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Genotypic versus Phenotypic diagnostic methods for detection of Streptococcus pneumoniae among children with Lower Respiratory Tract Infection in North India.

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Abstract

Introduction: Community acquired pneumonia (CAP) in the pediatric age group is caused by a myriad of bacteria and viruses. Streptococcus pneumoniae is a leading cause of severe bacterial infections. S. pneumonia has several virulence factors that allow it to cause infections in humans.

Material and Method: This study was descriptive observational study which was carried out in the Department of Microbiology in collaboration with Dept of Pediatrics, Mahatma Gandhi Medical College, Jaipur, Rajasthan. Sputum sample of 50 eligible children with Lower respiratory tract infection were included for the study. Two respiratory samples, one for culture and the other for PCR analysis and were collected from every child.

Results: Of the 50 samples processed, bacterial pathogens were detected in 55%. Streptococcus pneumoniae and Hemophilus influenzae were most frequently detected. The performance of PCR analysis and culture were identical for the typical bacterial pathogens.

Conclusion: We conclude that the study showed S. pneumoniae and H. influenza were the most detected organisms from respiratory secretions of children with community acquired pneumonia.

Keywords: Community acquired pneumonia, Real Time PCR, Streptococcus pneumoniae, Genotypic, Phenotypic.

Introduction

Streptococcus pneumoniae is an important human pathogen that causes a wide range of diseases. Respiratory tract illness is one of the primary manifestations of pneumococcal infection; however, microbiologic confirmation can be difficult. Traditionally, diagnoses of pneumococcal community-acquired pneumonia (CAP) and other lower respiratory tract infections (LRTIs) have been made through conventional culture of respiratory secretions, including sputum, bronchoalveolar lavage, or pleural fluid, or the

detection of pneumococcal bacteremia, but yields are low.[1]

According to the World Health Organization (WHO), Maternal and Child Epidemiology Estimates Group (MCEE), in 2015, India had the highest number of under-five deaths due to acute respiratory infection (ARI) in the world [2,3]. Pneumonia, defined as inflammation of the lung parenchyma, is the leading infectious cause of death globally among children younger than 5 years, accounting for an estimated 920,000 deaths per year. Streptococcus pneumoniae has been the most common pathogen to cause CAP worldwide historically. In the era before antibiotics, S. pneumoniae was 95% estimated for the cause of pneumonia. However, S. pneumoniae accounts for up to 15% of pneumonia cases in the United States and 27% of cases worldwide currently. Blood and sputum cultures are positive in only 20% to 25% of all pneumonia cases that are caused by S. pneumonia making it a challenging diagnosis for the clinician. [9,10,11]

In 2013, an estimated 2.6 million deaths worldwide were attributed to LRIs, while by 2015, this increased to 2.74 million [12]. Higher burden of LRIs is associated with low sociodemographic status, poor access to healthcare and nutrition [13,14]. Pneumococcal disease is a major public health problem in India. Almost a quarter of global pneumococcal cases and deaths occur in India [4,5]. Although pneumococcal disease is vaccine preventable, Streptococcus pneumoniae remains a major cause of acute lower respiratory infection (ALRI) in children, causing an estimated 2,94,000 under-five deaths [4].

Methods

Children aged 0 to 5 years satisfying the WHO criteria for pneumonia, severe pneumonia and with the presence of lung infiltrates on chest X-ray was enrolled. Two respiratory samples, one for culture and the other for PCR analysis was collected from every child. One respiratory sample was processed according to standard microbiological lab procedures. Routine microscopy of samples was done before processing with the help of Gram's staining. Primary inoculation was on blood agar and chocolate agar culture media as per standard protocols and incubated for 24-48 hours at 37°C in presence of 5-10% of CO2. Isolated Culture was then identified by colony morphology on media, growth on selective media, Gram's staining. Identification of all gram-positive isolates was done by VITEK-2 Compact system. RT-PCR was performed on all samples. DNA EXTRACTION-DNA was extracted by Qiagen viral DNA mini kit or as per standard protocol. DNA elute was stored at -80ºC till further processing. Real time Polymerase chain reaction was done from the extracted DNA in a BioRad CFX96 Thermal cycler.

Results

Out of the 50 cases studied, at least one bacterial pathogen was detected in cases. The respiratory sample was oropharyngeal swab in 06 and BAL in 44 children. The basic demographics data are showing in Table 1. Majority of the study subjects belonged to 0-12months age group (54%) and slightly higher preponderance to male gender (66%). Table 2 shows the Classification of study subjects based on the type of pneumonia according to WHO definition. 8 subjects were diagnosed with pneumonia, 30 subjects with severe pneumonia and 12 subjects with very severe pneumonia.(table) All the 50 subjects involved in the study had fever, cough, respiratory distress while, 32(64%) had presented with significant cough, 4(8%) had presented with seizures and 42(84%) had chest retractions. All 50 subjects were observed to have bronchial breath sounds on auscultation and signs of pneumonia on Chest X ray

examination.(table2) Sensitivity of VITEK= 46% (19% to 75% at 95% CI),Specificity of VITEK = 67% (50% to 82% at 95% CI)Likelihood ratio (Positive)= 1.4 (1.4 is >1, that is if the VITEK is positive, there is minimal increase in likelihood of the presence of infection, Likelihood ratio (Negative) = 0.79 (0.53 is <1, That is, if the VITEK is negative, there is minimal decrease in likelihood of the presence of infection)(table3)

