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Clinical and bacteriological profile of community acquired pneumonia in hospitalized children

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Abstract:
Community-acquired pneumonia (CAP) is the leading cause of mortality and morbidity with important clinical impact across the World. India accounts for 23 per cent of global pneumonia burden with case fatality rates between 14 and 30 per cent. Pneumonia is basically classified into typical and atypical pneumonia. Emerging evidence indicates that dual typical and atypical bacterial infections function synergistically in many cases and together likely enhance the severity of CAP. However, the optimal management of CAP in children is still not well defined and the diagnosis is challenging when based solely on clinical observations since the common symptoms of CAP, especially at an early stage, are similar to those of ordinary respiratory tract infections. So RT-PCR is a rapid and precise molecular technique is used for rapid detection of bacteria causing community acquired pneumonia. A total of 268 samples were tested for the respective bacterial pathogens, among the tested the most common pathogen was *Haemophilus influenzae* (18.3%, 49/268) followed by *S. pneumoniae* (14.6%,39/268), *M. catarhallis* (9.3%,25/268),*M. pneumoniae* (9%, 24/268), *B. parapertusis* (3.4%,9/268), *B. pertusis* (1.5%, 4/268), *C. pneumoniae* (1.5%, 4/268), *C. burnetti* (1.1%, 3/268)and *L. pneumophila* (0.74%, 2/268). *H. influenza* and *S. pneumoniae* were the most commonly detected organisms among the community acquired pneumonia patients.

Keywords: Community-acquired pneumonia; typical pathogens; atypical pathogens; Pneumonia; RT-PCR.

Background:
Community-acquired pneumonia (CAP) is considered to be a predominant cause of morbidity and mortality in developing countries **[1, 2].** According to the World Health Organization, more than 2 million children die from pneumonia every year worldwide, with 1.2 million of those deaths occurring in India**[3].**A number of factors contribute to the prevalence and severity of pneumonia in children, including prematurity, malnutrition, low socioeconomic status, exposure to tobacco smoke and child care facility **[4].** A timely diagnosis of the CAP-causing pathogen improves prognosis and helps in vaccine development. CAP has a unique bacteriological profile that varies by region over the time due to the overuse of antibiotics, environmental pollution, improved public awareness and variations in life expectancy **[5].** *Streptococcus pneumoniae* and *Haemophilus influenzae* cause the majority of CAP in children, while atypical bacteria such as *Mycoplasma pneumoniae*, are the most common in children with CAP. In spite of managing CAP with an etiological diagnosis, majority of the CAP patients require advance diagnostic testing **[6].** Diagnosis of CAP based only on clinical observations can be challenging since the signs and symptoms are often similar to those of common respiratory tract infections, particularly in the early stages **[7].** Standard microbiological investigations are not sensitive and effective in early stages of treatment, particularly in patients with mild symptoms of pneumonia, healthy individuals and pre-treated patients **[8, 9].**Further, data from healthcare settings are essential to determine the best course of action for the CAP treatment and management. Hence, clinical profile and aetiology of lower respiratory tract infections were determined in hospitalized children with CAP.

Materials and Methods:
This study is a cross sectional descriptive study and conducted at Department of Microbiology, Dr. ALM post graduate institute of Basic medical sciences. From September 2019 to August 2020, clinical samples were collected from children admitted at the tertiary care center, Chennai. Following approval from Institute Ethics Committee, informed consent and detailed filled-in questionnaires were collected from children's parent.

Inclusion criteria:
Samples were collected from hospitalized children aged 2 months to 12 years, with symptoms (fast breathing and chestindrawing) as per the World Health Organization.

Exclusion criteria:
Children with nasal bleeding, reduced level of consciousness, aspiration pneumonia, hospital acquired pneumonia, co-morbid conditions prone to cause recurrent pneumonia were excluded from the study.

Sample collection:
Induced sputum or expectorated sputum (in case of achievable condition) was obtained from the 268 children. DNA extraction from sputum specimens were performed using the QIAamp DNA minikit (Catalog no. 51104; Qiagen) following the manufacturer's protocol. DNA samples were quantified with UV spectrophotometer and stored at -20°C until further processing.

Real time PCR:
A real-time PCR was performed for *Streptococcus pneumoniae, Haemophilus influenzae Moraxella catarrhallis, Mycoplasma pneumoniae, Bordetellapara pertusiss, Bordetella pertusiss, Chlamydia pneumoniae, Coxiella burnetii* and *Legionella pneumophila* using primers and probe mentioned in Table 1. The PCR reaction was carried out in a total volume of 25µL includes, 5µL of KAPA probe fast Universal master mix(REF:KK4702), 200 nM each of primers and probes and 10ng DNA template, which was performed in QuantStudio3 Flex Real-Time PCR (Thermo Fisher Scientific, Inc.), using a thermal protocol (3 min at 95˚C, 3 sec at 95˚C followed by 20 sec at 60˚C and 30 sec at 60˚C for 40 cycles). All sigmoidal curves and cycle thresholds (CT) of <40 cycles were reported positive in the PCR results.

