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MICROBIAL ETIOLOGY OF COMMUNITY-ACQUIRED PNEUMONIA AMONG INFANTS AND CHILDREN ADMITTED TO THE PEDIATRIC HOSPITAL, AIN SHAMS UNIVERSITY

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Background: While recognizing the etiology of community-acquired pneumonia is necessary for formulating local antimicrobial guidelines, limited data is published about this etiology in Egyptian pediatric patients. *Objectives:* To determine the frequency of bacterial and viral pathogens causing community-acquired pneumonia (CAP) among immunocompetent Egyptian infants and preschool children. *Methods:* Ninety infants and preschool-age children admitted to our hospital with CAP were prospectively included in the study. Etiological agents were identified using conventional bacteriological identification methods and IgM antibodies detection against common atypical respiratory bacteria and viruses. *Results:* An etiology was identified in 59 patients (65.5%). Bacterial pathogens were detected in 43 (47.8%) of the cases while viral pathogens were detected in 23 (25.5%). Coinfection with more than one etiologic agent was evident in seven patients (7.8%). The most common typical bacterial cause of pneumonia was *Staphylococcus aureus* (n = 12, 13.3%), followed by *Streptococcus pneumoniae* and *Klebsiella pneumoniae* (n = 7, 7.8%, each). The commonest atypical bacterium was *Mycoplasma pneumoniae* (n = 10, 11.1%), whereas the commonest viral etiology was influenza viruses (n = 11, 12.2%). *Conclusion:* Although we could not determine the causative agent in some studied cases, this study provides preliminary data regarding the spectrum and frequency of microorganisms causing CAP in Egyptian infants and preschool children.

Keywords: pneumonia, respiratory infection, bacterial, viral, developing countries

Background

Respiratory infections have always been considered a worldwide health problem and a major cause of morbidity and mortality, with infants and young children especially susceptible [1]. Among these infections, pneumonia stays the predominant cause of childhood mortality, causing nearly 1.2 million deaths each year in children younger than 5 years. Most of these deaths occur in developing countries [2]. In Egypt, it was estimated that 10% of children deaths below the age of 5 years is likely caused by pneumonia and other acute respiratory infections [3]. Community-acquired pneumonia (CAP) is one of the most common serious infections in children. Its incidence among children aged less than 5 years in developing countries reached 0.29 #child per year, with a mortality rate of 1.3–2.6% [4]. Pediatric CAP is defined as the presence of signs and symptoms of pneumonia in a previously healthy child due to an infection which has been acquired outside hospital [5]. Determining the etiology of CAP is still difficult in routine clinical settings considering the difficulty in obtaining appropriate lower respiratory tract specimens from children. A considerable seasonal and geographical difference in such etiology has been reported. In most studies, *Streptococcus pneumoniae* has been the most common etiologic agent identified [6]. Available therapeutic guidelines for the empirical treatment of CAP rely on studies from the Western world. There is little information about the prevalence of microorganisms causing CAP in Egypt.

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Aim of the work

This study aimed to elucidate the common bacterial and viral pathogens causing CAP among immunocompetent Egyptian infants and preschool children admitted to the pediatric hospital of Ain Shams University and clarify the associated clinical characteristics in order to contribute to establishment of local guidelines for empirical antimicrobial therapy.

Materials and methods

Study design and patient selection

This prospective descriptive study was conducted on children, aged 1–72 months old, consecutively admitted to the children's unit of Ain Shams university hospitals from February 2012 to March 2013 with CAP. Children were eligible if they presented with clinical signs of pneumonia according to the World Health Organization criteria [7].

Exclusion criteria included children aged less than 1 month and those with an underlying chronic disease, immunosuppressed status, history of recurrent attacks of pneumonia, antibiotic intake within the last month, intake of pneumococcal vaccine, healthcare associated and hospital acquired pneumonia.

Data collection

Data was collected from each patient using a standardized data collection form. Recorded data included demographic characteristics, the date of the beginning of the current respiratory symptoms, antimicrobial intake, vaccination status, comorbidities, and presenting symptoms and signs.

Radiology

Chest x-ray was performed and interpreted according to Cherian et al. [8].

Routine laboratory investigations

On admission, a blood sample was taken for assessment of total white blood cell count with manually verified differential count, hemoglobin, platelet count, and qualitative assessment of serum C-reactive protein (CRP).

Microbiologic workup

Specimens for bacteriologic culture were collected before starting antimicrobial therapy.

