

# Survey of *Mycoplasma pneumoniae* in Iranian children with acute lower respiratory tract infections

## ABSTRACT

**Objectives:** *Mycoplasma pneumoniae* is an atypical pathogen, which is one of the major causes of lower respiratory tract infections (LRTIs) worldwide. This study was performed to determine the role of *M. pneumoniae* in acute LRTIs in children, who were referred to main pediatric hospitals in Shiraz, Iran, with the diagnosis of LRTI. Polymerase chain reaction method on a throat-swab specimen was utilized to detect *M. pneumoniae*. **Results:** One hundred patients with acute LRTIs were investigated in this study. There were 10 positive PCR for *M. pneumoniae* (10%), including 6 of 62 hospitalized patients and 4 of 38 outpatients. All patients with LRTIs due to *M. pneumoniae* had cough. Fever, flu like symptoms, dyspnea, pulmonary rales, wheezing, and conjunctivitis were other common signs and symptoms. **Conclusions:** The percentage of cases with *M. pneumoniae* infection in our population is similar to the reported in other parts of Asia. Precise and early detection of pathogen and appropriate antibiotic therapy are the key points in management of patients with LRTIs.

**Keywords:** atypical bacteria; *Mycoplasma pneumoniae*; PCR; respiratory infection;

[Braz J Infect Dis 2011;15(2):97-101 ]©Elsevier Editora Ltda.

## INTRODUCTION

Lower respiratory tract infections (LRTIs) continue to be a common cause of morbidity and mortality, especially in the pediatric age group,<sup>1,2</sup> contributing to about 1.9 million children's deaths yearly.<sup>3</sup> Determination of the putative pathogens is one of the main difficulties in LRTIs, as the diagnostic tests of respiratory samples are not sensitive enough to identify the causative microorganisms in most patients.<sup>4,5</sup> It is estimated that antigen screening, culture and serological methods can be helpful in only one third of the cases.<sup>6</sup> Therefore therapy is usually empiric in most cases.<sup>7,8</sup>

Atypical pathogens, particularly *Mycoplasma pneumoniae*, are major causes of acute LRTIs. Although *M. pneumoniae* usually causes pneumonia and bronchitis, other clinical manifestations such as rhinitis, pharyngitis, sinusitis and otitis media can also be seen.<sup>9,10</sup> There are some reports indicating significant role of *M. pneumoniae* as cause of LRTIs in children of all ages,<sup>11,12</sup> which underscores the importance of specific pathogen diagnosis, as the use of proper antibiotics, such as macrolides, can significantly reduce duration of the illnesses.<sup>13,14</sup>

Unfortunately, as result of the difficulty to detect *M. pneumoniae* due to insensitive culture and time-consuming and impractical in clinical practice, specific etiologic diagnoses have remained unknown in the majority of cases.<sup>15</sup> Several methods were utilized for detection of this pathogen, including cold agglutination test,<sup>16</sup> serological methods (*Mycoplasma* IgM antibody, complement fixation, ELISA)<sup>17,18</sup> and microparticle agglutination test.<sup>19</sup> However, there were a number of limitations with these methods, especially regarding sensitivity and specificity. Therefore polymerase chain reaction (PCR) is suggested as of the most practical typing method for rapid and sensitive detection of *M. pneumoniae* in throat swabs.<sup>20,21</sup> The PCR method could provide rapid diagnosis of *M. pneumoniae* infection, especially in younger children, and is a valuable method considering epidemiological, clinical, economic aspects in addition to turnaround time of results.<sup>22-24</sup> The sensitivity and specificity of the PCR method has been estimated about 70% and 80%, respectively.<sup>24</sup>

This study was performed to determine the role of *M. pneumoniae* in acute LRTIs in children, with use of PCR analysis.

