Original Research Article

Early diagnosis of atypical pneumonia among children by using immunofluorescence biochip method

Bewin Oral J[1*], Usha H C[2], Madhuri Kulkarni[3], Goldy S J[4], Rema Devi S[5], Jobin S R[6]

{[1]Assistant Professor, [5]Professor & HOD, [6]Assistant Professor, Department of Microbiology} {[4]Assistant Professor, Department of Obstetrics & Gynaecology} Dr. Somervell Memorial CSI Medical College Hospital, Karakonam, Thiruvananthapuram Dist., Kerala, INDIA.
[2]Clinical Microbiologist, Cauvery Heart & Multispecialty Hospital, Mysuru, INDIA.
[3]Retired Professor, Department of Microbiology, JSS Medical College, Mysuru, INDIA.
Email: bewin.grace@gmail.com

Abstract

Background: Atypical pneumonia, which is caused by *Mycoplasma pneumoniae*, *Legionella* spp., *Chlamydia pneumoniae* and *viruses*, has been thought to account for 7%-30% of community-acquired pneumonia (CAP). The treatment for the pathogens that cause atypical pneumonia is different from that of other bacterial pneumonia. The severity of pneumonia can range from mild to life-threatening, with uncomplicated disease resolving with outpatient antibiotics and complicated cases progressing to septic shock, acute respiratory distress syndrome (ARDS) and death. Therefore this study helps in early detection of the atypical pathogens causing CAP and also in initiation of appropriate treatment which reduces morbidity and mortality. **Methods:** This is a prospective study conducted in Department of Microbiology, JSS hospital, Mysore for a period of 18 months from January 2014 to August 2015. **Results:** 70 patients (children) of age less than 15 years with no evidence of typical pathogens causing pneumonia were included in our study. Among the 70 samples tested for atypical pathogens, 44 were positive (63%). *Mycoplasma pneumoniae* was the predominant atypical pathogen identified in 32.9% of the patients followed by *Respiratory syncytial virus* (17.1%). *Chlamydophila pneumoniae and Influenza B* were the next common pathogens detected in 4.3% of the patients. Mixed infections by *Legionella pneumophila, Adenovirus and Influenza A and B virus* were detected among the atypical pneumonia patients. **Conclusion:** Atypical pneumonia is in high incidence among CAP, even our study proved their high rate of positivity of 63%**.** Hence our study facilitates early diagnosis of atypical pneumonia patients by detecting IgM antibodies using Indirect Immunofluorescence and in starting the appropriate treatment in the early phase of infection, which helps in uplifting the public health of community.
Key Words: Atypical Pneumonia, Immunofluorescence, IgM antibodies

***Address for Correspondence:**
Dr Bewin Oral J, Assistant Professor, Department of Microbiology, Dr. Somervell Memorial CSI Medical College Hospital, Karakonam, Thiruvananthapuram Dist., Kerala, INDIA.
Residential Address: B 9, Ngo B Colony, Perumalpuram Post, Tirunelveli, Tamil Nadu - 627007. INDIA.
Email: bewin.grace@gmail.com
Received Date: 11/12/2019 Revised Date: 19/01/2020 Accepted Date: 03/02/2020
DOI: https://doi.org/10.26611/10081336

Access this article online

Quick Response Code:

Website: www.medpulse.in

Accessed Date: 28 March 2019

INTRODUCTION

Pneumonia is an inflammation of the lung parenchyma, often due to infection. It is a major cause of morbidity and mortality with an incidence of 20-30% in the developing countries and 3-4% in developed countries [1]. Clinically, Pneumonia is often classified as "Typical" or "Atypical pneumonia". In Typical pneumonia, there is sudden onset of fever, chills, pleuritic chest pain and productive cough. Typical pneumonia is usually caused by bacterial pathogens like *Streptococcus pneumoniae*, *Staphylococcus aureus, Klebsiella pneumoniae and Haemophilus influenzae.* Atypical pneumonia is a form of pneumonia

How to cite this article: Bewin Oral J *et al.* Early diagnosis of atypical pneumonia among children by using immunofluorescence biochip method. *MedPulse International Journal of Microbiology.* March 2020;13(3): 41-49. https://www.medpulse.in/Microbiology/