Respiratory specimens

Respiratory specimens were collected either by sputum induction or cough swab technique. Induced sputum samples were taken as previously described by Zar et al. [9]. Patients were pretreated with inhaled salbutamol delivered by a nebulizer device and then hypertonic saline 5.0% for 10 min. Sputum samples were then obtained by aspirating the nasopharynx through the nostrils with a disposable mucus extractor or by expectoration if the child was old enough to produce an adequate sputum sample. Cough swab was done by nebulization with normal saline first, and then, gag reflex was stimulated by irritation of uvula to initiate cough in the same time a sterile swab was put in front of the mouth droplets without touching the posterior pharynx [10].

Blood for blood culture and serology

Appropriate amount (according to the age) of venous blood was collected aseptically by venipuncture. Two milliliters of the sample was retained for serum separation, and the rest was evacuated into the blood culture bottle (Vacsera, Cairo, Egypt).

All samples were transferred immediately after collection to the Infectious Diseases Research and Infection Control Unit at Medical Microbiology and Immunology Department of Ain Shams University for further processing.

Processing of specimens

Respiratory specimens were subjected to the following:

1. Inoculation on blood agar, heated blood agar, and MacConkey's agar media;
2. Direct smear staining with Gram stain for microscopic examination.

All sputum cultures were screened for interpretability; only those with >25 leukocytes and <10 epithelial cells per low power field were selected [11]. Inoculated blood and MacConkey's agar plates were incubated at 37 °C aerobically while inoculated heated blood agar plates were incubated at CO_2 10% by the candle jar method. Blood cultures were incubated overnight at 37 °C. A blind subculture was done on blood agar plates after overnight incubation; if no growth was obtained, the bottles were examined daily for 7 days. Any sign of growth was followed by subculture. Isolates obtained from respiratory and blood cultures were completely identified using standard techniques [12].

Serological diagnosis

The clotted blood samples were centrifuged at 1000 *g* for 10 min. Sera were separated and stored at −20 °C until assayed by indirect immunofluorescent technique for the presence of specific IgM antibodies against common re-

spiratory pathogens, namely, *Legionella pneumophila* serogroup 1, *Mycoplasma pneumoniae, Coxiella burnetii, Chlamydophila pneumoniae,* adenovirus, respiratory syncytial virus (RSV), influenza A, influenza B, and parainfluenza viruses serotypes 1, 2, and 3, using Pneumoslide-M test (Vircell, Granada, Spain). Serum samples were diluted 1:1 with phosphate buffered saline (PBS) and then treated with antihuman IgG sorbent. The sorbent-treated diluted serum was incubated for 90 min at 37 °C with the ten slide wells. The slide was washed twice with PBS. A fluorescent secondary IgM antibody was added to the wells and incubated at 37 °C for 30 min, and then washed twice with PBS. Positive and negative controls were included in each test run. The slide was read using Zeiss fluorescence microscope at 400× magnification [13].

Statistical analysis

Continuous variables are expressed as mean and standard deviation (SD). Categorical variables are expressed as frequencies and percent. Chi-square and Fisher's exact tests were used to examine the relationship between categorical variables. Student *t*-test was used to assess the statistical significance of the difference between two study group mean. A significance level of $p < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc., Chicago, IL, USA).

Ethics statement

Informed consents were obtained from informed parents or guardians. The work has been approved by ASU Ethics Committee and was carried out in accordance with the ethical guidelines of the Declaration of Helsinki, 1975.

Results

Ninety infants and preschool children with CAP were included in the study. The mean age of the studied population was 21.30 ± 16.93 months (range 1–72 months), 60 (66.7%) were below 2 years and 30 (33.3%) ranged between 2 years and 5.5 years old. Fifty-seven (63.3%) were male, and 65 (72.2%) came from rural areas. The demographic and clinical characteristics of all cases are summarized in *Table 1*.

Table 1. Demographic, clinical and radiologic characteristics of all patients with identified and unidentified etiologies

