

Original Research Article

A study to assess the magnitude of atypical pneumonia by serum polymerase chain reaction in children aged 3 years to 18 years at Kempegowda Institute of Medical Sciences hospital, Bengaluru, Karnataka, India

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ABSTRACT

Background: Atypical organisms are a common causative agent of pneumonia in children more than 3 years of age, causing around 10-30% of the cases. Though atypical pathogens are said to cause relatively milder form of pneumonia as compared to other bacterial and viral pathogens, severe manifestations can also occur. Early identification of these pathogens can help in starting treatment with macrolides, which can reduce the length of hospital stay and mortality. Hence in this study the prevalence of atypical organisms was identified by using serum Polymerase chain reaction (PCR).

Methods: This was a prospective observational study conducted in children between 3 years to 18 years of age with clinical diagnosis of pneumonia admitted in wards and PICU in KIMS hospital. Authors excluded Immunocompromised children. Detailed history and clinical examination was done. Investigations - complete hemogram, Chest Xray, blood Culture and sensitivity and serum PCR was done for a sample size of 100 children.

Results: In this study among the three atypical organisms, Legionella pneumoniae was identified in 2% of the cases by serum PCR.

Conclusions: In this study among the three atypical pathogens authors could only identify Legionella pneumoniae. Prevalence of Legionella pneumoniae was 2%. While treating children aged 3 years to 18 years with pneumonia, a high degree of suspicion of atypical pathogens is required, especially in Legionella because of its high mortality rate. In such circumstances adding macrolides along with other antibiotics will be beneficial to the patients outcome and duration of hospital stay.

Keywords: Atypical pneumonia, Legionella, PCR

INTRODUCTION

Atypical organisms are identified as the causative agent of pneumonia in 10-30% of pediatric cases, most commonly affecting children more than 3 years.¹ Atypical organisms include Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumoniae. They are characterised by the presence features such as a

less virulent course, presence of signs more than symptoms (walking pneumonia), patchy infiltrates on chest radiographs and lack of response to beta lactam antibiotics.

Although they cause less severe infections commonly sometimes especially Legionella can cause severe

manifestations requiring ICU admissions and cause extra pulmonary manifestations.

Usually atypical organisms have a clinical picture similar to other bacterial and viral pathogens and cannot be distinguished by routine investigations. Each atypical pulmonary organism has a predilection for few extrapulmonary organs.

The characteristic pattern of extra pulmonary manifestation is what that distinguishes them each other.² Multiplex PCRs for throat and nasal swabs that include a panel of viruses as well as bacterial pathogens are now being used to increase etiological yield in Pneumonia.^{3,4}

N Rodrigues et al, in showed that though molecular testing has greatly improved sensitivity in the detection of bacterial pathogens in pneumonia from respiratory secretions, its role in discriminating between infection and colonization is less clear.⁵ Hence PCR from serum samples can rule out colonization as it is drawn from a sterile site.

The sensitivity and specificity of serum Polymerase chain reaction in diagnosis of typical pathogens was found to be between 50-70%.⁶⁻⁸

Polymerase chain reaction is one of the most sensitive and specific methods to identify respiratory pathogens, and also yield rapid results which can help in the early recognition of the etiological agent for appropriate treatment.

Very few studies are available in the Indian scenario in studying the prevalence of atypical organisms in children. Hence in this study authors assess the magnitude of atypical pneumonia in children by using serum PCR.

METHODS

Aim of the study is to identify atypical organisms (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumoniae*) as the causative agent of pneumonia in children aged 3 years to 18 years.

Objective of the study is early identification of atypical organisms to help in treatment with appropriate antibiotics and reduce the duration of hospital stay.

Children with clinical diagnosis of pneumonia between 3 years to 18 years admitted to wards and PICU of KIMS hospital Bangalore. Study duration is 18 months. Sample size is 100. This is prospective observational study.

Inclusion criteria

- Children aged 3 years to 18 years with clinical diagnosis of pneumonia admitted as inpatients in Kempegowda institute of medical sciences wards and PICU.

Exclusion criteria

- Immunocompromised children (including those on steroids >6 months, immune suppression therapy).

This was a prospective observational study conducted in children between 3 years to 18 years of age with clinical diagnosis of pneumonia admitted in wards and PICU in KIMS hospital. Authors excluded Immunocompromised children.

The study was approved by the Institutional Ethics Committee of the institute. Informed consent was obtained from the parents or the legal guardians of the study participants. Assent was taken for children more than 7 years.