Table 1: Basic characteristics of study subjects

Basic characteristics		Frequency (n=50)	Percentage (%)
Age group	0-12months	27	54
	12-24months	8	16
	24-60months	15	30
Sex	Male	33	66
	Female	17	34

Figure 1: Age Distribution of Study Subjects

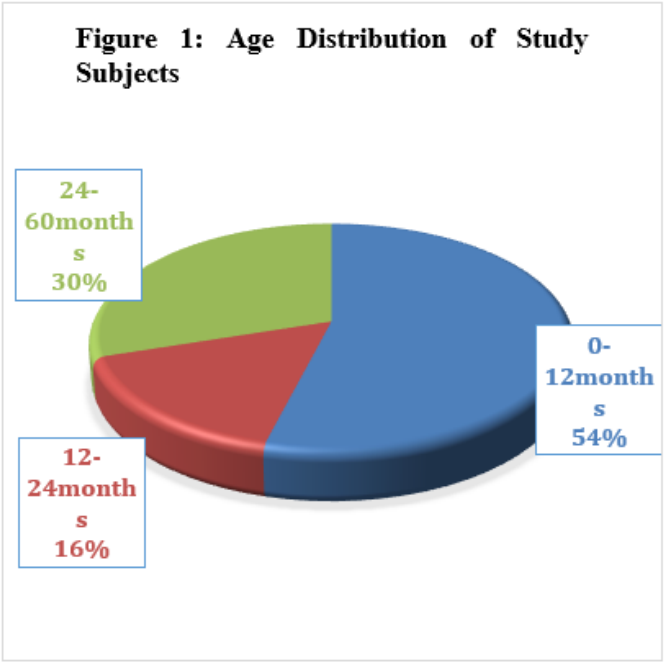


Figure 2: Sex Distribution of Study Subjects

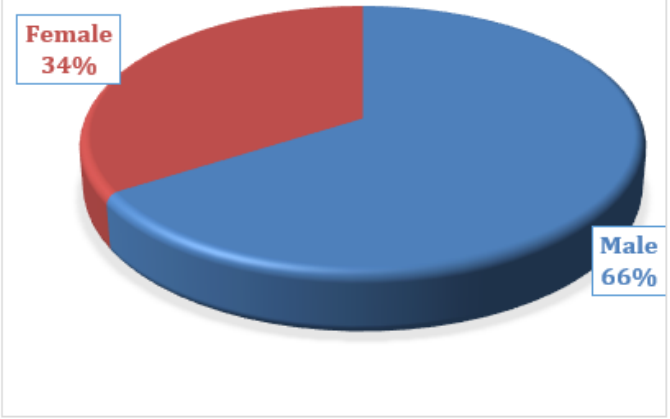


Table 2: Classification of study subjects based on the type of Pneumonia:

Type of Pneumonia	Frequency (n=50)	Percentage (%)
Pneumonia	8	16
Severe Pneumonia	30	60
Very severe Pneumonia	12	24

Figure 3: Severity of pneumonia among study subjects

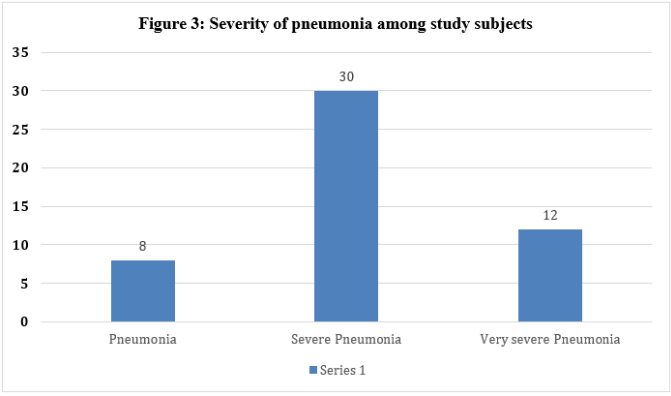
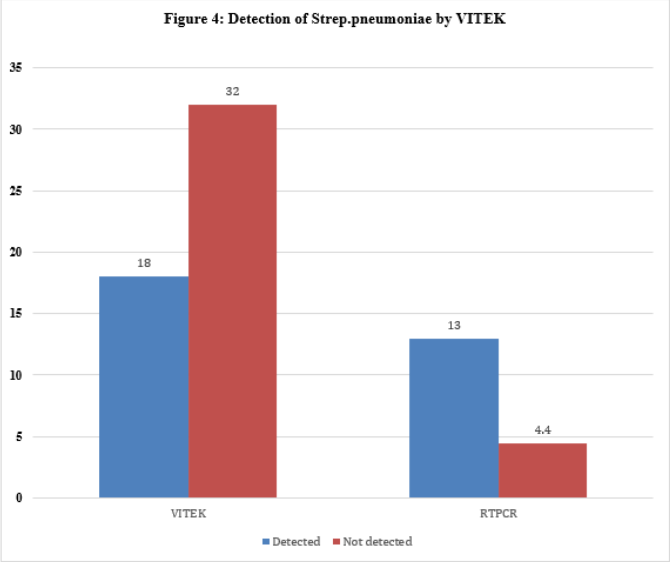


