

1-1-2013

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GHOTASLOU, REZA; SHARIFI, SIMIN; AKHI, MOHAMMAD TAGHI; and SOROUSH, MOHAMMAD HOSSEIN (2013) "Epidemiology, clinical features, and laboratory detection of *Mycoplasma pneumoniae* infection in East Azerbaijan, Iran," *Turkish Journal of Medical Sciences*: Vol. 43: No. 4, Article 5. <https://doi.org/10.3906/sag-1202-80>

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Epidemiology, clinical features, and laboratory detection of *Mycoplasma pneumoniae* infection in East Azerbaijan, Iran

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Received: 22.02.2012 • Accepted: 17.12.2012 • Published Online: 29.07.2013 • Printed: 19.08.2013

Aim: *Mycoplasma pneumoniae* is involved in the etiology of many respiratory tract infections. This prospective study describes the epidemiology and clinical properties of *M. pneumoniae* infections in hospitals of the Tabriz University of Medical Sciences.

Materials and methods: From May 2009 to November 2010, 200 patients with respiratory tract infections were enrolled into this study. Due to antibiotic use 51 patients were excluded. *M. pneumoniae* from respiratory infections was detected by culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) assays.

Results: *M. pneumoniae* infection was detected in 9 (6.15%), 8 (5.3%), and 3 (2.01%) of patients by PCR, ELISA, and culture methods, respectively. *M. pneumoniae* infection was mostly prevalent in patients 21–80 years old. Most infections (92%) were community-acquired and coughs, fever, dyspnea, and malaise were among the most common symptoms.

Conclusion: Pneumonia due to the *M. pneumoniae* infection is infrequent in Tabriz and the clinical symptoms of the patients were determined to be mild. The PCR method also proved to be more sensitive and reliable than ELISA and culture assays in the detection of *M. pneumoniae*.

Key words: *Mycoplasma pneumoniae*, pneumonia, epidemiology

1. Introduction

Mycoplasma pneumoniae infections happen both endemically and epidemically worldwide (1). *M. pneumoniae* is a member of a group of organisms, including *Chlamydia pneumoniae* and *Legionella pneumophila*, that cause atypical pneumonia (2). This bacterium is currently responsible for 10%–20% of all radiographically proven pneumonias and up to 25% of all pediatric primary atypical pneumonias (3). Although it is extremely contagious, most infections from *M. pneumoniae* are relatively mild and consist of low-grade fever, malaise, headache, dyspnea, fatigue, nonproductive cough, pharyngitis, tracheobronchitis, and bronchiolitis (4). Only 3%–10% of infected cases result in bronchopneumonia and death from *Mycoplasma* infection is uncommon, different from cases of *Streptococcus pneumoniae* infection (5,6). Myalgia and gastroenteritis are infrequent, and secondary complications include otitis media, erythema multiform, hemolytic anemia, myocarditis, pericarditis, and neurological abnormality (4). Pneumonia due to *M. pneumoniae* occurs worldwide all through the year but it is more common during summer and fall, and illness is most

prevalent in people 5–20 years old (7). The main problem with pneumonia due to *M. pneumoniae* is early diagnosis. Therefore, recently there has been a tendency for early detection of *M. pneumoniae* using very sensitive and fast techniques, like the polymerase chain reaction (PCR) assay (8). However, there has not been adequate research about *M. pneumoniae* infections in East Azerbaijan, Iran. The purpose of this study was thus to investigate the epidemiology, laboratory detection, and clinical features of infections caused by *M. pneumoniae* in this region.

2. Materials and methods

This randomized consecutive prospective study describes the epidemiology and clinical properties of *M. pneumoniae* infections in Tabriz hospitals. Clinical samples were collected from 200 patients who were diagnosed with upper respiratory tract infections (178 patients) and lower respiratory tract infections (22 patients) based on clinical symptoms, signs, and radiography. Patients complaining of cough, fatigue, respiratory distress, fever, chilliness, sore throat, sputum, malaise, chest pain, rales, and wheezes were considered for the study. Written informed consent

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was obtained from the patients before they were enrolled in the research, and the study protocol was approved by the ethics committee of Tabriz University of Medical Sciences. Due to their use of antibiotics, 51 patients were excluded. If *M. pneumoniae* isolates were recovered from nonhospitalized patients or within 48 h of entrance to the hospital, they were considered as community-acquired pneumonia. Pneumonia was identified based on clinical signs and symptoms as well as radiodiagnostic data of pneumonia in the case of some patients.