Characteristics	Total number (%)	Typical bacterial	Atypical bacterial	Viral	Mixed	Unidentified pathogen
Patients	90 (100%)	26 (28.9%)	9 (10%)	17 (18.9%)	7 (7.8%)	31 (34.4%)
Demographic characteristics						
Age in months (mean ± SD)	21.30 ± 16.93	21.4 ± 16.56	24.88 ± 14.58	20.71 ± 14.15	15.85 ± 15.2	22.5 ± 20.05
Age group						
≤2 years	60 (66.7%)	17	5	11	6	21
>2 years	30 (33.3)	9	4	6	1	10
Sex						
Female	33 (36.7%)	9	6	7	4	7
Male	57 (63.3%)	17	3	10	3	24
Residence						
Urban	25 (27.8%)	8	4	5	4	4
Rural	65 (72.2%)	18	5	12	3	27*
Clinical findings						
Fever	83 (92.2)	24	9	15	7	29
Rhinitis	86 (95.6)	24	9	17	7	31
Cough	67 (74.4)	21	6	10	6	24
Grunting	42 (46.7%)	12	10	5	9	6
Cyanosis	22 (24.4%)	6	6	2	6	2
Diminished air entry	84 (93.3%)	30	24	9	15	7
Fine crepitation	89 (98.9%)	26	9	17	7	30
Rhonchi	55 (61.1%)	20	15	5	10	5
Wheezes	44 (48.9)	12	5	7	4	16
Rapid breathing	90 (100)	26	10	18	5	31
R.R. (mean ± SD)	55.78 ± 7.08	55.62 ± 6.00	54.40 ± 6.50	52.78 ± 5.49*	59.0 ± 4.18	57.58 ± 8.69
Severe respiratory distress	24 (26.7)	4	6	2	6*	6*

Table 1. *(cont'd)*

Characteristics	Total number (%)	Typical bacterial	Atypical bacterial	Viral	Mixed	Unidentified pathogen
Laboratory findings						
Leukocytosis	41 (45.6%)	10	5	10	5	11
Neutrophilia	26 (28.9%)	6	4	9*	5*	2
Neutropenia	0 (0.0%)	0	0	0	0	0
Lymphocytosis	7 (7.8%)	2	0	2	1	2
Anemia	29 (32.2%)	11	3	4	3	8
Thrombocytopenia	5 (5.6%)	1	1	2	1	0
Positive CRP	79 (88.9%)	24	9	15	7	25
Radiological findings						
Patchy consolidation	73 (81.1%)	21	9	13	5	25
Lobar consolidation	14 (15.6%)	4	0	4	2	4
Interstitial consolidation	3 (3.3%)	1	0	0	0	2

*p value < 0.05
R.R.: respiratory rate; CRP: C-reactive protein.
Data are presented as mean ± standard deviation or median for continuous variables and as number (percentage) for categorical variables

An etiologic agent was identified in 59 patients (65.5%). Bacterial pathogens were detected in 43 (47.8%) of the cases while viral pathogens were detected in 23 (25.5%). Coinfection with more than one etiologic agent was found in seven patients (7.8%). The most common typical bacterial cause of pneumonia was *Staphylococcus aureus* (n = 12; 13.3%) followed by *S. pneumoniae* (n = 7; 7.8%) and *Klebsiella pneumoniae* (n = 7; 7.8%). The commonest atypical bacterium was *M. pneumoniae* (n = 10; 11.1%), whereas the commonest viral etiologies were influenza A and B viruses (n = 11; 12.2%) *(Table 2)*.

The most common presenting symptoms were rhinitis (n = 86; 95.6%) and fever (92.2%). With respect to examination, fast breathing was found in 100% of patients and 22 (24.4%) with cyanosis. The commonest auscul-

Table 2. Etiological agents identified in the study population (n = 90)

Patients	Number	%
Unidentified	31	34.4
Identified	59	65.5
Bacterial	43	47.8
Staphylococcus aureus	12	13.3
Streptococcus pneumoniae	7	7.8
Klebsiella pneumoniae	7	7.8
Haemophilus influenzae	2	2.2
Escherichia coli	2	2.2
Pseudomonas aeruginosa	1	1.1
Atypical bacteria	12	13.3
Mycoplasma pneumoniae	10	11.1
Chlamydophila pneumoniae	1	1.1
Legionella pneumophila	1	1.1
Viral	23	25.5
Influenza A virus	7	7.8
Influenza B virus	4	4.4
Para influenza virus 1–3	5	5.5
RSV	4	4.4
Adeno virus	3	3.3

Table 2. *(cont'd)*

Patients	Number	%
Mixed	7	7.8
Bacterial – bacterial	2	2.2
Escherichia coli and *Mycoplasma pneumoniae*	1	1.1
Pseudomonas aeruginosa and *Mycoplasma pneumoniae*	1	1.1
Bacterial – viral	4	4.4
Escherichia coli and RSV	1	1.1
Staphylococcus aureus and influenza A virus	1	1.1
Klebsiella pneumoniae and adeno virus	1	1.1
Mycoplasma pneumoniae and adeno A virus	1	1.1
Viral – viral	1	1.1
Influenza A and parainfluenza virus	1	1.1

RSV: Respiratory Syncytial Virus

tatory findings were fine crepitation (n = 89; 99%) and diminished breath intensity (n = 84; 93.3%).

