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Motasem Al-Masri

Aseel Thaher

Nael Abu-Hasan



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# Mycoplasma pneumoniae Respiratory Tract Infections in Nablus District


## Abstract

The lack of rapid and standardized tests for diagnosing Mycoplasma pneumoniae (M. pneumoniae) respiratory tract infections is a common problem in many countries. This pro-spective study was conducted to determine the prevalence of M. pneumoniae using classical enzyme-linked immunosorbent assay (ELISA) and nested polymerase chain reaction (PCR) techniques among patients with respiratory tract infections in Nablus District. The study in-cluded 129 patients who visited or were admitted to the involved clinics. At first visit to clin-ical settings, a throat swab was collected from all participants and serum specimens were col-lected from 103 patients. A second serum specimen was available for 16 patients. Throat swab specimens were tested by nested PCR for the detection of M. pneumoniae. Serum samples were tested for the presence of IgG and IgM antibodies by ELISA. Out of 129 exam-ined throat swab specimens, M. pneumoniae was detected by PCR in 15(11.6%) samples. M. pneumoniae specific IgM was detected in 4(3.9%) out of the 103 first serum samples. A total of 47(45.6%) patients possessed IgG in the first and/or second serum specimens. Ac-cording to the applied diagnostic criteria (at least 2 positive tests), 10(7.8%) patients were di-agnosed with current M. pneumoniae infection. The sensitivity of PCR was higher (100%) than that of ELISA-IgM (40%) in the first serum specimen. Most of M. pneumoniae infec-tions were diagnosed during winter (8 out of the 10 cases). The highest percentage of M. pneumoniae respiratory infection (11.3%) was found in patients with the age range 25-64 years. There were no significant differences in the frequency of signs and symptoms in pa-tients with M. pneumoniae infection compared to those with other infectious agents. The frequency of increase in WBCs and granulocytes counts were significantly lower in patients with M. pneumoniae infection ($P = 0.006$ and $<0.001$, respectively) compared to other infec-tious agents. While, frequency of increase in lymphocyte count in patients with M. pneu-moniae infection was significantly ($P=0.001$) higher. In conclusion, M. pneumoniae seems to be an important etiological agent in respiratory tract infections in the area, thus more attention is required in adopting health policy for diagnosis and used medication policies.



## Keywords

ELISA., PCR, Nablus District, Respiratory infection, Mycoplasma pneumoniae

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# *Mycoplasma pneumoniae* Respiratory Tract Infections in Nablus District[†]

*Motasem Al-Masri[1,*]; Aseel Thaher[2] & Nael Abu-Hasan[2]*

[1]Department of Microbiology, Immunology and Pathology, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine; [2] Department of Biology and Biotechnology, Faculty of Science, An-Najah National University, Nablus, Palestine

*Corresponding author: Motasemm@najah.edu

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## ABSTRACT

The lack of rapid and standardized tests for diagnosing *Mycoplasma pneumoniae* (*M. pneumoniae)* respiratory tract infections is a common problem in many countries. This prospective study was conducted to determine the prevalence of *M. pneumoniae* using classical enzyme-linked immunosorbent assay (ELISA) and nested polymerase chain reaction (PCR) techniques among patients with respiratory tract infections in Nablus District. The study included 129 patients who visited or were admitted to the involved clinics. At first visit to clinical settings, a throat swab was collected from all participants and serum specimens were collected from 103 patients. A second serum specimen was available for 16 patients. Throat swab specimens were tested by nested PCR for the detection of *M. pneumoniae*. Serum samples were tested for the presence of IgG and IgM antibodies by ELISA. Out of 129 examined throat swab specimens, *M. pneumoniae* was detected by PCR in 15(11.6%) samples. *M. pneumonia*e specific IgM was detected in 4(3.9%) out of the 103 first serum samples. A total of 47(45.6%) patients possessed IgG in the first and/or second serum specimens. According to the applied diagnostic criteria (at least 2 positive tests), 10(7.8%) patients were diagnosed with current *M. pneumoniae* infection. The sensitivity of PCR was higher (100%) than that of ELISA-IgM (40%) in the first serum specimen. Most of *M. pneumoniae* infections were diagnosed during winter (8 out of the 10 cases). The highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients with the age range 25-64 years. There were no significant differences in the frequency of signs and symptoms in patients with *M. pneumonia*e infection compared to those with other infectious agents. The frequency of increase in WBCs and granulocytes counts were significantly lower in patients with *M. pneumonia*e infection (P = 0.006 and <0.001, respectively) compared to other infectious agents. While, frequency of increase in lymphocyte count in patients with *M. pneumonia*e infection was significantly (P=0.001) higher. In conclusion, *M. pneumonia*e seems to be an important etiological agent in respiratory tract infections in the area, thus more attention is required in adopting health policy for diagnosis and used medication policies.

