Easy and rapid diagnosis of Mycoplasma pneumonia: is it possible?

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Background Atypical pneumonia (AP) with its different pathogens comprises a reasonable ratio of community-acquired pneumonia. *Mycoplasma pneumoniae* (*M. pneumoniae*) constitutes a known pathogen causing AP with pulmonary and extrapulmonary symptoms that necessitate early diagnosis and treatment. Serology and culture give diagnosis but after few days of infection onset.

Aim Study the incidence of *M. pneumonia* using PCR and relation to clinical symptoms.

Settings and design Comprehensive, prospective study.

Materials and methods A total of 80 patients with suspected AP were examined for clinical symptoms and signs such as cough, crepitations, arrhythmia and conscious level, and sputum was investigated using PCR for *M. pneumoniae*. Those with dry cough were subjected to fiberoptic-bronchoscopic bronchoalveolar lavage and the fluid was examined by PCR.

Statistical analysis Data were analyzed with the SPSS 22 software package.

Results Using the PCR method; *M. pneumonia* was 42%, mostly by bronchoscopic lavage because of dry cough, with significant correlation to arrhythmia, disturbed consciousness, and positive radiologic infiltrations (74, 65,76%, respectively).

Conclusion PCR is considered a highly specific diagnostic method for *M. pneumonia*. AP incidence is high in our region with special consideration to *M. pneumonia* as a causative agent with high percentage.

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Introduction

Atypical pneumonia (AP) comprises a reasonable ratio of pneumonia requiring hospital admission because of unusual presentation and complications [1,2]. It is 'atypical' because of unusual causative organisms and atypical clinical picture [3].

It previously included *rickettsia*, viruses, and fungi [4], but by time it became restricted to three pathogens: *Mycoplasma pneumoniae* (*M. pneumoniae*), *Chlamydophila pneumoniae*, and *Legionella pneumophila* [3].

AP represents a rising cause of pneumonia either single [5,6] or mixed with other bacteria [7] and the highest percentage of AP is caused by *M. pneumonia* [6,8].

Patients with *M. pneumonia* may complain of dry cough, dyspnea, exacerbation of chronic obstructive pulmonary disease [9], and otitis media [10] in addition to nonspecific symptoms such as myalgia, heart affection [6] and leukopenia [11], all of which may aggravate disease severity.

Accurate diagnosis of *M. pneumonia* rarely depends on the clinical picture [12], but necessitates other diagnostic methods like culture and serological confirmation. However, they are time consuming and require complicated techniques [13] that limit their commercial use [14].

It is crucial to diagnose the causative pathogens early, rapidly, and accurately to avoid unnecessary antibiotic use and to treat and protect the patient from serious complications [15].

Real-time PCR is a recent technique that varies in reliability according to age, disease severity, and specimens' type [16,17]. It is a rapid technique with high sensitivity [18].

Aim

The aim was to evaluate the incidence of *M. pneumoniae* in patients with AP using real-time PCR in addition to its relation to clinical picture.

Patients and methods

In this comprehensive prospective study, 80 nonredundant clinical specimens were collected between January 2017 and January 2018, from the Chest Department, Tanta University Hospital of Egypt.

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Inclusion criteria: in the form of suspicion of AP infection and clinical and radiological data in the form of low-grade fever, cough with scanty sputum, cardiac arrhythmia, increased heart rate, wheeze, crepitations, pleural rub, chest radiography patchy infiltrations with absent consolidation or pleural effusion [19–21].

Patients were excluded from the study if they were on mechanical ventilation, with lung tumor, tuberculosis, or hospital-acquired pneumonia (infection after 48 h of admission).

All patients were subjected to complete history taking, full clinical examination, general and local chest examination, routine laboratory investigation, for example, complete blood picture, erythrocyte sedimentation rate, C-reactive protein for collected samples and chest radiology [chest radiography and computed tomography (CT) when needed].