Comparing the demographic characteristics of patients in different etiological categories (typical bacterial, atypical bacterial, viral, mixed, and unidentified) revealed no significant differences among etiological categories except for rural residence which was significantly higher in patients with unidentified etiology ($p < 0.05$). As for the clinical findings, grunting was also significantly higher in patients with unidentified etiology and a significantly high respiratory rate was observed among patients with nonviral etiology. Severe respiratory distress was significantly higher in patients who had at least one pathogen detected than those with no identified etiology as well as in those with mixed etiologies (more than one pathogen detected) compared to the rest of the patients ($p < 0.05$).

The recorded routine laboratory results showed that CRP was positive in 80 (88.9%) patients. The commonest blood picture abnormality was leukocytosis (45.6%) followed by anemia (32.2%) and neutrophilia (28.9%) which was significantly evident in children with nonviral CAP ($p < 0.05$) *(Table 2)*.

According to chest radiography, patchy consolidation was the predominant finding (81.1%), followed by lobar (15.6%) and interstitial (3.3%) patterns of consolidation. There was no correlation between radiological findings and etiologies *(Table 2)*.

As for the diagnostic techniques employed in the study, culture of respiratory specimens was positive in 31.1% of cases, whereas Pneumoslide immunofluorescent IgM test yielded 36.7% positive result. Blood culture was done for 89 patients and was found positive in only 3.33% *(Table 3)*.

Table 3. Results of diagnostic techniques employed in the study

Diagnostic technique	Number	%
Culture of respiratory specimens		
Negative	62	68.9
Positive	28	31.1
Staphylococcus aureus	10	11.1
Streptococcus pneumoniae	7	7.8
Klebsiella pneumoniae	6	6.7
Haemophilus influenzae	2	2.2
Escherichia coli	2	2.2
Pseudomonas aeruginosa	1	1.1
Blood culture		
Negative	85	94.4
Positive	3	3.3
Staphylococcus aureus	2	2.2
Klebsiella pneumoniae	1	1.1
Contamination	1	1.1
Not done	1	1.1

Table 3. *(cont'd)*

Diagnostic technique	Number	%
Pneumoslide-M test		
Negative	57	63.3
Positive	33	36.7
Mycoplasma pneumoniae	9	10.0
Chlamydophila pneumoniae	1	1.1
Legionella pneumophila	1	1.1
Mycoplasma pneumoniae and influenza A virus	1	1.1
Influenza A virus	5	5.6
Influenza B virus	4	4.4
Para influenza virus 1–3	4	4.4
Respiratory syncytial virus	4	4.4
Adeno virus	3	3.3
Influenza A virus and parainfluenza virus	1	1.1

Discussion

The present study included 90 immunocompetent infants and preschool children hospitalized for CAP in a trial to identify the causative microbial etiology. The use of conventional methods of bacterial isolation parallel to the serological detection of specific IgM antibodies against common respiratory pathogens led to identifying at least one organism in 65.5% of the patients. In similar worldwide studies, the rate of pathogen detection varied widely ranging from as low as 38.4% [14] and 48% [15] to >80% [16–18]. The high rates of detection in the later studies could not be achieved in our study, considering their employment of invasive methods of specimen collection in some cases and the wide armamentarium of microbiologic diagnostic methods including viral isolation, antigen detection, and molecular techniques. Besides that, the negative history of antimicrobial intake before presentation could not be guaranteed, considering the fact that antimicrobials are readily purchased in Egypt without prescription.

Bacterial and viral pathogens were detected in 47.8% and 25.5% of the cases, respectively. These figures came very close to those reported previously [16, 19, 20]. Most of the identified bacteria were isolated by culture of respiratory specimens. Blood cultures yield was very limited (3.3%). Contamination was found in 1.1%. These results were consistent with those of other investigators who found rates of positive blood cultures range from 1 to 8% [15, 21]. Thus, the real benefit of routine blood culture in pediatric CAP remains in question because of the contrasting published conclusions. Most investigators suggest that blood cultures are likely to be unhelpful for management and can be omitted especially in mild and moderate CAP for cost–benefit reasons, while others still find that positive blood cultures can guide antibiotic therapy to narrow-spectrum [21–23]. The findings in this study were also conflicting, as, though the yield of blood culture was relatively low, the three isolated pathogens were not identified by respiratory specimen cultures. Therefore, the role of blood culture in redirecting the antimicrobial therapy still needs to be elucidated.

