

Community-Acquired Pneumonia in the Childhood: Analysis of the Diagnostic Methods

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Immunological assays such as CIE, LA, and Dot-ELISA were compared in order to diagnose community-acquired pneumonia. Serum, pleural fluid and urine samples were comparatively employed for bacterial antigen detection. Dot-ELISA proved to be an original and practical alternative procedure for detecting bacterial polysaccharide antigens from pleural fluid and/or concentrated urine samples, providing a rapid diagnosis for pediatric patients with community-acquired pneumonia.

Key-Words: Community-acquired pneumonia, *Streptococcus pneumoniae*, *Haemophilus influenzae*, bacterial pneumonia, immunological methods.

Community-acquired pneumonias (CAP) are associated with elevated rates of morbidity and mortality. Worldwide, about 4.5 million children younger than 5 years old are yearly affected by acute respiratory infections, and 80% to 90% of these cases are pneumonia [1]. Pneumonia has been defined as the presence of fever, acute respiratory symptoms, or both, plus evidence of parenchymal infiltrates on chest radiography. Even this definition overlaps somewhat with that of viral bronchiolitis and leaves some room for disagreement among clinicians [2]. About 80% of all patients with CAP are treated in an ambulatory setting, most of them without any diagnostic testing, which makes it harder to know the true etiology of pneumonia affecting these patients [3]. However, clinical and laboratory diagnosis using bacterial culture from blood, pleural fluid and pulmonary aspirate has showed that *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Staphylococcus aureus* and *Mycoplasma pneumoniae* are the main etiological agents of the pneumonias. Bacterial cultures of the nasopharynx or throat correlate poorly with cultures of lung tissue and confound more than clarify the etiology. Culture of blood or pleural fluid is clearly an insensitive method, but there are yet any established alternatives in children. Immunological assays have been employed to detect bacterial antigens of *S. pneumoniae* and/or *H. influenzae* type b, the most common bacteria causing pneumonia. The polysaccharide antigens have been detected in serum, urine, and/or pleural fluid samples from infants and children with suspected bacterial pneumonia [4-8].

Materials and Methods

Paired samples of pleural fluid, serum and urine from 550 children with clinical diagnosis of bacterial pneumonia were assayed by counterimmunoelectrophoresis (CIE), latex agglutination (LA), and dot-enzyme-linked immunosorbent assay (Dot-ELISA) for antigen detection according to

previously standardized procedures [4-8]. Anti-*Streptococcus pneumoniae* omniserum against 90 serotypes (pneumococcal omniserum) from Seruminstitut, Copenhagen, Denmark [9] and anti-*Haemophilus influenzae* type b produced according to routine procedure [10] were employed in the immunological methods. Aliquotes of 200 μ L of pleural fluid and/or serum samples were heat-treated (100°C/10 min) with 300 μ L of 0.1 M sodium EDTA pH 7.5, followed by centrifugation (2,500 rpm/15 min), and the supernatant was used in the immunological tests. Pleural fluid samples were also submitted to bacterial culture [4]. Urine samples were left to stand overnight (4°C) with ethanol (v:v) and centrifuged at 4,000 rpm/15 min for polysaccharide antigen precipitation. The pellet was dissolved in 0.5 mL of sterile distilled water plus 0.5 mL of 0.1 M sodium EDTA pH 7.5, followed by heat-treatment (water-bath, 100°C/10 min). After new centrifugation (2,500 rpm/15 min), the supernatant was stored until use in the immunological tests [4-8].

Parametric levels for evaluation of CIE, LA and Dot-ELISA in the use of pleural fluid, serum and urine samples were calculated according to standard method [11].

Results

Of the 550 pleural fluid samples, 146 (26.5%) had positive bacterial culture results for *S. pneumoniae* (15.4%) and *H. influenzae* (11.1%). Positivities for CIE, LA, and Dot-ELISA, using the same pleural fluid samples, were 36.0%, 50.2%, and 63.7%, respectively. Positivities (*S. pneumoniae* plus *H. influenzae*) obtained with serum samples in CIE, LA and Dot-ELISA were 24.2%, 38.2%, and 58.6%, respectively, while with urine samples the values of 37.0%, 47.3% and 58.0%, respectively, were obtained (Table 1).

