RESEARCH PAPER

Bacterial Pathogens Associated with Community-acquired Pneumonia in Children Aged Below Five Years

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Objectives: To determine the spectrum of bacterial pathogens causing community-acquired pneumonia in children below 5 years of age.

Methods: Children aged below 5 years satisfying the WHO criteria for pneumonia, severe pneumonia or very severe pneumonia, and with the presence of lung infiltrates on chest *X*-ray were enrolled. Two respiratory samples, one for culture and the other for PCR analysis, and a blood sample for culture were collected from every child.

Results: Of the 180 samples processed, bacterial pathogens were detected in 64.4%. *Streptococcus pneumoniae* and *Hemophilus influenzae* were most frequently detected. The performance of PCR analysis and culture were identical for the typical bacterial pathogens; atypical pathogens were detected by PCR analysis only.

Conclusion: *S. pneumoniae* and *H. influenza* were the most commonly detected organisms from respiratory secretions of children with community acquired pneumonia.

Keywords: *Epidemiology, Etiology, Polymerase chain reaction, Streptococcus pneumoniae.*

Community-acquired pneumonia (CAP) in the pediatric age group is caused by a myriad of bacteria and viruses. The present study aims to determine the spectrum of bacterial pathogens causing CAP in children aged below 5 years, using conventional and molecular methods.

METHODS

The study population comprised of 180 cases of CAP in children below 5 years of age, admitted in the Department of Pediatrics of a medical college hospital in Dibrugarh, Assam betweem June 2013 and May 2014. Cases were included as per WHO criteria for pneumonia, severe pneumonia or very severe pneumonia [1], and with the presence of lung infiltrates on chest *X*-ray. All cases of hospital-acquired pneumonia were excluded. 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.

The respiratory sample in majority of the cases was oropharyngeal swab. Bronchoalveolar lavage (BAL) was collected wherever possible. Blood samples were collected, and processed in Trypticase soy broth (HiMedia Labs, Mumbai, India) for culture. Oropharyngeal swab samples were collected with sterile flocked nylon swabs.

The first respiratory sample and the blood sample were processed according to standard microbiological procedures [2]. The second respiratory sample was used for PCR analysis. Total DNA was extracted by using the QIAamp DNA Mini Kit (Qaigen, Valencia, CA) according to manufacturer's instructions. Conventional singleplex PCR reaction was carried according to previously described techniques with slight modifications [3-6]. The PCR end products were analyzed by agarose gel electrophoresis and visualized under UV transilluminator. Cultured stocks of ATCC strains were used as controls for the cultivable strains. For the difficult to culture or unculturable organisms, in-house control DNA previously verified by sequence analysis was used.

Data entry, database management and statistical analysis were done using Epi-Info software version 7. A *P*-value of <0.05 was considered to be satistically significant.

RESULTS

Out of the 180 cases studied, at least one bacterial pathogen was detected in 116 (64.4%) cases. The respiratory sample was oropharyngeal swab in 163 and BAL in 17 children. A single pathogen was detected in 105 (58.3%) and multiple pathogens were detected in 11

(6.1%) cases. A total of 131 bacterial pathogens were detected from the 116 positive cases.

Majority of the children in whom bacterial pathogen was detected were in the 0-12 months age group (*n*=75) and males (*n*=115). The mean (SD) age was 16.9 (15.1) months (***Table* I**). A higher frequency of bacterial pathogens were detected in the cases presenting with very severe pneumonia in comparison to severe pneumonia and pneumonia.

The most commonly detected bacterial pathogen was *S. pneumoniae*, followed by *H. influenza* (***Table* II**). None of the *H. influenzae* strains were of type b. All the *S. aureus* strains were methicillin sensitive (MSSA). Non-pathogenic viridans streptococci were detected in 19 and mixed bacterial growth suggesting normal flora of the throat were detected in 33 cases (excluded from final analysis).

The overall mortality rate was 8.9%, and a bacterial pathogen was detected in 15 of these cases; the bacterial pathogens detected were *S. pneumoniae* in eight cases, and *K. pneumoniae* in four cases.

In case of *S. pneumoniae, K. pneumoniae* and *S. aureus,* all cases detected by PCR analysis of the respiratory samples were also detected by culture. The detection rate by blood culture was low (***Table* II**).

DISCUSSION

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 bacterial pathogens (*M. pneumoniae* and *C. pneumoniae*) accounted for about 10% of cases. The atypical pathogens could be detected only by PCR whereas typical bacterial pathogens could be detected as well by conventional culture methods.

