



## *Mycoplasma pneumoniae* lower respiratory tract infections among children

### Microbiology

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### ABSTRACT

*Mycoplasma pneumoniae* (*M.pneumoniae*) is a common pathogen in pediatric respiratory tract infections and is responsible for nearly 40% of community-acquired pneumonia in children. The present study was conducted to detect *M.pneumoniae* in children with lower respiratory tract infections. A total of 75 children aged 6 months to 12 years with clinically suspected acute lower respiratory tract infections (LRTIs) were selected for detection of *M.pneumoniae* by enzyme linked immunosorbent assay (ELISA) and particle agglutination (PA) test. Our study showed higher prevalence of *M.pneumoniae* in children  $\geq 5$  years of age and this was statistically significant ( $P=0.03$ ). None of the clinical signs, symptoms and radiological findings were found to be associated with *M.pneumoniae* infection ( $P>0.05$ ). ELISA/ PA test together detected *M.pneumoniae* infection in 23(30.7%) children. Our study underlines the role of *M.pneumoniae* in children with LRTIs and more particularly in  $\geq 5$  years of age.

### KEYWORDS:

*Mycoplasma pneumoniae*, diagnosis, serology.

### Introduction

Lower respiratory tract infections (LRTIs) are a common cause of morbidity and mortality among young children, overwhelming majority occurring in developing countries.<sup>1</sup> *Mycoplasma pneumoniae* (*M.pneumoniae*) is a common pathogen in pediatric patients with respiratory tract infections and is responsible for nearly 40% of community-acquired pneumonia in children.<sup>2</sup> The organism is not sensitive to  $\beta$ -lactam antibiotic treatment of infections, whereas the use of macrolides can markedly reduce the duration of illness. Diagnosis of infection based on clinical symptoms alone is not reliable, therefore, correct rapid and cost effective point of care diagnostic testing is needed to initiate appropriate antibiotic treatment.<sup>3</sup> Serological testing is the most widely used method for detection of *M.pneumoniae* respiratory infections in clinical practice.<sup>4</sup> Serological tests such as PA test and enzyme immunoassays remains the most practical and convenient methods for laboratory diagnosis of recent *M.pneumoniae* infections. The aim of this study was to detect *M.pneumoniae* in children with lower respiratory infections employing serological assays i.e. enzyme linked immunosorbent assay (ELISA) and particle agglutination (PA) test.

### Materials and methods

A total of 75 children aged 6 months to 12 years with clinically suspected acute lower respiratory tract infections admitted to Pediatrics ward of Lok Nayak Hospital, Maulana Azad medical college, New Delhi India were selected for this study.

The criteria for inclusion were the presence of cough and fever with breathlessness of less than 30 days duration, increased respiratory rate (with or without features of respiratory distress) on examination, presence of signs of consolidation or bronchopneumonia with or without wheeze on auscultation. Criteria for exclusion were the hospital acquired pneumonia i.e. pneumonia that developed 72 hours after hospitalization or within 7 days of discharge.

Written informed consent from the parents or legal guardian was taken of all children before them being enrolled in the study. Detailed history and clinical examination were performed for all cases. Blood samples were collected by venepuncture following all the usual sterile precautions. A convalescent phase sera was obtained after 4-6

weeks of enrollment.

Enzyme linked immunosorbent assay (ELISA) was performed for serum immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against *M.pneumoniae* by using commercially available ELISA based kits following the manufacturer's instructions (Calbiotech Inc. Austin Dr Spring Valley, CA Germany). The interpretative criteria were consistent with the recommendations of the manufacturer as outlined on the package insert. Particle agglutination (PA) test (Serodia Myco II, Fujirebio, Japan) was performed according to the manufacturer's instructions. PA test is an *in vitro* diagnostic test for the detection of antibodies to *M.pneumoniae* which is manufactured using artificial gelatin particles sensitized with cell-membrane components of *M.pneumoniae* (Mac strain). Definite compact button in center of well with a smooth round outer margin were read as negative agglutination patterns, and a definite large ring with firmly agglutinated particles spread within the ring as positive. Titres of  $\geq 40$  were regarded as positive for *M.pneumoniae* antibody.

Statistical analysis was performed using the statistical software Epi info version 3.5.3, CDC, Atlanta, GA, USA. The difference of proportion between the qualitative variables was tested using the Chi square test and the Fischer exact test.  $P \leq 0.05$  was considered as significant.

### Results

A total of 23(30.7%) patients with respiratory tract infections were diagnosed with *M.pneumoniae* infection. The maximum number 56(74.6%) children were  $< 5$  years and 19(25.3%) children were  $\geq 5$  years of age group. In the age group of  $< 5$  years 13(23.2%) children were positive and 43(76.7%) were negative for *M.pneumoniae* infection whereas in  $\geq 5$  years of age group 10(52.6%) children were positive and 9(47.3%) were negative for *M.pneumoniae* infection and this difference was statistically significant ( $P=0.03$ ; The table). There were 45 (60%) males and 30 (40%) females and male to female ratio was 1.5 with a male preponderance. The percentage of *M.pneumoniae* positive patients were slightly higher (33.3%) in females than (28.8%) in males and this difference was statistically insignificant ( $P=0.08$ ; The table). No significant association of *M.pneumoniae* infection with clinical signs and symptoms was found

( $P>0.05$ ;The table). Documentation of consolidation, consolidation & pleural effusion, bronchopneumonia, hyperinflation, interstitial infiltrates, hyperinflation + infiltrates and normal chest X-ray were numerically comparable and differences were statistically insignificant in *M. pneumoniae* positive and negative categories ( $P>0.05$ ;The table).

