IgG paired sera seroconversion

Table 3
Sensitivity, specificity, and positive and negative predictive values of *Mycoplasma pneumoniae* PCR using serology as a diagnostic standard.

PCR	Serology positive n (%)	Serology negative n (%)	Total n (%)
PCR positive	11 (4.4%)	8 (3.2%)	19 (7.7%)
PCR negative	57 (23.2%)	169 (68.9%)	226 (92.2%)
Total	68 (27.7%)	177 (72.2%)	245 (100%)

Serology is assumed to be the diagnostic standard. Sensitivity: 16.18%; specificity: 95.48%; positive predictive value: 57.89%; negative predictive value: 74.78%. The overall agreement between serology and PCR was slight (kappa = 0.149, agreement 73.47%).

Table 4
Sensitivity, specificity, and positive and negative predictive values of *Mycoplasma pneumoniae* serology using PCR as a diagnostic standard.

Serology	PCR positive n (%)	PCR negative n (%)	Total n (%)
Serology positive	11 (4.4%)	57 (23.2%)	68 (27.7%)
Serology negative	8 (3.2%)	169 (68.9%)	177 (72.2%)
Total	19 (7.7%)	226 (92.2%)	245 (100%)

PCR was assumed to be the diagnostic standard. Sensitivity: 57.89%; specificity: 74.78%; positive predictive value: 16.18%; negative predictive value: 95.48%. The overall agreement between serology and PCR was slight (kappa = 0.149, agreement 73.47%).

and/or rise in antibody titer is a definitive diagnosis of *M. pneumoniae* infection, convalescent phase sera are not always available.

5. Conclusions

Serological and molecular detection in combination is useful for rapid and reliable diagnosis of *M. pneumoniae* infections in children with LRTIs.

Conflicts of interests

All the authors report no conflict of interest relevant to this article.

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