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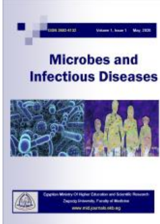

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Original article

Pneumococcal pneumonia in children less than 5 years: Are we underdiagnosing?

Sherif Moustafa [1], Nehal Abdelhamid [1], Nadia Moustafa [2], Nihal El Rifai [2], May sherif [3], Manal Kandil [1] , Amani Elkholy[*3]*

1- *Department of Pediatrics, National Research Centre, Cairo, Egypt*
2- *Departments of Pediatrics, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt*
3- *Department of Clinical Pathology, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt.*

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ABSTRACT

Background: *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia in children. Diagnosis of *Pneumococcal pneumonia* by culture methods is difficult, if antibiotics were taken before culture. The objectives of this study were 1-to evaluate the diagnostic yield of (BinaxNOW) antigen test, and culture methods compared to quantitative PCR, for the identification of *Streptococcus pneumoniae* in community-acquired pneumonia (CAP) children under 5 years, 2- and to assess the severity of *Pneumococcal pneumonia*. **Methods:** We enrolled 60 children with CAP, and 30 age and sex-matched healthy controls. For all patients, blood culture, respiratory specimen culture, qPCR, complete blood count and CRP were done. For both the patient group and control group, the urinary antigen test (BinaxNOW) was done. Patients were evaluated by RDS score. **Results:** The median age for patients and control were 9 ± 9.3 and 9.5 ± 13.5 months, respectively. PCR was positive in 12 (20%) out of 60 patients, indicating *Pneumococcal pneumonia*. Of them, 9 (15%) tested positive by BinaxNow, and one by positive culture of *Streptococcus pneumoniae* (1.2%). The RDS risk score identified significantly severe disease, and children higher TLC, more shift to the left and higher CRP, in *Pneumococcal pneumonia* group. **Conclusions:** *Pneumococcal pneumonia* presented with more severe disease. Culture methods were inferior to PCR and antigen test. The antigen test can be used as point of care rapid diagnostic test, for discriminating between children with and without *Pneumococcal pneumonia*; with the potential to impact patient care and improve antibiotic stewardship.

Introduction

Streptococcus pneumoniae (pneumococcus) is a leading cause of bacterial pneumonia as well as invasive diseases such as sepsis and meningitis. Pneumococcal infections lead to high morbidity and mortality rates worldwide, especially in children under 5 years of age and the elderly. It has been estimated that most cases of serious pneumococcal disease occur in low-income countries [1]. The annual worldwide incidence of community-acquired pneumonia (CAP) has been estimated to be between 1000 and 12 000 per 100 000 children; accounting for approximately one in five child deaths globally [2]. In 2015,

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* *Corresponding author:* Amani Ali El-Kholy
E-mail address: aelkholy@kasralainy.edu.eg
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approximately 700,000 children under five years died from pneumonia worldwide, especially in areas with poor living conditions, under-nutrition, unavailability of vaccines [3].

For appropriate treatment of pneumonia, antimicrobials should target the causative agent. However, the ability of physicians to identify the causative pathogen of pneumonia based on history, clinical examination and imaging is limited [4].

Making a precise diagnosis of *Pneumococcal pneumonia* has relied mainly on culturing of *Streptococcus pneumoniae* from blood and respiratory specimens. However, culture results could be negative in many pneumonia patients. The main reasons for failing to identify the pathogens of bacterial pneumonia are: (i) the failure to obtain an adequate sputum sample for culture; and (ii) collection of the respiratory specimen after starting antibiotics [5]. Blood cultures are positive in only about 25% of cases of *Pneumococcal pneumonia* [6].

In Egypt diagnosis of pneumococcal diseases is a challenge, because of the difficulty in isolation of *Streptococcus pneumoniae* by culture. In the study of **El-Kholy et al.** (2020), Only 19 out of 40 specimens from invasive pneumococcal diseases grew by culture, and the rest were diagnosed by PCR. The authors attributed this to the antibiotic intake before the collection of microbiological cultures, especially before presenting to the hospital [7].