For detection of *M. pneumoniae*, respiratory tract specimens were processed by 3 methods: culture, serology, and PCR (3,7,8). Specimens for PCR and culture methods were acquired from throat swabs (117 samples), sputum (15 samples), endotracheal aspiration (3 samples), bronchial washing (8 samples), and bronchoalveolar lavage (6 samples). Blood samples were taken from patients for serologic analyses. Laboratory diagnostic criteria were according to results of PCR, serology, and culture assays.

In summary, broth and agar media were composed of pleuropneumonia-like organism broth (PPLO, Difco), horse serum, freshly prepared yeast extract supplemented with thallium acetate (Sigma), glucose, and phenol red. Specimens were inoculated in broth media and incubated aerobically at 37 °C. Broth media were subcultured on agar medium when the color changed to yellow. The colonies were observed at 100× magnification in agar medium. All the colonies obtained on the culture were identified by Dine's stain and by biological, biochemical, and PCR assays. A commercially available enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN Inc.) was used for quantitative and semiquantitative determination of antibody titers (IgG and IgM) according to the kit instructions. DNA was extracted from clinical samples by boiling, and classical PCR was performed using *M. pneumoniae* attachment protein (P1 primer) (7). To increase the reliability of PCR results, positive PCR product was sent for sequencing. Data were analyzed using the chi-square and Fisher exact tests by SPSS 16.

3. Results

Of the 149 patients studied over the 10-month study period, a total of 9 (6.15%) respiratory specimens were PCR-positive for *M. pneumoniae*, while IgM ELISA and culture methods revealed 8 (5.3%) and 3 (2.01%) positive cases, respectively. It was interesting that all of the culture- and IgM ELISA-positive patients were also PCR-positive. Elevated IgG anti-*M. pneumoniae* was detected in 79 (53%) of the patients. The mean age of patients was 25 ± 2 years and the age range of patients was from 1 to 81 years old. The highest prevalence rate of *M. pneumoniae* respiratory infection was diagnosed in patients 21–80 years old. All 9 patients with *M. pneumoniae* infection had a cough; thus, coughs were the most common symptom, and only 3 patients had sputum production with mild

severity. Epidemiological and clinical manifestations of the patients in this study are shown in the Table. Of all the isolates, 88.9% were community-acquired and 11.1% were nosocomial. Mean hospitalization duration was 17 ± 9 days. Eighty-six of the suspected cases (58%) were associated with pneumonia and 63 cases (42%) were observed to involve upper respiratory tract infections.

4. Discussion

A variety of techniques are used to assess *M. pneumoniae*, each with different advantages and disadvantages. In this study, PCR, ELISA, and culture methods were employed for the detection of *M. pneumoniae* infection in 149 suspected patients. The culture method for the isolation of *M. pneumoniae* requires 2–4 weeks, which limits its clinical usefulness. Moreover, culturing of *M. pneumoniae* is expensive and time-consuming (2,3,9,10). Serological methods are more extensively used than culture, because they are easier to carry out and more affordable (7,11,12). However, they are generally nonspecific, retrospective, and need 2 samples for titration (2). It should be noted that in our study, the most reliable result was obtained by the PCR method, which proved to be highly sensitive, specific, and faster than other methods. The specificity of the culture method was 100%, while its sensitivity barely reached 33%, relative to the results of the PCR method as a gold standard. All of the culture and IgM ELISA test positive patients were PCR-positive, too. The PCR method is more sensitive and is the gold standard currently being used for diagnosis of this organism in some laboratories (13).

In a similar study carried out by Al-Rashed in Saudi Arabia, *M. pneumoniae* was found in 6% of 112 adults (14). In another study of community-associated pneumonia in adult patients from Finland, the bacterium accounted for 30% of all episodes (15). In Turkey, *M. pneumoniae* was diagnosed in 13% of patients by the real time-PCR method (16). Naghipour et al. from Iran studied 261 children with acute respiratory infections during 2003 to 2004 and detected *M. pneumoniae* in 2 cases (1%) by the PCR method (17).