caused by the atypical pathogens and presents clinically in a pattern which is inconsistent with typical pneumonia [2]. "Atypical pneumonia" is caused by atypical organisms. These Atypical organisms may include bacteria, virus, fungi, and protozoa. The Atypical pneumonia patients usually present with scanty to moderate amount of sputum production, limited consolidation, insignificant raise in white cell counts and no alveolar exudates. Even though these infections are called "atypical," they are not uncommon.[3] Atypical pneumonia comprises 20% of all cases of pneumonia and prompt diagnosis is essential for proper clinical management and for better clinical outcome. Childhood Acute Respiratory Infection (ARI) is the largest cause of morbidity among under-five aged children across the world. Pneumonia amounts for almost one-fifth of total mortality in vulnerable age groups[4]. *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* may exacerbate asthma and are also associated with coronary artery disease and multiple sclerosis (MS). These atypical pathogens are known to cause community acquired pneumonia (CAP) resulting in major public health threat. The importance about the atypical pneumonia patients are the difficulty in early diagnosis and non-responsiveness to β-lactam therapy. Clinical presentation is often indistinguishable from pneumonia due to classical pathogens. Culture identification of these pathogens are technically demanding and time-consuming and even has a low sensitivity, these drawbacks further affect our early and accurate diagnosis of the infected patients, hence affects the management of these patients resulting in high morbidity and mortality. Thus there is a need for advanced diagnostic tools to detect these atypical pathogens causing atypical pneumonia. Hence the main aim of our study was in early detection of Specific *IgM* antibodies in the early phases of the infection using biochip Indirect Immunofluorescence method in order to initiate appropriate treatment which reduces the morbidity, hospital stay and mortality among the infected individuals.

AIMS AND OBJECTIVES OF THE STUDY

1. Early diagnosis of different atypical pneumonia causing pathogens.
2. Detecting the rate of different rare pathogens.

MATERIALS AND METHODS

The present study is a prospective study conducted in the Department of Microbiology, JSS hospital, Mysore for a period of 18 months from January 2014 to August 2015.

Selection of patients:

Inclusion criteria:

Patients (children) below 15 years of age with clinical signs and symptoms of pneumonia (productive or non-productive cough, fever and positive lung signs in chest radiography) and children who were not diagnosed of typical pneumonia caused by typical agents were included in this study.

Exclusion criteria

The patients with culture reports yielding positive result for growth of typical pathogens causing typical pneumonia were excluded from our study. And patients more than 15 years of age were excluded from study.

Sample collection

Whole blood samples were collected and serum separation was done by centrifugation. The serum samples were then subjected to indirect immunofluorescence method using Biochip (Pneumoslide -M -Vircell slide-Granada, Spain). Manufacturer's test kit protocol was followed during testing and for interpretation of results.

Testing kit

It is an indirect immunofluorescence assay (IFA) kit used for the detection of specific IgM antibodies against the main etiological agents causing atypical pneumonia namely,

Legionella pneumophila serogroup 1, Mycoplasma pneumoniae, Coxiella burnetii, Chlamydophila pneumoniae, Adenovirus, Respiratory Syncytial Virus, Influenza A, Influenza B and Parainfluenza serotypes 1,2 and 3.

Principle of the IFA test

The IFA method is based on the reaction between the IgM antibodies present in the patient's sample and the antigen which is already adsorbed on the slide surface. The specific IgM antibodies present in the sample combines with the antigen in the slide forming an antigen- antibody complex. In the following step, the antigen-antibody complex combines with the fluorescein-labelled anti-human globulin and they can be seen under fluorescent microscope.

VIRCELL PNEUMOSLIDE M SLIDE: 10 slides of 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. *Mycoplasma pneumoniae* in McCoy cells.
3. *Coxiella burnetii* in phase II suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.
4. *Chlamydophila pneumoniae*, elementary bodies.
5. *Adenovirus* in HEp-2 cells.
6. *Respiratory syncytial virus* in HEp-2 cells.
7. *Influenza A* in LLC-MK2 cells.
8. *Influenza B* in LLC-MK2 cells.

9. *Parainfluenza* serotypes 1, 2 and 3 in LLC-MK2 cells.
10. Cell control.

All the antigens in the slide are obtained in cell culture except *L .pneumophila*.
Each viral well contains infected cells inactivated with formaldehyde and also the non-infected cells and they are fixed using acetone.

Assay procedure

Blood should be collected under aseptic precautions by venipuncture. Serum samples are to be refrigerated at 2-8ºC or even can be stored at -20ºC in a deep freezer, if there is an expectation of delay in performing the test for 7 days. Samples should not be repeatedly frozen and thawed, since it might decrease the immunoglobulin titre (especially IgM antibodies). Hyperlipidemic or contaminated sera should be avoided. Serum or plasma samples are the compatible samples for this kit.

Procedure:

1. All reagents are brought to room temperature before use. Slides are allowed to reach room temperature before opening.
2. Half dilution of serum samples are prepared by adding 25 µl of sample to 25 µl of Phosphate buffered saline (PBS). The control sera should not be diluted.
3. Diluted patient's serum is treated with anti-human IgG sorbent by adding 30 µl of sera to 150 µl of sorbent and mixed thoroughly, Control sera must not be sorbent treated. The treated sera should be centrifuged to remove the precipitate, which interfere with the test.
4. 15 µl of sorbent-treated serum is added in every slide well. 15 µl of non-diluted positive control is added to each well of a slide and 15 µl of non-diluted negative control to each well of another slide.
5. Slide is placed in a humid chamber and incubated at 37ºC for 90 minutes.
6. Slide is rinsed briefly with a gentle stream of PBS **(**directing PBS at wells is avoided) and is immersed in PBS while shaking gently on a shaker, for ten minutes. Slide is dip washed briefly in distilled water.
7. Slide is allowed to air dry.
8. 15 µl of anti-human IgM FITC conjugate solution is added to each well. (No dilution required).
9. Incubated in a humid chamber for 30 minutes at 37ºC.
10. Steps 6 and 7 are repeated
11. A small drop of mounting medium is added to each well and carefully covered with a coverslip.
12. Observation of slide is done immediately under 400x magnification using fluorescence microscope. If any delay in observation, these slides can be allowed to be kept in dark at 2-8ºC for 24 hours.

