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

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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 immunoflourescent 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*.

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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 *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 *Legionella pneumophila* serogroup 1, *Mycoplasma pneumoniae*, *Coxiella burnetii* phase II, *Chlamydia pneumoniae* and *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: *Legionella pneumophila* serogroup 1, *Mycoplasma pneumoniae*, *Coxiella burnetii* phase II, *Chlamydia pneumoniae* and *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:

- *L. pneumophila* serogroup 1, suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.
- *M. pneumoniae* in McCoy cells.
- *C. burnetii* in phase II, suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.

- *C. pneumoniae*, elementary bodies .
- *C. psittaci*, elementary bodies.

2-VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.

3G-VIRCELL PNEUMOBACT IgG POSITIVE CONTROL: 300 µl of positive control serum for IgG, containing sodium azide.

3M-VIRCELL PNEUMOBACT IgM POSITIVE CONTROL: 300 µl of positive control serum for IgM, containing sodium azide.

4-VIRCELL PNUMOBACT NEGATIVE CONTROL: 600 µl of negative control serum, containing sodium azide.

5G-VIRCELL ANTI-HUMAN IgG FITC CONJUGATE: 2 vials with 1.1 ml of fluorescein-labeled anti-human IgG fluorescein conjugate in a phosphate buffer containing Evan´s blue, sodium azide and a protein stabilizer.

5M-VIRCELL ANTI-HUMAN IgM FITC CONJUGATE: 2 vials with 1.1 ml of fluorescein-labeled anti-human IgM fluorescein conjugate in a phosphate buffer containing Evan´s blue, sodium azide and a protein stabilizer.

6-VIRCELL MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.

7-VIRCELL ANTI HUMAN IgG GLOBULIN (SORBENT): 1 vial with 1.5 ml of sorbent (goat anti-human IgG, containing sodium azide).

Pneumobact IgM Determination :

- Allowing all reagents and slides to reach room temperature before opening.
- Prepartion 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 centrifugated 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 37ºC 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 37ºC.
- 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 2-8ºC 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.

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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.

Bacteria	Sample no.=59			
	Positive		Negative	
	No.	%	No.	%
L. pnemophila serogroup 1	4	6.78	55	93.22
M. pneumoniae	24	40.68	35	59.32
C. burnetii phase II	0	0	59	100
C. pneumoniae	12	20.34	47	79.66
C. psittaci	1	1.69	58	98.31

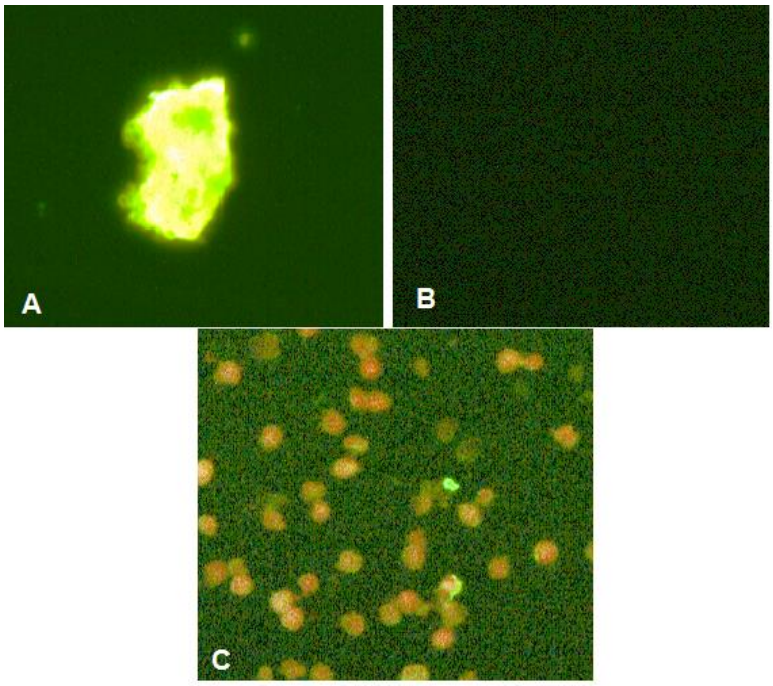

Figure (1): (A) represent Positive control, (B) represent negative control and (C) represent positive results for *Legionella pneumophila* serogroup 1.

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 immunoflourecent 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].