Various rapid tests, using nucleic acid amplification and antigen detection, have been introduced for the diagnosis of pneumococcal infections [8]. Polymerase chain reaction (PCR) assays, has provided cost-effective, timely and precise diagnosis of pneumonia pathogens that supported therapy. Different types of PCR became available in the clinical laboratory and were included in the laboratory diagnostic work flow [9].

Urinary antigen detection (BinaxNOW) is an immune-chromatographic test for the presence of the pneumococcal C-polysaccharide coat protein in urine and can produce results within 15 minutes [8]. Studies showed that using BinaxNOW antigen detection kit and PCR for detection of *Streptococcus pneumoniae* would improve identification of pneumococci from respiratory specimens of children having *Pneumococcal pneumonia* [9-11].

The aim of this study was: 1. to assess the diagnostic yield (BinaxNOW) antigen detection test and culture methods for the identification of *Streptococcus pneumoniae* as the cause of CAP in children, compared to quantitative PCR. 2. And to assess the severity of *Pneumococcal pneumonia*, compared to non- *Pneumococcal pneumonia* in children less than 5 years.

Methods

Patients

The study protocol was approved by the Research Ethics Committee of National Research Centre, Egypt and carried out in compliance with the Helsinki Declaration (2008). Informed consents were taken from the parents and/or caregivers before carrying out the procedure.

Patients: We enrolled 60 children between 6 months to 6 years of age, with CAP recruited from the inpatient wards and ICU of Specialized New Children Hospital (Abou El- Reesh), Cairo University, between January and April 2020. The children had signs and symptoms suggestive of lower respiratory tract infection [12]. Patients' demographic data, clinical manifestations and chest radiographic findings were recorded. Children with pre-existing chronic chest disorder (e.g. tuberculosis, cystic fibrosis, etc.) or chronic cardiac disease were excluded.

Controls

We enrolled 30 healthy infants and children matched for age and sex as a control group. Neither the patient nor the control groups received pneumococcal vaccines.

Cases were evaluated for the severity of community acquired pneumonia (CAP) by CAP Severity of illness in children **(Table1)** [13]. Admission to ICU or intermediate care unit with continuous cardio-respiratory monitoring, were indicated for children having ≥1 major or ≥ 2 minor criteria of CAP severity of illness. Data were collected on five components: respiratory rate, wheezing, accessory muscle use, blood oxygen saturation (SpO2), and feeding difficulties. Each component was given 0 or 1 point, and the total score was classified as mild (0–1 points), moderate (2–3 points), or severe (4–5 points) [14].

Laboratory investigations and culture

All patients were tested for complete blood picture (CBC), and C- reactive protein detection (CRP), and culture of blood, pleural fluid and induced sputum. Induction of sputum was done by administration of hypertonic saline.

Culture of specimens

The quality of sputum for culture was assessed in accordance with accepted criteria [15], and only sputa of acceptable quality were cultured. Each sputum sample was streaked on a sheep blood agar plate (BAP). BAPs were incubated at 35°C in a CO2 enriched atmosphere using a candle jar for 18 to 24 hours. Plates were examined and alpha-hemolytic colonies were tested for optochin susceptibility and bile-solubility. Optochin-susceptible bile-soluble isolates were identified as *Streptococcus pneumoniae* [7].

Quantitative PCR (qPCR) assay [10,16]

The lytA qPCR assay was done. Samples were considered positive for pneumococcal lower respiratory tract infection with a cutoff value of 105 DNA copies/ml [16].

Specimens: Nebulized sputum samples were collected from the patients on admission to hospital and were stored at −80 °C until tested. Only sputa of acceptable quality were cultured.