## Authors

Nahal Hadi<sup>1</sup>

Sara Kashef<sup>2</sup>

Mohammad Moazzen<sup>3</sup>

Michel Shamoone Pour<sup>4</sup>

Nima Rezaei<sup>5</sup>

<sup>1</sup>Department of Bacteriology and Virology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>2</sup>Allergy Research Center, Department of Pediatrics, Division of Immunology and Allergy, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>3</sup>Department of Pediatrics, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>4</sup>Department of Bacteriology and Virology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>5</sup>Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Submitted on: 3/8/2010

Approved on: 6/16/2010

## Correspondence to:

Nahal Hadi

Academic Unit of Infection & Immunity,

School of Medicine & Biomedical Sciences, F Floor,

Medical School, University of Sheffield, Beech Hill Road,

Sheffield, S10 2RX, UK

Phone: +44 755 602 0485

hadina@sums.ac.ir

We declare no conflict of interest.

## PATIENTS AND METHODS

### Setting

This prospective study was performed in 2006 on pediatric patients, who were referred to Namazee and Dastgheib Hospitals (Shiraz, Iran) because of acute lower respiratory tract infections. This study was approved by Ethics Committee of the Hospital, Shiraz University of Medical Sciences. Written informed consent was taken from the parents of enrolled patients.

### Patients enrollment

One hundred children (64 boys and 36 girls) were included in this study. Demographic data and patients' history were documented in the standardized questionnaire, designed for this study. All symptoms and detected signs in the physical examination, including cough, flu-like symptoms, dyspnea, pulmonary rales, wheezing, and conjunctivitis were also recorded. Initial laboratory investigations, including complete cell blood count and chest X-ray, were done before throat-swab sampling. The patients with the following criteria were enrolled in this study: respiratory symptoms associated with abnormal chest X-ray suggestive of pneumonia, respiratory distress, and presence of rales in lung examination. Fever was defined as body temperature higher than 37.8°C.

### Exclusion criteria

Patients with chronic respiratory problems, such as asthma and cystic fibrosis, and other chronic diseases, such as malignant disease, tuberculosis and autoimmune diseases, were excluded from this study. Those who had history of blood transfusions or immunosuppressive therapy were also excluded. Enrolled patients were not receiving antibiotics during the 72 hours before sampling.

### PCR method

The polymerase chain reaction (PCR) method on a throat-swab specimen was utilized to detect *M. pneumoniae*. Although all the patients were prescribed a 10-day course of macrolides (erythromycin or azithromycin at the recommended dose), throat-swab specimens were taken at the first day prior to antibiotic therapy. The samples were transported in PPLO broth to the microbiology laboratory and were incubated at 37°C for a few hours after removing the swabs. The cultures were centrifuged at 1,000 g for 5 minutes. The supernatant then was collected in a sterile 1 mL Eppendorf tube and centrifuged at 20,000 g for 20 minutes. After decanting the supernatant the pellet was suspended in 20 µL of sterile Mili-Q water and boiled for 10 minutes.

The latter was then used as DNA template in PCR reactions which were performed by the *M. pneumoniae* PCR kit (Genekam Biotechnology AG, Germany), based on the protocol. The negative and positive controls provided in the kit were used in each assay. DNA fragments were visualized on 1% agarose gel by electrophoresis.

### Statistical analysis

Epi Info 6 program (version 6.2, World Health Organization, Geneva, Switzerland) was used for statistical analyses. Association between categorical variables were assessed by Chi-square or Fisher's exact test. P-value of less than 0.05 was considered statistically significant.

## RESULTS

### Characteristics of patients

One hundred patients with acute LRTIs (64 male and 36 female), including 62 hospitalized and 38 outpatients, were investigated in this study. Patients' ages ranged from 6 months to 17 years.

### Positive PCR patients

Among 100 investigated patients, PCR for *M. pneumoniae* turned out positive in 10 (10%) patients, being 5 boys (7,8%) and 5 girls (13,8%) [ $p = 0.48$ ]. *M. pneumoniae* positivity rates among hospitalized patients (9.5%) and outpatients (10.5%) were similar ( $p = 0.57$ ).

### Seasonal variations

Half of the identified *M. pneumoniae* cases were referred during the autumn season, while only 3 cases occurred during the winter and 2 cases in the spring. No *M. pneumoniae* was detected during the summer season.