TABLE I PROFILE OF CHILDREN WITH COMMUNITY ACQUIRED PNEUMONIA

Parameters	*Total cases of CAP* (*n*=180)	*Bacterial pathogen detected, No. (%)*
Age 0 -12 mo	75	53 (70.7)
Age 12-24 mo	61	38 (62.3)
Age 24-60 mo	44	25 (56.8)
Male gender	115	80 (69.6)
WHO classification		
Pneumonia	31	11 (35.5)
Severe pneumonia	81	49 (60.5)
Very severe pneumonia	68	56 (82.4)
Death	16	15 (93.8)

The main limitation of this study was that isolates from the oropharyngeal swab samples might represent the normal oropharyngeal flora, and might not necessarily be the cause of CAP. Moreover, the study used conventional singleplex PCR for the detection of pathogens. 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 [7-9]. *S. aureus* and some Gram-negative bacilli like *K. pneumoniae* were detected as the leading cause of pneumonia by Johnson, *et al*. [10]. 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 [11]. The detection of infection by blood culture was lower in the present study as compared to few other studies [9,10].

We conclude that *S. pneumoniae* and *H influenzae* are the most common bacterial organisms associated with community acquired pneumonia. PCR with pathogen-specific primers 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.*

TABLE II PROFILE OF SAMPLES POSITIVE FOR BACTERIAL PATHOGENS

Organism	*Total*	*Sample type*		*Blood*
	(n=131)	*OPS (n=116)*	*BAL (n=15)*	*Culture (n=4)*
S. pneumonia	32	22	10	3
H. influenzae (non type b)	28	28	0	0
K. pneumonia	23	19	4	1
S. aureus	15	15	0	0
P. aeruginosa	9	8	1	0
M. pneumonia	8	8	0	-
B. pertussis	7	7	0	-
C. pneumonia	5	5	0	-
Acinetobacter species	3	3	0	0
Citrobacter koseri	1	1	0	0

OPS: Oropharyngeal swab, BAL: Bronchoalveolar lavage.

WHAT THIS STUDY ADDS?

- In addition to *Streptococcus pneumoniae*, atypical bacteria (*M. pneumoniae* and *C. pneumoniae*) were detected from respiratory secretions of 10% children with community acquired pneumonia.

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REFERENCES

1. Park K. Park's Textbook of Preventive and Social Medicine. 21st ed. Jabalpur: M/S Banarsidas Bhanot; 2012. p. 156.
2. Collee JG, Marr W. Culture of bacteria. *In*: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburg, UK: Churchill Livingstone; 2006. p. 113-30.
3. Morozumi M, Nakayama E, Iwata S, Aoki Y, Hasegawa K, Kobayashi R, *et al*. Simultaneous detection of pathogens in clinical samples from patients with community-acquired pneumonia by real-time PCR with pathogen-specific molecular beacon probes. J Clin Microbiol. 2006;44: 1440-6.
4. Ozen NS, Ogunc D, Muthu D, Ongut G, Baysan BO, Gunseren F. Comparison of four methods for the rapid identification of *Staphylococcus aureus* directly from BACTEC 9240 blood culture system. Indian J Med Microbiol. 2011;29:42-6.
5. Lind-Brandberg L, Welinder-Olsson C, Lagergard T, Taranger J, Trollfors B, Zackrisson G. Evaluation of PCR for the diagnosis of *Bordetella pertussis* and *Bordetella parapertussis* infection. J Clin Microbiol. 1998;36:679-83.
6. Lam WY, Yeung ACM, Tang JW, Margaret IP, Chan EWC, Hui M, *et al*. Rapid multiplex nested PCR for detection of respiratory viruses. J Clin Microbiol. 2007;45:3631-40.
7. Hortal M, Estevan M, Iraola I, De Mucio B. A population-based assessment of the disease burden of consolidated pneumonia in hospitalized children under five years of age. Int J Infect Dis. 2007;11:273-7.
8. Gratten M, Mongomery J. The bacteriology of acute pneumonia and meningitis in children in Papua New Guinea: assumptions, facts and technical strategies. PNG Med J. 1991;34:185-98.
9. Nantanda R, Hildenwall H, Peterson S, Kaddu-Milindwa D, Kalyesubula I, Tumwine JK. Bacterial aetiology and outcome in children with severe pneumonia. Ann Trop Paediatr. 2008;28:253-60.
10. Johnson AW, Osinusi K, Aderele WI, Gbadero DA, Olaleye OD, Adeyemi-Doro FA. Etiologic agents and outcome determinants of community-acquired pneumonia in urban children: a hospital-based study. J Natl Med Assoc. 2008;100:370-85.
11. Stralin K, Tornqvist E, Kaltoft MS, Olcen P, Holmberg H. Etiologic diagnosis of adult pneumonia by culture and PCR applied to respiratory tract samples. J Clin Microbiol. 2006;44:643-5.