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Original article

Rapid Diagnosis of Bacterial Pneumonia in Under-five Children by Latex Particle Agglutination Test in Urine

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Abstract

***Streptococcus pneumoniae* and *Haemophilus influenzae* type b are the main causes of community-acquired bacterial pneumonia in developing countries including Bangladesh. This study was carried out to find a rapid diagnostic method for early detection of bacterial pneumonia. Latex Particle Agglutination Test (LPAT) was done in urine for quick detection of capsular antigens of *S. pneumoniae* and *H. influenzae* type b. Three hundred children were enrolled based on clinical criteria as defined by the WHO. Among them, 70 pneumonic children, who had total white blood cell counts around 20000/ mm^3, Neutrophils ≥ 75% and consolidation on chest X-rays, were selected for LPAT in urine. Some 20 children of the same age group, who had chest diseases other than pneumonia, were selected as sick control group. Among 70 pneumonic cases, 36 (51.43%) were positive by LPAT in urine. Out of 36 LPAT-positive cases, 19 (52.7%) were positive for *S. pneumoniae* and 17 (47.3%) were positive for *H. influenzae* type b by specific anti-sera. The LPAT is thus appears as a quick and reliable test for the diagnosis of bacterial pneumonia cases caused by *S. pneumoniae and H. influenzae* type b.**

Key words: Emerging resistance, Common bacteria

Introduction

Pneumonia is one of the leading causes of morbidity and mortality in children worldwide, mainly in developing countries.[1,2] In 1990, 12.9 million children under the age of five died, a third of this deaths were due to acute respiratory tract infection (ARI), mainly pneumonia.[3] It is estimated that at least three million children die from pneumonia each year in developing countries.[4] Mortality from pneumonia is approximately 10-15 times higher in developing countries than in developed countries.[5,6] In Bangladesh, acute respiratory tract infection (ARTI) is one of the most frequent cause of death.[7] The Community Acquired Pneumonia (CAP) presents both a diagnostic and therapeutic challenge to clinicians.[8] Determination of the etiology of lower respiratory tract infection is a complex task.[9] Definite clinical diagnosis is based on X-Ray findings, culture of lung aspirates and measurement of blood oxygen level.[10] The chest radiograph considered the "gold standard" for the diagnosis of pneumonia but cannot differentiate the bacterial and nonbacterial origin.[11] In recent years, blood cultures have been utilized to obtain information on the bacterial etiology of pneumonia in young children but its sensitivity is low, because less than 8% of the children with Acute lower respiratory tract infection (ALRI) are bacteremic.[12,13] The ALRI is one of the major causes of morbidity and mortality of the children under five years in the United States and throughout the world.[14] *S. pneumoniae* and *H. influenzae* type b are main the agents among community-acquired pneumonia in developing countries.[15]

Organisms responsible for pneumonia occasionally cause bacteremia. Lung aspiration culture is the most sensitive and reliable method for isolation of bacterial agents in children but lung aspiration is invasive and exposes the child to potentially dangerous complications.[8,16,17] Several studies on blood culture among children with pneumonia showed that

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positive culture ranged between 1% to 27%.[18] Etiological diagnosis of bacterial pneumonia is difficult in small children, as collection of blood for culture is difficult or already started antibiotics, as is widely practiced in Bangladesh. Thus the true burden of the disease is still unknown in many developing countries.[19, 20] In this condition, detection of antigen in urine samples is a simple useful tool for diagnosis of bacterial pneumonia.[21] One study in India revealed that blood culture was positive in only 10 (9.09%) of 110 children who had features of ALRI, whereas by latex agglutinations assay on serum and urine, 52 (47.2%) cases were found positive.[22] The present study was undertaken to detect antigen in urine for rapid diagnosis of bacterial pneumonia among children of under-five years.

Methods

The study was carried out in the department of Microbiology, Dhaka Medical College during the period of November, 2004 to November, 2005. It was a cross-sectional study. A total of 300 children of under-five years, who were suffering from pneumonia clinically, were included in this study. The patients were selected from the pediatrics ward of Dhaka Medical College Hospital and Azimpur Matrnity Hospital. A detailed medical history, socio-economic status of parents was taken and physical examination was done on each patient. All data were recorded in a pretested data sheet. According to WHO guidelines, patients were classified into three groups: a) pneumonia; b) severe pneumonia; and c) very severe pneumonia.

Among the 300 pneumonic children, 70, who had total WBC counts around 20,000, neutrophil counts ≥75% and consolidation on chest X-rays, were selected for LPAT in urine.

Twenty children of the same age group with similar socio-economic background, suffering from heart/chest disease, other than pneumonia, were selected as sick control. Symptoms like cough, temperature, respiration rate, chest indrawing, stridor, wheezing and creeping, cyanosis, unable to drink, were considered to diagnose the cases of pneumonia.[21,22] Patients suffering from renal failure, foreign body aspiration, respiratory paralysis, chromosomal anomalies were excluded from this study.