DNA extraction and PCR amplification

Between 0.5 and 1 ml of thawed specimens underwent DNA extraction with the QIAGEN DNA Mini kit (QIAGEN Inc., Valencia, CA). according to the manufacturer's instructions. DNA was eluted in 100 µl of QIAGEN elution buffer and stored at −20°C. The concentrations of the DNA extracted from the bacterial cultures were determined by the Nanodrop method (Nanodrop Technologies, Wilmington, DE).

Screening Qualitative real-time PCR amplification

The pneumococcal lytA gene sequence was detected using the lytA-CDC forward primer (5′-ACGCAATCTAGCAGATGAAGCA-3′) and reverse primer (5′-TCGTGCGTTTTAATTCCAGCT-3′) as previously described and a FAM-labelled lytA-CDC reversed probe (5′-6FAM–CTCCCTGTATCAAGCGTTTTCGGCA–BBQ) with a reverse strand modification of the CDC probe used in previous publications [16].

Real-time duplex PCR assays were performed in a final volume of 25 µl, containing 2.5 µl DNA, on the ABI 7500 RT-PCR system (Applied Biosystems) with Abbott optical 96-well reaction plates and adhesive covers. The PCR mixture contained 2× Universal TaqMan Master Mix (Applied Biosystems) with each primer at a concentration of 0.2 µM, and probes were used at a concentration of 0.2 µM. A positive *Streptococcus pneumoniae* extraction, and a 'no template' control (molecular grade water) were included in each amplification run. Cycling conditions were as follows:, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Analysis of qualitative results was performed with ABI 7500 software version 2.0.1.

Quantitative real-time-PCR amplification [10]

All positive samples underwent a quantitative single-plex RT-PCR assay with the same PCR conditions and lytA-targeting primers and probes. An internal control was not included at this stage. However, 50× Rox dye fluorochrome was included in each run to verify PCR efficiency across the optical 96-well plate. The standard curve was set up using 10-fold dilutions of a known concentration of Streptococcus pneumoniae, established by colony plate count. The standard included six concentrations from 1.8×106 to 1.8×101 CFU/ ml. Analysis of quantitative data was performed with ABI 7500 software v 2.0.1

BinaxNOW urine antigen test [8]

The BinaxNOW was performed on urine from both patients and control groups, according to the manufacturer instructions, without knowledge of the patients' results of culture or qPCR. Briefly, clean catch midstream urine specimens were collected in sterile containers. Stored at room temperature (15-30ºC) if tested at the same time of collection. Alternatively, urine was stored at 2-8 ºC, or frozen for up to 2 weeks, prior testing. A negative result gives a single pink to purple colored control line in the top half of the window, indicating a presumptive negative specimen. This control line means that the detection part of the test was performed correctly, but no *Streptococcus pneumoniae* antigen was found. A positive specimen gives two pink or purple-colored lines. This means that antigen was detected. Specimens with low levels of antigen may give a faint patient line. Any faint line was considered positive. If no lines were seen, or if just the sample line was seen, the test was invalid and was repeated.

Statistical analysis

Statistical package SPSS version 15.0 for windows (SPSS, Chicago and IL., USA) was used for statistical analysis. Data were represented as median and range, mean ± standard deviation; or frequency and percentages.

Results

The patients' median age was 9 ± 9.3 months and ranged from 6-44 months; 61.7% (n= 37) were males. Ninety percent of the patients (n=54) were admitted into wards and 10% (n=6) were admitted into pediatric ICU. Regarding the control group, the median age was 9.5 ± 13.5 months and ranged from 6-48 months; 53.3% (n=16) were males.

