Asian Pac J Trop Med
2009;2(2):1-8

Original article

Atypical pathogens in community acquired pneumonia of Egyptian children

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Abstract

Objective: Diagnosis of atypical pathogens as an aetiology for community-acquired pneumonia (CAP) in children is a challenge world wide. The aim of this study was to detect the frequency of atypical pathogens as a cause of community-acquired pneumonia (CAP) in Egyptian children. **Methods**: From 50 children (with age ranged from 2 months to 12 years) hospitalized for community-acquired pneumonia; respiratory sputum samples were collected by induction or spontaneously. All samples were subjected to conventional cultures and Polymerase Chain Reaction(PCR) technique DNA extraction for identification of *Mycoplasma*, *Chlamydia pneumoniae* and *Legionella pneumophila*. **Results**: A definite pathogen was identified in 78% of the studied children; 30% typical bacteria, 8% candida albicans and atypical bacteria in 40% of the pneumonic children. Chlamydia pneumoniae was isolated from 26% of the children while Mycoplasma pneumoniae was isolated from 14%, whereas Legionella pneumophilla was not isolated at all. **Conclusion**: Atypical pathogens are evident as a potential aetiology for community-acquired pneumonia in (13.3%) of young and (80%) of older Egyptian children.

Keywords: Atypical pathogens; Community-acquired pneumonia; Children; Egypt

INTRODUCTION

Community-acquired pneumonia (CAP) is a common and potentially serious infection that afflicts children throughout the world; it is fundamentally different in children and in adults[1]. In the developing world, pneumonia is not only more common than it is in Europe and North America, it is also more severe and is the largest killer of children[2].

Age was the best predictor of the microbial aetiology of pneumonia, dominating atypical pathogens in older children[3]. However recently, atypical bacteria play a greater role in causing lower respiratory tract infections than had been thought previously[4-6]. This carries a special importance in prescribing the specific CAP management for atypical pathogens at the proper time[7,8].

The primary aim of this study is to detect the frequency of *Mycoplasma pneumoniae*, *Chlamydia pneumophilia* and *Legionella pneumophilla* as atypical pathogens in the aetiology of community-acquired pneumonia (CAP) in the Egyptian pediatric age group.

MATERIALS AND METHODS

It is a cross sectional observational study, performed from December 2007 to June 2008.

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Subjects

The present study was conducted on 50 children admitted to the Pediatric Hospital, Ain Shams University. They were diagnosed clinically and radialogically as community-acquired pneumonia according to British Thoracic Society criteria 2002 (fever > 38 ℃, tachypnea, cough and / or findings of crackles, bronchial breathing or diminished breath sounds on auscultation) together with radiological infiltrations consistent with pneumonia[3].

Selection criteria

The ages of the included children ranged from 2 months to 12 years with a mean (3.7 years ± 3.5). They were 28 males and 22 females newly admitted to the hospital. They were classified into 2 groups according to age: group I including children with CAP aged less than 5 years old: 30 children, group II including children with CAP aged more than 5 years old: 20 children.

Exclusion criteria

Children with asthma; chronic chest problems; children with hospital-acquired pneumonia or children with TB pneumonia were excluded.

Methods

All the studied children were subjected to the following: detailed history taking, thorough clinical examination, plain chest radiogrophy, complete blood counts and respiratory samples investigation; The respiratory samples were subjected to conventional culture, atypical pathogens. Besides, sampling investigations include:

(A) Blood sampling: Through vein-puncture to obtain blood sample on EDTA for complete blood counts using coulter counter T 660.

(B) Respiratory secretions samples: Spontaneous sputum was collected from older and cooperative children. They were asked to expectorate immediately before breakfast. Induced sputum (with concentrated saline inhalation) was done for young children -who can't expectorate[9]. The expectorated sputum was collected into sterile disposable plates, and then incubated at 37°C for 1 hour.

Microbiological processing of sputum samples

They were transferred immediately after collection to the Infection Control Unit at Microbiology and Immunology Department, Ain Shams University for further preparing.

The sample was divided into two portions for: 1-Routine culture. 2-Other portion of the specimen was then digested, decontaminated, concentrated and stored at −70℃ till they were further analyzed by PCR for the presence of atypical pathogens.

I Culture of liquefied sample:

The sputum samples were vortexed for 1 minute, thereafter samples were cultured according to standard procedures[9,10] on the following media: blood agar, Mc Conkey's agar and choclate agar supplemented with Vitox supplement (Oxoid).

The isolated microorganisms were identified by colony morphology, Gram smear as well as biochemical and enzymatic reactions.

II Preparation and storage of specimen for the PCR:

Sputum digestion, decontamination and concentration processing of sputum samples were done according to Murayama et al[9].