Sample collection

(1) Sputum samples: patients sit upright or at 45°, inspired deeply and hold and then coughed with expectoration into a clean container.
(2) Patients with dry cough were subjected to fiberoptic bronchoscopy (PENTAX EB-1970UK Europe GmbH Julius Vosseler Strasse 104, Hamburg, Germany) and bronchoalveolar lavage: under complete aseptic technique; bronchoscope was introduced until peripheral bronchioles, 30–50 ml of sterile saline was injected throughout. Saline was aspirated and collected in a sterile plastic container with a firmly fitted cover.

All samples were located in tubes containing PBS, vortexed and then stored at −20°C until processed [22].

Processing

Quantitative real-time PCR was done for *M. pneumoniae* in different respiratory samples using Microbial DNA QPCR assay kit (*M. pneumoniae*) (catalog no. 330033; QIAGEN Sciences, Germantown, USA). Sensitivity and specificity of real-time PCR assays in the detection of *M. pneumoniae* is 100%. As reported by Templeton *et al.* [23], the sensitivity of the real-time PCR assay reduces with the delay in collection of samples from the onset of the disease.

(1) Nucleic acid extraction from 200 µl of the specimen was done. The volume was reduced to 100 µl, and 7 µl aliquots were stored at −70°C.
(2) Purification: DNA concentration was measured at 260 nm and was greater than 10 ng/ml and the A260/A280 ratio was greater than 1.8.
(3) Preparation of master-mix: for each sample, four separate PCR reactions were prepared, including controls for positive PCR control, no template control, microbial DNA positive control, and microbial DNA QPCR assay.

The kit was utilized to calculate the load of *M. pneumoniae* by targeting the cytadhesin P1 gene (130bP) which is particular for *M. pneumoniae*.

Real-time PCR primers for P1 cytadhesin gene of *M. pneumoniae*:

Forward primer: CCAACCAAACAACAACGT TCA.

Reverse PRIMER: TAACGGCAACACGTAATC AGGTC.

Total volume per sample was 25 ml. The PCR mixture consisted of 12.5 ml microbial qPCR 12.5 ml, 1 ml microbial DNA qPCR assay, 5 ng genomic DNA sample, and variable amounts of microbial DNA-free water.

Plate preparation

After vortexing for 1–2 s (for mixing), the mix was put into the PCR wells. A plastic PCR plate was sealed with an adhesive optical cover and was inserted into the PCR instrument.

Performing real-time PCR

(1) Cycling condition: amplifications were done using a Light-Cycler (step 1; Applied Biosystem, Foster City, California, USA).
(2) The threshold (CT) was calculated for each well using the cycler's software (Roche Diagnostics Ltd, Forrenstrasse, CH-6343 Rotkreuz, Switzerland).

Standard curve generation

With absolute standard curve, the copy number of the target was known. The template was accurately quantified and the sample is accurately determined to contain 1×10^{11} copies and diluted 10-fold eight times down to 1×10^{3} and the PCR was performed on each dilution for three replicates.

The standard curve correlated the copy number with a particular CT. The copy number values for the unknown samples were derived by comparison to this standard curve.

Informed consent was taken from patients or relatives for the procedures and for usage of their medical data in the present study.

Tanta university ethics committee approved the study protocol.

Statistical analysis

The data were analyzed with the SPSS 22 software package (SPSS Inc., Chicago, Illinois, USA). Quantitative variables presented as mean±SD and range. Qualitative variables presented as percentages. Comparisons between groups were done with χ^2 or t independent test. A P value of less than 0.05 was considered statistically significant.

Results

The present study included 80 patients [32 (40%) men and 48 (60%) women] with clinical and radiological suspicion of AP (Table 1).

Concerning the clinical data of patients with AP, most of them had pleural rub and crepitations while a small number had wheeze, arrhythmia, disturbed consciousness, and chest radiography infiltration. The incidence of *M. pneumonia* was 42% in all patients with AP. In all studied patients, only 36 (45%) patients had productive cough while 44 (55%) patients had dry cough, so they were subjected to bronchoscope and bronchoalveolar lavage (Table 2).

According to PCR performed for *M. pneumonia*, there were a positive PCR group (34 patients) and negative PCR group (46 patients) with no significant difference between the two groups as regards age, sex, bronchial asthma, and steroid intake as risk factors for *M. pneumonia* infection (Table 3) and also regarding erythrocyte sedimentation rate and leukocytic count in both groups (Table 4).

