Research Journal of Pharmaceutical, Biological and Chemical Sciences

Rapid Investigation of Uncultivable Respiratory Tract Bacteria Among Tuberculosis Patients in Hilla City, Iraq.

Hussein Oleiwi Muttaled Al-Dahmoshi*

Ph.D. Microbiology, University of Babylon, College of Science, Biology Dept., Iraq.

ABSTRACT

During a period of 3 months, a fifty nine serum sample were collected from patients (Male with age mean±SD (45.50±16.01) with tuberculosis who visit tuberculosis center in Hilla City-Iraq. One serum sample were collected from patients after confirmation of tuberculosis by acid fast staining and culturing on Lowenstein- Jensen medium. All serum samples were subjected to indirect immunofluorescent assay (IFA) to investigate presence of IgM antibodies specific for a five pulmonary pathogens includes (Legionella pneumophila serogroup 1, Mycoplasma pneumoniae, Coxiella burnetii phase II, Chlamydia pneumoniae and Chlamydia psittaci). The results revealed presence of M. pneumoniae (40.68 %), C. pneumoniae (20.34%), L. pneumophila (6.78%) and C. psittaci (1.69%). All samples were negative for C. burnetii phase II. This study conclude that the possibility of emerging pneumonia during or post tuberculosis and push a need for seeking for pulmonary pathogen for tuberculosis patients and take in account the treatment regimen.

Keywords: Tuberculosis, uncultivable, IFA, Mycoplasma, Chlamydia.

*Corresponding author
INTRODUCTION

Tuberculosis is an airborne transmitted bacterial disease. The causal agent is Mycobacterium tuberculosis (the tubercle bacillus). Although pulmonary tuberculosis is the most frequent site of involvement; tuberculosis can affect any organ in the body but less frequently. The main reservoir of \textit{M. tuberculosis} is the patient with pulmonary tuberculosis. Such patients may have pulmonary “cavities” that are rich in bacilli (100 million bacilli in a cavity of approximately 2 cm in diameter)[1], and those patients highly infectious. Tuberculosis can affects about 9.4 million individuals and is responsible for the death of 1.8 million individuals across the world annually. It dwell about one third of worlds[2,3]. Iraq occupy the fifth order among middle east countries infected with TB and about 3 thousands die due TB[4]. TB is a public health priority in Iraq. The country is among 7 of the countries of the Region with a high burden of TB, and accounts for 3% of the total number of cases. Iraq is one of the five countries (Egypt, the Netherlands, the United Kingdom and Yemen) that account for 0.5% of the estimated global number of incident cases in 2014. There are an estimated 20 000 TB patients in Iraq. Estimated deaths due to TB are more than 4000 annually and the last report for TB in Iraq about 8268 cases[5].

There is a possibility to get secondary infection among tuberculosis patients and this will render them hard and need long time to get rid tuberculosis. The probability to catch pneumoniae expected and cannot be noted especially with uncultivable bacteria. The most common causative uncultivable bacterial agents of pneumoniae are \textit{Legionella pneumophila} serogroup 1, \textit{Mycoplasma pneumoniae}, \textit{Coxiella burnetii} phase II, \textit{Chlamydia pneumoniae} and \textit{Chlamydia psittaci}[6,7,8,9,10]. The treatment regimen different for patients with tuberculosis from those with pneumoniae. This study aims to investigate the uncultivable bacterial causes of pneumoniae among patients suffering from pulmonary tuberculosis.

MATERIALS AND METHODS

Sample collection:

Fifty nine serum sample were collected from patients (Male with age mean±SD (45.50±16.01) with Tuberculosis who visit Tuberculosis center in Hilla City-Iraq. All patients undergo tuberculosis and the sample collected after performing all test to confirm tuberculosis including acid fast staining, and positive culture on Lowenstein-Jensen medium.

Identification and Diagnosis:

Ziehl-Neelsen staining were performed for each sputum sample (after digestion, decontamination, and concentration of sputum) to investigate acid fast bacilli and when the staining is positive then the sputum samples prepared for cultivation on Lowenstein-Jensen medium[11].

Indirect immunofluorescent assay (IFA):

The IFA assay used in the current study called Pneumobact (Vircell/Spain) and it is kit for the simultaneous diagnosis in human serum of IgM antibodies of the main ethiological bacterial agents of infectious diseases of the respiratory tract: \textit{Legionella pneumophila} serogroup 1, \textit{Mycoplasma pneumoniae}, \textit{Coxiella burnetii} phase II, \textit{Chlamydia pneumoniae} and \textit{Chlamydia psittaci}[12].