The DNA was then purified with a QIA amp DNA blood Kit (article 51104; Quiagen, Basel, Switzerland) according to the instructions of the supplier, except that the election steps was done with 100 μL (instead of 200uL) AE election buffer. Extracts were stored at −70℃ until they were required for analysis.

Amplification by PCR and analysis of the amplified products were performed as described Gullsby et al[4].

Detection of *Mycoplasma pneumoniae* by PCR

The PCR primers used were MPP1 (sense): 5′TGCCATCAACCCGCGCTTAAC and MPP2 (antisense): 5′CCTTTGCAACTGCTATAGTA. Using Biometra thermocycler, a denaturation step of 95℃ for 3min then 36 cycles of primer annealing at 95℃ for 1min, 55℃ for 1min and 72℃ for 1min then an extension step at 72℃ for 5 min. The amplified products were detected by gel electrophoresis, these two primers amplify a fragment of 466bp.

Detection of *Chlamydia Pneumophilla* by PCR

A C. pneumoniae species specific primer set used; forward primer HL-1 (5′-GTTGTTCATGAAGGCCTACT-3′); reverse primer HR-1 (5′-TGCATAACCTACGGTGTGTT-3′).

Samples were amplified for 40 cycles. Each cy-

cle consisted of the following denaturation at 94℃ for 1min, annealing at 55℃ for 1mn and primer extension at 72℃ for 1 min. Amplification products were analyzed by electrophoresis through a 1.5% agarose gel stained by ethidium bromide.

Detection of *Legionella pneumophila* by PCR

A *Legionella pneumophila* species specific primer set used; this primer set amplifies a 800 bp fragment and consists of the following primers; forward primer LEG-1 (5′- GTCATGAGGAATCTCGCTG-3′); reverse primer LEG-2 (5′-CTGGCTTCTTCCAGCTTCA-3′). Samples were amplified for 35 cycles. Each cycle consisted of the following denaturation at 93℃ for 1mn, annealing at 55℃ for 1min, 74℃ for 1.5 min and then an extension step at 74℃ for 7 min. Amplification products were analysed by electrophoresis through a 1% agarose gel stained by ethidium bromide[5].

Statistical analysis

Standard computer program SPSS for Windows, release 11.0 (SPSS Inc, USA, 2005) was used for data analysis. All numeric variables were expressed as mean standard deviation (SD). Comparison of different variables in various groups was done using student *t* test and Mann Whitney test for normal and nonparametric variables respectively. Chi-square (2) test was used to compare frequency of qualitative variables among the different groups. For all tests a probability (*P*) less than 0.05 was considered significant. Graphic presentation of the results was also done.

RESULTS

A definite pathogen was identified in 78% (39/50) of the studied children, out of these typical bacteria was defined in 30% (15/50) and in 8% (4/50) Candida Albicans using non conventional culture, atypical pathogens were identified in 40% (20/50) of the children using PCR technique (Figure 1).

Typical bacteria were isolated from 30% of the patients as follows; they were (13%) Gram -ve organism, (6.7%) *staphylococcus viridans*, (3.3%) *streptococcus pneumoniae*, (3.3%) *heamolytic streptococci*, (3.3%) *pseudomonas aeruginosa*.

In Children with atypical pneumonia, *Chlamydia pneumoniae* was isolated from 26% of the children while *Mycoplasma pneumoniae* was isolated from and 14%, whereas *Legionella pneumophilla* was not isolated in any case of pediatric pneumonia.

Still the mean age of the patients with atypical pneumonia was significantly higher than that with typical pneumonia. Atypical pathogens were evident in only 13.3% of children below 5years, compared to 80% of older children (Table 1). Clinical and radiological details are found in a demographic table (Table 2)

Regarding the clinical manifestations (Table 3), cough and expectoration were significantly higher in the atypical bacterial pneumonia group while dyspnea was significantly higher in the typical bacterial pneumonia group. Concerning the radiological findings, there was non significant difference in the pneumonic infiltration between children with typical pneumonia versus children with atypical pneumonia however the pneumonic shadowing was usually unilateral in both groups. Laboratory findings cleared a significant higher total and differential leucocytic counts in PCR negative children compared to PCR positive children for atypical pathogens (Figure 2).

