Etiology of community acquired pneumonia among children in India with special reference to atypical pathogens

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ABSTRACT

Objectives: The aim is to identify the etiology of community acquired pneumonia in children with special reference to atypical bacteria and viruses. Materials and Methods: A total of 94 pneumonia children were enrolled in the study. Sixty-seven did not have an etiological diagnosis by conventional culture. These children were subjected to immunofluorescence assay by Pneumoslide IgM. Results: Ninety-four children were evaluated for etiology by conventional culture. Twenty-seven of them had the bacteriological diagnosis. Rest 67 were further analyzed for causative organism using Pneumoslide immunofluorescence test. Among this group, 38 (56.7%) had etiological diagnosis. Atypical bacteria were identified in 23 cases, most common being *Mycoplasma pneumoniae* and which was more common between 5 months and 2 years of age. Viruses were identified in 19 cases, and the most common virus was Respiratory syncytial virus. Mixed pathogens were identified in five children., *M. pneumoniae* was the common offending agent. Conclusions: Atypical bacteria and viruses play an important role as etiological agents in pneumonia in children. Pneumoslide IgM is useful for rapid detection of atypical bacteria and viruses.

KEY WORDS: Atypical bacteria, etiology, pneumonia, Pneumoslide IgM, viruses

INTRODUCTION

Acute respiratory tract infections are the largest cause of morbidity and mortality among under-five children worldwide.\(^{[1-5]}\) In India, pneumonia is the single most important cause of death among children in the postneonatal period, accounting for 27.5% of mortality among under-five children.\(^{[3]}\) In India, there are only a few systematic review or studies regarding the etiology of pneumonia in children.\(^{[1,4]}\) In general, the etiology can be identified in only 30%-50% of cases using conventional methods.\(^{[5]}\) This lack of information leads to inappropriate use of antibiotics with consequent antibiotic resistance and increased cost.\(^{[5]}\) In community acquired pneumonia (CAP) it is not possible to clinically or radiologically to distinguish between the etiological agents\(^{[6,7]}\) and hence, serological diagnosis would be very helpful. IgM antibodies which appears after infection are more valuable for the early diagnosis in children.\(^{[5]}\) Pneumoslide-M test (indirect immunofluorescence assays [IFAs]) which detects the IgM antibodies against nine pathogens has reasonable sensitivity and specificity.\(^{[8]}\) The aim of this study is to find the etiology of CAP in children with special reference to atypical bacteria and viruses by utilizing Pneumoslide-M test.

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MATERIALS AND METHODS

This study was conducted in the Department of Paediatrics at JSS Hospital, Mysore. Children aged 2 months to 16 years admitted with clinical diagnosis of pneumonia as defined by WHO,[9] were included after obtaining the informed written consent of the parents/guardians. Clearance for the study was obtained from JSS Medical College Ethical Committee.

Sample size
Indian studies have shown the prevalence of atypical organisms in CAP to be about 10%. Considering this prevalence, margin of error of 5% and confidence level of 90%, we had to recruit 97 patients for this study. Sample size was calculated using online calculator raosoft available on http://www.raosoft.com/© 1996–2011 by Raosoft, Inc. We were able to recruit 94 pneumonia children in the study. Of them, 67 did not grow any bacteria through different conventional culture methods and were processed for Pneumoslide IgM test [Figure 1].

Study period
From July 1, 2015 to September 2015, all the CAP cases were included till the sample size was attained. Nosocomial and aspiration pneumonias were excluded from the study.

Sample collection
Blood sample was taken for complete blood count, erythrocyte sedimentation rate, C-reactive protein and blood culture. Sputum was collected on 3 consecutive mornings after giving saline nebulization/nasogastric aspiration cultures collected on 3 consecutive mornings. Nasal/ear discharge swabs were collected if required for cultures. Chest X-ray was taken at the time of admission.

If the conventional cultures revealed a typical bacteria, they were excluded. Rest of the cases were processed for Pneumoslide IgM to identify atypical bacteria and viruses [Figure 1].

Pneumoslide-M
Blood was collected, serum separated and was subjected to indirect SIR test. Pneumoslide-M-Vircell slide-Granada, Spain 10 slides of 10 wells (9 etiological + 1 control). Manufacturer's test protocol was followed during testing and result interpretation. IFA detects IgM antibodies of the etiological agents of respiratory tract infection (Legionella pneumophila serogroup 1, Mycoplasma pneumoniae, Coxiella burnetii, Chlamydia pneumoniae, adenovirus, respiratory syncytial virus (RSV), influenza A and B and parainfluenza serotypes 1, 2, and 3). The specific antibodies react with the antigen, the antigen-antibody complexes react with the fluorescein-labeled anti-human globulin. It can be examined using an immunofluorescence microscope [Figure 2].