**Keywords:** *Mycoplasma pneumoniae*, Respiratory infection, Nablus District, PCR, ELISA.

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## INTRODUCTION

The most common reasons for doctor visits are respiratory tract infections and common cold, which result in missed work or school [1]. *M. pneumoniae* infection is associated with mild acute respiratory infections such as sore throat, pharyngitis, rhinitis, and tracheobronchitis, however, it can also cause more critical infections including pneumonia or lung abscess [2, 3].

*M. pneumoniae* respiratory tract infection occurs in persons of all ages, but is most common among children and young adults [4, 5]. *M. pneumoniae* lack a cell wall [2], which makes the bacteria resistant to β-lactam antibiotics, are not stained with Gram stain [6] and makes these organisms pleomorphic [7]. Culture of *M. pneumoniae* from clinical specimens is rarely performed in a routine context because it requires specialized media and its time consuming, where it takes up to 21 days to obtain a visible colony [8, 9].

Patients with *M. pneumoniae* respiratory infection show clinical presentations not significantly different from those of patients with respiratory infections caused by other respiratory pathogens such as *Chlamydia pneumoniae* [8, 10]. Thus, the diagnosis of *M. pneumoniae* respiratory infection depends on laboratory tests. However, culture and Gram stain are not suitable for the diagnosis of *M. pneumoniae* respiratory infection. The combined use of polymerase chain reaction (PCR) and serology for the diagnosis of *M. pneumoniae* respiratory infection was recommended [9].

The incidence of *M. pneumoniae* in respiratory tract infections in Palestine was not evaluated. The present study was conducted to determine the prevalence of *M. pneumoniae* infection among patients with respiratory tract infection in Nablus District on the basis of both serology (IgM and IgG) and PCR. To our knowledge we are the first to study *M. pneumoniae* infections in our region. This study will provide doctors with new information about the prevalence of the *M. pneumoniae* in Nablus District and availability of new diagnostic tools in our region they can use for correct diagnosis.

## METHODS

### *Study design*

In the present prospective cross-sectional study, the patients were selected consecutively among patients who visited or were admitted to participating clinical settings and were diagnosed by physicians to have respiratory tract infection. Patients with evidence of cancer, disorders of the pulmonary or cardiovascular system, and those who had received antibiotics active against *M. pneumoniae* were excluded. Each patient or parents of patient were informed about the research (informed consent letter) before he/she gave their consent. The study proposal was approved by Protocol for Human Subjects Research Institutional Review Board (IRB**)** of An-Najah National University

### *Population*

The study included patients with respiratory tract infections admitted to or visited the participating clinical settings from September 2015 to April 2016. Participated clinical settings included: two governmental hospitals (Al-Watani and Rafidya), the Palestinian Red Crescent Center in town of Asira and An-Najah National University clinic at new campus. All of these clinical settings are in the Nablus district.

A specially designed questionnaire was used for data collection of the concerned patients. The questionnaire was filled by the researcher with the help of the treating physician or an assistant nurse. The questionnaire included a set of questions concerning the following information: age, gender, place of residence, number of family members and smoking status, date of onset of the disease and time of admission to hospital, clinical data (reported symptoms of cough, sputum production, vomiting, abdominal pain, headache, difficulty of breathing, shortness of breath and fever $\geq 38\ ^{\circ}C$) and available laboratory findings such as CBC

### *Sample collection*

At the time of hospital admission or first visit to the clinic, from each patient, a throat swab specimen was obtained with the help of a doctor or a nurse. Medical stuff was asked to provide the first serum specimen. Second serum specimens were taken after 7-15 days after initial sample collection when possible. Throat swab specimens were placed in 2ml normal saline and immediately placed on ice. Samples were then transported to the laboratory and placed in sterile Eppendorf tubes and stored at -20°C until the time of examination.

The serum specimens were placed in Eppendorf tubes and kept at -20°C until examination. In case of the presence of 2 serum specimens, the first and second serum specimens were examined together.