### Clinical manifestations

All patients with acute LRTI due to *M. pneumoniae* complained of cough lasting for a mean of 9.5 days, compared to a mean duration of 7 days among PCR negative patients ( $p = 0.35$ ). Although 8 out of 10 patients with positive PCR had fever at the time of admission, and all but one had low-grade fever. Half of the cases presented with productive cough, while the remaining half had dry cough, irrespective of being positive and negative PCR for *M. pneumoniae*. Flu-like symptoms were present in 40% of the patients with *M. pneumoniae*, which was lower than the 63.3% seen in the negative patients. However, this difference was not significant ( $p = 0.18$ ). In contrast, dyspnea was more common among PCR-positive patients for *M. pneumoniae* (50% vs. 36.7%,  $p = 0.49$ ). Other characteristics of the patients are presented in the Table 1, which shows no significant difference on clinical manifestations between these two groups.

**Table 1. Characteristics of patients with acute lower respiratory tract infections**

Characteristics	PCR-Positive for <i>M. pneumoniae</i> (n = 10)	PCR-Negative for <i>M. pneumoniae</i> (n = 90)	p-value *
Gender (male/female)	5/5	59/31	0.49
Cough	10 (100%)	90 (100%)	-
Fever	8 (80%)	57 (63.3%)	0.49
Flu like symptoms	4 (40%)	57 (63.3%)	0.18
Dyspnea	5 (50%)	33 (36.7%)	0.49
Pulmonary rales	3 (30%)	34 (37.8%)	0.74
Wheezing	1 (10%)	23 (25.6%)	0.68
Conjunctivitis	2 (20%)	11 (12.2%)	0.34
Hospitalized cases	6 (60%)	56 (62.2%)	0.57
Outpatient cases	4 (40%)	34 (37.8%)	0.57

\*Not significant in all these comparisons.

### Para-clinical findings

Complete blood cell count was similar in the two groups of positive and negative PCR for *M. pneumoniae*. Patients with positive PCR for *M. pneumoniae* had a mean white blood cells (WBC) count of 10,200/m<sup>3</sup> with 65% polymorphonuclear cells (PMN), whilst mean WBC in the PCR-negative cases was 10,000/m<sup>3</sup> with 54% PMN. Expert radiologist reports of chest X-ray were available for 70 patients; among PCR-positive patients for *M. pneumoniae* there were 2 cases of lobar infiltrations and 3 of interstitial infiltrations. Positive radiological findings among PCR-positive patients for *M. pneumoniae* was 71.4% (5 of 7), lower than the 77.8% (49 of 63) found among PCR-negative patients, but this difference was not significant (p = 0.66).

### DISCUSSION

Respiratory infections with *M. pneumoniae* are common in most areas of the world, which cause atypical pneumonia and other respiratory tract diseases with outbreaks occurring every 4-7 year intervals. The annual rate of infection is estimated between 1.3% in endemic periods and 50% in epidemic periods. About 15-20% of community-acquired pneumonia was associated with *M. pneumoniae*, with an incidence of 2 cases per 1,000 population annually.<sup>25,26</sup>

The role of *M. pneumoniae* in respiratory infections was investigated in different regions, but its prevalence varies greatly from study to study. Although geographical regions can alter the prevalence of *M. pneumoniae* in respiratory infections, type of study, age of studied patients and the methods used for detecting *M. pneumoniae* can be responsible

for the discrepant results of previous studies. Our study revealed that 10% of patients with LRTIs could be attributed to this atypical bacteria, which is similar to that one reported in other parts of Asia,<sup>27,28</sup> in spite of the fact that our study was conducted in a different location and at a different time. Ngeow *et al.* carried on a large surveillance study in Asia, which showed the prevalence of *M. pneumoniae* in about 12% among children with community-acquired pneumonia.<sup>27</sup> In an earlier study from South-eastern Asia, Ouchi *et al.* studied 1,104 Japanese children with acute LRTIs and found *M. pneumoniae* in 13% of the patients,<sup>28</sup> also in agreement with our study.

