



Original Article

## Diagnostic Accuracy of Genotypic Versus Phenotypic Techniques for Identification of Streptococcus pneumoniae in Pediatric Lower Respiratory Tract Infections

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### ABSTRACT

**Background**-Evaluation of Genotypic versus phenotypic diagnostic methods for detection of Streptococcus Pneumoniae among children with lower respiratory tract infection

**Objective**-To determine the diagnostic accuracy of phenotypic and genotypic method for detection of *Streptococcus Pneumoniae* among children with lower respiratory tract infection.

**Methods**-Detailed relevant history was taken as age, gender, history of any consumption of antibiotic, in-dwelling medical devices and the duration of ward and ICU stay. All the samples were collected from various IPD and OPD wards. The sample was collected after, properly labelling the container with the patient's name, ID number etc. Two respiratory samples, one for culture and the other for PCR analysis were collected from children of age group 0 to 5 years, presenting with symptoms of Fever, cough, rapid breathing. Samples were collected with universal precautions by prescribed sterile techniques and transported to the laboratory as soon as possible maintaining optimum transportation conditions.

**Results**- VITEK-2 system was used in the current study to identify isolates. Of all the organisms identified 20% were *K. pneumonia*, 18% *Paerogninosa*, 12% *S. pneumonia*, 8% *S. aureus*, and 6% *Acinetobacter spp.* All these organisms have been known to cause or are secondary invaders in children suffering with pneumonia and in RT-PCR method identified *S. pneumoniae* in 26% and *H.influenzae* in 12%, samples.

**Conclusion**-*Streptococcus pneumoniae* which has been implicated as causative agent of pneumonia in majority of such cases, can be identified by RT-PCR. We found this to be highly sensitive method with other advantages as being much faster than culture technique and if multiplex PCR is done, there is a possibility of identifying other fastidious organisms and targeted therapy can be started early in the disease. Due to COVID-19 pandemic molecular labs have been established at mostly all diagnostic centers, with lot of Human resource training and availability of kits even for multiplex PCR, at cheaper rates. Advantage of this can be taken for early identification of pathogens for other diseases. Hence we conclude Detection of *S. pneumoniae* infection by PCR is a helpful tool with high sensitivity for early detection of cases. This will help in the timely management of patients and the achievement of a better clinical outcome.