Kit Componenets:

1-VIRCELL PNEUMOBACT SLIDE: 10 slides with two rows of 5 wells (each slide used to test 2 serum samples) with the following antigens:

- \textit{L. pneumophila} serogroup 1, suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.
- \textit{M. pneumoniae} in McCoy cells.
- \textit{C. burnetii} in phase II, suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.
Pneumobact IgM Determination:

- Allowing all reagents and slides to reach room temperature before opening.
- Preparation of a 1/2 dilution of serum samples by adding 25 μl of sample to 25 μl of PBS. The positive and negative control sera should not be diluted.
- Treating of diluted sera with anti-human IgG sorbent, by adding 15 μl of sera to 75 μl of sorbent and thoroughly mix. Positive and negative control sera must not be diluted nor sorbent treated. The treated sera can be used directly, or centrifuged to remove the precipitate, which does not interfere with the test.
- Addition of 15 μl of sorbent-treated serum in every slide well. Addition of 15 μl of non-treated non-diluted positive control to the wells of the upper row of a slide and 15 μl of non-treated non-diluted negative control to the wells of the lower row of the same slide.
- Placing the slide in a humid chamber and incubation at 37°C for 30 minutes.
- Rinsing the slide briefly with a gentle stream of PBS and immerse in PBS while shaking gently on a shaker, for ten minutes. Dip wash slide briefly in distilled water.
- Allowing the slide to air dry.
- Addition of 20 μl of anti-human IgM FITC conjugate solution to each well. (No dilution required).
- Incubation of slide in a humid chamber for 30 minutes at 37°C.
- Repeats steps 6 and 7.
- Addition a small drop of mounting medium to each well and carefully cover with a coverslip.
- Reading the slide as soon as possible in a fluorescence microscope at 400x magnification. If this is not possible, store in the dark at 2-8°C up no more than 24 hours, until observation.

RESULTS AND DISCUSSION

Pneumonia is an infection of the lungs that can cause mild to severe illness in people of all ages. The most common atypical pneumonias are caused by three zoonotic pathogens, *Chlamydia psittaci* (psittacosis), *Francisella tularensis* (tularemia), and *Coxiella burnetii* (Q fever), and three nonzoonotic pathogens, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* [13]. The results revealed that *L. pneumophila* present in 6.78% male with tuberculosis (table 1). The positive IFA results displayed in figure (1). *Legionella pneumophila*, a gram-negative rod normally inhabiting aquatic environments, transmitted to human via aerosols and cause pneumonia (legionellosis) that can be acquired in the community or in hospitals.
Legionella pneumonia cannot be differentiated from other pneumonia according to the clinical manifestation and need for appropriate microbiological testing[14]. Traditional risk factors for legionellosis include smoking, corticosteroid use, and chronic lung disease[15]. Munder and Yu (2002)[10] and Yu et. al. (2002)[16] state the possibility of infection with L. pneumophila serogroup 1 among immunocompromised people.

Table (1): Percentage of Uncultivable Bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Sample no.=59</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>L. pneumophila serogroup 1</td>
<td>4</td>
<td>6.78</td>
<td>55</td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>24</td>
<td>40.68</td>
<td>35</td>
</tr>
<tr>
<td>C. burnetii phase II</td>
<td>0</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>C. pneumonia</td>
<td>12</td>
<td>20.34</td>
<td>47</td>
</tr>
<tr>
<td>C. psittaci</td>
<td>1</td>
<td>1.69</td>
<td>58</td>
</tr>
</tbody>
</table>

Figure (1): (A) represent Positive control, (B) represent negative control and (C) represent positive results for Legionella pneumophila serogroup 1.

Figure (2) show positive IFA results for Mycoplasma pneumoniae. It compile largest percentage (40.68%) among tuberculosis patients. Mycoplasma pneumoniae is a common causative pathogen of respiratory infections all age groups and cause about 10-30% of all cases of community-acquired pneumonia [17,18]. It can cause severe pneumonia among immunocompromised patients like those with tuberculosis. Their diagnosis cannot be based on clinical symptoms only and rapid laboratory diagnosis is very important[19].

Ruiz-Gonzalez et.al.[20] specified that a major factor contributing to the unknown etiology of CAP is the difficulty of identifying “atypical” respiratory tract pathogens, which include Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella species. Hirai et.al. (1991)[21] stated reliability of indirect immunoflourecnet assay for diagnosis of M. pneumoniae infections. Concern occurrence of Coxiella burnetii phase II among tuberculosis, all samples were negative for specific IgM antibody for Coxiella burnetii phase II and this may be due to fact that the usual reservoir for this bacteria are cattle, sheep, and goats[8].
Figure (2): (A) represent Positive control, (B) represent negative control and (C) represent positive results for *Mycoplasma pneumoniae*.

Figure (3): (A) represent Positive control, (B) represent negative control and (C) represent positive results for *Chlamydia pneumoniae*.

C. psittaci were positive among (1.69%) of tuberculosis. *Chlamydia psittaci* is a bacterium that can be transmitted from pet birds to humans. In humans, the resulting infection is referred to as psittacosis (also known as parrot disease, parrot fever, and ornithosis). Psittacosis often causes influenza-like symptoms and can lead to severe pneumonia and nonrespiratory health problems. With proper treatment, the disease is
rarely fatal. During a ten years period CDC received reports of 831 cases of psittacosis[25]. Positive IFA results for C. psittaci shown in figure(4).

![Image](image_url)

**Figure (4)**: (A) represent Positive control, (B) represent negative control and (C) represent positive results for Chlamydia psittaci.

**CONCLUSION**

This study conclude that the possibility of emerging pneumonia during or post tuberculosis and push a need for seeking for pulmonary pathogen for tuberculosis patients and take in account the treatment regimen.

**ACKNOWLEDGMENTS**

I warmly thank the personnel of the Microbiology lab., the personnel of Tuberculosis center in Hilla City-Iraq for their contributions.

**REFERENCES**