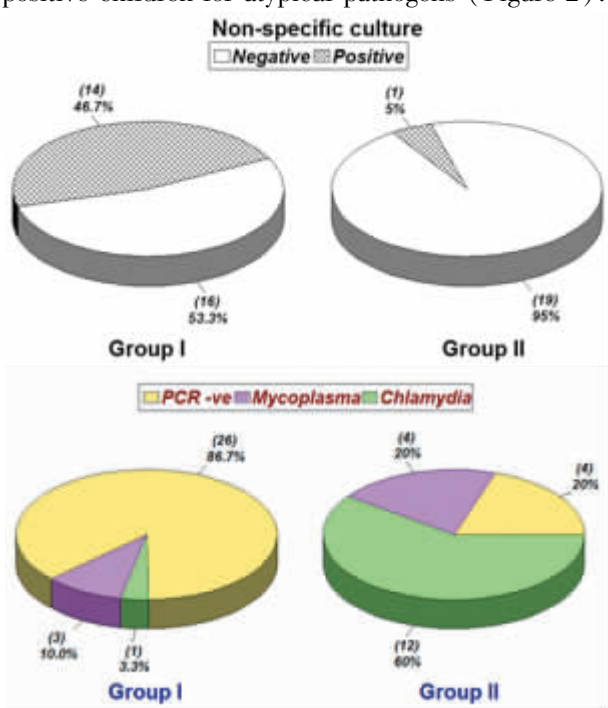

Figure 1 Isolated pathogens in Egyptian children with CAP using both bacterial cultures and PCR isolation pathogens.
a-Bacterial non specific Cultures.
b-PCR isolation of atypical pathogens.

Table 1 PCR results for atypical pathogens diagnosis in Egyptian children with CAP.

PCR	Group I $N=30$		Group II $N=20$		χ^2	*P*-value
	No	%	No	%		
Positive	4	13.3	16	80 *	24.5	0.000 *

* Highly significant result.

Table 2 Clinical characteristics of group I and group II.

Characteristics	Group I $n=30$ No (%)	Group II $n=20$ No (%)	χ^2/z#	*P*-value
I- History				
-Previous admission	6(20%)	7(21%)	0.0	0.9
-Previous pneumonia	24(80%)	7(35%)	0.3	1
II- Symptoms				
- Cough	26(86%)	20(100%)	2.9	0.8
- Expectoration	17(56.7%)	14(70%)	0.9	0.3
- Dyspnea	24(80%)	7(35%)	13	0.00 *
- Wheeze	27(81%)	11(55%)	8.6	0.03 *
III- Signs				
- Tachypnea	23(76%)	10(50%)	1.9	0.1
- Fever	15(50%)	14(70%)	6.6	0.3
-Rales(sibilent rhonchi)	7(24.1%)	10(50%)	3.7	0.06
- Bronchial breathing	20(69%)	8(40%)	6.2	0.04 *
- Fine crepitation	18(62.1%)	12(60%)	0.0	0.8
- Decreased air entry	5(17.2%)	8(40%)	3.1	0.07
- Respiratory distress	24(80%)	10(50%)	8.1	0.01 *
IV- Radiologically				
Pulmonary infiltrate	30(100%)	20(100%)	0.8	
V- Laboratory				
- WBC count $\times 10^9$/L	11.3 ± 6.9	9.8 ± 1.6	6#	0.5
- Lymphocyte %	43.6% ± 19.3	31 ± 8.3	2#	0.01 *
- Neutrophil %	51.4% ± 15.5	61.3% ± 13.3	2#	0.009 * *

* Singificant. * * Highly significant.

Table 3 Clinical Manifestations in positive and negative groups for atypical pathogens

Clinical characteristics	PCR positive $N=20$		PCR negative $N=30$		χ^2	P-value
	No	%	No	%		
Cough	20	100	26	86.7	2.89	0.08
Expectoration	15	75	16	53.3	2.39	0.12
Dyspnea	6	30	25	83.3	14.6	0.001 **
Wheeze	11	55	27	89.9	8.6	0.03 *

* Singificant. * * Highly significant.