Quality control:

The reaction is considered positive when apple green nuclear, cytoplasmic and/or peripheral fluorescence in 1-15% of the cells for positive sera *to adenovirus, influenza, RSV*. Apple green fluorescence noticed all over the organism in samples infected with *Legionella, Chlamydophila or Coxiella.* In case of mycoplasma infection, Apple green fluorescence is noticed in periphery. The reaction is negative, when no fluorescence noticed in cells of *Legionella,Chlamydophila and Coxiella* and Red cellular pattern noticed for *Mycoplasma, adenovirus, influenza A and B, RSV and parainfluenza*. The presence of fluorescence in all the cells or in the well no.10 involves the presence of antinuclear or anticellular antibodies and the result should not be considered as positive, then an alternative technique is required. Be sure to check in all the cases the absence of fluorescence in the cell control well. As cross-reactive antibody is frequently found in patients with non-Legionella infection, positive results are evaluated considering the symptomatology and the IgM reports. Due to these reasons that IgM positive Legionella sera at 1/12 were diluted in titres from1/12 to 1/192 and only titres higher than or equal to 1/96 were considered as significant for *Legionella* infection.

OBSERVATION AND RESULTS

The present study was conducted in the Department of Microbiology, JSS hospital, Mysore for a period of 18 months from January 2014 to August 2015. In this study period, patients who presented with features of pneumonia to the hospital were screened for microbiological diagnosis for typical organisms adopting standard laboratory techniques. The patients with positive result for diagnosis of typical pathogens causing typical pneumonia were excluded from the study. 70 patients with no evidence of typical pathogens causing pneumonia were included in the present study. Serum samples from these patients were tested for the presence of IgM antibodies by Indirect Immunofluorescence(IF) to various organisms causing atypical pneumonia, which include *Legionella pneumophila serogroup 1 (LP1), Mycoplasma pneumoniae (MP), Coxiella burnetii (COX), Chlamydophila pneumoniae (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV), Influenza A (INFA), Influenza B (INFB) and Parainfluenza 1, 2 and 3 (PIVs).* The study included 70 children of age less than15 years. Among the 70 samples tested for atypical pathogens, 44 samples yielded positive report for atypical infections. The

rate of positive cases with atypical infection is 63%. Out of these 44 positive samples, 28 were male children and 16 were female children. Chest X-ray of those patients with positive result for atypical infection did not show any positive findings for pneumonia. Around 61.5% of children tested were associated with diarrhoea and vomiting. And around 50% of the children who presented with severe pneumonia were detected with specific IgM antibodies of atypical pathogens, hence diagnosed of atypical pneumonia. Malnutrition was a risk factor found in three children (4.3%) who were diagnosed with atypical pneumonia. Around 59.5% of children had leucocytosis whereas 4.7% had leukopenia. *Mycoplasma pneumoniae* (32.9%) was the predominant atypical pathogen detected followed by *Respiratory syncytial virus* (17.1%). *Chlamydophila pneumoniae and Influenza B* (4.3%) were the next commonly detected atypical pathogens in our study. Mixed infections were noticed among the atypical pneumonia patients. Four patients showed positivity for mixed infection. One child with mixed infection presented with positive result for *Legionella pneumophila, Adenovirus and Influenza A* virus. Two among the four children with mixed infection had co -infection of *Mycoplasma pneumoniae* and *Adenovirus.* And the other child had co -infection of *Mycoplasma pneumonia and Influenza A&B*. The common age of *Mycoplasma* infected patients ranges from 2-4 years and of *RSV* infected children was less than 1 year, suggesting that *RSV* is more common among infants. Among 70 children in our study, two of them died. One who died had co- infection of *Mycoplasma pneumoniae* infection and H1N1 infection. The other child who died had pulmonary hypertension in addition to the current *RSV* infection.