Data entry, database management, and statistical analysis were performed using Epi-Info software version 7 (Atlanta, GA, USA). All qualitative variables were expressed as proportions with confidence intervals and quantitative variables were expressed as mean ± standard deviation. Chi-square was used for finding association between qualitative variables.

RESULTS

Ninety-four CAP were enrolled in our study and most of the cases belong to 5 months to 2 years of age [Table 1]. Of the 94 pneumonia children, in 27 cases bacterias were isolated from different conventional culture methods (ear/nasal/ discharge swab cultures/sputum/nasogastric aspiration cultures) and the common bacterias isolated being Streptococcus pneumoniae, staphylococcus haemolyticus and pseudomonas aeroginosa. Blood cultures were sterile in all 94 cases. Rest of the 67 cases were processed for immune florescence assay using Pneumoslide IgM. Of which, 38 (56.7%) children were positive for various atypical bacterial and viral agents causing CAP [Figure 1].

Atypical bacterial agents were identified in 23 cases, most common being M. pneumoniae and which was more common between 5 months and 2 years of age [Tables 1 and 2].
In general, atypical bacteria are difficult to identify, and it is a challenge for both clinician and microbiologist to determine the etiology of pneumonia due to the relative inaccessibility of infected tissue (lung) and the difficulty in obtaining noncontaminated the upper airway samples. Other practical problems encountered include, low yield of blood cultures and difficulty in obtaining adequate sputum/bronchoalveolar lavage in children. In general, atypical bacteria are difficult to isolate. Since 2000, the diagnostic yield has improved with a pathogen identified in 65%–86% of CAP. In the recent years with the development of new laboratory technologies, it was observed that the role of virus and atypical bacteria as the cause of respiratory tract infection was underestimated. None of the blood cultures were positive in the present study. As such blood cultures are positive in 10%–20% of children with pneumonia and S. pneumoniae is cultured in the blood in <5%. In a study from India, out of 2345 CAP children, nasopharyngeal aspiration (NPA) and blood cultures yielded bacteria in only 13.9% and 2.1% of children, respectively. We excluded a few cases from the Pneumoslide IgM test as they grew organisms from airways/nasal secretions using conventional methods and however, they may be just commensals and not represent the causative organism. In another Indian study, out of the 100 CAP in under-five children, at least one bacterial pathogen was detected in 116 (64.4%) cases using conventional and molecular methods. S. pneumoniae and Haemophilus influenzae were the most common bacterial pathogens and atypical bacteria accounted for about 10% of cases. Ji et al. could identify at least one type of pathogen in 5871 (57.32%) out of 10,243 hospitalized acute respiratory infections (ARI) children by utilising the NPA and serum samples for multi-pathogen detection. They found that 32.47% were due to virus, which were detected by direct IFA, 28.02% bacteria and 26.94% due to atypical pathogens detected by fluorescent quantitative PCR. M. pneumoniae was the most common pathogen identified (25.74%).

### DISCUSSION

The primary pathogens as the cause of CAP include typical bacteria, atypical bacteria, and viruses. Etiology varies from country to country, with respect to age and could be due to differences in seasons as well. Ideally, it is desirable to isolate the responsible organism. However, the pathogen is not identified in a significant proportion of cases that otherwise meet the clinical definition of CAP. The etiological diagnosis cannot be made in 50% of the patients even after extensive diagnostic tests. The pathogen was isolated only in 29% of the patients with standard sputum and blood cultures in a study carried out by Shah et al. It is a challenge for both clinician and microbiologist to determine the etiology of pneumonia due to the relative inaccessibility of infected tissue (lung) and

### Table 1: Age distribution in community acquired pneumonia children and the common pathogens identified by using Pneumoslide IgM

| Age categories | CAP children enrolled for the study (n=94) | CAP Children who didn’t grow any organism on conventional culture (n=67) | CAP children positive for Pneumoslide IgM (n=38) | Most common pathogens identified (n=38) |
|----------------|------------------------------------------|-------------------------------------------------------------|-----------------------------------------------|--------------------------------------|
| 5 months to 2 years | 68                                       | 52                                                          | 26                                             | Mycoplasma (13), RSV (9)             |
| 2-5 years       | 20                                       | 11                                                          | 9                                             | Mycoplasma (3), chlamyphila (3), RSV (1) |
| 5-10 years      | 4                                        | 3                                                           | 3                                             | Mycoplasma (3)                        |
| 10-15 years     | 2                                        | 1                                                           | 0                                             | -                                    |

CAP: Community acquired pneumonia, RSV: Respiratory syncytial virus

### Table 2: Pathogens identified by using Pneumoslide IgM in community acquired pneumonia children (n=67)

| Pathogens identified by Pneumoslide IgM | n (38) |
|----------------------------------------|--------|
| M. pneumoniae                          | 15     |
| RSV                                    | 10     |
| C. pneumoniae                          | 3      |
| Influenza B                            | 3      |
| L. pneumophila                         | 1      |
| Adenovirus                             | 1      |
| Mixed pathogens                        |        |
| M. pneumoniae + adenovirus             | 2      |
| M. pneumoniae + influenza A            | 1      |
| Influenza A + influenza B              | 1      |
| M. pneumoniae + H1N1                   | 1      |