**Keywords:** Genotypic, Phenotypic, PCR, *Streptococcus pneumoniae*.

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## INTRODUCTION

Pneumonia is an infection of the lung parenchyma including the alveoli. It affects all ages, but causes increased morbidity and mortality in infants, neonates and the elderly.<sup>1</sup> Pneumonia in the paediatric age group is caused by a myriad of bacteria and viruses.<sup>2</sup> Pneumonia, defined as inflammation of the lung parenchyma, is the leading infectious cause of death globally among children younger than 5 yrs., accounting for an estimated 920,000 deaths each year. Pneumonia mortality is closely linked to poverty. More than 99% of pneumonia deaths are in low- and middle-income countries, with the highest pneumonia mortality rate occurring in poorly developed countries in Africa and South Asia. According to the World Health Organization (WHO) and 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.<sup>3,4</sup> Pneumococcal disease is a major public health problem in India. Almost a quarter of global pneumococcal cases and deaths occur in India.<sup>5,6</sup> Common widespread diseases of the respiratory system occur when microorganisms invade the respiratory tract. Infectious respiratory diseases are globally seen as a major health concern because they can rapidly become severe and lead to death. Respiratory diseases are categorized into upper and lower respiratory tract infections (LRIs). LRIs are more severe because pathogens infect sterile parts of the respiratory system such as the lungs, trachea, and bronchi.<sup>7</sup> In 2013, an estimated 2.6 million deaths worldwide were attributed to LRIs, while by 2015, this increased to 2.74 million. Higher burden of LRIs is associated with low socio-demographic status, poor access to healthcare and nutrition.<sup>8</sup> Community-acquired pneumonia (CAP) is the seventh leading cause of death in the United States, and the cost of these hospitalizations is estimated to cost up to \$9 billion in the United States (US) dollars each year. Thirty-day hospital mortality associated with CAP has been estimated to be as high as 22% and is the leading cause of death amongst all infectious diseases. In the United States, mortality from pneumonia in children declined by 97% between 1939 and 1996. This decline likely resulted from the development of antibiotics and vaccines and the expansion of medical insurance coverage for children. Effective vaccines against measles and pertussis contributed to the decline in pneumonia-related mortality during the 20th century.<sup>9</sup> *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. *Streptococcus pneumoniae* is a leading cause of acute respiratory infection (ARI) and is responsible for substantial morbidity and mortality rates especially in developing countries. Worldwide, annually 1.1 million children die under the age of five years from pneumococcal infections and nearly all of these deaths occur in children from low and lower-middle income countries.<sup>10</sup> *Streptococcus pneumoniae* is the bacterium that has historically been the most common pathogen to cause Pneumonia worldwide. In the era before antibiotics, *S. pneumoniae* was estimated to be the cause of 95% of all cases of pneumonia. Currently, however, *S. pneumoniae* accounts for up to 15% of pneumonia cases in the United States and 27% of cases worldwide today. Blood and sputum cultures are positive in only 20% to 25% of all pneumonia cases that are caused by *S. pneumoniae* making it a challenging diagnosis for the clinician.<sup>11,12,13</sup> Although pneumococcal disease is vaccine preventable, *Streptococcus pneumoniae* remains a major cause of acute lower respiratory infection (ALRI) in children, causing an estimated 294,000 under-five deaths. 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.<sup>1</sup> This stems from the lack of sensitive, specific, and cost-effective diagnostics. While retrospective studies have shown favorable outcomes with empiric therapy of CAP, failure to specifically identify *S. pneumoniae* as the causative agent has consequences for the individual patient and the population at large. The individual patient may not receive adequate antimicrobial therapy or may receive excessively broad-spectrum and expensive treatment. At the population level, the lack of a pathogen-based diagnosis decreases our ability to accurately assess disease burden, to evaluate the effects of interventions such as immunization, to track antimicrobial resistance, and to investigate new therapeutic agents.<sup>14</sup> Antimicrobial resistance among *S. pneumoniae* has emerged as a problem. The emergence of drug resistant *S. pneumoniae* (DRSP), resistant to important antimicrobials such as penicillin, cephalosporins and macrolides, is a problem of global concern that has made treatment of the disease most difficult.<sup>15,16</sup>

## METHODS

The present study was a Descriptive observational study. This study was carried out in the Department of Microbiology in collaboration with Dept of Pediatrics, Mahatma Gandhi Medical College, Jaipur, and Rajasthan. The test group selected was pediatric patients of 0 to 5 yrs. of age having symptoms of respiratory tract illness with fever, and cough. Sputum samples were collected from patients admitted with the symptoms as per our inclusion criteria and from outpatient's department (OPD) regardless of their, sex, religion and ethnicity. A structured Performa was filled with the patient information. Where necessary patient was visited again for gathering information especially regarding the inclusion and exclusion criteria adopted in our study. The samples were transported at the earliest for processing in the lab.

Ethical consideration: Institution Ethics Committee approval was obtained before the start of the study. (MGUMST/Aead./PhD./REG/603)

### Inclusion criteria: -

- Children of age group 0 to 5 years, presenting with symptoms of Fever, cough, rapid breathing (as per WHO Criteria).

- Children whose parents gave consent.

**Exclusion criteria: -**

- Children already on antibiotics. Children having congenital respiratory tract malformations.
- Children having broncho pulmonary dysplasia.
- Children having history of choking / suspected foreign body inhalation
- Children having cardiac diseases.