Table 1: Age wise distribution

Age in years	Children
0-5	66 (94%)
6-15	4 (6%)
Total	70 (100%)

Table 2: Gender wise distribution

Gender	Children
Male	43 (61%)
Female	27 (39%)
Total	70 (100%)

Table 3: Based on Clinical diagnosis

Clinical Diagnosis	Children
Bronchopneumonia/ Pnemononia	67 (96%)
Pneumonia with ARDS/Shock/Sepsis	3 (4%)
Total	70 (100%)

Table 4: On the basis of Symptoms presented

Symptoms	Children (n=70)
Cough	70 (100%)
Fever	70 (100%)
Dyspnoea	57 (81%)
Chest pain	4 (6%)
Expectoration	0 (0%)
Head ache	0 (0%)
Confusion	3 (4%)
Diarrhea vomiting	31 (44%)
Cold with running nose	70 (100%)

Table 5: X-Ray findings

X-Ray	Children
Nil	22 (31%)
Bilateral pneumonic patches	19 (27%)
Para cardiac infilterations	8 (11%)
Left Mid zone pneumonia	7 (10%)
Right lower lobe consolidation	5 (7%)

Diffuse b/l patchy opacities	**3 (4%)**
Cardiomegaly with interstitial patches	**2 (3%)**
Extensive infilterates	**0 (0%)**
Middle lobe consolidation b/l	**2 (3%)**
Hilar opacities	**0 (0%)**
Mid zone pneumonia	**1 (2%)**
ARDS	**1 (2%)**
Multifocal consolidation	**0 (0%)**
Total	**70 (100%)**

Table 6: Pneumoslide M- Organism detected

Pneumoslide M	Children (n=70)
No organism detected	**26(37.1%)**
Organism detected	**44(62.9%)**
Legionella	**1(1.4%)**
Mycoplasma	**23(32.9%)**
Coxiella	**0(0%)**
Chlamydophila	**3(4.3%)**
Adenovirus	**1(1.4%)**
RSV	**12(17.1%)**
Influenza-A	**1(1.4%)**
Influenza-B	**3(4.3%)**

Pneumoslide M	Children (n=70)
No infections	**26(37.1%)**
Infections	**44(62.9%)**
One infections	**40(57.1%)**
Two Infections	**4(5.7%)**

DISCUSSION

The atypical respiratory pathogens namely *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae, Legionella pneumophila and viruses like RSV, Adenovirus ,Influenza virus* cause acute respiratory-tract infections and community acquired pneumonia in children especially in developing countries. They can also commonly occur as co pathogens in mixed infectious pneumonia patients resulting in high mortality [5]. The prevalence of each pathogen varies from country to country and could be due to the differences in the geographic areas or due to the different diagnostic procedures used in different studies [6]. Despite the progress made in the diagnosis of Lower Respiratory Tract Infection, it takes a few days to identify the causative microorganism in blood or sputum samples [7]. Cell cultures for viral and atypical bacterial isolation are usually sensitive and detect a broad spectrum of organisms. But the time taken to get results may be as long as 14 days [8]. Recently PCR technique has been reported as a rapid method for detection of atypical pathogens. PCR assays need specialized equipments and reagents which are expensive [9]. The aim of the present study was to detect various atypical pneumonia causing pathogens by using rapid indirect immunofluorescent assay in facilitating the early initiation of the appropriate treatment for better prognosis. In our study, out of 70 patients (children) selected, 44 samples (63%) yielded positive result proving atypical pneumonia. The positivity rate for atypical pneumonia is 63% in our study. This observation was similar to many other studies conducted throughout the world [10, 11]. An Egyptian study by Zaki and Godal has reported a high incidence of atypical pathogens compared to our study and also noted that co-infections of atypical pathogens with agents causing classic pneumonia were high in their study [12]. This was not observed in our study, where only atypical pathogens were detected, probably due to the fact that our study population included only those patients who were tested negative for classic pneumonia causing agents by standard microbiological methods. Various other studies which reported the incidence of atypical pneumonia from all parts of the world show considerable variation in the frequency of their occurrence [11, 13]. The present study correlates with the study done in USA whose incidence was 54%. Asian countries have lesser incidence rates compared to our study while Nordic countries show similar data [10, 14]. Incidence of Bacterial causes of atypical pneumonia also showed comparable results with a study conducted by Agmy *et al.* in Egypt and was shown to be 34%[18,19], whereas a Thailand study by