Low frequency of *M. pneumoniae* was reported in only few studies, such as the study by Elkholy *et al.* involving 111 Egyptian children with positivity rate of only 4.5%<sup>29</sup> and the study by Meijer *et al.* on 557 Dutch patients with positivity rate of only 1.3%.<sup>30</sup> However, there are several studies reporting higher frequency of *M. pneumoniae*. In the study by Principi *et al.* on 613 Italian children from 21 different centers, who were hospitalized because of community acquired LRTIs, this pathogen was found in 34% of the patients.<sup>31</sup> In the study by Liu *et al.* on 256 Taiwanese patients with clinical suspicion of atypical pneumonia, *M. pneumoniae* was positive in 32% of the cases.<sup>24</sup> In another study by Maltezou *et al.* on 65 Greek children, *M. pneumoniae* infection was confirmed in 27.5% of them.<sup>32</sup> As expected, the frequency of *M. pneumoniae* pneumonia should be much higher at the time of outbreaks. The study by Kim *et al.* in Seoul, Korea, on 234 hospitalized children with community-acquired LRTIs during two outbreaks revealed about 65% of patients with *M. pneumoniae* pneumonia.<sup>33</sup>

However, it should be emphasized that some of these studies reflect the prevalence of *M. pneumoniae* in hospitalized patients, while we also included outpatients, albeit with similar frequency with hospitalized cases. Although the study by Sidal *et al.* in Istanbul, Turkey on 284 patients, seen at an outpatient clinic with respiratory symptoms, showed positive antibody for *M. pneumoniae* in about 30% of cases.<sup>34</sup> Another study by Butun *et al.* in Izmir, Turkey on 100 children, receiving care at the outpatient department with respiratory symptoms, revealed positive antibody for *M. pneumoniae* in only 8% of cases.<sup>35</sup>

In our study, the *M. pneumoniae* in autumn season was found higher compared to other seasons, which contrasts with the Turkish study indicating significantly higher frequency of this pathogen in winter season.<sup>34</sup> Meanwhile in the study by Foy *et al.* in Seattle during an 11-year period, no significant seasonal variation was documented.<sup>36</sup>

Atypical pneumonia could be distinguished from other types of pneumonias by its mild clinical course and its ability to cause different symptoms in different people. Cough and throat pain are common symptoms, whilst fever is usually a symptom of hospitalized patients diagnosed with pneumonia. However, we did not find any specific clinical characteristic that could accurately identify patients with *M. pneumoniae*, similarly to other studies.<sup>35,37</sup> In fact, none of the signs and symptoms was unique to *M. pneumoniae* infections enabling to predict atypical pathogens based on clinical characteristics. Such findings can emphasize the point that clinical and laboratory features alone cannot predict the etiology of community-acquired pneumonia in children and therefore are not useful in therapeutic decision-making.<sup>38,39</sup> It shows the importance of effective laboratory diagnosis tools to detect such pathogens. As clinical symptoms are nonspecific, persistent cough lasting more than a week, refractory to conventional antibiotic therapy should prompt clinicians to suspect the diagnosis of LRTIs by atypical microorganisms.<sup>40</sup> However, initiation of effective antibiotic therapy is usually delayed. Such delay may lead to some complications, while the patients are not appropriately treated.<sup>31</sup>

Therefore, detection of microorganisms and appropriate treatments are the key points in the management of patients with LRTIs. Although *M. pneumoniae* can be isolated from cultures, this is a difficult and time-consuming method which limits its clinical usefulness. Therefore, the PCR method for detecting *M. pneumoniae* in throat swabs could be considered as the most practical method for rapid diagnosis, particularly in younger children and in early stage of disease.<sup>20,24</sup>

*M. pneumoniae* is an atypical bacterium that could cause LRTIs worldwide, which requires adding a macrolide in the antibiotic regimen.<sup>40</sup> Prompt diagnosis by appropriate technique and starting appropriate antibiotic therapy could prevent the use of unnecessary antibiotics and further complications.