Quantitative real-time-PCR for detection of Streptococcus pneumoniae:

Out of 60 specimens, qPCR was positive in 12 (20%). These constituted the *Pneumococcal pneumonia* group. PCR-negative patients were considered as the non- *Pneumococcal pneumonia* group (n=48). All of the patients had fever, tachypnea, tachycardia and cough on examination and none of them had attacks of hemoptysis. No statistical difference was found between the 2 groups **(Table 2).**

Comparison between pneumococcal and non-pneumococcal groups, according to the severity indices

Pneumococcal pneumonia patients were categorized according to the RDS score into mild and moderate (16.7%), and severe (83.3%), which was of significance when compared to the non-*Pneumococcal pneumonia* group (*p*-value <0.05). Using the community acquired pneumonia (CAP) Score, the patients at risk were 91.7%, and patients at no risk were 8.3%, which was of statistical significance compared to the non-*Pneumococcal pneumonia* group (*p*-value <0.05) **(Table 3).**

Chest X-ray findings

Imaging of the *Pneumococcal pneumonia* group revealed lobar pneumonia in 8 (66.7%) and bronchopneumonia in 4(33.3%). The non-*Pneumococcal pneumonia* group showed lobar pneumonia in 23(48%), and bronchopneumonia in 25(52%); cavitation was seen in 1 patient. No statistically significant difference was found between the 2 groups.

Laboratory tests

The *Pneumococcal pneumonia* children showed lower hemoglobin, higher total leucocytic count, more shift to the left and higher CRP, compared to non- *Pneumococcal pneumonia* group **(Table 4).**

Sputum and blood culture

The sputum culture was positive for *Streptococcus pneumoniae* in only one patient (1.2%) among the *Pneumococcal pneumonia* group (PCR positive patients). All blood cultures were negative for *Streptococcus pneumoniae.*

BinaxNOW urine antigen test

Nine out of 60 patients (15%) tested BinaxNow positive, and no positive results were in the control group. All BinaxNow positive patients had positive PCR results. BinaxNow positive patients had more severe pneumonia according to the severity indices **(Table 5).**

Binax Now sensitivity and specificity:

Comparing the Binax Now to the PCR as a gold standard for diagnosing *Pneumococcal pneumonia*; revealed that Binax Now has sensitivity of 75% and specificity of 100%.

Table 1. Criteria for CAP Severity of illness in children with CAP [13]

Major criteria	Minor criteria
• Invasive mechanical ventilation • Fluid refractory shock • Acute need for NIPPV • - Hypoxemia requiring FiO2 greater than inspired concentration or flow feasible in general care area	• Respiratory rate higher than WHO classification for age • Apnea • Increased work of breathing (eg. Retractions , dyspnea , nasal flaring , grunting) • Multilobar infiltrates • PEWS score > 6 • Altered mental status • Hypotension • Presence of effusion • Comorbid conditions

Abbreviations: CAP community acquired pneumonia, FiO2, fraction of inspired oxygen; HgbSS, Hemoglobin SS disease; NIPPV, noninvasive positive pressure ventilation; PaO2, Arterial Partial Pressure of Oxygen; PEWS, Pediatric Early Warning Score

Table 2. Clinical characteristics of pneumonia patients with positive and negative PCR

Variable		(*Pneumococcal pneumonia*) **n=12**	**(non-***Pneumococcal pneumonia*) **n=48**	***P*-Value**
		Frequency n (%)	**Frequency n (%)**	
Fever		12 (100)	48 (100)	1.000
Tachypnea		12 (100)	48 (100)	1.000
Heamoptysis		0 (0)	0 (0)	1.000
Cough		12 (100)	48 (100)	1.000
Vomiting		8 (66.7)	35 (72.9)	0.631
Behavior	Playing	4 (33.3)	15 (31.3)	0.910
	Quite	5 (41.7)	30 (62.5)	0.492
	Sleepy	1 (8.3)	0 (0)	0.321
	Lethargic	0 (0)	2 (4.2)	0.796
	Irritable	2 (16.7)	1 (2.1)	0.104
SpO2	≥95%	3 (25)	20 (41.7)	0.529
	≤95%	9 (75)	28 (58.3)	0.619
Use of accessory muscles		5 (41.7)	35 (72.9)	0.463
Chest indrawing		10 (83.3)	40 (83.3)	1.000
Grunting		5 (41.7)	4 (8.3)	0.291
Diminished air entry		12 (100)	46 (95.8)	0.812
Wheezing		7 (58.3)	40 (83.3)	0.621
Bronchial breathing		10 (83.3)	22 (45.8)	0.471
Crepitations		6 (50)	22 (45.8)	0.819
Need for nebulizer		7 (58.3)	40 (83.3)	0.642
Tachycardia		12 (100)	48 (100)	1.000
Hypotension		7 (58.3)	18 (37.5)	0.620
Dusky skin colour		1 (8.3)	4 (8.3)	1.000
Capillary refill time	1-2 sec	7 (58.3)	20 (41.7)	0.792
	3 sec	4 (33.3)	27 (56.3)	0.529
	4 sec	1 (8.3)	1 (2.1)	0.422