But there was statistically significant differences between the two groups as regards pleural rub, chest wheeze, crepitations, state of consciousness, heart rate, and arrhythmia (Table 3). In addition, there was a significant difference between the type of examined sample for *M. pneumonia* (Table 4).

The PCR positive group and the PCR negative group were significantly different as regards chest radiography infiltration and C-reactive protein results (Table 4).

The higher percentage of disturbed consciousness, chest radiography infiltrations, arrhythmia, and wheeze in patients with AP were mainly found in those with *M. pneumoniae*; in addition most of the patients subjected to BAL sampling were those with *M. pneumonia* (Table 5).

Positive and negative result curves show amplification and no amplification, respectively (Figs 1–4).

Table 1 Quantitative variables of the studied group

	Mean±SD	Range
Age	46.41±11.8	18–65 years
Heart rate	95.53±17.29	65–125 beats/min
CRP	12.46±2.17	6–96 mg/l
ESR	25.61±22.22	8–105 mm/h

Discussion

AP constitutes a considerable percentage of community-acquired pneumonia in both adults and children [6]. *M. pneumonia* represents 20–40% of community-acquired pneumonia [24].

The present study included 80 patients with suspected AP without previous identification of the causative agents. Patients with productive cough were subjected to sputum examination by PCR for *M. pneumonia*, and those with dry cough were examined

Table 2 Percentage in the studied group according to each of clinical characters and investigations

		N (%)		
Chest radiography infiltrations	Positive	28 (35)	Negative	52 (65)
Wheeze	Wheezy	27 (34)	Not wheezy	53 (66)
Pleural rub	Positive	48 (60)	Negative	32 (40)
Crepitations	Positive	44 (55)	No crepitations	36 (45)
Arrhythmia	Positive	34 (42)	No arrhythmia	46 (58)
Conscious level	Conscious	70 (87.5)	Disturbed	10 (12.5)
Past history of bronchial asthma	Positive	38 (47.5)	Negative	42 (52.5)
History of receiving steroids	Positive	28 (35)	Negative	52 (65)
Type of sample	Sputum	36 (45)	BAL	44 (55)
PCR for *Mycoplasma pneumoniae*	Positive	34 (42%)	Negative	46 (58%)

Table 3 Distribution of demographic and clinical characters in relation to PCR results

	Positive PCR For *Mycoplasma pneumoniae* (34 patients) [*n* (%)]	Negative PCR For *Mycoplasma pneumonia* (46 patients) [*n* (%)]	*P* value
Age	44.44±11.94 (18–62)	48.03±11.61 (20–65)	0.245
Sex			
Male	18 (53)	14 (31)	0.126
Female	16 (47)	32 (69)	
Pleural rub			
Present	22 (65)	10 (22)	0.002
Absent	12 (35)	36 (78)	
Chest wheeze			
Wheezy	15 (44)	12 (26)	0.0672
Not wheezy	19 (56)	34 (74)	
Crepitations			
Present	19 (56)	25 (54)	0.714
Absent	15 (44)	21 (46)	
Heart rate	110.88±9.69 (95–125)	82.97±10.66 (65–105)	<0.001
Arrhythmia			
Present	25 (74)	–	<0.001
Absent	9 (26)	46 (100)	
Conscious level			
Conscious	24 (70.5)	46 (100)	<0.001
Disturbed	10 (29.5)	–	
History of asthma			
Positive	14 (41)	24 (52)	0.176
Negative	20 (59)	22 (48)	
Receiving steroids			
Receiving	11 (32)	17 (37)	0.234
Not receiving	23 (68)	29 (63)	