Nuanchan *et al.* [16] on the contrary showed less incidence of bacterial atypical pneumonia. Studies in various Asian countries including Kuwait have shown similar results for atypical pathogens with incidence ranging from 24-39 % [14, 15]. Few INDIAN studies have been done to assess the incidence of atypical pathogens in the community and the causative agents were identified to be of low frequency compared to the present study [7, 17]. The lower incidence may be due to the reason that only bacterial pathogens were diagnosed, unlike in our study, where both bacteria and virus were detected. Most studies showed atypical virus as the leading cause of respiratory tract infections in children. However in our study bacterial infections were more common compared to viral among the study population. In our study viral aetiology was detected in 24% of the children, whereas bacterial aetiology was detected in 39 % of children. More male children were affected in our study, concurrent with most studies. *Legionella pneumophila* is known to cause Legionnaire's disease which is a systemic infection involving lungs and presents as severe pneumonia requiring hospitalisation and intensive care [18]. Mortality with *Legionella pneumophila* pneumonia is usually high, compared with other atypical pathogens. Kristopher *et al.* have reported mortality rate of 14 % [19]. Our study showed much higher mortality (in 3 out of 4 patients, it was fatal). Co morbid conditions present in our patients' i.e. pulmonary hypertension, rheumatoid arthritis, and seizure disorder were probably responsible for this trend, as also shown in several other studies [19]. Most of our patients presented with cough, expectoration, fever and dyspnoea as the chief complaints, with few of them also had complaints of headache and chest pain. Clinical picture, routine laboratory tests and chest X-ray did not help in predicting the aetiology. Various other studies have also shown that, different etiologic causes for pneumonia could not be distinguished on clinical basis, or radiological or various routine laboratory methods [20, 21, 11]. Hence the need for laboratory confirmation in the diagnosis of atypical pneumonia forms a milestone in optimum management. *Coxiella burnetti* was detected in one patient, who had pleural effusion in addition to bronchopneumonia. She did not have other serious complications like myocarditis, endocarditis or cerebral involvement. Carlos *et al.* has described that the zoonotic causes of atypical pneumonia may involve pleura, and patients presenting with history of exposure to known risk factors and unexplained pleural effusion, may be suspected to have pneumonia caused by zoonotic atypical pathogens like *Coxiella or Francicella.* However, there was no history of intimate contact with animals in our patient [22]. In the present study, 63% of the hospitalised children aged less than 16 years with acute respiratory tract infection were infected with at least one atypical respiratory pathogen. *M. pneumoniae and RSV* were the predominant agents causing pneumonia. Multiple pathogens were detected in 5.7% of the patients. Two studies conducted in China by Ji liu *et al.* and Chen *et al.* for the detection of atypical pathogens in children, reported causative agents of pneumonia in 25.7% and 58.4% respectively [23,24]. In another study Sally *et al.* had shown prevalence of atypical pathogens in children to be 45 %. [5]. however our study showed much higher prevalence compared to these studies. Similar high prevalence was also seen in a Brazilian study, which reported 78% prevalence with viral infections accounting for 60 %. [25]. An INDIAN study conducted by Jyostsna *et al.* in Lucknow showed low prevalence of atypical pathogens with only 10% of the children being diagnosed with atypical pathogens and the only pathogen identified was *Mycoplasma pneumoniae* [26]. Our study also showed high concurrence with the various other studies regarding the most common etiological agent detected, which was found to be *M pneumoniae* followed by *RSV.* Chinese studies differ in this matter where the predominant viral pathogens were *adenovirus and Influenza B virus*. [27, 28, 25, 29.30, 23, 24, 31] This could be due to geographical variation in prevalence of various pathogens [6]. *Chlamydophila pneumoniae* was the etiological agent present in 4.3% of children. The results are in concordance with a study from King George's Medical University Lucknow, which showed prevalence of *Chlamydophila pneumoniae* to be 5.5 % [32, 33]. *Influenza B, Influenza A and Adenovirus* were the other viral pathogens detected in 6% of children, which correlated with similar prevalence in other studies [23, 24]. Our study had only one child with *Legionella pneumophila* antibodies. This low prevalence has been reported in other studies also [23,24]. Mixed infections (5.7%) found in our study, correlated well with the study conducted by Chen *et al.* who reported mixed infections in 6.5% of children [24]. The main co-pathogen found in mixed infection in our study was *Mycoplasma pneumoniae* which was also observed in other studies [24, 34, 35, and 36]. Sally *et al.* have hypothesised that *Mycoplasma pneumoniae* often participated in mixed infections, and it may enhance susceptibility to other infectious agents [21]. Patients with features of atypical pneumonia with detectable pathogens, had less severe breathlessness compared to those in whom organisms were not detected (Undiagnosed category), indicating less severe pneumonia and better outcome in them. All the children in our study had similar clinical pictures with cough, fever, and rhinitis. Diarrhoea and vomiting were also common extra-pulmonary symptoms present in significant number of children. Most other studies also recorded similar findings. Esposito *et al.* and Wubel *et al.* had made similar observations, and concluded that clinical and routine laboratory parameters did not help in differentiating typical

and atypical pneumonia in children [16, 37, 30, 20, and 11]. In majority of the studies, *M. pneumonia* was found in all age groups, with higher incidence in children aged 5-14 years [24]. However, in our study, though all age groups were affected, it was most common in children below 5 years. Most of the *RSV i*nfection in our study was seen in children below 1 year of age, which was in concordance with other studies [30]. Mortality was observed in children (2.9%). *RSV* pneumonia was reported in this child, however, the associated severe primary pulmonary hypertension in this child contributed to the mortality. Several studies have documented that comorbid conditions worsens the outcome in these children [38]. The other child had co-infection with M.pneumoniae and H1N1.