Table 3: Evaluation of conventional VITEK testing in detection of Streptococcus pneumoniae with that of genotypic RTPCR testing:

Detection of Streptococcus pneumoniae by VITEK		RTPCR results		Total
		Streptococcus pneumoniae detected	Streptococcus pneumoniae not detected	
	Detected	6(33%)	12(67%)	18(100%)
	Not detected	7(22%)	25(78%)	32(100%)



Discussion

The detection of Streptococcus pneumoniae, H. influenzae from respiratory secretions in children with community-acquired pneumonia (CAP) suggests the involvement of these pathogens in causing respiratory infections. In this study we are trying to summaries the information provided and discuss this great clinically relevant topic of North Indian population. Streptococcus pneumoniae in the given data detected in respiratory secretions from 10% of children with CAP. Streptococcus pneumoniae is a common bacterial pathogen associated with pneumonia, particularly in children. It can cause various respiratory infections, ranging from mild to severe. Detection of S. pneumoniae in respiratory secretions indicates its likely involvement in the development of pneumonia in these children [14].

In general clinical practice for infectious disease, diagnosis and treatment for CAP must be started based on clinical symptoms, radiological findings, and laboratory results at the time of hospitalization, considering the severity of the symptoms. One of the measures to decrease health care costs and to improve benefits for patients is to identify the causative microorganisms rapidly and precisely, thus enabling the appropriate antibiotic to be selected at the beginning of hospitalization. However, conventional culture techniques for microorganisms, still the “gold standard” in clinical laboratories, are time consuming. For identification of S. pneumoniae, H. influenzae, the culture method takes a week or more, and therefore, CFX96 real-time PCR, and nucleic acid sequence-based amplification have been used, improving the sensitivity and the specificity. With molecular genetic assays, it is possible to detect micro-organisms with high sensitivity and specificity, since bacteria damaged by antibiotics and remaining DNA of dead bacteria killed by antibiotics are detected. Thus, simultaneous detection of the CAP pathogens S. pneumoniae, H. influenzae, is desirable for rapid diagnosis of CAP and for the selection of appropriate antibiotics, supporting traditional laboratory methods. Real-time PCR with MB probes allows us to monitor in vitro DNA amplification successively, eliminating nonspecific amplification and the need for gel electrophoresis, and with the remarkable progress of PCR machines and reagents, real-time PCR is now available in clinical laboratories [15].

In this study, S. pneumoniae and H. influenzae were the most commonly detected bacterial pathogens in the under-five children with CAP in this region. The atypical pathogens could be detected only by PCR whereas

typical bacterial pathogens could be detected as well by conventional culture methods. It is important to note that the given information states that these pathogens were detected in respiratory secretions of 10% of children with CAP. This implies that there may be other causative agents or factors contributing to the remaining 90% of cases [15]. Proper diagnosis and identification of the pathogens involved in CAP are crucial for appropriate treatment and management of affected children. Although the method is highly sensitive for detection, it cannot give an estimate of the bacterial load as compared to conventional culture. Also, there was no provision of testing the antibacterial sensitivity of the bacterial isolates following a PCR reaction. Convenience sampling and lack of asymptomatic controls were other limitations. Various other studies have found S. pneumoniae and H. influenzae to be the most common isolate from respiratory samples of children with CAP [2,3]. S. aureus and some Gram-negative bacilli like K. pneumoniae were detected as the leading cause of pneumonia by Johnson, et al. [4]. The comparable performance of conventional methods and PCR in detection of typical organisms such as S. pneumoniae, K. pneumoniae and S. aureus has been reported earlier [8]. The detection of infection by blood culture was lower in the present study as compared to few other studies [4,5].

Conclusion

In the conclusion of this study, we are showing that S. pneumoniae and H. influenzae are the most common bacterial organisms associated with community acquired pneumonia. RT-PCR with pathogen specific primers and probes can improve the diagnostic yield by increasing the detection of fastidious organisms such as H. influenzae, and atypical agents like M. pneumoniae and B. pertussis etc. Our data demonstrate that real-time PCR with pathogen specific probes can detect microorganisms within few hours; and by this assay it is possible to assess the time course of empirical therapy, thus supporting infection can be managed. Finally, we expect that the real-time PCR technique described here will be expanded to a multiplex RT-PCR for the community acquired pneumonia in the pediatric age group is caused by a myriad of bacteria and viruses.

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