Table 4 Investigation results in the studied patients in relation to PCR result

	Positive PCR For *Mycoplasma pneumoniae* (34 patients) [*n* (%)]	Negative PCR For *Mycoplasma pneumonia* (46 patients) [*n* (%)]	*P* value
Chest radiography infiltrations			
Infiltrations	26 (76.5)	2 (4.5)	<0.001
No infiltrations	8 (23.5)	44 (95.5)	
Type of sample			
BAL	27 (79.5)	17 (37)	<0.001
Sputum	7 (20.5)	29 (63)	
CRP	19.25±21.42 (6–96)	6.9±8.8 (6–48)	0.004
ESR	32.45±36.66 (10–105)	22.7±20.4 (10–85)	0.354
CBC			
WBCs	12.27±35.26	14.64±42.16	0.248
Neutrophils	43.5±27.8	54.5±36.62	0.163

BAL, bronchoalveolar lavage; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBCs, white blood cells.

bronchoscopically for BAL that was examined also for *M. pneumonia* using PCR.

The patients were classified retrospectively to positive and negative groups regarding the result of PCR for *M. pneumonia*, and each group was correlated to recorded clinical parameters of the patients.

In the present study, the mean of patients' age with AP was 46.41 (60%) years and most of them were women. However, the mean age in *M. pneumonia* positive group was 44.44 (18%) years and most of them were men.

These findings were concomitant with that of Shangguan *et al.* [15], who reported a higher percentage of *M. pneumoniae* in men than women. And also in a study by Dash *et al.* [20] for 130 patients with AP, women were 35 (27%) while men were 95(73%).

In the present study, BAL constituted 79.5% while sputum was 20.9% of the examined samples in *M.*

Table 5 Clinical data of *Mycoplasma pneumoniae* positive group regarding the studied patients

	Patients with atypical pneumonia (*n*=80) (*n*)	Patients with positive *Mycoplasma pneumonia* (*n*=34) (*n*)	% of *Mycoplasma pneumonia* to atypical pneumonia
Male	32	18	56%
Female	48	16	33%
Radiography infiltrations	28	26	93%
Wheeze	27	15	56%
Pleural rub	48	22	46
Crepitations	44	19	43
Arrhythmia	34	25	74
Conscious	70	24	34
Disturbed consciousness	10	10	100
Past history of bronchial asthma	38	14	37
History of receiving steroids	28	11	39
Sputum sample	34	7	21
BAL sample	44	27	61

BAL, bronchoalveolar lavage.

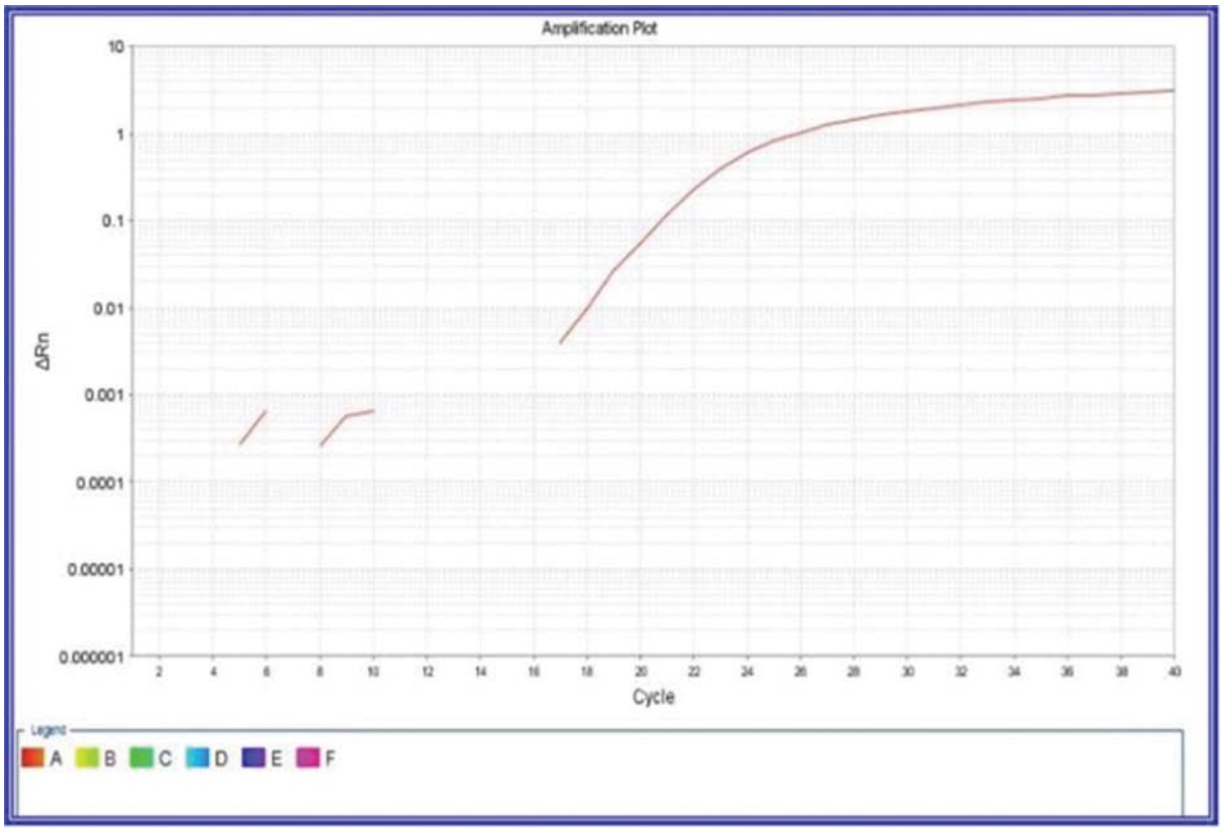
pneumoniae positive group. The PCR results may differ according the type of sample used; for example, sputum, lavage, or swab [22].