ELISA detected *M.pneumoniae* infection in 22(29.3%) patients: specific IgM antibodies alone were detected in 8(36.3%) patients; specific IgM and IgG antibodies together in 6(27.2%) patients; specific IgG antibodies in acute phase in 6(27.2%) patients; four fold rise in IgG antibodies alone in 2(9%) patients in convalescent phase sera. PA test was positive in 10(13.3%) patients; 9(90%) were ELISA positive and 1(10%) positive by PA test alone. ELISA/ PA test together detected *M.pneumoniae* infection in 23(30.7%) children.

### Discussion

*M. pneumoniae* is an important cause of acute respiratory tract infection, reported to be uncommon in children aged <5 years and most frequent among school aged children 5-15 years of age with decline after adolescence and tapering off in adulthood.<sup>5</sup> Our study showed higher prevalence of *M.pneumoniae* in children >5years of age and this was found to be statistically significant ( $P=0.03$ ). Our findings are in agreement with the previous studies which reported a higher rate of *M.pneumoniae* infections in children >5 years of age<sup>6,7</sup> in contrast Kumar et al (2011) reported *M.pneumoniae* infection relatively higher in patients <1 year old with LRTIs.<sup>8</sup> The percentage of *M. pneumoniae* positive patients were slightly higher in (33.3%) females than in (28.8%) males and the incidence of *M. pneumoniae* infection with sex was statistically insignificant ( $P=0.08$ ). Our study is in agreement with Vervloet *et al* (2010)<sup>9</sup> and is in contrast to previous findings by Kashyap et al (2008).<sup>10</sup>

In the present study, none of the signs and symptoms were correlated with *M. pneumoniae* infection emphasizing the need for laboratory confirmation which is in accordance with previous studies which cast doubt on the specificity of clinical and laboratory features for predicting the microbial cause of LRTIs.<sup>11</sup> In our study, none of the radiographic findings were statistically significant 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.<sup>8</sup> Korppi et al (2008)<sup>12</sup> concluded that radiographs are not helpful for differentiating between viral, pneumococcal and atypical bacterial aetiology of community-acquired pneumonia in children.

Serology for *M.pneumoniae* infection was positive in 23(30.7%) children in agreement with previous studies by Kumar et al (2011)<sup>8</sup> which reported 34% and Kashyap et al (2008)<sup>10</sup> reported 21.3%. *M.pneumoniae* positivity by serology in children with community-acquired lower respiratory tract infections. Liu et al (2007)<sup>13</sup> reported serological positivity of 30%. Our study detected 10(13.3%) cases by PA test. Templeton et al (2003)<sup>14</sup> reported positivity by PA test in acute sample and convalescent samples (5.6%) and (8.5%), respectively. ELISA/ PA test together detected *M. pneumoniae* infection in 23(30.7%) children, one additional case was picked by PA test which was ELISA negative. The sensitivity of serological tests depends on the time point of first serum and on the availability of paired sera for seroconversion to IgG and/or rise in antibody titer. The discrepancy in serologic values could be accredited to the variation in the time of sampling and the difference in kinetics of IgG and IgM toward *M pneumoniae*. It is well known that the serum immunoglobulins produced during an *M pneumoniae* infection are heterogeneous and that their kinetics are related to the type of antigen.<sup>15</sup>

### Conclusion

In conclusion, our data underline that *M.pneumoniae* plays an important role in children with lower respiratory tract infections and more particularly in children  $\geq 5$  years of age.

**The table : Association of *M.pneumoniae* with demographic, clinical and radiological features of the patients**

Character	M.P.positive No. (%)	M.P.negative No. (%)	Total(n=75) No.(%)	P value
Age				0.03
<5 years	13(23.2%)	43(76.7%)	56(74.6%)	
$\geq 5$ years	10 (52.6%)	9(47.3%)	19(25.3%)	
Sex				0.08
Males	13(28.8%)	32(71.1%)	45(60%)	
Females	10(33.3%)	20(66.6%)	30(40%)	
Wheezing	12 (36.3%)	21(63.6%)	33(44%)	0.48
Coryza	12(25%)	36(75%)	48(64%)	0.24
Crepitations	10 (29.4%)	24 (70.5%)	34(45.3%)	0.97
Rhonchi	6 (26%)	17 (73.9%)	23(30.6%)	0.76
Consolidation	4 (36.3%)	7 (63.6%)	11(14.6%)	0.72
Consolidation & pleural effusion	2 (40%)	3 (60%)	5(6.66%)	0.63
Bronchopneumonia	2 (33.3%)	4 (66.6%)	6(8%)	1.00
Hyperinflation	3 (25%)	9 (75%)	12(16%)	0.74
Interstitial infiltrates	9 (33.3%)	18 (66.6%)	27(36%)	0.90
Hyperinflation+ Infiltrates	1 (20%)	4 (80%)	5(6.66%)	1.0
Within Normal Limit	2(22.2%)	7(77.7%)	9(12%)	0.71

M.P.: *Mycoplasma pneumoniae*

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