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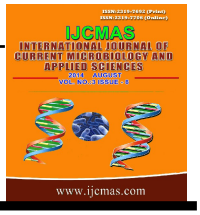

Original Research Article

Serological and Molecular detection of *Mycoplasma pneumoniae*

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Keywords

Mycoplasma pneumonia, RT-PCR, ELISA

A B S T R A C T

Mycoplasma pneumonia is one of most commonest infectious agents for respiratory system that infect all ages groups of both sexes. This study was conducted to compare the serodiagnosis test depended on IgM measured by an indirect enzyme linked immunosorbent assay (ELISA) and real time polymerase chain reaction(RT-PCR) for detection of *M.pneumoniae* infections. There are two type of specimens were collected from patients included sera and throat swabs for 127 patients admitted to the hospitals in period from January to June 2013.The ELISA and RT-PCR techniques were used for the diagnosis of *M. pneumonia* in clinical specimens. Forty two samples (33.07 %) were positive for *M.pneumonia*e IgM. Whereas 19(14.96%) were positive by RT-PCR. Serodiagnosis appear to be more sensitive for detection of *M. pneumoniae* than molecular diagnosis.

Introduction

Mycoplasma pneumoniae; the smallest self-replicating organism, was identified as the causative of primary atypical pneumonia in early 1960s. Since then, *M. pneumonia*e has become one of the most common pathogens of community acquired pneumonia in children and young adults. *M. pneumoniae* can be transmitted through aerosols in typical outbreak settings in which close atypical contact exists (Joo *et al*., 2012)[1]. Around 25% of people infected with *M. pneumoniae* may experience extra pulmonary complication and host responses that develop after *M. pneumoniae* infection likely contribute to autoimmunity. *M. pneumoniae* can be expected on average every 3-7 years but at any given time may account for as many as 40% of community acquired pneumonia cases[2,3]. *M.pneumoniae* infection cannot be distinguished clinically from infections caused by viral or other bacterial agents. Thus a rapid and accurate laboratory diagnosis is relevant for an effective therapy and for limiting the spread of infection in the community[4,5].A specific diagnosis is important because β-lactam antibiotics used empirically in the treatment of these infections are ineffective. Because of the fastidious nature of the *M.pneumoniae*, culture methods are relatively insensitive, time consuming, expensive and are successful in only 30-60% of the confirmed diagnosed cases[6,7].

Due to the vast reduction in time in comparison with culture, PCR has been used increasingly for *M. pnemoniae* detection. Difficulties exist in the detection of etiologic agents, including *M. pneumoniae* for lower respiratory tract infections in children (especially younger children) with regard to adequate sampling of respiratory materials for pathogen culture and polymerase chain reaction (PCR), and the need for paired blood sampling for serologic tests. In addition, it is known that in some patients, the diagnostic antibodies are not detected in the early stage of *M. pneumoniae*infection[7]. Thus this work aimed to check the sensitivity and specificity of ELISA technique in the diagnosis of *M. pneumonia* depending on the golden standard test which is RT-PCR.

Materials and Methods

Clinical specimens: A total of (127) specimens were collected from patients admitted to Al-Diwaniya Maternity and pediatric Teaching Hospital and patient come to Chest and Respiratory Consultant Clinic Diseases Center which suffering from respiratory infections from January 2013 to May 2013.The specimens include throat swab and 2-3 ml freshly drawn blood .Swabs were placed in 2 ml phosphate buffer saline(P.B.S) and put in ice bag until be taken to the laboratory for extracted DNA immediately or store at -20ºC for other time. Blood samples were collected in gel tubes and stored at room temperature for 20 minutes to separate serum by centrifugation (Lewis *et al*., 2001)

Serodiagnosis of *M. pneumoniae*: Serum specimens which were collected in acute phases from patients were used to detect IgM antibodies against *M. pneumoniae* by commercial ELISA kit (Vircell/ Spain) according to the manufacturer's instructions.