Table 3. Severity indices of pneumococcal positive and negative pneumonia patients.

Variable		*Pneumococcal pneumonia* **n=12**	**Non-***Pneumococcal pneumonia* **n=48**	***P*-Value**
		Frequency (%)		
RDS Score	Mild & Moderate	2 (16.7)	35 (72.9)	*0.001
	Severe	10 (83.3)	13 (27.1)	
CAP Score	Risk	11 (91.7)	22 (45.8)	*0.004
	No risk	1 (8.3)	26 (54.2)	

*P-value significant if <0.05

CAP: Community Acquired Pneumonia**;** RDS score: Respiratory distress Severity Score

Table 4. Complete Blood Count and CRP of pneumococcal and non-*Pneumococcal pneumonia* patients.

Variable	*Pneumococcal pneumonia* **n=12**	**Non-** *Pneumococcal pneumonia* **n=48**	***P*-value**
	Mean ± SD		
Hemoglobin	9.5 ± 1.5	10.8 ± 1.4	*0.007
White blood cells count	18.3 ± 3.0	11.6 ± 4.1	*<0.001
Staff	6.6 ± 1.6	2.6 ± 2.4	*<0.001
Segmented	32.5 ± 6.6	29.1 ± 8.6	0.038
Frequency of Shift to left	11(91.7)	9 (18.8)	*<0.001
CRP mg/L	136.3±56	58.5±31.5	*<0.001

**P*-value significant if <0.05

Table 5. BinaxNow compared to severity indices

		Binax Now		***P*-value**
		Yes (n=9)	**No (n=51)**	
RDS score	Mild-moderate	1	36	*<0.001
	Severe	8	15	
CAP score	Risk	8	25	*<0.033
	No risk	1	26	

**P*-value significant if <0.05

Discussion

Pneumonia is associated with high morbidity and mortality in young children, especially in developing countries. *Streptococcus pneumoniae* represents the main pathogen of pneumonia in children worldwide [1-3]. The rapid and accurate diagnosis of *Streptococcus pneumoniae* is challenging and hence, improving the accuracy of diagnosis and hence accurate management is mandatory [14].

Until recently, the diagnosis of pneumonia depended on clinical picture and imaging. Identification of pathogens depended on culture methods, which were limited by failure to isolate pneumococci after antibiotic therapy and by overgrowth by other microorganisms from the patient's flora. In our study only one out of 60 sputum specimens grew *Streptococcus pneumoniae* (1.2%), compared to 20% by PCR. This is in keeping with previous reports from Egypt. In their hospital-based surveillance for laboratory-confirmed *Streptococcus pneumoniae* cases in children younger than 5 years, from 2008 to 2011, **Draz et al.** isolated *Streptococcus pneumoniae* from 12 patients: four invasive pneumococcal disease (IPD), and eight non-IPD out of more than 22,000 cultured specimens [17]. The low detection rate may be attributed to antibiotic intake prior to collection of the microbiological cultures, especially before presenting to the hospital. Antibiotics are available over the counter in Egyptian pharmacies, and self-medication and purchasing without medical prescriptions are common practice in Egypt. [18-19]. However, *Streptococcus pneumoniae* was more frequently identified from children with severe pneumonia in Upper Egypt. [20]. In a study on 100 children with the severe community-acquired pneumonia admitted to ICU in Assiut (in Upper Egypt), *Streptococcus pneumoniae* was isolated from 36% of the cases [20].