## REFERENCES

- Mulholland K. Magnitude of the problem of childhood pneumonia. *Lancet* 1999; 354:590-2.
- Vuori E, Peltola H, Kallio MJT *et al.* Etiology of pneumonia and other common childhood infections requiring hospitalization and parenteral antimicrobial therapy. *Clin Infect Dis* 1998; 27:566-72.
- Klig JE, Shah NB. Office pediatrics: current issues in lower respiratory infections in children. *Curr Opin Pediatr* 2005; 17:111-8.
- Thomson RB. Laboratory diagnosis of respiratory infections. *Curr Opin Infect Dis* 1999; 12:115-9.
- Menéndez R, Córdoba J, de la Cuadra P *et al.* Value of the polymerase chain reaction assay in noninvasive respiratory samples for diagnosis of community-acquired pneumonia. *Am J Respir Crit Care Med* 1999; 159:1868-73.
- George H, Mccracken JR. Diagnosis and management of pneumonia in children. *Pediatr Infect Dis J* 2000; 19:924-8.
- Mandell LA, Wunderink RG, Anzueto A *et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; 44:S27-72.
- Stolza D, Christ-Craind M, Gencaya MM *et al.* Diagnostic value of signs, symptoms and laboratory values in lower respiratory tract infection. *Swiss Med Wkly* 2006; 136:434-40.
- Tan JS. Role of atypical pneumonia pathogens in respiratory tract infections. *Can Respir J* 1999; (Suppl 6):15-9.
- Wadowsky RM, Castilla EA, Laus S *et al.* Evaluation of Chlamydia pneumoniae and *Mycoplasma pneumoniae* as etiologic agents of persistent cough in adolescents and adults. *J Clin Microbiol* 2002; 40:637-40.
- File TM, Tan JS, Plouffe JF. The role of atypical pathogens: *Mycoplasma pneumoniae*, Chlamydia pneumoniae, and Legionella pneumophila in respiratory infection. *Infect Dis Clin North Am* 1998; 12:569-92.
- Block S, Hedrick J, Hammerschlag MR *et al.* *Mycoplasma pneumoniae* and Chlamydia pneumoniae in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. *Pediatr Infect Dis J* 1995; 14:471-7.
- Taylor-Robinson D, Bebear C. Antibiotic susceptibilities of mycoplasmas and treatment of mycoplasmal infections. *J Antimicrob Chemother* 1997; 40:622-30.
- Principi N, Esposito S. Comparative tolerability of erythromycin and newer macrolide antibacterials in paediatric patients. *Drug Safety* 1999; 20:25-41.
- Harris R, Marmion BP, Varkanis G *et al.* Laboratory diagnosis of *Mycoplasma pneumoniae* infection. 2. Comparison of methods for the direct detection of specific antigen or nucleic acid sequences in respiratory exudates. *Epidemiol Infect* 1988; 101:685-94.
- Lind K, Bentzon MW. Ten and a half years seroepidemiology of *Mycoplasma pneumoniae* infection in Denmark. *Epidemiol Infect* 1991; 107:189-99.
- Petitjean J, Vabret A, Gouarin S, Freymuth F. Evaluation of four commercial immunoglobulin G (IgG)- and IgM-specific enzyme immunoassays for diagnosis of *Mycoplasma pneumoniae* infections. *J Clin Microbiol* 2002; 40:165-71.
- Uldum SA, Søndergård-Andersen J, Skov Jensen J, Lind K. Evaluation of a commercial enzyme immunoassay for detection of *Mycoplasma pneumoniae* specific immunoglobulin G antibodies. *Eur J Clin Microbiol Infect Dis* 1990; 9:221-3.



