

Original Research Article

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Prevalence of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* in LRTI Patients in a Tertiary Care Center, Karimnagar

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ABSTRACT

“Atypical pneumonia” has been defined as “any Community acquired pneumonia (CAP) that is different to that caused by *Streptococcus pneumoniae*”, to indicate “a CAP caused by one of several identified pathogens including *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Coxiella burnetti*” and Viruses. *Mycoplasma pneumoniae* is the most important and common cause of community-acquired pneumonia (CAP). The conventional detection methods (culture) lack sensitivity and takes longer duration of time for detection. The study was undertaken during November 2018 to April 2019, 102 patients with respiratory tract infections were enrolled into this study. *M. pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* from respiratory tract infections was detected by enzyme-linked immunosorbent assay (ELISA). Atypical bacteria isolated from pneumonia patients are *Chlamydia pneumoniae* in 5(4.9%), *Mycoplasma pneumoniae* 7 (6.8%), and *Legionella pneumophila* 15 (14.7%) of patients detected by ELISA. Atypical pneumonia infection was mostly prevalent in patients 16–76 years old. Most infections (90%) were community acquired and cough, fever, dyspnea, and malaise were among the most common symptoms. Pneumonia due to the Atypical bacterial infection is infrequent in Chalmeda Anand Rao institute of Medical sciences and the clinical symptoms of the patients were determined to be mild. The ELISA method also proved to be more sensitive and reliable than culture assays in the detection of *M. pneumoniae*.

Keywords

Atypical pneumonia, IgM, ELISA

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Introduction

The atypical respiratory pathogens *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* are now recognised as a significant cause of acute respiratory-tract infections, but they remain colorless after Gram-staining and are difficult

to identify by conventional bacterial culture tests. It is reported that patients with atypical pneumonia were more likely to have normal or reduced white blood cell counts. (Huang HH, 2003) However some published data showed that between atypical pneumonia and general bacterial pneumonia, there were no significant differences in the symptoms such

as fever, cough, productive sputum, and the sign of lung rales (Porath, 1997 and Monsieur, 1997).

Also, X-ray findings and the increase of white blood cell counts and percentage of neutrophils were similar between them. Especially, patients with pneumonia caused by *L. pneumophila* presented with the typical symptoms of Streptococcus pneumonia while patients with *S. pneumonia* also presented with the symptoms of atypical pneumonia (Yu, 2003). However, beta-lactams are not effective for atypical pneumonia. Therefore, laboratory detection methods and clinical biology research on the diagnosis and treatment of atypical pathogens infection is particularly important.

Atypical pathogens

Atypical organisms such as *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* are implicated in cases of community-acquired pneumonia (CAP). *M. pneumoniae* in 1962 was successfully isolated (Chanock, 1962). *M. pneumoniae* lacks a cell wall, but it can grow in artificial culture medium, and it is the most frequent pathogens found in patients with CAP. *C. pneumoniae* found in 1986 is intracellular organisms and is a common cause of acute and chronic respiratory tract infections (Grayston, 1990). *Legionella* was found in 1976 because it caused infection outbreak of America veterans. It has been confirmed more than 50 *Legionella* species, a total of 70 serotypes. *L. pneumophila* with 16 serotypes is intracellular organisms and closely related to human infection (Diederer, 2008).

Atypical pathogens in community acquired pneumonia

With the widespread use of antibiotics, the change of living environment and constantly

updated diagnostic methods, we found atypical pathogen played an important role in CAP. A prospective study was performed on 665 consecutive adult patients with CAP at 12 centers in 7 Chinese cities between 2003 and 2004 (Liu, 2006). The results showed that atypical pathogens caused 32.4% cases of CAP, of which 20.7% *M. pneumoniae*, 6.6% *C. pneumoniae* and 5.1% *L. pneumophila*. Of 195 patients with a bacterial pathogen, an atypical pathogen was identified in 10.2% cases. Survey of Cao et al. on the etiology and clinical outcomes of CAP treated in an ambulatory setting showed that the most common pathogens were *M. pneumoniae* (29.4%) and then virus copathogens (2.5%) (Cao, 2010). The previous research data of "pathogens monitoring network among adults with CAP in Beijing" showed that of 410 patients with CAP, ≥ 4 -fold increase of paired serum *M. pneumoniae* IgG antibody titer was observed in 18.8% of cases. (Qu, 2012). Atypical pathogens, rather than *S. pneumoniae*, become the most important pathogen of adult CAP.