Urine was collected aseptically, for antigen detection by latex particle agglutination test (LPAT). Urine samples were kept at -20°c if the test was not done immediately. The Wellcogen bacterial antigen kit (Bronidox) was used to detect antigen of *Haemophilus influenzae* type b and *Streptococcus pneumoniae* in urine.

Urine were heated at 100° C in a boiling water bath for 5 minutes and allowed to cool at room temperature before use. The samples were then centrifuged at 1400 rpm for 15 minutes, and the supernatant fluid was then tested. Positive test showed agglutination and negative test showed no agglutination. Positive control and negative control tests were done by the reagents included in the kits.

Results

Among 300 cases, 186 (62%) children were male and 114 (38%) were female, of them majority (63%) were between 3-11 months, 28% were within <12-23 months. Majority (65%) of the cases came from lower income group, whereas 35% cases were from middle-income group. There was none from higher income group. Among the cases, 70 (23.3%) children had both lung consolidation and high leukocyte count. Their WBC were around 20,000 and neutrophil count ≥75%. Some 38 (12.82%) cases had high leukocyte count with patchy opacity. The LPAT in urine was done with 70 (23.3%) cases, who had consolidation on X-ray chest and total WBC ±20,000 per mm^3 of blood and neutrophils ≥75%. (Table I)

Table I: X-ray findings, TLC* and neutrophil percentage among 300 clinically diagnosed pneumonia cases

No. of study subject	X-Ray findings	WBC/mm^3	Neutrophil
70 (23.3%)	Consolidation	≥ 20,000	≥75%
38 (12.6%)	Patchy opacity	15,000	≥70%
192 (64.0%)	Patchy opacity	≤15,000	≤70%

*TLC- Total leukocyte count

Out of 70 cases, LPAT in urine was positive in 36 (51.9%) cases who had high leukocyte count and lung consolidation. Whereas, among 20 cases of control group, only 1 (5%) was positive by LPAT. (Table II)

Table II: Latex Particle Agglutination Test (LPAT) in urine samples of children with pneumonia (n=70)

	No (%) of LPAT result		
Study Subjects	Positive	Negative	Total
Cases	36 (51.9%)	34 (48.1%)	70 (100.0%)
Control	01 (5.0%)	19 (95.0%)	20 (100.0%)

Out of 36 LPAT positive cases, 19 (52.7%) were *S. pneumoniae* and 17 (47.3%) were *H. influenzae* type b. (Figure 1)

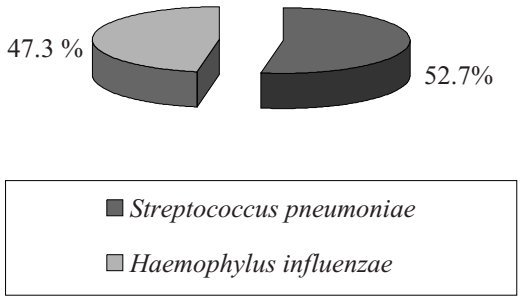

Figure 1: Bacterial agents detected by LPAT

Discussion

Various community- and hospital-based studies in Bangladesh noted that mortality from pneumonia among under-five children was 10% to 30%.[22] A positive blood culture provides the basis for a specific etiologic diagnosis but it is time-consuming and the sensitivity of this method is low. The method of direct examination and culture of sputum have lower sensitivity and specificity. Antigen detection is a promising noninvasive approach for diagnosis of pneumonia. Bacterial antigens produced at site of infection are distributed to body fluids and can be detected by various test procedures like Latex particle agglutination, Co-agglutination, ELISA and PCR.[14] In this study, urine was tested for detection of antigens of *H. influenzae* type b and *S. pneumoniae* by LPAT.

Among 70 cases selected from 300 clinically diagnosed pneumonic children, LPAT in urine were positive in 36 (51.9%) and rest 34 (34.1%) were negative. Similarly, Nunes *et al* (2004) reported that among 45 children with pneumonia, who had positive X-ray findings and high leukocyte count, LPAT were positive in 77.3% cases and negative in 22.7%.[15] Another study in India by Bahl *et al* (1995) showed the highest diagnostic yield with LPAT showing 52 (41.2%) cases positive by LPAT.[23] One study in USA found 24% antigenuria, in children with acute lower respiratory tract infection.[12] In our study, among 70 pneumonic children, 19 (52.7%) *S. pneumniae* and 17 (47.3%) *H. influenzae* type b were detected by LPAT.

The LPAT is a quick and reliable test for the diagnosis of bacterial pneumonia caused by *S. pneumoniae* and *H. influenzae* type b, even in partially-treated patients where cultures are often negative. It is a simple, rapid test and can be carried out in an ordinary laboratory setup. Detection of bacterial pneumonia cases by LPAT in urine may be higher if the kit would provide reagent for other capsulated *H. influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae.*

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