Figure 1

Figure 2

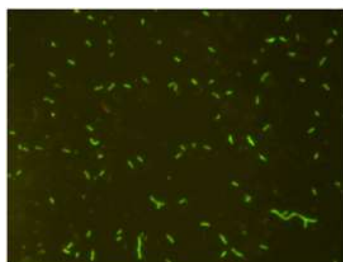

Figure 3

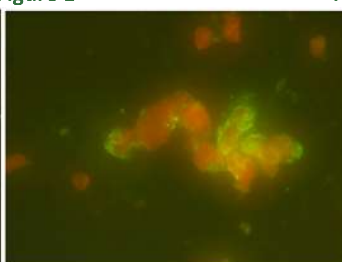

Figure 4

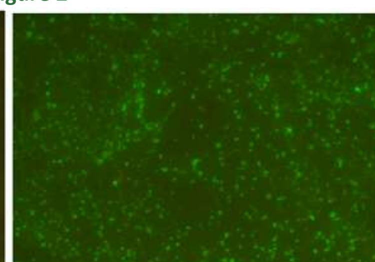

Figure 5

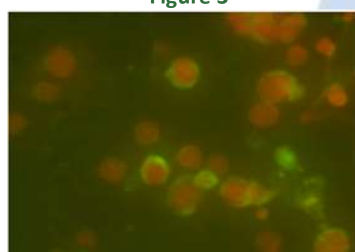

Figure 6

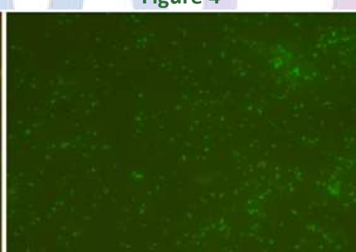

Figure 7

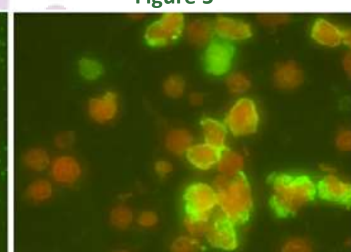

Figure 8

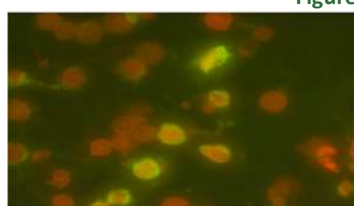

Figure 9

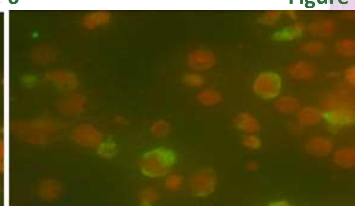

Figure 10

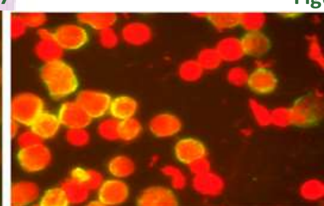

Figure 11

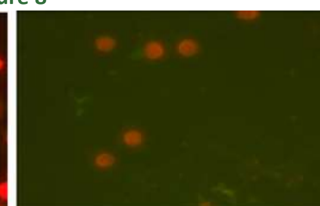

Figure 12

Figure 1: PneumoslideM Indirect Immunofluoresence Kit for IgM detection of atypical pneumonia; **Figure 2:** Fluorescent Microscope; **Figure 3:** Positive for *Legionella pneumophila* antibodies; **Figure 4:** *Adenovirus* positivity showing perinuclear fluorescence; **Figure 5:** Positive for *Chlamydophila pneumonia*e antibodies; **Figure 6:** Positive for *Influenza B* antibodies showing perinuclear fluorescence; **Figure 7:** Positive *for Coxiella burnetti* antibodies; **Figure 8:** IgM antibodies to *Mycoplasma pneumoniae* showing perinuclear fluorescence; **Figure 9:** Perinuclear fluorescence showing presence of antibodies to *RSV;* **Figure 10:** Antibodies to *Parainfluenza virus* showing positive IgM antibodies; **Figure 11:** Antibodies to *Influenza A virus* showing perinuclear fluorescence; **Figure 12:** Cell control

SUMMARY

Atypical pneumonia is one of the major causes of community acquired pneumonia in children. The present study titled "Early diagnosis of atypical pneumonia among children by using immunofluorescence biochip method" was taken up with the objective of early diagnosis of different atypical pneumonia causing pathogens and also rate of different rare pathogens. 70 children were included in our study, these children were clinically diagnosed patients of pneumonia yet negative for typical pathogens causing pneumonia. In these 70 serum samples tested for the presence of specific IgM antibodies to various atypical pathogens causing atypical pneumonia, only 44 serum samples yielded positive result. Details of clinical history, examination, associated radiological and routine laboratory findings were noted. The positivity rate in our study was 63%. Predominant atypical pathogens detected in children were *Mycoplasma pneumoniae* and *Respiratory syncytial virus.* Clinical history/examination, radiological findings and routine laboratory investigations did not differentiate between atypical pneumonia and typical pneumonia. *Legionella pneumophila* was associated with severe pneumonia and increased mortality. *Mycoplasma pneumoniae* was the predominant aetiological atypical agent causing atypical pneumonia in children below five years of age. *Respiratory syncytial virus* was found to be affecting more infants in our study. *Chlamydophila pneumoniae* and *Adenovirus* were causing severe lower respiratory tract infection among children resulting in increasing community acquired pneumonia. Mortality due to atypical pathogens was less in children and the observed mortality rate may be due to associated co morbid conditions.