### *DNA extraction and polymerase chain reaction for detection of M. pneumoniae in throat swabs*

DNA was extracted by a simple boiling method as described by Waring *et al*. [11]. Aliquot of the sample lysate was then used directly for PCR amplification or kept at -20°C until use.

DNA extractions from throat swab samples were tested for *M. pneumoniae* by nested

PCR. The DNA sequences of the used primers targeting the P1 adhesion were obtained from Lam *et al*. [12]. PCR Ready mix (SIGMA-ALDRICH, USA) was used and PCR was performed with a total reaction mixture volume of 25µl. The mixture contained 1x PCR buffer [1.5 units Taq DNA polymerase, 10mM Tris-HCl, 50 mM KCl, 1.5 $MgCl_2$, 0.001% gelatin, 0.2mM deoxynucleoside triphosphate (dNTP)]. A concentration of 0.8pmole/µl was used for each of the used primers.

PCR reaction was performed using AmpliTaq Gold (Tprofessionla Biometra thermocycler, Germany). First and second rounds of nested PCR were the same and carried out as follows: one cycle of three minutes at 94°C (for denaturation), 35 cycles of amplification (one minute at 94°C [for denaturation] and one minute at 64°C [for annealing] and one minute at 64°C [for extension]), and final extension of 10 minutes at 72°C. A negative control with no template was included and specimens known to be positive were used as positive control in each PCR run. PCR products were detected by 2% agarose gel electrophoresis with ethidium bromide staining and the bands were visualized under UV light.

### *Detection of IgM and IgG antibodies against M. pneumoniae*

Enzyme-Linked Immunosorbent Assay (NovaTec Immundiagnostica GmbH, Germany) was used for the determination of IgM and IgG antibodies in serum specimens of the patients. The procedure was carried out according to the manufacture instructions. Results were calculated in Nova Tec Unit (NTU) and interpreted according to manufacturer instructions. The cut-off value was first calculated, which was equal to the mean of the absorbance value of the 2 wells to which cut-off control was applied. Nova Tec Unit equal to the patient absorbance value × 10 ÷cut-off. The interpretation of the results using this unit was negative when NTU was smaller than 11 and positive when it was bigger than 11.

### *Diagnosis of M. pneumoniae infection*

In the present study, the laboratory diagnosis of *M. pneumoniae* infection was carried out similar to what was previously described [9]. Current or recent infection of *M. pneumoniae* was definitely diagnosed if at least two of the following criteria were found:

1. positive PCR in throat swab specimen
2. positive IgM antibodies (in the first and/or the second sample)
3. seroconversion or significant increase of IgG antibodies (twofold increase), or IgG titers >40 Nova Tec Unit

Probable cases of recent or current *M. pneumoniae* infection were diagnosed when only one of the three diagnostic criteria mentioned above was found.

### *Statistical analysis*

A specialized statistician has performed statistical analysis. SPSS version 21 for Windows was used for comparison of the prevalence of *M. pneumoniae* infections between genders, different age ranges, and seasons. In addition, the frequencies of clinical and laboratory parameters in patients with *M. pneumoniae* infections were compared with those without such infection. In the statistical analysis, Chi-square and Fisher's exact were used for the comparison of frequencies in different groups, while t-test was used for mean comparison in different groups. P value < 0.05 was considered significant.

## RESULTS

### *Patients and specimens' collection*

The study included 129 patients admitted to or visited one of the participating clinical settings with respiratory tract infection symptoms (diagnosed by physicians) from September 2015 to April 2016. The patients were inpatients and out patients at Al-Watani National Hospital (n=70), Rafidya Hospital (n=11), out patients of An-Najah National University clinic (n=18), and out patients in Red Crescent center in Asira (n=30). All of these clinical settings are in Nablus district. Throat swabs were obtained at the time of admission or first visit from all of the 129 patients, and the first serum specimens were obtained from 103 patients. Second serum specimens were obtained from 16 of 103 patients 7 to 15 days later after the first serum sample collection.

### *Results of PCR and Serology*

Table 1 shows the results of the examined throat swab specimens by nested PCR and serum specimens by ELISA (IgM and IgG). Among the 129 throat swab specimens, DNA of *M. pneumoniae* was detected in 15(11.6%) patients. ELISA assays for the detection of IgM and IgG were carried out for 103 patients. *M. pneumoniae*-specific IgM was detected in 4(3.9%) of the first serum samples. Collected second serum (16) showed 2 additional IgM positive samples. Therefore, 6(5.8%) of the involved patients had positive IgM in the first and/or second serum specimen.