Other studies reported high levels of IgG antibody titer in healthy people, perhaps because of previous infection with *M. pneumoniae*, while IgM titer was elevated in active infections (12,13). In our study 53% of the patients had previous infection and 5.3% of them had active *M. pneumoniae* respiratory infection. Our study demonstrated that *M. pneumoniae* is one of the relatively uncommon respiratory infections in East Azerbaijan, Iran. However, the results of this study showed that the number of *M. pneumoniae* infections has been underestimated, because in the majority of community-acquired pneumonia cases, individuals are treated as outpatients.

Climate, seasonality, and geography are not thought to be of paramount significance; however, infection takes place most commonly during fall and winter (2). Likewise,

Table. Epidemiological and clinical characteristics of 9 patients with *M. pneumoniae* infections

Characteristics	Total patients, n (%) [*]	Patients with <i>M. pneumoniae</i> infections, n (%) ^{**}
Sex		
Male	77 (52)	4 (44.4)
Female	72 (48)	5 (55.5)
Symptoms/signs		
Cough	134 (90)	9 (100)
Fever	57 (38)	5 (55.5)
Malaise	52 (35)	5 (55.5)
Dyspnea	70 (47.9)	5 (55.5)
Fatigue	68 (45.6)	4 (44.4)
Skin manifestations	18 (12)	2 (22.2)
Chills	33 (22.1)	2 (22.2)
Myalgia	36 (24)	2 (22.2)
Diarrhea	16 (10.7)	2 (22.2)
Chest pain	34 (23)	1 (11.1)
Sputum production	55 (37)	3 (33.3)
Underlying disease		
Asthma	4 (2.7)	4 (44.4)
Diabetes	4 (2.7)	2 (22.2)
Liver diseases	1 (0.7)	1 (11.1)
No underlying disease	125 (83.9)	2 (22.2)

*: 149 patients; **: 9 patients with *M. pneumoniae* infections detected by PCR.

in our study, *M. pneumoniae* infection was more prevalent in the colder months, and this finding is consistent with other studies (2,5,14). Our result revealed that most of the infections were community-acquired (88.9%) and only 11.1% of patients had nosocomial infection; this result is in agreement with other studies (2,5,9,14). It is interesting to note that *M. pneumoniae* mainly affects people between 21 and 80 years old in this region. This finding is inconsistent with reports from temperate countries, where the infection is most frequent in school-aged children (7,15). The identification of pneumonia was based on fever, dyspnea, tachypnea, cough, and bronchopneumonic infiltration distinguished via X-ray (18). The course of the disease was varied, and cough persisted for several weeks. Overall, the clinical features of infections with *M. pneumoniae* in our cases were mild. However, some researchers have reported that *M. pneumoniae* infections had a poor prognosis, with a few fatal cases (5,7). We found that febrile patients had a mild to moderate fever of 38 °C or less, while a high-grade fever of more than 39 °C was rare. Cough was the most common sign in patients and fever, malaise, dyspnea, and fatigue were found in most patients in this study. Huong et al. reported a fever of less than 38 °C and pharyngitis in all patients, and a dry cough and dyspnea were found in most cases (19). In our study, leukocytosis was also common (67%) and the mean white blood cell count was 10,533 ± 5000 cell/mm³. Clyde showed that *M. pneumoniae* infection has a positive correlation with leukocytosis

(5). Interestingly, 44.4% of the patients suffered from asthma along with *M. pneumoniae* respiratory infection. Waites and Talkington and Nisar et al. reported that *M. pneumoniae* infection may be related to asthma (10,20). Smoking is globally related with pulmonary diseases (21), but the results of this study did not show any significant rise in *M. pneumoniae* infection among cigarette smokers ($P = 0.3$).

In the Azerbaijan state of Iran, there is no national epidemiological surveillance system for *M. pneumoniae* infections; hence, the incidence of this infection was unclear. To the best of our knowledge, this is the first report of *M. pneumoniae* isolation from the northwest of Iran. We found that none of the signs or symptoms were sufficient to differentiate precisely *M. pneumoniae* infection, and *M. pneumoniae* detection must be done by specific laboratory tests. This research has shown that PCR is a highly sensitive test, but the results from the current study suggest that using a combination of tests may provide better diagnostic information.

Acknowledgments

This article was written based on the dataset of an MSc thesis, registered at the Tabriz University of Medical Sciences. We would like to thank the Department of Microbiology and the hospitals of Tabriz University of Medical Sciences.

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