Previous studies have stated that the PCR used for sputum is superior to that for nasopharyngeal swabs followed by throat swabs that give least results using PCR. Raty *et al.* [25] described 69% sensitivity for sputum samples and 50% and 37.5% for throat swabs and nasopharyngeal swabs, respectively, but no distinctive differences were stated by Stralin *et al.* [26] between sputum samples and throat swabs.

In the present study; the used real-time PCR technique had diagnosed *M. pneumoniae* in 34% of cases with AP that constituted a high diagnostic value with high sensitivity and specificity of this new method.

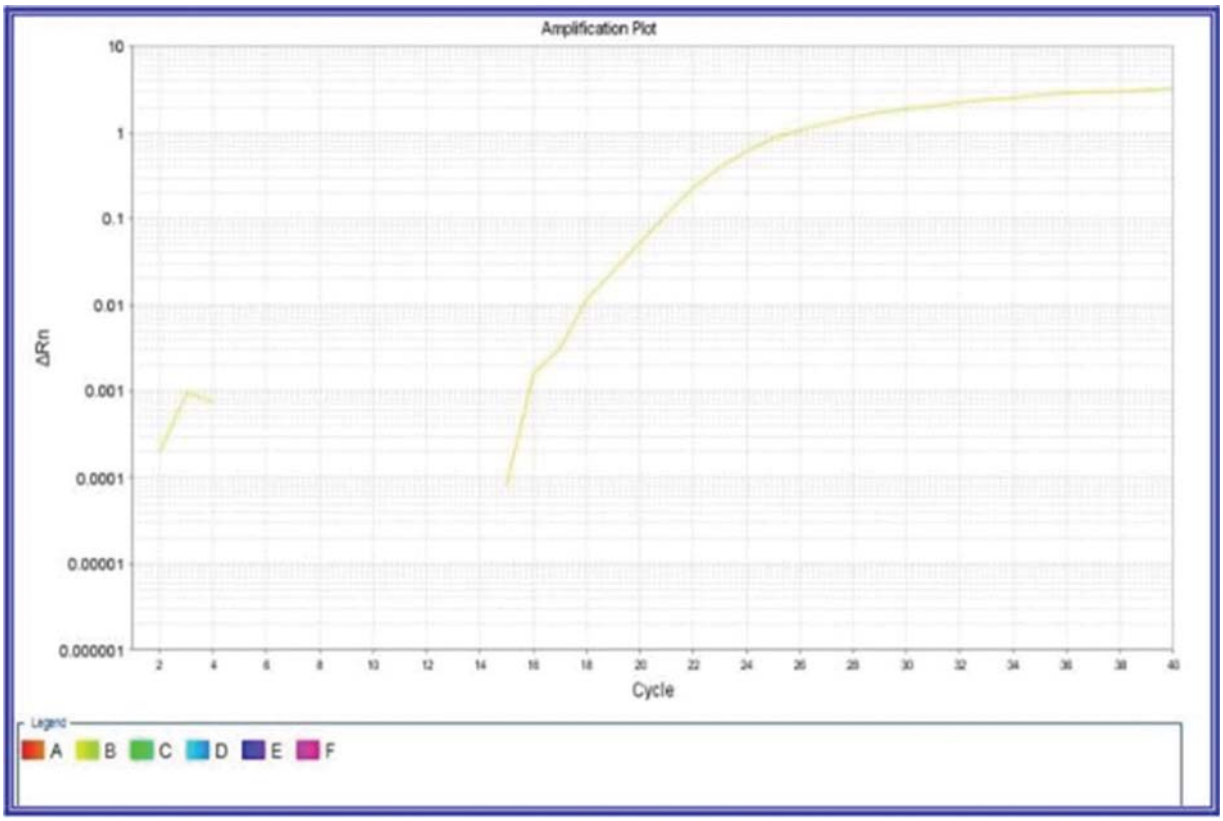
This agrees with the results published by Zhao *et al.* [27] who investigated 60 *M. pneumoniae* clinical specimens in one of the China hospitals by using MpP1 real-time PCR. Also, in a study by Chaudhry *et al.* [28] when compared serology with sputum secretions for *M. pneumonia* diagnosis, the PCR result was 19.4% while serology detected only 6.7% of cases.