In the first serum specimen of 103 patients, IgG antibody was detected in 47 (45.6%), while in the collected (16) second serum specimen, IgG antibodies were detected in 11(68.8%) cases. No seroconversion was detected (negative IgG in the first and positive IgG in second), thus the patients with positive IgG in the first and/or in the second serum specimen were 47 (45.6%).

**Table (1):** PCR and ELISA (IgG and IgM) findings in tested samples.

| Patients with positive results in 1st &/or 2nd [a] serum specimens No. (%) | Positive results in 2nd serum specimens No. (%) | Positive results in 1st a Serum specimens No. (%) | Positive PCR No. (%) | No. of the examined specimens No. (%) | Assay |
|---|---|---|---|---|---|
| - | - | - | 15(11.6%) | 129 | **PCR** |
| 6(5.8) | 2 (12.5)[c] | 4(3.9) | - | 103+16[b] | **ELISA-IgM** |
| 47(45.6) | 11(68.8) | 47(45.6) | - | 103+16 | **ELISA-IgG** |

[a] 1st , First serum sample; 2nd , second serum sample
[b] 103 first serum sample and 16 second serum sample
[c] percentage calculated using 16 second serum sample

### *Patients diagnosed with M. pneumoniae infection*

According to the diagnostic criteria of *M. pneumoniae* current infection, mentioned in the methods, 10 (7.8%) patients were diagnosed with the infection. Table 2 shows the PCR, IgM and IgG results of the 10 diagnosed cases. *M. pneumoniae* bacterium was detected by PCR in throat swabs in all of these 10 patients diagnosed with *M. pneumoniae* current infection, indicating high sensitivity of PCR. IgM antibodies were detected in 4 cases (3.9%) in the first serum sample. Seroconversion of IgM antibody (negative IgM in the first and positive IgM in second) was detected in one patient indicated by number 9 in Table 2. IgG antibodies were positive in all of the 10 cases in the first serum sample. A significant increase (twofold increase) in IgG antibodies was detected in one case (patient number 9). PCR appears to be more sensitive (100%) compared to IgM in the first serum specimen (40%).

Eight (7.7%) patients did not meet the diagnostic criteria of current *M. pneumoniae* infection and were considered as probable cases. Among these patients, 5 cases (number 11-15) had positive PCR without serology confirmation. Seroconversion of IgM was detected in one case (patient 16) with negative PCR and no significant increase in IgG titer. In 4 cases (number 11, 12, 17, 18) IgG antibodies were detected in 4 first serum samples.

Evidence of possibly previous *M. pneumoniae* infection was detected in 33(25.6%) patients who were negative by PCR and negative for IgM in the first and in the second samples, but IgG antibodies were positive in the first or/ and the second samples.

**Table (2):** Patients diagnosed with current, probable, or possible previous *M. pneumoniae* infection.

| Sample number | PCR | First serum sample | | Second serum sample | |
|---|---|---|---|---|---|
| | | IgM (NTU) | IgG ( NTU) | IgM ( NTU) | IgG ( NTU*) |
| Patients with current *M. pneumoniae* infection | | | | | |
| 1 | P* | N* | P(43) | NA* | NA |
| 2 | P | N | P(54) | NA | NA |
| 3 | P | P (28.3) | P(97) | NA | NA |
| 4 | P | N | P(102) | NA | NA |
| 5 | P | P(12.6) | P(15) | NA | NA |
| 6 | P | N | P(50) | NA | NA |
| 7 | P | N | P(83) | NA | NA |
| 8 | P | P (23.6) | P(12) | NA | NA |
| 9 | P | N | P (27) | P (15) | P(74) |
| 10 | P | P (35) | P (15) | NA | Na |
| Patients with probable *M. pneumoniae* infection | | | | | |
| 11 | P | N | P(28) | NA | NA |
| 12 | P | N | P(15) | NA | NA |
| 13 | P | N | N | NA | NA |
| 14 | P | N | N | NA | NA |
| 15 | P | N | N | NA | NA |
| 16 | N | N | P(34) | P(13) | P(31) |
| 17 | N | N | P(49) | NA | NA |
| 18 | N | N | P(40) | NA | NA |
| Patients with evidence of possible *M. pneumoniae* previous infection | | | | | |
| 19-26 | N | N | P(15-28) | N | P(15-30) |
| 27-51 | N | N | P(12-37) | NA | NA |
| Patients with no evidence of *M. pneumoniae* infection | | | | | |
| 52-57 | N | N | N | N | N |
| 58-129 | N | N | N | NA | NA |

*P, positive; N, negative; NA, not available; NTU, Nova Tec Unit.

