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Case Study

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Diagnosis of Bacteria Atypical Pneumonia Causative Agents by Using Indirect Immune Fluorescent Assay

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Introduction

Pneumonia causes significant morbidity and mortality in children worldwide, especially in resource-poor settings. Accurate identification of bacterial etiology leads to timely antibiotic initiation, minimizing overuse, and development of resistance(Gunaratnam *et al*,2021). Although chest radiography (CXR) is frequently used to diagnose pneumonia, it has important limitations including poor interobserver agreement, decreased accessibility in resource-poor settings, and most importantly for this analysis, an inability to distinguish bacterial pneumonia from viral pneumonia (Elemraid *et al*,2014)

Diagnosis of pneumonia in chest X-ray images can be difficult and error-prone because of its similarity with other infections in the lungs(Ayan, E., 2022)

pneumonia was ranked frst among the causes of child mortality under the age of fve-year-old children, with 19%, 18%, and 16%, respectively. The late diagnosis of the disease due to limited resources makes pneumonia the most dangerous disease that causes childhood deaths especially in low and middle-income countries (McAllister, D.A., *et al*,2019)

The World Health Organization (WHO) describes some symptoms of pneumonia as the presence of fast breathing, chest indrawing, cough, cold and difculty breathing (Drake, D.E., *et al*,2007). A great number of bacterial and viral pathogens can cause CAP but the etiology of pneumonia cases is rarely detected in clinical practice (Gilani *et al*, 2012)

As a result of the existence of high morbidity and difficulty of identify the causative agent of pneumonia infection, the role of this study to diagnosis of some of important bacteria agents by Immunofluorescence IgM

Subjects and study design

This is a case-control study. A total of 80 individual were involved in this study, with age ranging from (1 to 36) months.

Subjects: A 40 patients suffering from pneumonia And 40 health individuals as control, who were observation in pediatrics hospitals in Babel governorate during the period from September 2019 to February 2020

by helping of clinicians in diagnosis of pneumonia patients.

The sample are whole blood were collected from each participant, noting that the sera were used to determine of causative agent of atypical bacterial Pneumonia for all specimens,.

Ethical considerations

The approvals were obtained from all the participants (patients and healthy) and also agreed to study scientifically and morally by the medical committee in the department of pediatric for pediatric hospitals in Babel governorate.

Materials and Methods

Materials of Indirect Immune Fluorescent Assay Kits

IgM kits

1.Vircell Pneumoslide Slide: each kit have10 slides and each slide have 10 wells with the following antigens:

1. *L. pneumophila* serogroup 1 suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.

2. *M. pneumoniae* in McCoy cells.

3. *C. burnetii* in phase II suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.

4. C. *pneumoniae*, elementary bodies.

6. Cell control.

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

3. Vircell Pneumoslide IgM Positive control: 500 µl of positive control serum, containing sodium azide.

4. Vircell Pneumoslide IgM Negative control: 500 µl of negative control serum, containing sodium azide.

5. **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 of 1.65 ml of sorbent (goat anti-human IgG, containing sodium azide).

Methods Diagnosis of causative agents of pneumonia

3.2.4.1.IIF assay procedure:

1. All reagents was putted to room temperature before use. Allow the slides to reach room temperature before opening.

2. Prepared a 1/128 and 1/256 dilution of samples by added 10 µl of sample to 1270 and 2550 µl of PBS 2 (1/128 and 1/256 dilution).The control sera 3 and 4 should not be diluted.

3. Applied 20 µl of 1/128 dilution into the wells 1, 2, 3 and 4 and of 1/256

dilution into the wells 5, 6, 7, 8, 9 and 10. Added 20 µl of non-diluted positive control 3 to each well of a slide and 20 µl of non-diluted negative control 4 to each well of another slide.

4. slide was incubated in a humid chamber for 30 minutes at 37ºC.