Figure 1



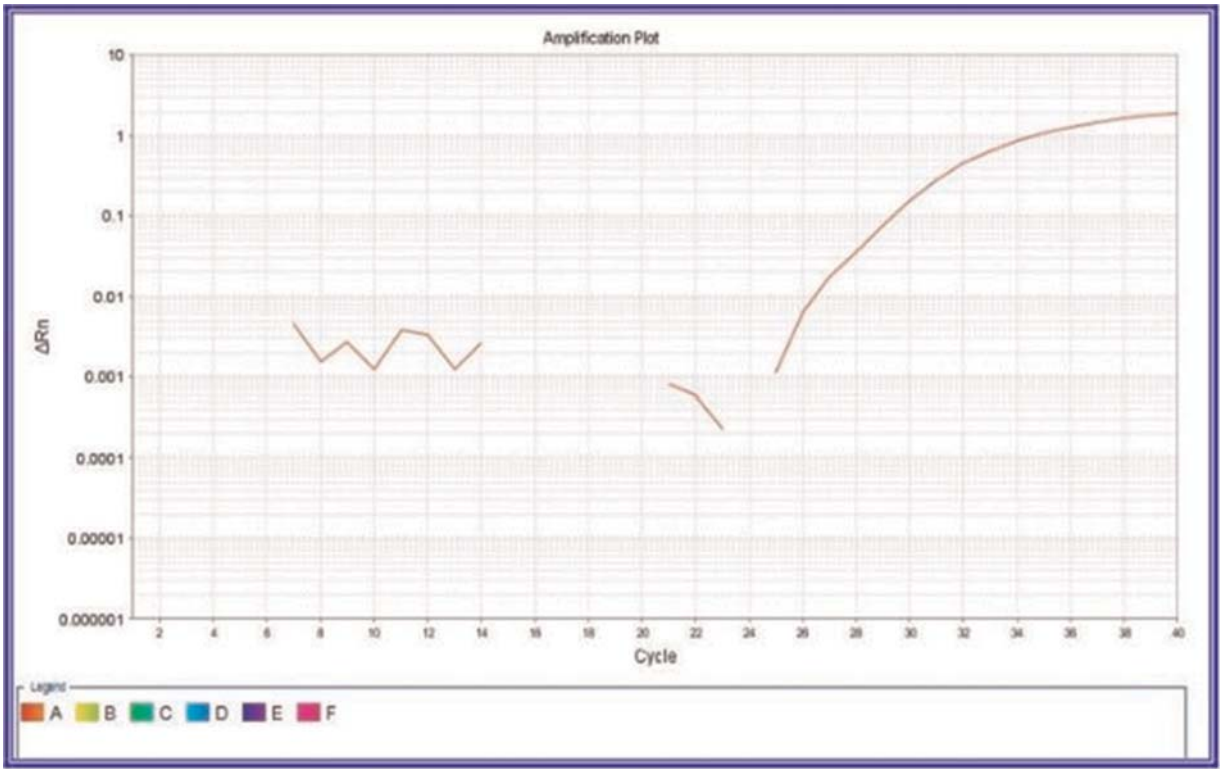
Positive PCR control curve (human DNA to assure the extraction and amplification process).