No evidence of *M. pneumoniae* infections by any of the applied methods (both PCR and ELISA) were found in 78(60.5%) of the studied cases.

### *M. pneumoniae infection in relation to different factors*

The infection rate in males (8.6%) was found to be insignificantly (P =0.753) higher than females (7.1%). Patients' age included in the present study ranged from 1 to 91 years with a mean age of 32 years.

The highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients with the age ranged 25-64 years, which was insignificantly higher than that of patients ranging 0-9 years (9.1%, P=0.663) and 10-24 years (7%, P=0.383). Furthermore, *M. pneumoniae* infection was not diagnosed in 12 patients with age ≥ 65 years.

Table 3 shows the frequency of clinical signs and symptoms reported in patients with or without *M. pneumonia*e infection. No significant difference was found in the compari-

son of frequencies of signs and symptoms between patients with *M. pneumonia*e infection and those with other causatives of respiratory infections, however, some variations were noted. Cough, sputum production, fever, and abdominal pain were insignificantly higher in cases diagnosed to have *M. pneumonia*e infection. On the contrary, headache, difficulty of breathing, shortness of breath, vomiting, and average days of disease onset were insignificantly lower in patients with *M. pneumonia*e infection.

**Table (3):** Signs and symptoms among patients with or without *M. pneumoniae* infection.

| Signs and symptoms | *M. pneumoniae* positive (n= 10) No. (%) | *M. pneumoniae* negative (n=119) No. (%) | P value |
|---|---|---|---|
| Cough | 10 (100) | 110 (92.4) | 0.287 |
| Sputum production | 8(80) | 93 (78.2) | 0.212 |
| fever ≥ 38 °C | 6(60) | 54 (45.4) | 0.367 |
| Abdominal pain | 4(40) | 28 (23.5%) | 0.627 |
| Headache | 4(40%) | 76(63.9%) | 0.563 |
| Difficulty of breathing | 8(80%) | 98(82.4%) | 0.602 |
| Shortness of breath | 8(80%) | 101(84.9%) | 0.653 |
| Vomiting | 2(1.6%) | 27(20.9%) | 0.125 |
| Average days of disease onset | 6.11± 2.83 | 6.7± 2.45 | 0.526 |

A comparison of means of parameters of complete blood count (CBC) among patients with *M. pneumoniae* infection and among those without this infection was carried out. In patients with *M. pneumoniae* infection, means of WBC count (7± 3.3), lymphocyte percentage (28.5± 13.5), granulocytes percentage (68.2±14.3) and platelets count (278.3±88.9) did not differ significantly from means of those patients without *M. pneumoniae* infection, where means of WBC count, lymphocyte percentage, granulocytes percentage and platelets count were 7.4± 3.2, 28.9±13.7 , 67.4±14.2 and 280.1±70.3, respectively.

Results of the CBC were compared to normal ranges in different ages using reference ranges [13, 14] to determine if there were any increases in the CBC values as shown in Table 4. In patients with infection by respiratory pathogens other than *M. pneumoniae*, the rates of increase of WBC (8.4%) and of granulocytes (40.3%) were significantly higher (P= 0.006 and <0.001, respectively) than that in patients with *M. pneumonia*e infection. On the contrary, the percentage of increase in lymphocytes among patients diagnosed with *M. pneumonia*e infection (20%) was significantly higher than that in patients infected by other respiratory pathogens (15.9%).