M. pneumoniae: Mycoplasma pneumonia, C. pneumoniae: Chlamyphila pneumonia, L. pneumophila: Legionella pneumonia, RSV: Respiratory syncytial virus

Viruses causing CAP was identified using Pneumoslide IgM in 19 cases, and the most common virus identified was RSV. Most of the RSV occurred between 5 months and 2 years of age (90%) [Tables 1 and 2]. Mixed pathogens were identified in four children, M. pneumoniae is the common offending agent [Table 2]. In one child with CAP, M. pneumoniae was isolated along with H1N1 influenza virus by polymerase chain reaction (PCR) in the nasopharyngeal secretions.

Out of 94 children enrolled, 92 cases were discharged and there were two mortalities. Pathogens isolated from the expired cases were RSV and another child of mixed infection (M. pneumoniae with H1N1).
consistency, with rates varies from 27% to 36%, whereas *Chlamydia pneumoniae* is responsible for 5% to 14% of cases.\[8\] *M. pneumoniae* is most often identified in older children.\[6\] *Mycoplasma* is not unusual in children aged 1–5 years, but Baer observed 22% incidence of *M. pneumoniae* in children aged 1–3 years.\[8\] In a study from Vietnam also, the highest proportion of atypical pathogens causing CAP and of particularly severe CAP due to atypical pathogens occurred in children younger than 2 years. *M. pneumoniae* was the most common pathogen in severe form of atypical CAP.\[5\] Of the 19 cases of *M. pneumoniae* identified in the present study, 68.4% were below 2 years of age which is comparable to Vietnam study.\[3\] *M. pneumoniae* is the common pathogen in all the age groups in various studies but was more frequent in children older than 1 year.\[3,5,8\]

Pathogens isolated using Pneumoslide IgM in CAP in various other studies are depicted in Table 3. *M. pneumoniae* was the most frequent pathogen isolated which is similar to our study. All these studies have not used conventional methods to detect typical bacteria but used only Pneumoslide-M test. Probably by not using conventional culture methods, they would have missed the typical pathogens of CAP.

The viruses were identified as etiological agents in 19 cases of CAP children and RSV was the most common virus identified in this study. Viruses cause a significant percentage of CAP infections, especially in children younger than 2 years and the prevalence of viral pneumonia decreases with age.\[6,8,13\] Viral etiologies accounts for CAP in up to 80% of children younger than 2 years.\[8\] RSV is consistently the most frequently detected virus, representing up to 40% in <2 years, but rarely identified in older children.\[18\] Similar observation was made in our study also and 90% of RSV occurred in <2-year-old. However, the highest detection rate of RSV, was seen in the 3–6-year-old group in a study by Wu et al.\[19\] In a study from China, out of 30,443 ARI children, 4428 (14.55%) were positive for at least one virus using direct immunofluorescence assay from nasopharyngeal swabs. The common viruses identified were RSV (68.11%), adenovirus (16.01%), and parainfluenza virus 3 (11%).\[10\]

Overall we found mixed infection in five children with CAP and *M. pneumoniae* was the most common pathogen associated with co-infection. Various studies have noted mixed viral and bacterial infections in 8%–40% of cases.\[2,5,8\] In most of the studies also mycoplasma is the most common offending agent similar to our study.\[2,5,8\] Liu et al. observed mixed infection in 2391 children out of 39,756 children and the most common agent observed in the co-infections were *M. pneumoniae* and influenza B virus.\[5\] Chen et al. also observed mixed infection in 6.56% of children.\[9\] Some viruses cause extensive damage to the epithelium of the respiratory tract which might promote super infection by another virus.\[5\] Probably infection by one (potential) pathogen facilitates other pathogens. This is well documented with influenza infection.\[9\] According to one hypotheses, patients infected with mycoplasma may be susceptible to other infectious pathogens.\[8\] Pathogens isolated from the death cases were RSV and another child of mixed infection (*M. pneumoniae* with influenza A H1N1 virus).

**Limitation of our study**

We excluded 27 cases from the Pneumoslide IgM test, as they grew organisms from airways using conventional methods, which may be commensals. We used only basic conventional methods (not molecular) to identify typical bacteria. The Pneumoslide IgM test could detect only viral and atypical bacterial pathogens. It could not identify bacterial pathogens.

**CONCLUSIONS**

Atypical bacteria and viruses play an important role as aetiological agents in CAP in children. The study highlights the usefulness of Pneumoslide IgM as a multi panel test for rapid detection of atypical bacteria and viruses, knowledge of which is valuable to ensure prompt initiation of targeted therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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