Materials and Methods

The study was conducted during the period November 2018 to April 2019. The study group consists of 100 patients attending medical outpatient department and admitted in Chalmeda Anand Rao Institute of Medical sciences, Karimnagar.

Inclusion criteria

Patients with Community Acquired Pneumonia.

Acute exacerbation of Bronchial asthma

Chronic Obstructive Pulmonary Disease

Age 16 to 65 years and

Patients presenting with symptoms and signs of fever, cough, dyspnea and headache.

Presence of new pulmonary infiltrates on chest x ray.

Exclusion criteria

Patients on Antibiotics
Hospital Acquired Pneumonia
Pulmonary Tuberculosis
Bronchiectasis
Interstitial lung disease
Age <16 years and >65 years

Sample collection and processing

Blood collection: The skin over the vein was cleaned with 70% alcohol and allowed to dry. Then povidone iodine was applied was allowed to dry for 1 minute. Blood sample was collected into a sterile bottle without anticoagulant. It was allowed to stand for formation of clot. Then it was centrifuged and supernatant was taken and stored at -20⁰C.

Processing

All samples were subjected to ELISA to detect IgM antibodies for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*.

Test was performed according to manufacturer's instructions:

(EUROIMMUN Anti-*Mycoplasma pneumoniae* ELISA (IgM),

EUROIMMUN Anti-*Chlamydia pneumoniae* ELISA (IgM),

EUROIMMUN Anti-*Legionella pneumophila* ELISA (IgM)

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: negative
Ratio >0.8: to <1.1: borderline
Ratio >1.1: positive

Results and Discussion

Seroprevalence was detected by IgM antibody test using ELISA (EUROIMMUNE) microplate wells coated with antigens (detergent extract of *Mycoplasma pneumoniae*, strain MAC ATCC 15531, MOMP of CWL-029 strain of *Chlamydia pneumoniae*, LPS *Legionella pneumophila* strain 1-7) for IgM detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*.

Of the 102 patients studied over the 6-month study period, IgM ELISA revealed 5 (4.9%) *Chlamydia pneumoniae*, 7 (6.8%) for *Mycoplasma pneumoniae* and 15 (14.7%) *Legionella pneumophila* positive cases, respectively. The highest prevalence rate of *M. pneumoniae* infection was diagnosed in patients of Age 16–76 years old.

The present study detected IgM antibodies for *Mycoplasma pneumoniae* in 6.8% patients. According to study by Rama Chaudhry *et al.*, 2013 India IgM antibodies for *Mycoplasma pneumoniae* detected in 4.47% of patients of Community acquired pneumonia (Rama Chaudhry *et al.*, 2013).

According to a study by Liu Fang Ching *et al.*, (2008) IgM antibodies for *Mycoplasma pneumoniae* were detected in 3.7% in adults. In year 2004 and 2.9% in year 2005 (Liu Fang Ching, 2008).

Present study shows similarity in prevalence to these two studies. In the present study IgM antibodies for *M. pneumoniae* has been noted in 5 cases of CAP and 2 cases of Bronchial asthma with acute exacerbations.

Blasi *et al.*, (2004) Europe: reported a role for *C. pneumoniae* and *M. pneumoniae* infection as a trigger for 5–30% episodes of wheezing or acute asthma exacerbations (Blasi, 2004).

Paraskevi Xepapadaki *et al.*, (2008): *M. pneumoniae* was associated with hospitalization for asthma exacerbation 18%.

Rama Chaudhry *et al.*, (2013) found that males are more commonly affected than females Male to female ratio is 1:6.

The present study showing Out of 7 cases positive for *M. pneumoniae* 4(57%) cases were males and 3(42%) female.