**Table (4):** Frequency of increase in CBC parameters in patients with *M. pneumoniae* infection and among those with other infectious agents.

| Parameters | *M. pneumonia positive N=10* No. (%) | *M. pneumoniae* negative *N=119* No. (%) | P value |
|---|---|---|---|
| Increase WBC count | 0(0) | 10 (8.4) | 0.006 |
| Increase lymphocyte | 2(20) | 19(15.9) | 0.001 |
| Increase granulocytes | 3(30) | 48(40.3) | <0.001 |
| Increase platelets count | 1(10) | 6(5.1) | 0.133 |

Among the 10 patients with *M. pneumoniae* infection, 7 (70%) were clinically diagnosed with upper respiratory tract infection and the rest 3 cases (30%) were diagnosed as bronchitis cases. The frequency of upper res-

piratory tract infection caused by *M. pneumoniae* (70%) was close to that caused by other pathogens (72.3%). However, bronchitis rate in patients infected by *M. pneumoniae* (30%) was insignificantly (P=0.334) higher than those infected by other pathogens (18.5%). On the contrary, frequency of pneumonia caused by *M. pneumoniae* (0%) was insignificantly lower (P=0.605) than other infectious agents (9.3%).

*M. pneumoniae* infection frequency was highest (10.4%) during winter followed by spring (4.5%), and no cases were detected during autumn. These variations were insignificant (Table 5).

**Table (5):** Seasonal distribution of *M. pneumoniae* infection.

| Non- *M. pneumoniae* No. (%) | *M. pneumoniae* infection No. (%) | Total number | Season |
|---|---|---|---|
| 69(89.6) | 8 (10.4) | 77 | Winter |
| 42(95.5) | 2(4.5) | 44 | Spring |
| 8(100) | 0 (0) | 8 | Autumn |

Among the included 129 patients, 15 had diabetes mellitus, 2 had anemia, 20 had hypertension, and 22 were smokers as shown in Table 6. No significant association between *M. pneumoniae* infection and smoking (P=0.230), anemia (P =0.85), hypertension (P =0.520), diabetes mellitus (P = 0.762) or number of siblings (P = 0.879) were found. The finding of a limited number of patients diagnosed with *M. pneumonie* infection makes it difficult to link such risk factors to this infection; however, it is worth noting that smoking status (13.6%) is a more pronounced risk factor in *M. pneumoniae* infection compared to other studied risk factors.

**Table (6):** Risk factors for acquiring *M. pneumoniae* infection.

| Risk factor | No. positive | *M. pneumonia No.(%)* | *Non M.pneumoniae No.(%)* | P Value |
|---|---|---|---|---|
| Smoking | 22 | 3(13.6) | 19(86.4) | 0.230 |
| Hypertension | 20 | 1(5) | 19(95) | 0.520 |
| Diabetes mellitus | 15 | 1(6.7) | 14(93) | 0.672 |
| Mean No. siblings | 129 | 4.5±1.1 | 4.7±1.4 | 0.762 |
| Anemia | 2 | 0(0) | 2(100) | 0.850 |

## DISCUSSION

Difficulty and high costs of diagnosis of *M. pneumonia*e infection by laboratory methods hinder the determination of its prevalence in most third world countries. To our knowledge, the present study represents the first determination of the prevalence of *M. pneumonia*e in respiratory tract infections in adults and children in Nablus district. For more accurate diagnosis of *M. pneumonia*e infections, a combination of PCR and serological testing using ELISA assay for the detection of IgM and IgG specific antibodies were used.

In the present study, 10 patients (7.8%) were diagnosed with current *M. pneumoniae* respiratory tract infection and 8 patients were diagnosed to be probably infected with M. *pneumoniae*. In a study carried out in Malaysia during 2011, a close percentage of M. *pneumoniae* infection rate (6.5%) was determined by serology among 17–80 years-old patients with community acquired pneumonia [15]. Similar findings were also reported from Iran in a study conducted during 2009 to 2010, where 6.15% of patients were diagnosed with *M. pneumoniae* respiratory tract infections based on PCR, culture and serology findings [16]. A higher frequency (21.6%) of *M. pneumoniae* in respiratory tract infections was reported by a study carried out in 12 teaching hospitals in Beijing during 2010 to 2011 for adult and adolescent patients (≥ 14 years of

age) with radiographically confirmed community acquired pneumonia (CAP). The findings of this study were based on IgM antibody testing, florescent quantitative PCR, and culture [17]. In a previous research carried out in Greece, *M. pneumoniae* infection was diagnosed as the only pathogen in 11.1% of cases of children with respiratory tract infection [10]. Variations in the frequency of *M. pneumoniae* infection rate in the respiratory tract in different geographical regions are expected to occur due to seasonal climate differences in temperature, geographic features, applied diagnostic methods, types of collected specimens, population spread on area, and antibiotics application policy [18].