5. Rinsed slide 1 briefly with a gentle stream of PBS 2 (avoid directing

PBS at wells) and immerse in PBS while shaking gently on a shaker, for ten minutes. Dip wash slide briefly in distilled water.

6. Slide was allowed to air dry.

7. A20 µl was added of anti-human IgG FITC conjugate solution 5G to each well. (No dilution required).

8. A steps 4, 5 and 6WAS repeated

9. Added a small drop of mounting medium 6 to each well and carefully cover with a coverslip.

10. The slide was read 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.

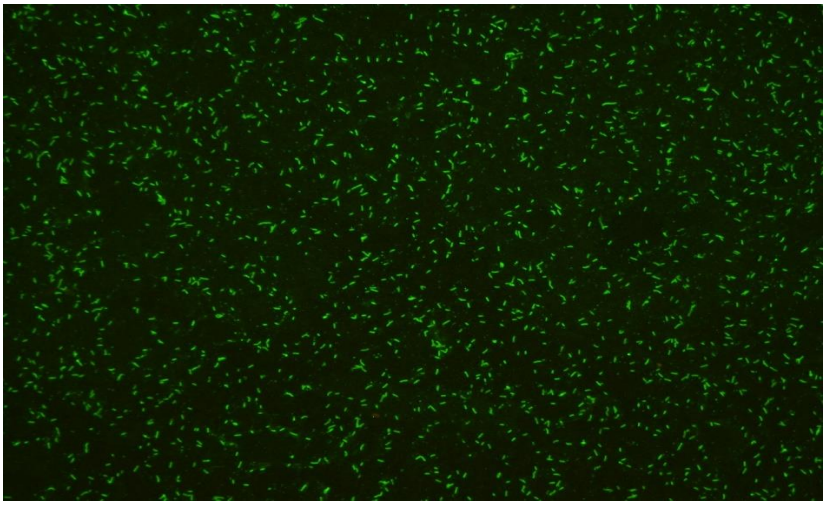

Figure 1: IFFA for *Legionella*

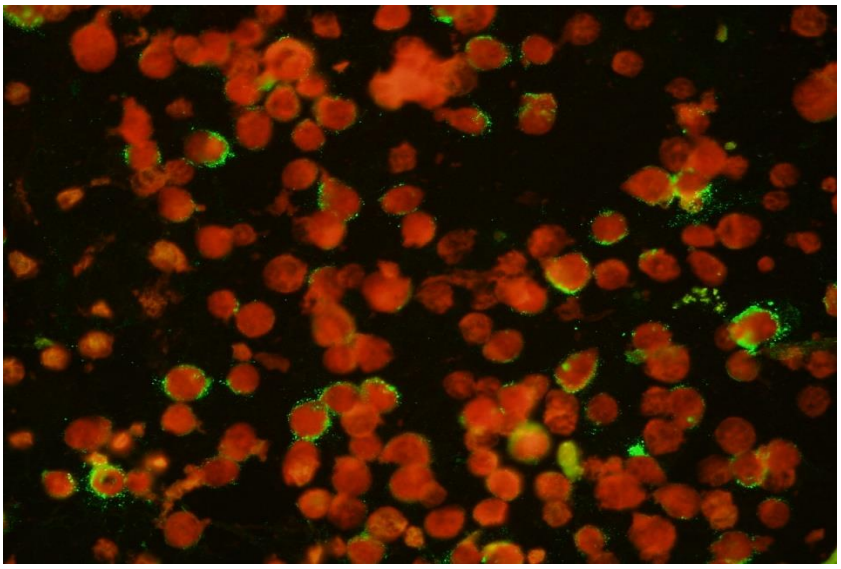

Figure 2: IIFA for *Mycoplasma*

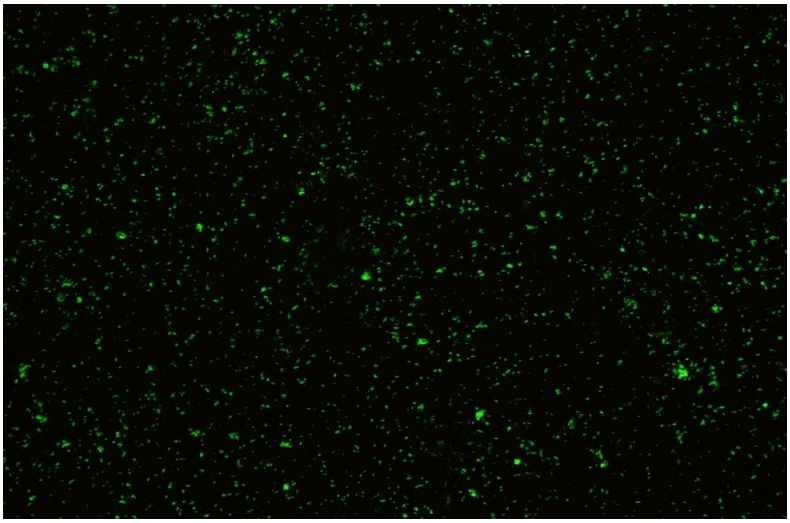

Figure 3: IIFA for *Coxiella*

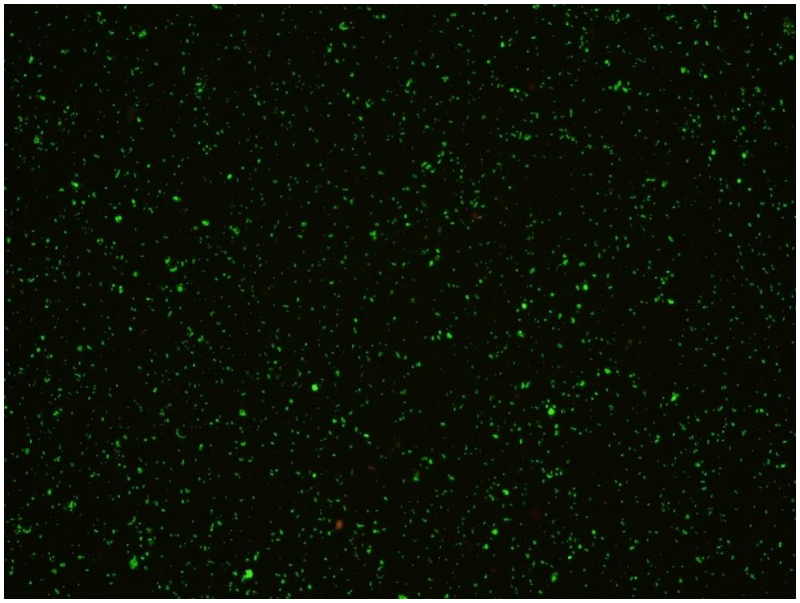

Figure 4: IIFA for *Chlamydia*

Result and Discussion

In the current study, the following bacteria were detected M. *pneumonia, C. pneumoniae, C. burnetii and L. pneumophila* were isolated from 11 (27.6 %), 4 (10 %), 2 (5%) and 2 (5 %), respectively which mean the totally 47.6 % are bacteria infection of atypical pneumonia while other may be viral or other causes.

In line with our study the Jiang, Z., *et al*,2021 study about *Mycoplasma pneumoniae* is a major causative agent of community-acquired pneumonia which can lead to both acute upper and lower respiratory tract inflammation, and extrapulmonary syndromes(Jiang, Z., *et al*,2021).

atypical" organisms do not have such properties and mostly caused by *Legionella*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* (Eisenhut & Mizgerd, 2008). The reason why in our study, bacteria was the most commonly encountered agent is that our study is a hospital based study and that most children admitted are severely ill children and severe pneumonia is usually seen bacterial causes and most bacterial infections are often secondary complication to primary viral pneumonia (Rodrigues and Groves, 2018).

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