Molecular diagnosis of M. pneumonia

DNA extraction from throat swab

DNA samples were extracted directly from clinical specimens for throat swabsusing genomic DNA purification kit supplemented byQIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Measurement of extracted DNA concentration andpurity was done using a nanodrop to estimate the concentration and purity of the extracted DNA according to the manufacturer's instructions.

Real time PCR reaction assay

Forty two extracted DNA from patients were giving positive results in IgM ELISA test were involved in this test.Amplification of DNA was carried out in a final volume 50µl as following (Table 1). The procedure for detection of *M.pneumoniae* by RT-PCR was done as following Table.2.

Results and Discussion

Serodiagnosis of *M.pneumoniae*

It was shown that 42 (33%) of (127) serum specimens give positive results for *M.pneumoniae* infection. The results revealed that no significant differences ($p<0.05$) in the percentage of *M.pneumoniae* infections among age groups table (3).

Serology is probably the most frequently method used in diagnosis of *M. pneumoniae* infection due to isolation of *M. pneumoniae* from atypical bacterial pneumonia requires 2-4 weeks which limits clinical usefulness[4].

Table.1 Content of the reaction mixture for polymerase chain reaction

Composition of RT-PCR reaction mixture		**Reaction volume**
Sample	Non-template control (NTC)	5 µl (PCR grade water)
	MP positive control DNA clinical sample	5 µl
Internal positive control		1 µl
PCR grade water		44 µl
Total		50 µl

Table.2 Protocol of *M.pneumpniae* amplification

Step	**Temp.**	**Running time**
Line 1: Pre-denaturation	**95°C**	**10 minutes**
Line 2: Denaturation	**95°C**	**20 seconds**
Line 1:Annealing & Extension	**55°C**	**30 seconds**
Scan	**FAM: Target / TAMRA Internal positive control**	
Go to	**Line 2, 40 Cycle**	

Table.3 The seropositive cases of *M. pneumoniae* infection from acute respiratory infection using IgM ELISA test according to age

Age (year)	Number of examined cases	number of positive cases	Percentage(%)
>5	59	15	11.8
<5	68	27	21.2
Total	127	42	33
X^2 = 2.911, P value = 0.087 non- significant			

Table.4 The positive cases of *M. pneumoniae* according seasons

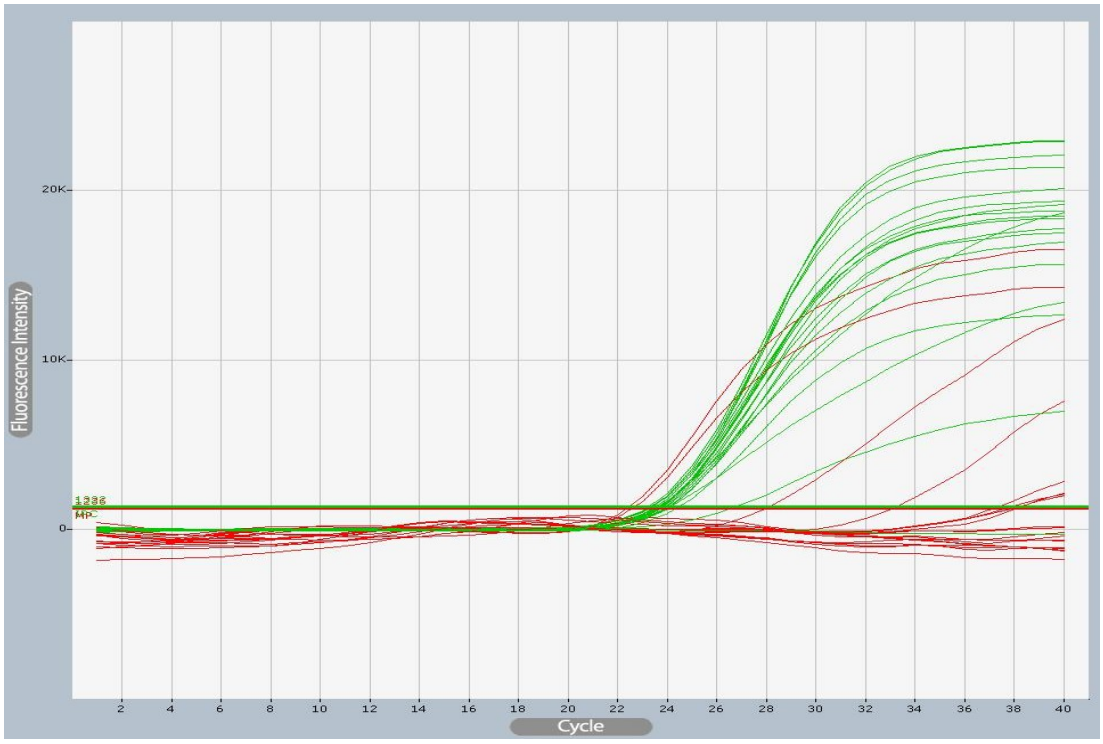
Month	**Number of examined cases**	**positive cases**	**%**
January	28	7	25
February	40	13	32.5
March	17	4	23.5
April	18	9	50
May	24	9	37.5
Total	127	42	33