In our patients, *Pneumococcal pneumonia* was presented with higher severity scores, compared to other pneumonia patients. This is in concordance with previous reports [21].

The WBCs and CRP were significantly higher in children with *Pneumococcal pneumonia*. A previous study concluded that CRP levels of ≥40 mg/L were associated with confirmed bacterial pneumonia particularly *Streptococcus pneumoniae* and H. influenzae, and negatively associated with respiratory syncytial virus pneumonia [22].

qPCR identified *Streptococcus pneumoniae* in 20% of pneumonia patients. Currently PCR is the gold standard for diagnosis of pneumonia pathogens. Unfortunately, blood culture was negative in all our patients. The microbial yield of blood culture in pediatric pneumonia varies according to the blood culture volume and prior antibiotic treatment; which reduce blood culture yield by approximately 45% [23]. In addition, bacterial growth is time-consuming. Hence the introduction of PCR for the identification of pneumococcal nucleic acid was a major advance in diagnosis and management of *Pneumococcal pneumonia*.

Real time PCR targeting lytA and piaB (permease gene of the pia ABC transporter) are currently considered the gold-standard, culture independent assay for *Pneumococcal pneumonia* detection. Quantitative PCR and proper specimen collection differentiate infection from colonization by pneumococci [24]. Multiplex PCR and film-array advanced diagnostics have been introduced to clinical laboratories, but they are too costly to use in resource- limited settings, and their results are difficult to interpret [24]. Some studies reported low concordance with culture in pathogen identification, and substantial discrepancies in identifying antimicrobial resistance gene targets compared to the susceptibility testing [24,25].

BinaxNow identified *Streptococcus pneumoniae* in 15% of the patients and was negative in all the control group. All BinaxNow positive patients had positive PCR results. In addition, BinaxNOW positive patients had significantly severe disease, according to the severity indices, and higher the CRP levels. The BinaxNow showed a sensitivity of 75% and a specificity of 100% compared to qPCR. In their study on 1154 adult CAP patients in China, of which 770 (66.7%) had received antibiotics before the BinaxNow test, the positive rate of the test was 3.3%, with sensitivity and specificity 57.9% and 96.6%, respectively [27]. A meta-analysis, published in 2013, of 27 studies comparing the BinaxNOW® assay with cultures in patients with CAP showed an overall assay sensitivity of 74.0% (CI 66.6–82.3%) and specificity 97.2% (CI 92.7–99.8%) [17]. This study concluded that the BinaxNOW® assay had a higher sensitivity than culture in the diagnosis of CAP, as well as a high specificity, making it a useful diagnostic tool in clinical practice [28]. Being rapid and non-invasive makes the rapid urinary antigen test a useful point of care diagnostic test, in resource-limited settings [27-29]. Whilst initial evidence suggested urine antigen testing had a high sensitivity, recently data have suggested the actual sensitivity is lower than expected, at approximately 60–65% [30].

In conclusion, *Pneumococcal pneumonia* presented with severe disease in children less than 5 years. Identification of the pathogen can be missed when depending on culture methods alone, especially in a setting of misuse of antibiotics. The challenging laboratory diagnosis of *Streptococcus pneumoniae* underestimates the disease burden. Advanced and rapid diagnostics are recommended to improve diagnosis and patient management. We also call for including the pneumococcal vaccines in the compulsory childhood vaccination to prevent serious pneumococcal infections.

List of abbreviations

CAP: Community Acquired Pneumonia

RDS score: Respiratory distress Severity Score

IPD: Invasive Pneumococcal Disease

CRP: C reactive protein

qPCR: Quantitative Polymerase Chain Reaction

Conflict of interest

Not declared.

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