Statistical analysis:
Using MS Excel, descriptive statistics was used to analyze the clinical factors and demographic data. Chi-square and fishers exact test was employed to assess the statistical significance of our study.

Results:
In our study, 44.8% (120/268) of samples collected from children with CAP were positive for bacterial infection (Figure 1A). Among

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the positive samples,71.7% (86/120) and28.3% (34/120) samples were found to be positive for typical and atypical bacteria,respectively.8.3% (10/120) were samples had both typical and a typical bacteria. The most common pathogen was *H. influenzae* 18.3% (49/268) followed by *S. pneumoniae* 14.6% (39/268), *M. catarhallis* 9.3% (25/268), *M. pneumoniae* 9% (24/268), *B. parapertusis* 3.4% (9/268), *B. pertusis* 1.5%(4/268), *C. pneumoniae* 1.5%(4/268), *C. burnetti* 1.1% (3/268) and *L. pneumophila* 0.74% (2/268).The association of positive infection status with gender and age of the children were not statistically significant in our study (P >0.005) (Table 2). In positive bacterial infection, clinical factors such as the increased respiration rate, cough and cold, temperature, Capillary Refill Time (CRT), Subcostal recession (SCR), consolidation in chest X-rays and increased heart rate were significantly associated with infected children (P <0.005).

Table 1: Primers and probes sequences used for the molecular detection of various bacterial pathogens:

Target	Organisms	Primer sequence(5′-3′)	References
lytA	*Streptococcus pneumonia*		
	Forward	5'-A GTC GTT CCA AGG TAA CAA GTC T-3'	**[10]**
	Reverse	5'-AC CAA CTC GAC CAC CTC TTT-3'	
	Probe	5'-FAM-ATCAGATTGCTGATAAAACGA-BHQ1-'3+	
smpB	*Haemophilus influenzae*		**[11]**
	Forward	5'-ATTAAATGTTGCATCAACGC-3'	
	Reverse	5'-GACTTTTGCCCACGCAC-3'	
	Probe	FAM-ACGRTTTTACCATAGTTGCACTTTCTC-BHQ1	
copB	*Moraxella catarrhalis*		**[12]**
	Forward	5′-CGTGTTGACCGTTTTGACTTT-3′	
	Reverse	5′-CATAGATTAGGTTACCGCTGACG-3′	
	Probe	FAM-ACCGACATCAACCCAAGCTTTGG-BHQ1	
IS481	*Bordetella pertussis*		Present study
	Forward	5′-GGTTGTATGCATGGTTCATCCGAA-3′	
	Reverse	5′-GCACACAAACTTGATGGGCGAT-3′	
	Probe	FAM-TCGCCAACCCCCCAGTTCACTCA-BHQ	
IS1001	*Bordetellapara pertussis*		**[13]**
	Forward	5′GATATCAACGGGTGACGGATC-3′	
	Reverse	5′-GTATGCCAACCCAGTTCGAA-3′	
	Probe	FAM-TGCTGCAATCGAGCAACGTGCA-BHQ1	
P1	*Mycoplasma pneumonia*		**[14]**
	Forward	5′-GTGAACGTATCGTAACACGAGCTTT-3′	
	Reverse	5′-TGGTTTGTTGACTGCCACTGC-3′	
	Probe	5′-TAM-TTGTCGCGCACTAAGGCCCACG-MGB-3′	
ompA	*Chlamydia pneumonia*		**[15]**
	Forward	5 -AAG GGC TAT AAA GGC GTT GCT-3	
	Reverse	5 -AGA CTT TGT TCC AGT AGC TGT TGC T-3	
	Probe	5-FAM/TCC CCT TGC CAA CAG ACG CTG G/3BHQ-3	
ompA	*Coxiella burnetti*		**[16]**
	Forward	5′-CAGAGCCGGGAGTCAAGCT-3	
	Reverse	5′-CTGAGTAGGAGATTTGAATCGC-3	
	Probe	FAM-5′-CAGCCCTGCAGCGAGGAGCC-3′BHQ1-3	
Mip	*Legionella pneumophila*		
	Forward	5′-GCAATGTCAACAGCAA 3′	**[17]**
	Reverse	5′-CATAGCGTCTTGCATG 3′	
	Probe	5′-FAM-CAACTTATCCTTGTCTGTAGCT-BHQ-3	

Table 2 Association of clinic-pathological characteristics of children's with positive and negative status of the bacterial infection