In the present study, all diagnosed cases of *M. pneumoniae* infection were PCR positive and only 4(40%) were positive by IgM ELISA in the first serum specimen. Thus, the sensitivity of nested PCR (100%) was higher than that of serological testing of IgM (40%) in the first serum specimen. In another study, *M. pneumoniae* IgM assay determined by capture ELISA was found to show a sensitivity of 66.7% in the first serum sample and conventional PCR sensitivity was 75% [9]. Variations in sensitivity of various testing techniques could be due to variations in the sensitivity of ELISA kits or PCR, sample type, and patient age (IgM more sensitive in children).

In the present study, the highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients within the age range of 25-64 years. This was followed by age range 0-9 (9.1%) and 10-24 (7%). In 2015, Keping Chen et al. [19] reported that *M. pneumoniae* infection was the most predominant (40.8%) in school-aged children 7-14 years. However, a very low prevalence of *M. pneumoniae* infection rate (1%) based on RT-PCR was found among Iranian children with acute respiratory infections during 2003 to 2004 [20]. During 2010-2015, a study in England found *M. pneumoniae* infection is predominantly found in children and adults <44 years of age [21]. Variations in the frequency of this infectious bacterial agent among different age groups may be due to the frequency of close contact with infected individuals as well as the development or decline of immune status in relation to age.

In the current study, the infection rate was highest during winter (10.4%) followed by spring (4.5%). It was reported that *M. pneumoniae* infection occurs most commonly during fall and winter [18]. On the contrary, a study in China reported that *M. pneumoniae* infection was relatively high in summer and autumn (45.08% and 47.14%, respectively) and relatively low in spring (38.3%) and winter (35.5 %) [19].

In our study, all of the 10 patients with *M. pneumonia*e infection had cough, thus coughing was the most common symptom. No significant difference was found in signs and symptoms between patients with *M. pneumonia*e infection and with other causatives of respiratory tract infections. Consistent with our findings on associated symptoms, a previous study reported fever and cough to be the most common symptoms (both 84%) in patients with *M. pneumoniae* infection [10]. Medjo et al. [3] found that cough was more frequent in children with *Mycoplasma pneumoniae* pneumonia compared to children with pneumonia caused by other pathogens.

In the present study, patients with infection by respiratory pathogens other than *M. pneumoniae* possessed rates of increase of WBC (8.4%) and of granulocytes (40.3%) significantly higher (P= 0.006 and <0.001, respectively) than those in patients with *M. pneumoniae* infection. On the other hand, the percentage of increase in lymphocytes among patients diagnosed to have *M. pneumoniae* infection (20%) was significantly higher than that in patients infected by other respiratory pathogens. It was reported in Beijing-China [17] that the mean of lymphocytes was 19.6±10.64 in patients with *M. pneumoniae* infection, which is insignificantly (P=0.614) higher than in patients without *M. pneumoniae* infection (18.5 ± 7.14). The lower frequency of increase of WBCs among patients infected by *M. pneumoniae* may be due to the lower antigenic stimulation caused by *M. pneumoniae* as this bacterium is the smallest free live one and possesses a very low division rate.

Among the investigated risk factors in the present study, the highest frequency of association with *M. pneumoniae* respiratory infection was smoking (13.6%). Similar findings

were reported by [22] as smoking was reported to be strongly associated with *M. pneumoniae* respiratory infections.

Two limitations of the present study should be mentioned. The first limitation is number of confirmed cases is small and consequently may limit the value of statistical analysis results. The second limitation is the small number of second serum specimens. Although we tried hard to obtain second serum specimens, many patients refused to come again after the recovery of their health for the second serum specimen. However, this problem is not expected to affect the results of the study to a considerable level because the results also depend on PCR. Nested PCR technique is very sensitive in detecting the pathogen in the acute phase before the development of antibodies. In our results, only 5 cases out of 129 (3.9%) were PCR positive without detecting a diagnostic immune response and these were called probable cases of infection.

In summary, *M. pneumoniae* plays an important role as an etiological agent of respiratory tract infections in Nablus district. *M. pneumoniae* respiratory infection rate was highest in the age range of 25-64 years. Most cases of *M. pneumoniae* respiratory infection were diagnosed during winter followed by spring. With the exception of an increase in lymphocyte percentage, symptoms, and signs appear to have a limited diagnostic value of *M. pneumoniae* infection.

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**Competing interests**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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