**Specimen:** Sputum sample.

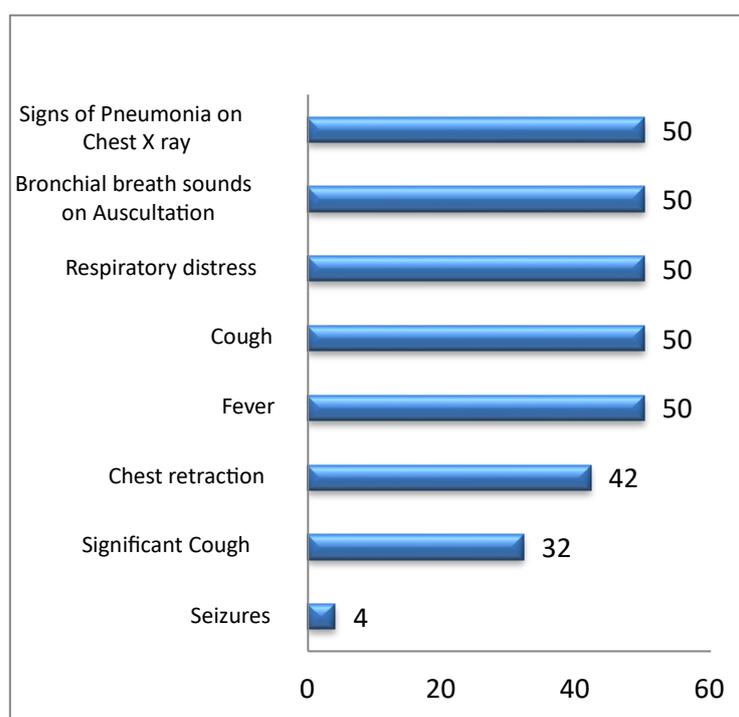
**Collection of specimens:** Detailed relevant history was taken as age, gender, history of any consumption of antibiotic, in-dwelling medical devices and the duration of ward and ICU stay. All the samples were collected from various IPD and OPD wards. The sample was collected after, properly labelling the container with the patient's name, ID number etc. Two respiratory samples, one for culture and the other for PCR analysis were collected from children of age group 0 to 5 years, presenting with symptoms of Fever, cough, rapid breathing. Samples were collected with universal precautions by prescribed sterile techniques and transported to the laboratory as soon as possible maintaining optimum transportation conditions.

**RESULTS**

A total of 50 subjects were included in the study that includes 44 Inpatients (88%) and 6 Outpatients (12%). Majority of the study subjects belonged to 0-12 months age group (27.54%) and had slightly higher preponderance to male gender 33.66%. 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. 8(16%) subjects were diagnosed with pneumonia and 42(84%) subjects with severe pneumonia. Figure 4 shows the severity of pneumonia among study subjects.

**Table 1: Basic characteristics of study subjects**

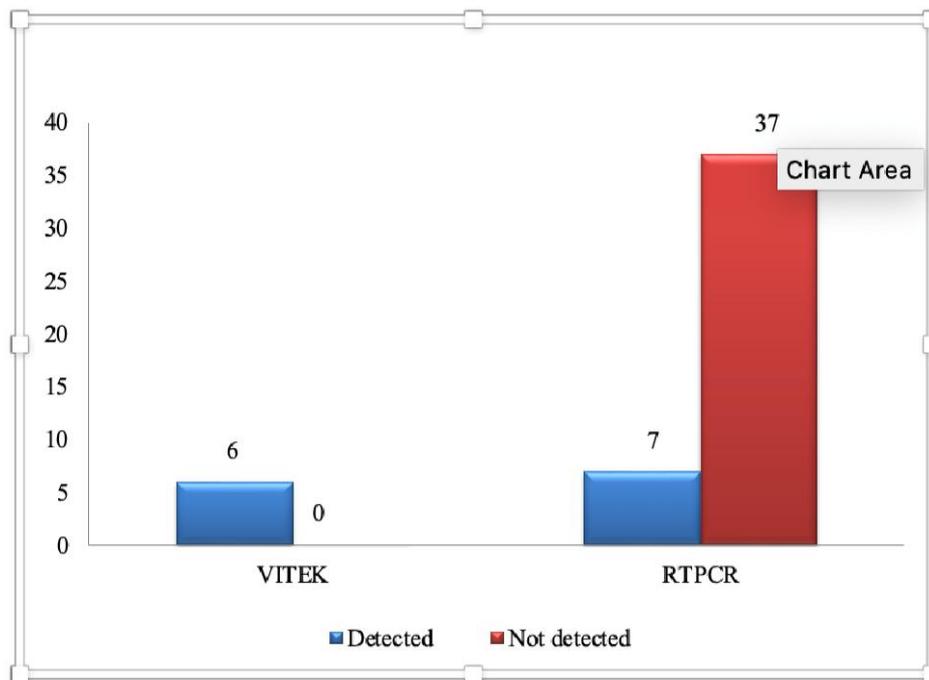
Basic characteristics		Frequency (n=50)	Percentage (%)
Age group	0-12months	27	54
	12-24months	8	16
	24-60 months	15	30
Gender	Male	33	66
	Female	17	34