S.No	Characteristics	Categories	Negative % (n= 148)	Positive % (n= 120)	P-value
1	Gender	Male	52.7	47.3	0.39
		Female	58.3	41.7	
2	Age	1-<2	60.9	39.1	0.68
		2-<5	52.9	47.1	
		5-≤12	55.2	44.8	
3	Fever	1-≤3	51.1	48.9	0.24
		>3	58.7	41.3	
		Nil	38.5	61.5	
4	Cough and cold	1-≤5	56.9	43.1	0.01
		>5	67.4	32.6	
		Nil	38	62	
5	Breathlessness	1-≤3	50.4	49.6	0.15
		>3	51.9	48.1	
		Nil	62.8	37.2	
6	Chest indrawing	1-≤2	48.9	51.1	0.34
		>2	57.9	42.1	
		Nil	58.4	41.6	
7	Temperature	97-≤100	69.6	30.4	0.003
		>100 - 102	50.9	49.1	
		No data	46.2	53.8	

8	CRT	<2sec	62	38	0.012
			46.6	53.4	
9	ICR	High	49.2	50.8	0.15
		Minimal	60	40	
		Nil	61.1	38.9	
10	SCR	High	46.7	53.3	0.014
		Minimal	100	0	
		Nil	63.6	36.4	
11	CREPTS	High	51.8	48.2	0.34
		Minimal	76.9	23.1	
		Occasional	50	50	
		Nil	57.3	42.7	
12	Wheeze	Present	50	50	>0.99
		Absent	55.4	44.6	
13	SpO_2	>95	55.9	44.1	0.5
		90-95	46.9	53.1	
		No data	64.3	35.7	
14	CXR	BHI	52.6	47.4	0.04
		Positive	88.2	11.8	
		Pneumonia	51	49	
		Negative	61.1	38.9	
15	Heart Rate	High	50	50	0.013
		Low	75	25	
		Normal	67.7	32.3	
		No data	46.6	53.4	
16	Respiration rate	High	61.2	38.8	0.041
		Low	46.6	53.4	
		Normal	63.5	36.5	
17	Treatment by antibiotics	Given	52.4	47.6	0.099
		Not Given	51.5	48.5	
		No data	69.6	30.4	
18	Outcome	Improved	52.5	47.5	0.095
		Not Improved	50	50	
		No data	70.7	29.3	

Discussion:

Lower respiratory tract infections still continue to be a significant cause of morbidity and mortality in developing countries **[18].** Antibiotic use by healthcare providers has changed the natural history of infectious disease in patients **[19].** CAP causing bacterial identification by culture or serology remains challenging in managing the infections. Molecular methods play an important role in rapid and accurate diagnosis of CAP. It also aids in the management of critical patients by prescribing appropriate antibiotic regimen with good clinical outcomes **[20].** Furthermore, molecular methods such as Real-time PCR have the advantage of directly detecting the causative agents in clinical specimens **[21, 22].**In the present study, 268 clinical samples were subjected to RT-PCR and 44.8% of children were tested positive for bacterial CAP. In contrast, previous study reported much lesser prevalence of bacterial CAP in children **[23].** The most common pathogen observed in our study was *Haemophilus influenza* (18.3%) which is in accordance with the previous study **[24].** From our investigation we observed the infection mainly occurs in children belongs to the age group of 2-5 years which was consistent with the previous reports published by **[25].** Similar to our findings, the World Health Organization published a report indicating an increase in the incidence of pneumoniae infection among children of the same age group. Prior studies demonstrated that male children are more prone to pneumonia **[26, 27].** Although no significant correlation was found between infection status, age, and gender in our investigation, we eventually found an increased incidence of pneumonia in male children.

The study also investigated the correlation of clinical manifestations with bacterial CAP and we found no significant association due to the similarity in signs and symptoms presented by typical and atypical bacterial infections. Previous studies reported cough, breathlessness and fever were the most common clinical symptom associated with bacterial CAP **[28, 29].** On the contrary, our study found that increased respiration rate, consolidation in chest X-rays and CREPTS were associated with severity of the disease. Novel diagnostic techniques are rapid and sensitive, potentially increasing the detection of respiratory infections. PCR methods, on the other hand, allow testing for multiple pathogens at the same time in a single analysis **[30, 31].** In our study, both typical and atypical bacterial pneumonia was observed among the children with CAP. A recent report suggests that dual antibiotic therapy may reduce the mortality of CAP patients **[32].** Broad-spectrum therapy may be opted for hospitalized children; however the overuse of such antibiotics may result in drug resistance; therefore, clinicians must choose the right antibiotic to treat CAP patients. The combination of b-lactam and macrolide antibiotics or fluoro-quinolone-mono-therapy would be the better option for treating children with CAP **[33, 34].**

Conclusion:

We would like to conclude that the rapid diagnosis of the causative CAP bacterial pathogen in children would allow for the early antibiotic treatment. Our data firmly establish the importance of broad spectrum empiric therapy that covers both typical and atypical bacterial pathogens responsible for CAP in children.

Further studies needed to develop appropriate antibiotic policies based on regional etiological spectrum.

Limitation:

This study has several limitations like it is a single centre study, no follow up was done on the study participants, limited sample size, lack of biochemistry data and information on prior antimicrobial agents.

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Reference 34 above seems unrelated to the context in which it is cited.