Parametric levels of sensitivity, specificity, positive (+PV) and negative (-PV) predictive values, prevalence and accuracy for CIE, LA, and Dot-ELISA using pleural fluid, serum and urine samples obtained in relation to the gold standard bacterial cultures of the pleural fluid samples are presented in the Table 2.

Discussion

Bacterial culture and several immunological assays have been proposed to diagnose community-acquired pneumonia. Bacterial culture of secretions from upper

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Table 1. Comparison among bacterial culture, counterimmunoelectrophoresis (CIE), latex agglutination (LA), and Dot-ELISA for detection of *S. pneumoniae* and *H. influenzae* type b antigens in the 550 paired samples of pleural fluid, serum and urine

Antigens	Culture	Pleural fluid			Serum			Urine		
		CIE	LA	Dot-ELISA	CIE	LA	Dot-ELISA	CIE	LA	Dot-ELISA
<i>S. pneumoniae</i>	85	120	164	216	76	120	192	119	151	188
(%)	(15.4)	(21.8)	(29.8)	(39.3)	(13.8)	(21.8)	(35.0)	(21.6)	(27.5)	(34.2)
<i>H. influenzae</i>	61	78	112	134	57	90	130	84	109	131
(%)	(11.1)	(14.2)	(20.4)	(24.4)	(10.4)	(16.4)	(23.6)	(15.4)	(19.8)	(23.8)
Negative	404	352	274	200	417	340	228	347	290	231
(%)	(73.5)	(64.0)	(49.8)	(36.3)	(75.8)	(61.8)	(41.4)	(63.0)	(52.7)	(42.0)
Total positive	146	198	276	350	133	210	322	203	260	319
(%)	(26.5)	(36.0)	(50.2)	(63.7)	(24.2)	(38.2)	(58.6)	(37.0)	(47.3)	(58.0)

Table 2. Parametric levels for counterimmunoelectrophoresis (CIE), latex agglutination (LA) and Dot-ELISA of pleural fluid, serum and urine samples in relation to bacterial cultures of 550 pleural fluid samples

Parameters	Pleural fluid			Serum			Urine		
	CIE	LA	Dot-ELISA	CIE	LA	Dot-ELISA	CIE	LA	Dot-ELISA
Sensitivity	100.0	100.0	100.0	91.1	100.0	100.0	100.0	100.0	100.0
Specificity	87.1	67.8	49.5	100.0	84.2	56.4	86.0	71.8	57.2
+PV	73.7	53.0	41.7	100.0	69.5	45.3	72.0	56.2	45.8
-PV	100.0	100.0	100.0	96.8	100.0	100.0	100.0	100.0	100.0
Prevalence	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5
Accuracy	90.6	76.4	63.0	97.6	88.4	68.0	89.6	79.3	68.5

(+PV) positive, and (-PV) negative predictive values.

respiratory tract is not recommended since the normal flora includes the bacteria commonly responsible for pneumonia [2]. On the other hand, blood bacterial culture proved to be a low-sensitive test that depends on viable microorganisms and on previous administration of antimicrobial agents [12]. Other microbiological method named polymerase chain reaction (PCR) has been proposed to diagnose CAP [13]. However, this technique is complex and time-consuming, it has not been yet fully standardized, and is very expensive for use in developing countries. Then, immunological assays such as CIE, LA and Dot-ELISA have been proposed as sensitive and specific methods in the diagnosis of CAP. Studies with CIE and LA have demonstrated that these methods may detect 20 to 50 ng/mL of polysaccharide bacterial antigens, while enzymatic immunoassays may detect 0.3 to 0.6 ng/mL [14]. Moreover, among these three methods, CIE is very expensive for developing countries due to the elevated cost of concentrated pneumococcal omniserum, and LA is also difficult to access due to elevated cost of commercial kits.

Our proposed Dot-ELISA proved to be a practical alternative procedure for bacterial antigen detection, in view of its execution without the need for reading equipment, and economy of pneumococcal omniserum and other bacterial antiserum, that may be used at 1:200 dilution [6-8]. Positivities obtained by Dot-ELISA are higher than that obtained by CIE and LA, when pleural fluid, serum, and/or urine samples are employed in the tests, despite the similar values of sensitivity.

Dot-ELISA proved to be an original and practical alternative procedure for detecting bacterial polysaccharide antigens from pleural fluid, serum, and/or concentrated urine samples, providing a rapid diagnosis for pediatric patients with community-acquired pneumonia.

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