Figure 2



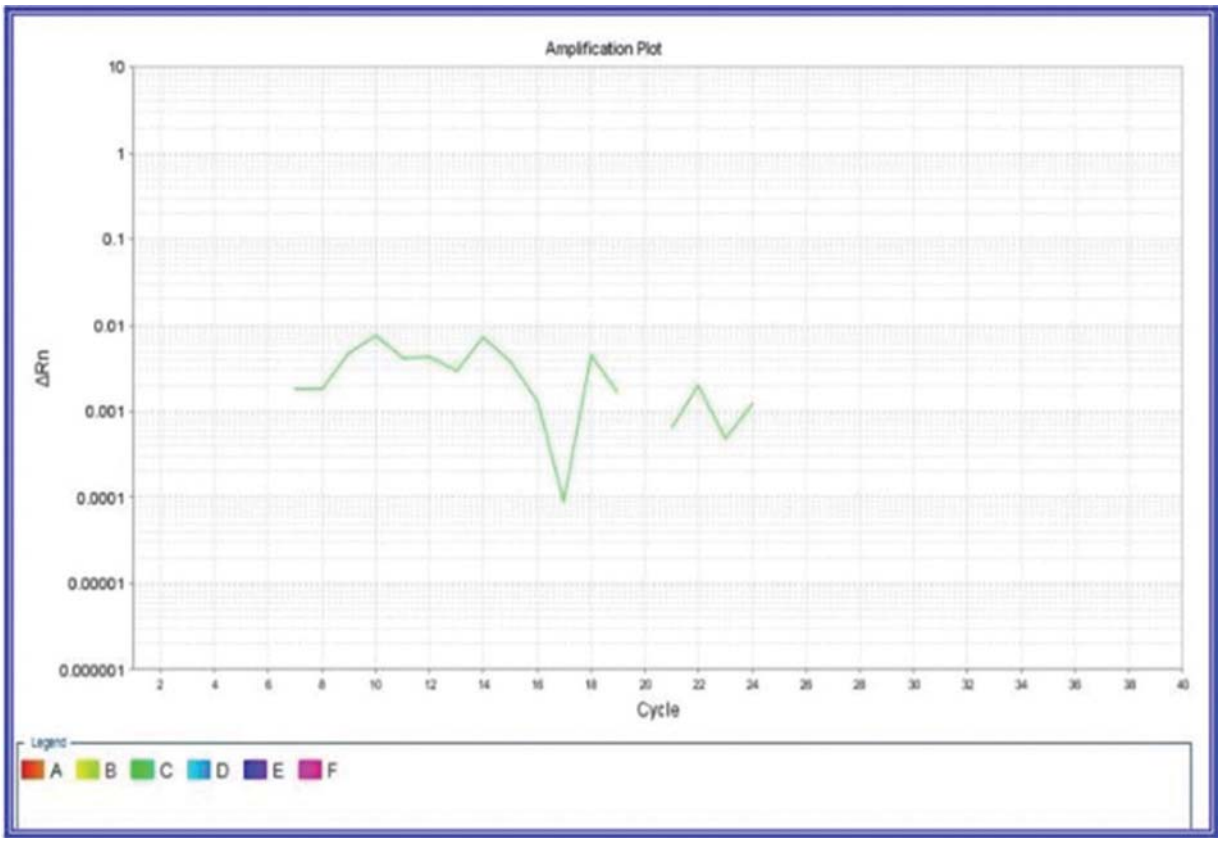
Positive *Mycoplasma pneumoniae* control curve.