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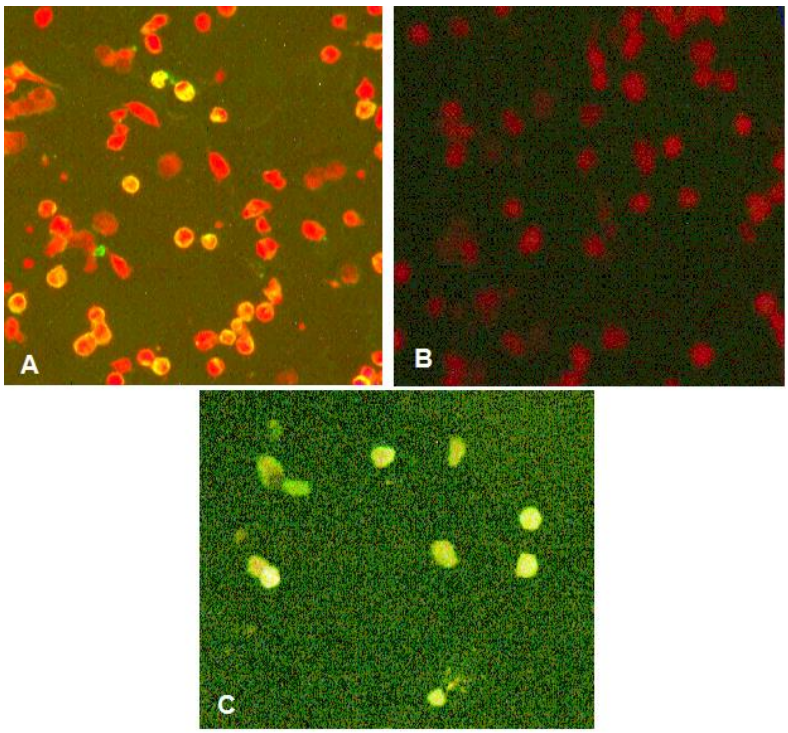

Figure (2): (A) represent Positive control, (B) represent negative control and (C) represent positive results for Mycoplasma pneumoniae.

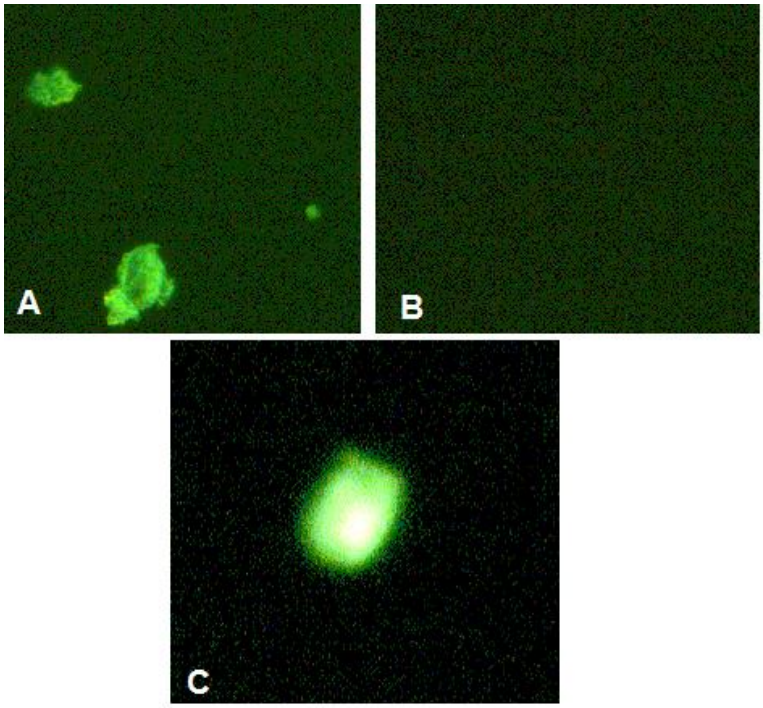

Figure (3): (A) represent Positive control, (B) represent negative control and (C) represent positive results for Chlamydia pneumoniae.

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).

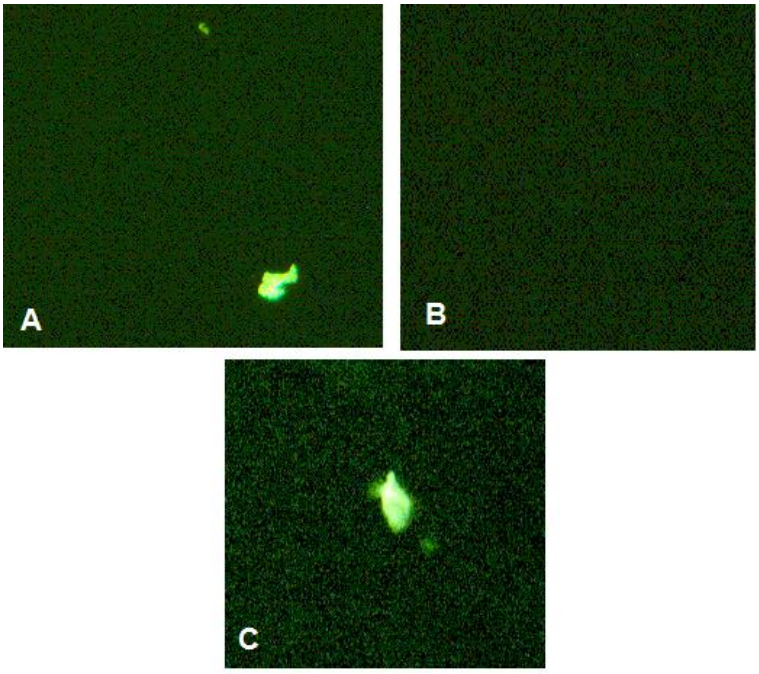

Figure (4): (A) represent Positive control, (B) represent negative control and (C) represent positive results for Chlamydia psittaci.

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.

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