Pneumoslide-M was used in the study as it has been previously evaluated as a rapid feasible multiple panel test for detection of several viruses and atypical bacteria with sensitivity comparable to polymerase chain reaction (PCR) [13, 24, 25]. In the light of fact that each community has its specific pathogens as a leading cause of CAP, the use of pneumoslide-M was a tool for expanding the panel of pathogen identification and we did not aim to evaluate the sensitivity of the test; however, results of this study revealed a lower rate of positive results using pneumoslide-M (33/90; 36.7%) when compared with other studies (31/60; 51.66%) [13] and (624/1204; 51.83%) [25].

In this study, *S. aureus* was found the most common typical respiratory pathogen causing CAP (13.3%) in contrast to other published reports in which it used to come after *S. pneumoniae* and *Haemophilus influenzae* in frequency [14, 17, 26]. However, our finding is supported by that of Atwa [27], who detected *S. aureus* as the most common isolated organism from sputum of children with CAP in Egypt. These findings highlight the potentially rising role of this pathogen in our community where methicillin resistance has been extensively reported lately [28–30]. The role of *S. aureus* in CAP has also been the focus of other studies [31, 32]. It was reported after influenza epidemics [33] and was found the most common bacterial coinfection among adults during influenza pandemic in 2009 with the majority being methicillin-resistant *S. aureus* and associated with unfavorable prognosis [34]. In the current study, only one patient had evidence of coinfection with *S. aureus* and influenza virus.

Pneumonia caused by atypical bacteria solely counted for 10% of the cases. It has well been established that

M. pneumoniae has always played a more crucial role in CAP among older age groups [35]. Evidence of *M. pneumoniae* infection in this study was detected in ten patients (11.1%); in three of them, it was found mixed with either bacterial or viral infection. Similar rates were recorded by Layani-Milon et al. and Chiang et al. [14, 36]. This finding drives the attention to the role of CAP caused by *M. pneumoniae* in children less than 5 years old even if the rates had not reached those recorded among older age groups [37].

The most common viral infection causing CAP was influenza viruses (12.2%). Earlier studies had named influenza virus as the best example of primary viral pneumonia [38]**.** Comparable rates were documented in the same age group by more recent studies [39, 40]. RSV was less reported in our study (4.4%) compared to other studies as we excluded children with bronchiolitis. Evidence of viral and bacterial coinfection was detected in four cases. However, the lack of viral isolation or antigen detection assays in the study halted the determination of the exact pathogenesis whether these were concomitant or secondary bacterial infections.

Interestingly, *Pseudomonas aeruginosa,* an uncommon cause of CAP [41], was isolated from a patient who had serological evidence of *M. pneumoniae* infection. It should be mentioned that the patient presented in bad general condition that necessitated admission to the intensive care unit and mechanical ventilation.

Supporting previous reports, we found that clinical manifestations, and laboratory and radiographic findings are poor indicators of the underlying etiology in childhood pneumonia [35]. Except for the severe respiratory distress that was significantly higher in patients with identified causative agent and also in patients with mixed infections when compared to other groups, all other clinical manifestations did not show association of significant importance in this study. Even the historical clinical differentiation between typical bacterial pneumonia and atypical pneumonia had been shown as an inaccurate reflection for the etiology [42]. Coinciding with Elemraid et al. [43], we found neutrophilia more significant in patients with non-viral etiology.

Limitation

There are some limitations in this study that should be noted, including the relatively small-sized sample that was restricted to hospital admitted patients. Wider scale studies that include ambulatory patients are needed to provide more information about the etiological agents implicated in the disease. Microbiological techniques used in the study were limited to conventional bacteriological identification methods and serological assay against most common respiratory pathogens. Yet, more diagnostic modalities as PCR and viral antigen detection could aid in identifying more etiologic agents.

Conclusion

S. aureus, M. pneumoniae, and influenza viruses were the most detected causative agents identified in the study. Local therapeutic and prevention guidelines for CAP should have special focus on these agents. Clinical manifestations and radiologic findings could not be relied upon to differentiate between bacterial and viral pneumonia, which necessitate the adoption of rapid feasible microbiologic diagnostic technique to aid in the diagnosis and management of hospitalized patients.

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Conflict of interest

The authors declare no conflict of interest.

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