**Figure 1:** Clinical presentations of study subjects

**Table 2:** Statistical analysis of general characteristics and risk factors for colonization with *S. pneumoniae* in children

Characteristics		All cases (n=50)	<i>S. pneumoniae</i> positive (n=13)	<i>S. pneumoniae</i> negative (n=37)	Statistical test results
Gender	Male	33(66%)	9(69.2%)	24(64.9%)	Fischer's exact test P=0.52 df-1 Not significant
	Female	17(34%)	4(30.8%)	13(35.1%)	
	Total	50(100%)	13(100%)	37(100%)	
Age group	0-12months	27(54%)	9(69%)	18(48%)	Chi square test P=0.36 df- 2 Not significant
	12-24months	8(16%)	2(15.4%)	6(16.2%)	
	24-60months	15(30%)	2(15.4%)	13(35.1%)	
	Total	50(100%)	13(100%)	37(100%)	



**Figure 2:** Detection of Strep. pneumoniae by VITEK

## DISCUSSION

Pneumonia is a leading cause of morbidity and mortality in children under the age of five years worldwide.<sup>16</sup> The majority of pediatric pneumonia deaths are reported in developing countries where the disease burden is high, whereas pneumonia is associated with higher healthcare costs in developed countries.<sup>17</sup>

In our study, Culture and RT-PCR were both used to identify *S. pneumoniae* in sputum samples, although mere detection does not establish a causal role.<sup>18</sup> Patients who are on antibiotics before the culture may give a false negative result. In our study, the performance of PCR and culture was not found similar, the rate of isolation of *S. pneumoniae* in culture is lower than its detection by PCR; the possible reason could be due to its fastidious nature and special culture requirements. Sometimes the presence of other bacteria can mask the isolation of *S. pneumoniae* in routine culture. The main difficulty in attributing etiology of pneumonia to *S. pneumoniae* is due to its frequent presence in asymptomatic children.

Real-time PCR, which is a sensitive and specific assay, to quickly identify *S. pneumoniae* from samples. The intrinsic benefit of this technique lies in its adaptability to direct detection from clinical specimens. Infectious organism detection using PCR techniques has gained recognition as a useful clinical diagnostic tool. The development of effective DNA purification techniques is required to separate genetic material from biological components that are known to hinder DNA polymerase activity during the PCR, to avoid false-negative findings.

In the present study 13 (26%) out of the 50 sputum samples examined by PCR had *Streptococcus pneumoniae*. 6 out of these 13 samples were positive, while the remaining seven cases were negative on culture. Our findings are comparable with the other studies of Dangan R *et al.*<sup>19</sup>, (13%) and Nomanpour *et al.*, (9.38%)<sup>20</sup> Whereas Greiner O *et al.*<sup>21</sup> reported a higher positivity (34.7%).

*Streptococcus pneumoniae* which has been implicated as causative agent of pneumonia in majority of such cases, can be identified by RT-PCR. We found this to be highly sensitive method with other advantages as being much faster than culture technique and if multiplex PCR is done, there is a possibility of identifying other fastidious organisms and targeted therapy can be started early in the disease. Due to COVID-19 pandemic molecular labs have been established at mostly all diagnostic centers, with lot of Human resource training and availability of kits even for multiplex PCR, at cheaper rates. Advantage of this can be taken for early identification of pathogens for other diseases. Detection of *S. pneumoniae* infection by PCR is a helpful tool with high sensitivity for early detection of cases. This will help in the timely management of patients and the achievement of a better clinical outcome.

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