Our study detected IgM antibodies for *Legionella pneumophila* in 14.7% of patients.

According to study by Ewing *et al.*, (2002) in Europe: present data on a large population, hospitalized with acute exacerbations of COPD, which provides evidence for the first time for *Legionella* spp. infection as a potential underlying pathogen in as many as 16.7% of cases detected by serology (Ewing, 2002).

According to study by Sabah Javed *et al.*, (2010) IgM antibodies for *Legionella pneumophila* detected in 15.92% of cases. most common presenting symptoms are fever 80.6%,cough 96.7%,dyspnea 58%,headache 16.1% (Sabah Javed, 2010).

The patients included in our study showing fever (76.6%) 69, cough (87.7%) 72, dyspnea

(38.8%) 35, head ache (8.8%) 8. Almudenarojas *et al.*, (2005) reported 29.7% positive for IgM *L. pneumophila* and that most cases of legionellosis are caused by serogroup-1 (Almudenarojas, 2005).

Direct methods of diagnosis include culturing, direct fluorescent staining, and antigen detection in urine. While the first two methods display low and variable sensitivities. The latter has become a reference technique in most laboratories, enabling easy and early diagnosis of legionellosis. Indirect immune fluorescence is the most common method for serological diagnosis. Although serology yields good sensitivity and specificity data. The delay in the development of a measurable antibody response constitutes a major drawback for diagnosis in the acute patient. Immunoglobulin M (IgM) detection is widely used in infectious serology, since IgM appears earlier in the course of a disease; however, despite its reported validity for the diagnosis of legionellosis, its use is not widespread and some authors consider it of limited value. The Enzyme-linked immunosorbent assay (ELISA) technique, which generally shows higher sensitivity and better characteristics in terms of both automation and objective measurement than immuno fluorescence (Table 1).

Table.1 Ig M antibodies detection of atypical pathogen by ELISA (No= 102)

Pathogen	No	%
<i>Chlamydia pneumoniae</i>	5	4.9
<i>Mycoplasma pneumoniae</i>	7	6.8
<i>Legionella pneumonia</i>	15	14.7

According to a study by Agarwal *et al.*, 2008 India *Chlamydia pneumoniae* has been discussed as a possible co factor causing

chronic obstructive pulmonary disease (COPD) and asthma (Agarwal, 2008). They detected IgM antibodies for *Chlamydia*

pneumoniae in 18.3% of patients. in contrast to above study our study detected only 4% of chlamydia pneumonia by ELISA. According to Miyashita *et al.*, (1998) and Gencay *et al.*, (2001), the rate of 3% compares favourably to serological studies of *Chlamydia pneumoniae* in COPD and asthma (Miyashita, 1998 and Gencay, 2001).

According to study by Nagesh *et al.*, (2004) found that Serological testing is considered the most useful means of determining the prevalence of *C. pneumoniae* infection MIF is currently the standard in *C. pneumoniae* serology, but is subjective and requires an expert microscopist to interpret the results. Inter-laboratory variation of MIF shows an overall agreement with reference standard titres of c. 80%. In comparison, ELISA is more objective than MIF. It can be automated, with the advantages of high throughput and electronic records. ELISA is therefore easier to standardise and is the preferred diagnostic method ELISA may become a preferred objective test in the sero epidemiological study of *C. pneumoniae* infection and its link with atherosclerotic vascular disease (Nagesh, 2004).

According to study by Surinder Kumar *et al.*, 2011 India tachypnea was documented in 9 (75%) cases; cough and coryza in 12 (100%) cases; fever in 8 (66.67%), while 4 (33.33%) cases were a febrile (Kumar, 2011).

Five (41.67%) cases documented audible/auscultable wheezing and 3 (25%) crepitations. The presence of *C. pneumoniae* antibody was higher in 10 (7.87%) males than in 2 (2.74%) females.

Our study detected chlamydia pneumonia antibodies in 5 male patients and none in the female patients.

Urinary antigen detection has been treated as the most specific reference test for diagnosis

of legionellosis, this test has not been utilized in our study due to cost effective.

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