Detailed history and clinical examination was done. Investigations included complete hemogram, Chest X ray, blood Culture and sensitivity and serum PCR was done for a sample size of 100 children from January 2018 to April 2019. The diagnosis of pneumonia in children clinically was made according to the WHO guidelines, tachypnea >40/min if child is less than 5 years and >30/min if child is more than 5 years and/cough and/or difficulty in breathing, fever >39.0 degree Celsius).

On the day of admission to the hospital, samples were collected by sterile technique and PCR samples were stored in appropriate conditions. Chest Xray findings were reported by radiologist. Serum RT-PCR (Real time polymerase chain reaction) was done using a kit to detect 33 respiratory pathogens (Fast track diagnostics kit) by trained professional.

The respiratory pathogen kit was a quantitative in vitro nucleic acid amplification test. It can detect viruses like-influenza A, H1N1, Influenza B, influenza C virus, human rhino virus, human corona virus 229E, NL63, HKU1, OC43, Human parainfluenza virus 1 to 4, human metapneumomo virus A and B, human boca virus, respiratory syncytial virus, parechovirus, enterovirus and human adeno virus, bacteria-staphylococcus aureus, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Hemophilus influenza B*, *Klebsiella pneumoniae*, *Legionella pneumoniae*, *Legionella longbeachae*, *Moraxella catarrhalis*, *Pneumocystis jirovecii*, *Bordetella* Species and *Salmonella*.⁹

Statistical analysis

Statistical analysis was done using software stata version 14, p value of less than 0.05 was considered significant.

RESULTS

Of the 100 cases enrolled 51% were males and 49% were females. 69% of the cases were between 3-5 years, 16% between 6-10 years and 15% between 10-18 years of age.

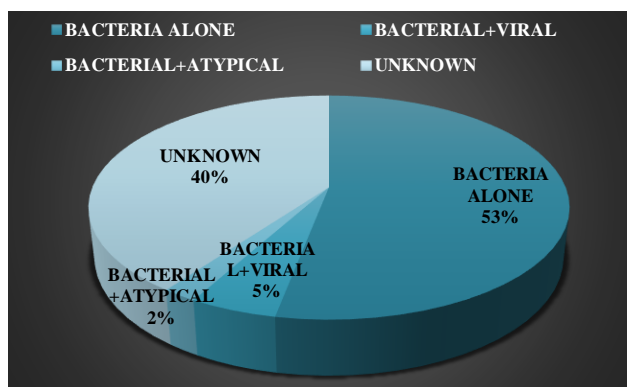


Figure 1: Distribution of etiological agents by serum PCR.

Most of the cases were during the months of June to September 2018 which is in the rainy season. Peak was during August 2018. The two cases of atypical pneumonia were during the months of August and November 2018. Among the atypical pathogens only *Legionella pneumoniae* was identified in 2% of the cases. *Mycoplasma* and *Chlamydia* were not detected. 53% of the cases were caused by bacterial organisms alone, 5% by coinfection of bacterial and viral organisms and 2% by coinfection of bacterial and atypical pathogens (Figure 1).

Table 1: Distribution of etiological agents identified by serum PCR.

Name of the organism	Percentage
<i>S. pneumoniae</i> + <i>Legionella</i>	1%
<i>S. pneumoniae</i> + <i>S. aureus</i> + <i>Legionella</i>	1%
<i>Streptococcus pneumoniae</i>	37%
<i>Staphylococcus aureus</i>	7%
<i>S. pneumoniae</i> + <i>Klebsiella pneumoniae</i>	2%
<i>S. pneumoniae</i> + <i>S. aureus</i>	3%
<i>S. pneumoniae</i> + <i>B. pertussis</i>	2%
<i>S. pneumoniae</i> + Human adeno virus	2%
<i>S. pneumoniae</i> + Human reovirus	1%
<i>S. aureus</i> + Human Metapneumo virus	1%
<i>S. aureus</i> + <i>K. pneumoniae</i>	2%
<i>K. pneumoniae</i> + <i>S. aureus</i> + Human adeno virus	1%
Unknown	40%
Total	100

(*S. pneumoniae*- *Streptococcus pneumoniae*, *S. Aureus*-*Staphylococcus aureus*, *B. pertussis*- *Bordetella pertussis*, *K. pneumoniae*-*Klebsiella pneumoniae*).

Table 2: Demographic and clinical characteristics associated with bacterial, bacterial with atypical, bacterial with viral, or unknown pathogens.