X^2= 4.073, P value = 0.396, non-significant P>0.05

Table.3-3 Positive results of *M. pneumoniae*according ELISA andRT-PCR tests.

test	No. of cases	No. of positive	%	X^2 =7.03 P value = 0.008 Significant
IgM ELISA	127	42	33	
RT-PCR	127	19	14.96	

Figure.1 Detection of *M.pneumoniae* DNA by RT-PCR in which the green curves represent internal positive control (TAMRA) while red curves represent target (FAM) .There are 6 positive sample in which the curve.



The results of the current study are agreement with Mattie *et al.* about the age of infection, in which 27% of the age of the patients diagnosed as having *M. pneumoniae* was < 5 years[8]. This observation is in contrast to the published Canadian guidelines for diagnosis and management of community acquired pneumonia, which recommend detection of *M. pneumoniae* IgM only in children less than five years. Also, other studies have shown that *M. pneumoniae* infections in young children are detectable by IgM serology[9]. Other study in Thailand indicated that the most positive titer were found in patients 5-9 years followed by patient 1-4 years[10,11,12].

The seropositivity in age group less than five years with asymptomatic cases in contrast to the seropositivity associated with symptomatic cases in large age group

may be attributed to the immunopathalogical effect of immune response to M. pneumonia that is well developed in large age groups than small age groups.

Moreover, seropositive cases were classified according to the seasonal months. The infection with *M. pneumoniae* occurred throughout the year, but the infection rate reaches a maximum in spring months, the peak number of *M. pneumoniae* infection was in April and May (50%,37.5%) respectively (table 4).

This result is agreement with study carried out in China that refers to the rate of infection in Spring more than Winter (11% and 7.5% respectively)[2].

Molecular diagnosis of *M. pneumoniae*

Among 127 throat swabs (19) samples (14.96%) give positive result by using RT-PCR technique table 3-3 & figure.1.

A high serologic titer can be a clue for presence of *M. pneumoniae* confirmed by RT-PCR. From 42 cases that were positive in ELISA, only 13 (30.95%) give positive in RT-PCR. Whereas, only six (7.05%) cases were RT-PCR among 85 seronegative cases. These may be due to difficult obtained adequate specimen especially from children that used in DNA extraction because of difficult sampling or the bacteria were eradicated from the body after antibiotic treatment while the IgM persist for several weeks after infection.

As reported by Thurman *et al*.,[13] the sensitivity of the RT-PCR assay reduces with the delay in collection of samples from the onset of diseases**.** The results of the present work were agreement with Rama *et al*., [14] when they reported sensitivity and specificity of RT-PCR compared to serology assay (ELISA) was 34.6 and 84.2 per cent, respectively**.** Also the results of this study found that the PCR assay have a lower sensitivity than serology and this agreed with Menendez *et al*. [15] when he found of 12 serological positive only 3 were positive by PCR.

In conclusion the low sensitivity of the PCR leads to recommend the use of both serology and RT-PCR in parallel to confirm *M. pneumonia*e infection.

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