CONCLUSION

The main objective of the study was rapid diagnosis of atypical pneumonia by IgM antibody detection using Indirect Immunofluorescence biochip method. Atypical agents (bacteria and viral) causing atypical pneumonia were detected among 63% of children selected in our study. Clinical features, routine laboratory findings and radiological features could not differentiate the typical and atypical pneumonia in our study thereby emphasising the importance of microbiological diagnosis of atypical pneumonia. *Mycoplasma pneumoniae* and *Respiratory syncytial virus* are the major pathogens causing atypical pneumonia in children. *Mycoplasma pneumoniae* and *RSV* had good clinical outcome whereas *Legionella pneumophila* pneumonia had worst outcome. Lower respiratory tract infections are fairly common in children with atypical pathogens being implicated in good number of cases. The clinical presentation can range from mild to severe in children with no significant radiological and laboratory findings. *Respiratory syncytial virus* is more common in infants whereas *Mycoplasma pneumoniae* cause disease in older children. *Adenovirus* can also cause severe respiratory disease in children and have to be considered while treating children with lower respiratory infections. Most of the children recover from atypical pneumonia, while children with co morbid conditions have very severe manifestations of disease and sometimes even fatal consequences are encountered.

REFERENCES

1. Shrestha R, Paudel N, Barakoti B, Dhungana D,Sharma P. Etiology and clinical profile of inpatients with community acquired pneumonia in Manipal teaching hospital, Pokhara, Nepal. Nepal Journal of Medical sciences 2012;1(2):84-8.)
2. Bedi RS. Community acquired pneumonia – Typical or atypical. Lung INDIA 2006;23:130-1
3. Centers for Disease Control and Prevention. (2014). Atypical Pneumonia. Retrieved from http://www.cdc.gov/atypical/
4. Joseph L Mathew, Ashok K Patwari, Piyush Gupta,Dheeraj Shah, Tarun Gera, *Siddhartha Gogia, , *et al.*. UNICEF-PHFI Series on Newborn and child health, INDIA: Methodology for systematic reviews on child health priorities for advocacy and action. INDIAn Pediatr 2011;48:191-218.
5. Jahnz-Rózyk K[1], Targowski T, Jurkiewicz D, Zielnik-Jurkiewicz B. Atypical bacteria in respiratory tract infections --pathogenesis and diagnosis .Pol Merkur Lekarski. ;25(149):412-
6. Maitreyi, R. S., Broor, S., Kabra, S. K., Ghosh, M., Seth, P., Dar, L. and Prasad, A. K. Rapid detection of respiratory viruses by centrifugation enhanced cultures from children with acute lower respiratory tract infections. J Clin Virol 2000; 16:41–47.
7. Bansal S, Kashyap S, Pal L S, Goel A, "Clinical and bacteriological profile of Community Acquired Pneumonia in Shimla, Himachal Pradesh" INDIAn J Chest Dis Allied Sci. 2004;46(1):17-22.
8. Jennings LC, Anderson TP, Beynon KA, Chua A, Laing RT, Werno AM, *et al.* Incidence and characteristics of viral community-acquired pneumonia in adults. Thorax. 2008;63(1):42–8.
9. Syrmis MW, Whiley DM, Thomas M, Mackay IM, Williamson J, Siebert DJ, *et al.*. A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. J Mol Diagn. 2004;6(2):125–31.
10. Ragnar Norrby S: Atypical pneumonia in the Nordic countries: Aetiology and clinical results of a trial comparing fl eroxacin and doxycycline. Nordic Atypical Pneumonia Study Group. J Antimicrob Chemother 1997; 39:499–508.
11. Gleason PP. The emerging role of atypical pathogens in community-acquired pneumonia. *Pharmacotherapy* 2002; 22: 2S–11S.
12. El Sayed Zaki M, Goda T. Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study. J Infect Dev Ctries. 2009;3:199–205.