Variable	Bacterial (n=53)	Bacterial + Atypical (n=2)	Bacterial + Viral (n=5)	Unknown (n=40)	p value
Age in years, md (range)	4 (4-7)	4 (3-5)	4 (4-5)	4 (3-6)	0.61
Sex, male n (%)	26 (51.0)	1 (2.0)	2 (3.9)	22 (43.1)	0.94
Fever duration before admissions, md (range)	5 (3-6)	4 (4-4)	2 (2-3)	4 (3-5)	0.06
Cough duration before admissions, Md (range)	5 (3-6.5)	4 (4-4)	5 (3-5)	5 (3-6)	0.64
Total duration of fever, md (range)	6 (5-8)	6.5 (6-7)	5 (4-5)	6 (5-7)	0.14
Previous antibiotics, n (%)	28 (60.9)	0	3 (6.5)	15 (32.6)	0.18
Chest retractions, n (%)	28 (53.9)	2 (3.8)	2 (3.8)	20 (38.5)	0.50
Elevated total count (>11,000/mm ³)	26 (51.0)	1 (50)	2 (3.9)	23 (45.1)	0.43
Need for ventilator, n (%)	4 (44.4)	0	0	5 (55.6)	0.73
Duration of hospital stay, md (range)	5 (3-5)	5 (3-7)	5 (3-5)	5 (3-5)	0.82

Table 1 shows the exact distribution of etiological agents. Etiological agent was identified in 60% of the cases, with the most common causative agent being *Streptococcus pneumoniae*.

Table 2 shows the baseline clinical and demographic pattern of the study population.

The mean age of the population was 5.5 years.

As it can be seen from the above table there is no statistically significant difference between the various parameters such as Age, sex, duration of fever, duration of cough, total duration of fever, duration of hospital stays, severity of pneumonia, elevated total count. This

shows that it is not possible to distinguish the various organisms based on clinical presentation.

There is no increase in duration of hospital stay between pneumonia caused by bacteria alone and by coinfection.

The mean duration of hospital stay was 4.5 days. Around 46% of the patients had received treatment with antibiotics oral or injectables prior to admission.

Fever and cough was seen in all the cases and chest retractions in 59% of the cases. Both the cases with Legionella and bacterial coinfections had chest retractions hence belongs to severe pneumonia.

Clinical features of cases with legionella

Both the cases had fever, cough and hurried breathing at admission, wheeze more than crepitations on auscultation, past history of bronchial asthma and had coinfection with streptococcus pneumoniae.

The first case was a 3-year-old female, who in addition had vomiting and diarrhoea which is one of the extra pulmonary manifestations and had coinfection with Staphylococcus aureus also.

The second case was a 5-year-old female, who presented with severe respiratory distress and needed PICU admission.

Both the cases were treated, and were discharged with no mortality. The duration of hospital stay was 5-6 days.

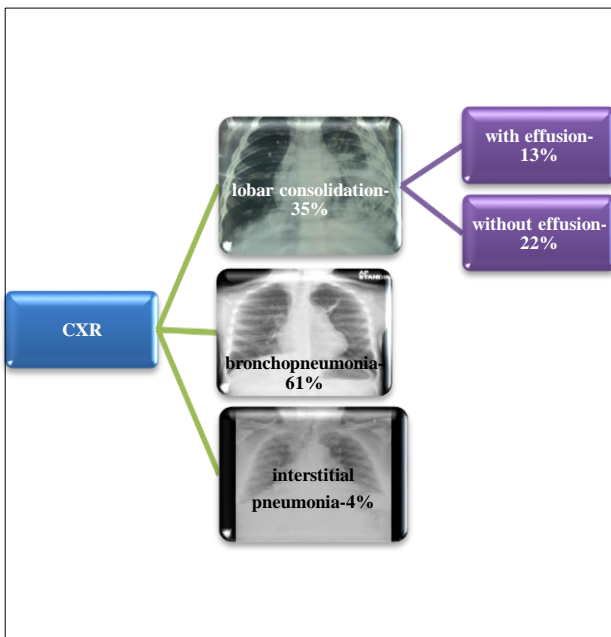


Figure 2: Distribution of chest radiograph findings.

The most common chest radiographic finding was bronchopneumonia (Figure 2).

Table 3: Duration of hospital stay.

Duration of hospital stay	Percentage(%)
Less than 3 days	0
3-5 days	77
6-9 days	18
More than or equal to 10 days	5

Most of the children with pneumonia had a mean hospitalstay for 3-5 days (Table 3).