Figure 3



Positive result curve shows amplification.

Figure 4



Negative result curve shows no amplification.

Moreover, Sondergaard *et al*. [29] searched 746 patients with respiratory tract infection using the PCR test and found 134 of patients had positive results for *M. pneumoniae*.

Nilsson *et al.* [30] studied 164 patients during a community outbreak of *M. pneumonia* infection and compared patients' serology with PCR results of oropharyngeal swabs and stated the high diagnostic value of PCR especially in the early stage of the disease in comparison to serology results.

In our PCR-positive group for *M. pneumoniae*, wheeze was auscultated in 44% of cases, crepitations in 56%, and arrhythmia in 74% of cases; 79.5% of patients had dry cough and just 20.5% of patients had productive cough.

In partial similarity, Shangguan *et al.* [15] stated dry and productive cough in 54.5% and 26.1% out of 88 patients, respectively, and 25% of the studied patients had crepitations.While Rahimian and Hosseini [31] stated after his study (for the Hajj pilgrims) that absence of cough made the diagnosis of *M. pneumoniae* unlikely. Dash *et al.* [20] stated a significant presence of cough (90%), dyspnea (63%), chest pain (57%), and sore throat (27%) in *M. pneumonia* positive group when they studied different diagnostic methods for *M. pneumonia* detection.

Chen *et al*. [32] recorded 24.4% of cases had wheeze and 6.5% had dyspnea and positive radiological finding in 36% cases.

All of that proved the importance of clinical presentation especially cough and extrapulmonary symptoms in early suspicion of *M. pneumoniae*.

In the present study, the incidence of *M. pneumonia* (in those with suspected AP) was 42%; this ratio is not a low percentage and was related or even higher than those obtained by other physicians in their related studies.

However, Sondergaard *et al.* [29] in their study recorded that 17.96% of cases had *M. pneumoniae* as a cause of AP. Nilsson *et al*. [30] recorded a ratio of 11.02% out of 8157 studied patients.

Moreover, Chen *et al*. [32] stated the incidence of *M. pneumonia* as 14.58% in 2009 and 13.99% in 2010

when they studied its incidence in respiratory secretions of admitted patients with suspected AP for 3 consecutive years.

In their study, Chaudhry *et al*. [28] concluded 19% incidence of *M. pneumonia* in 134 patients with pneumonia. In addition, Touati *et al*. [33] studied 540 patients with lower respiratory tract infection and found that 23.3% of cases had *M. pneumoniae* as a cause of pneumonia.

This implies the high percentage of *M. pneumoniae* in patients with lower respiratory tract infections in relation to other causative agents causing AP.

This relatively high ratio in the present study may be related to the socioeconomic status of our developing country and the need for more improvement in hygienic measures to avoid or even lessen these infections.

More studies are suggested to survey the incidence of pneumonia including all suspected causative agents as an important step in the national controlling programs concerning human health in developing countries that suffer poverty of the recording system for patients' data.

One of the limitations of this study is its neglect of other diagnostic tools for *M. pneumonia* such as culture or serological methods to correlate with the methods used in the study.

Another limitation is not declaring the percentage of other isolated organisms causing pneumonia other than *M. pneumonia*.

Conclusion

M. pneumoniae incidence is not low among patients with AP.

Chest radiology and clinical presentation are undoubtedly important in early suspicion and detection of *M. pneumoniae*, but with no comparable diagnostic accuracy to serology and culture. But serology does not give the required high sensitivity in the early days of the infection period. Moreover, culture needs specific precautions and long time to give the required colonies, that delay early specific and effective treatment.

PCR is a considerable specific method for the diagnosis of *M. pneumoniae* especially when time factor is an essential matter in early and successful treatment strategies. It gives specific and accurate results with the required quantitative calculation of bacterial infection.

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Conflicts of interest

There are no conflicts of interest.

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