19. Echevarría JM, León P, Balfagón P *et al.* Diagnosis of *Mycoplasma pneumoniae* infection by microparticle agglutination and antibody-capture enzymeimmunoassay. *Eur J Clin Microbiol Infect Dis* 1990; 9:217-20.
20. Bernet C, Garret M, de Barbeyrac B *et al.* Detection of *Mycoplasma pneumoniae* by using the polymerase chain reaction. *J Clin Microbiol* 1989; 27:2492-6.
21. Ieven M, Ursi D, Van Bever H *et al.* Detection of *Mycoplasma pneumoniae* by two polymerase chain reactions and role of *M. pneumoniae* in acute respiratory tract infections in pediatric patients. *J Infect Dis* 1996; 173:1445-52.
22. Abele-Horn M, Busch U, Nitschko H *et al.* Molecular approaches to diagnosis of pulmonary diseases due to *Mycoplasma pneumoniae*. *J Clin Microbiol* 1998; 36:548-51.
23. Skakni L, Sardet A, Just J *et al.* Detection of *Mycoplasma pneumoniae* in clinical samples from pediatric patients by polymerase chain reaction. *J Clin Microbiol* 1992; 30:2638-43.
24. Liu FC, Chen PY, Huang F *et al.* Rapid diagnosis of *Mycoplasma pneumoniae* infection in children by polymerase chain reaction. *J Microbiol Immunol Infect* 2007; 40:507-12.
25. Foy HM, Cooney MK, McMahan R, Grayston JT. Viral and mycoplasmal pneumonia in a prepaid medical care group during an eight-year period. *Am J Epidemiol* 1973; 97:93-102.
26. Foy HM. Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. *Clin Infect Dis* 1993; 17:S37-46.
27. Ngeow YF, Suwanjutha S, Chantarojanasriri T *et al.* An Asian study on the prevalence of atypical respiratory pathogens in community-acquired pneumonia. *Int J Infect Dis* 2005; 9:144-53.
28. Ouchi K, Komura H, Fujii M *et al.* Chlamydia pneumonia infection and *Mycoplasma pneumoniae* infection in pediatric patients. *Kansenshogaku Zasshi* 1999; 73: 1177-82.
29. Elkholy A, Elkarakasy H, Fattouh A *et al.* Acute lower respiratory tract infection due to Chlamydia and *Mycoplasma* spp. in Egyptian children under 5 years of age. *J Trop Pediatr* 2009; 55:195-7.
30. Meijer A, Dagnelie CF, De Jong JC *et al.* Low prevalence of Chlamydia pneumoniae and *Mycoplasma pneumoniae* among patients with symptoms of respiratory tract infections in Dutch general practices. *Eur J Epidemiol* 2000; 16:1099-106.
31. Principi N, Esposito S, Blasi F, Allegra L, Mowgli Study Group. Role of *Mycoplasma pneumoniae* and Chlamydia pneumonia in children with community-acquired lower respiratory tract infections. *Clin Infect Dis* 2001; 32:1281-9.
32. Maltezou HC, La-Scola B, Astra H *et al.* *Mycoplasma pneumoniae* and Legionella pneumophila in community-acquired lower respiratory tract infections among hospitalized children: diagnosis by real time PCR. *Scand J Infect Dis* 2004; 36:639-42.
33. Kim NH, Lee JA, Eun BW *et al.* Comparison of polymerase chain reaction and the indirect particle agglutination antibody test for the diagnosis of *Mycoplasma pneumoniae* pneumonia in children during two outbreaks. *Pediatr Infect Dis J* 2007; 26:897-903.
34. Sidal M, Kilic A, Unuvar E *et al.* Frequency of Chlamydia pneumoniae and *Mycoplasma pneumoniae* infections in children. *J Trop Pediatr* 2007; 53:225-31.
35. Bütün Y, Köse S, Babayiğit A *et al.* Chlamydia and *Mycoplasma* serology in respiratory tract infections of children. *Tuberk Toraks* 2006; 54:254-8.
36. Foy HM, Kenny GE, Cooney MK *et al.* Longterm epidemiology of infections with *Mycoplasma pneumoniae*. *J Infect Dis* 1979; 139:681-7.
37. Esposito S, Blasi F, Bellini F *et al.* *Mycoplasma pneumoniae* and Chlamydia pneumoniae infections in children with pneumonia. *Eur Respir J* 2001; 17:241-5.
38. Wubbel L, Muniz L, Ahmed A *et al.* Etiology and treatment of community-acquired pneumonia in ambulatory children. *Pediatr Infect Dis J* 1999; 18:98-104.
39. Korppi M, Heiskanen-Kosma T, Jaloner E *et al.* Aetiology of community-acquired pneumonia in children treated in hospital. *Eur J Pediatr* 1993; 152:24-30.
40. Esposito S. Importance of acute *Mycoplasma pneumoniae* and Chlamydia pneumoniae infections in children with wheezing. *Eur Respir J* 2000; 16:1142-6.