DISCUSSION

This study showed a prevalence of Legionella pneumoniae to be 2%, which is little higher than the CDC study in 2006 which showed a prevalence of 1.7%. Legionella pneumonia was the only atypical organism identified in this study, amongst the other two atypical organisms like Mycoplasma and Chlamydia.¹⁰

Mycoplasma and chlamydia are said to be the most common cause of pneumonia in children more than 3 years, but it could not be detected in this study population probably because most cases of Mycoplasma present with mild symptoms and hence would have been treated on out-patient basis.¹¹

In this study, the two cases with Legionella were female children. Compared to previous studies done by Victoria Ng et al, in Canada, which showed a male to female ratio of 1.5:1.¹²

These two above cases were admitted in august and November 2018, which was during rainy season where the environmental condition favours the growth of the organism. A study done by Hicks et al, in in the United States, heavy rainfall was associated with increased risk for legionellosis and hence clinicians should be aware of the increased risk for legionellosis during periods of heavy rainfall.¹³

Authors found out that the cases with Legionella where healthy children with significant comorbidity of bronchial asthma. In a study done by Beer et al, in the higher prevalence of bronchial asthma in Legionella has been established, where 17% of asthmatic children compared to 1% of the children from the control group had positive serology which was statistically significant.¹⁴ Bronchial asthma is a well-known co morbidity which can predispose to bacterial and viral infections due to underlying immune dysfunctions and airway inflammation which suppress the physiological airway defences. Association of Mycoplasma and Chlamydia as a trigger to bronchial asthma is also well known.¹⁵

Legionella positive Case 1, was a 3-year-old female, admitted with complaints of cough for 5 days, fever for 4 days, hurried breathing for 2 days, vomiting and diarrhoea for 2 days. At admission was child had

tachypnea with chest retractions and respiratory system examination revealed wheeze more than crepitations. Chest Xray had features of bronchopneumonia. Child had anaemia with Hb of 10g/dl, Total counts were normal. Child received antibiotics and was discharged in 5 days. PCR report showed that child had coinfection with streptococcus pneumonia.

Legionella positive Case 2, was a 7-year-old female, admitted to PICU with complaints of Fever, cough and hurried breathing for 1 day. Child had tachypnea with chest retractions, saturation of 94% and extensive expiratory wheeze on auscultation. Chest Xray showed features of bronchopneumonia. Investigations done showed elevated total count of 17,000/mm³, with neutrophilic leucocytosis. Child recovered and was discharged after 6 days. PCR showed that this child had coinfection with Streptococcus pneumoniae and Staphylococcus aureus.

Both the cases had coinfection with bacteria. Bacterial coinfection must have caused reduced immunity which would have lead to infection with Legionella. In a study done by Brown et al, in Canada, the first case description of coinfection of Streptococcus pneumoniae with Legionella was observed.¹⁶

Hence in situations where already typical organisms have been identified as a causative agent of pneumonia, a high degree of suspicion is needed when there is a delay in response to treatment, specific investigations to rule out atypical organisms can help in instituting appropriate therapy.

Currently, Real-time PCR is regarded as the molecular method of choice for detection of Legionella, because of its high specificity (100%), sensitivity (80%) and rapidity of results. There are a very few studies which have been done where *Legionella pneumoniae* was isolated from serum, as Legionella is a intracellular organism making its isolation difficult. Data from studies by Lindsay et al, and Helbig et al, shows that PCR for Legionella DNA can be detected in urine and sera at high sensitivity (>80%).^{17,18}

As the prevalence of Legionella is relatively low but mortality is high, hence highly sensitive and specific test is needed, necessitating a rapid test. PCR is the only diagnostic test that meets all the above requirements.

In this study the causative respiratory pathogen was identified in 60% of the cases.

The etiological agent could not be identified in 40% of the cases probably because, though the organism would have caused infection in the lower respiratory tract, bacteremia would not have occurred during the time of sample collection or they might not have been a part of the respiratory panel of 33 organisms by PCR.

The mortality due to legionellosis is 10%. Among the atypical organisms, it can cause severe pneumonia indistinguishable from Streptococcus pneumoniae and other bacterial organisms. The study conducted by Alexander et al, in the United States, mortality was 23% for even those patients who received correct therapy and was 70% for those who did not. These show the importance of appropriate therapy for legionellosis.¹⁹

CONCLUSION

The aim of this study was to assess the magnitude of atypical pneumonia in children aged 3 years to 18 years by serum polymerase chain reaction.

In this study among the three atypical pathogens authors could only identify Legionella pneumoniae. Prevalence of Legionella pneumoniae was 2%. In comparison to this study, earlier study done at CDC in 2006 showed prevalence to be 1.7% in children.

If the facilities are available for Polymerase chain reaction test in the vicinity of the treating place, even though its expensive, it would be an ideal test to identify atypical organisms, where the results can be obtained within 8-12 hours.

While treating children aged 3 years to 18 years with pneumonia, a high degree of suspicion of atypical pathogens is required, especially in Legionella because of its high mortality rate and clinically legionellosis is not easily distinguishable from other bacterial and viral pathogens. In such circumstances adding macrolides along with other antibiotics will be beneficial to the patients outcome and duration of hospital stay.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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