13. Arnold FW, Summersgill JT, LaJoie AS, *et al.*. A worldwide perspective of atypical pathogens in community-acquired pneumonia. Am J Respir Crit Care Med 2007;175:1086–1093.
14. Ngeow YF, Suwanjutha S, Chantarojanasriri T *et al.*. An Asian study on the prevalence of atypical respiratory pathogens in community acquired pneumonia. Int J Infect Dis 2005; 9:144-53.
15. N. Behbehani, A. Mahmood, E. M. Mokaddas *et al.*, "Significance of atypical pathogens among community-acquired pneumonia adult patients admitted to hospital in Kuwait," *Medical Principles and Practice*. 2005 :14, (4), 235–240.
16. Prapphal, N., Suwanjutha, S., Durongkaveroj, P., *et al.*. (2006): Prevalence and clinical presentations of atypical pathogens infection in community acquired pneumonia in Thailand. J. Med. Assoc. Thai., 89, 1412-1419
17. Udwadia ZF, Doshi AV, Joshi JM. Etiology of community acquird pneumonia in INDIA. Eur Respir J 2003;22:5445.
18. Cunha BA. Severe legionella pneumonia: rapid diagnosis with Winthrop-UniversityHospital's weighted point score system (modified). Heart Lung 2008;37:312–21.
19. Thibodeau, Viera JA. Atypical pathogens and challenges in community-acquired pneumonia. *Am Fam Physician* 2004;69:1699.
20. Esposito S, Blasi F, Bellini F, Allegra L, Principi N. Characteristics of Mycoplasma pneumoniae and Chlamydia pneumoniae infections in children with pneumonia. Mowgli Study Group. Eur Respir J 2001; 17: 241–5.
21. Sally A.F *et al.*, Pneumoslide-M Technique for Rapid Detection of Atypical Pathogens in Critically ILL Children with Lower Respiratory Tract Infections, Journal of Medical Sciences;2006:6(5):793-799
22. Carlos E Kummerfeldt,* John T Huggins, Steven A Sahn. Unusual Bacterial Infections and the Pleura. Open Respir Med J. 2012; 6: 75–81.
23. Liu J, Ai H, Xiong Y, *et al.*. Prevalence and correlation of infectious agents in hospitalized children with acute respiratory tract infections in Central China. PLoS One 2015;10:e0119170
24. Keping Chen, Runqing Jia, Li Li, Chuankun Yang and Yan Shi. The aetiology of community associated pneumonia in children in Nanjing, China and aetiologicalPatterns associated with age and season. BMC Public Health .2015; 15:113.
25. Nascimento-Carvalho CM, Ribeiro CT, Cardoso MR *et al.*. The role of respiratory viral infections among children hospitalized for community-acquired pneumonia in a developing country. *Pediatr Infect Dis J* 2008; **27**: 939–941.
26. Agarwal J, Awasthi S, Rajput A, Tiwari M and Jain A. Atypical bacterial pathogens in community-acquired pneumonia in children: a hospital-based study. Trop Doct 2009, 39: 109– 111
27. Huong PL, Hien PT, Lan NT, Binh TQ, Tuan DM, Anh DD, *et al.*. First report on prevalence and risk factors of severe atypical pneumonia in Vietnamese children aged 1–15 years. BMC Public Health. 2014;14: 1304.
28. Tsolia, M.N., Psarras, S., Bossios, A., *et al.*. (2004): Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. Clin. Infect. Dis., 39, 681-686
29. Juven T, Mertsola J, Waris M, *et al.*. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000; 19:293–8
30. Wubbel L, Muniz L, Ahmed A, Trujillo M, Carubelli C, McCoig *et al.*. Etiology and treatment of community-acquired pneumonia in ambulatory children. Pediatr Infect Dis J 1999;18:98–104.
31. Bamba M, Jozaki K, Sugaya N, *et al.*. Prospective surveillance for atypical pathogens in children with community-acquired pneumonia in Japan. J Infect Chemother 2006;12:36-41
32. Hammerschlag MR. Chlamydia pneumoniae and the lung. Eur Respir J 2000; 16:1001–7.
33. Jain R, Jain A, Agarwal J, Awasthi S. Chlamydia sp. in hospitalised children with community acquired pneumonia. INDIAn Pediatr. 2007 Mar;44(3):216–18.
34. Vervloet LA, Marguet C, Camargos PA. Infection by *Mycoplasma pneumoniae* and its importance as an etiological agent in childhood community acquired pneumonias. Braz J Infect Dis. 2007;11:507–14.
35. Chen LL, Cheng YG, Chen ZM, Li SX, Li XJ, Wang YS: Mixed infections in children with Mycoplasma pneumoniae pneumonia. Zhonghua Er Ke Za Zhi 2012, 50(3):211–215.
36. Tristram DA, Miller RW, McMillan JA, Weiner LB. Simultaneous infection with respiratory syncytial virus and other respiratory pathogens. Am J Dis Child. 1998; 142: 834–836.
37. Masia M, Gutierrez F, Padilla S, Soldán B, Mirete C *et al.*. Clinical characterisation of pneumonia caused by atypical pathogens combining classic and novel predictors. *Clin. Microbiol. Infect.* 2007; 13: 153–61.
38. Jung JW. Respiratory syncytial virus infection in children with congenital heart disease: global data and iterim results of Korean RSV-CHD survey. Korean J Pediatr 2011;54(5):192-196.

Source of Support: None Declared
Conflict of Interest: None Declared