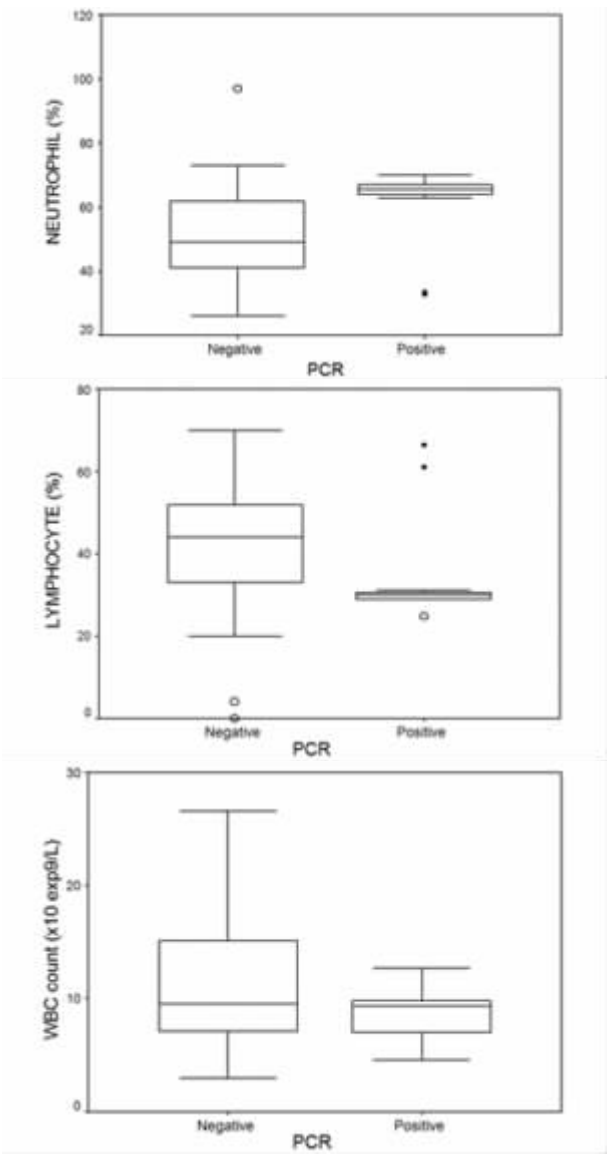

Figure 2 Laboratory mean values in children with PCR positive children for atypical pathogens compared to PCR negative group.
a-Neutophils.
b-Lymphocytes.
c- Total white blood cell count.

In the current study the mean age of Mycoplasmal cases was 4.7 years whereas in Chlamydial cases it was 7.6 years. Also, there was a significant statistical difference in sex distribution between *Chlamydia pneumoniae* (C. pn) group and *Mycoplasma pneumoniae* (M. pn) group where C. pn patients showed male preponderance.

As regard clinical manifestations of C. pn patients and M. pn patients, there were non significant difference noted except for respiratory distress and tachypnea which were significantly higher in C. pn group.

No specific radiological feature or laboratory parameter [as regard total and differential White blood cell (WBC) counts] allowed us to distinguish patients with *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* infection.

DISCUSSION

Community-acquired pneumonia is one of the most common serious infections in children[11]. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* often are the etiologic agents in children older than five years[12]. However; recent studies reported that both organisms *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* play a significant role in the pathogenesis of community-acquired pneumonia in children of all ages[4,7,11,13]. This highlights an important change of treatment policy of CAP in children to guarantee proper management in the form of specific macrolides at the proper time[14]. Always, one of the challenges in planning the treatment of respiratory tract infections in children is identifying the causative agent[2].

Despite major improvements in the diagnosis of

pathogenic organisms causing acute respiratory infections (ARI), details of infections caused by atypical pathogens are not well understood, particularly in developing countries[12].

The aim of this study was to determine the frequency of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella pneumophilla* species as an etiology of community-acquired pneumonia in Egyptian children. The predominance of the age incidence of pneumonia in children younger than 5 years (30/50) in our work was related to the fact that CAP is epidemiologically commoner in younger children compared to older children (20/50).

Comparison of the clinical manifestations in the studied groups revealed that; in children less than 5 years old, cough was the most frequent presenting symptom (86%), wheeze were the next frequent symptom (81%) followed by respiratory distress (80%), while expectoration was the least common. In children more than 5 years old (group II), cough was the most frequent presenting symptom (100%), followed by wheeze (55%), then expectoration (50%). These differences may be just age differences.

Regarding the clinical signs in group I (children less than 5 years old) tachypnea was the most frequent clinical sign (76%), followed by bronchial breathing (69%), then fine crepitations (62.1%). In group II, fever was the most frequent clinical sign (70%), followed by fine crepitation (60%). Unfixed signs may be explained by the presence of different stages of pneumonia resulting in different signs and symptoms for each stage, as in early onset, local chest findings may be minimal.

Our data showed no significant difference of TLC between the 2 groups, neutrophil % was significantly higher in group II (61.3% ±13.3) (t = -2, P = 0.009) while lymphocyte % was significantly higher in group I (51.4% ± 15.5) (t = -2, P = 0.01). These results represent Lymphocytic predominance which is normally found in early years of life

A definite pathogen was identified in 78% of the studied Egyptian children, out of those, typical bacteria was defined in 30% using conventional culture, atypical bacteria was identified in 40% using PCR technique and 8 % revealed Candida albicans.

In contrast to our findings Huang et al[15] studied the epidemiology and clinical characteristics of community-acquired pneumonia in Chinese hospitalized children, where identifiable pathogen was present in only 38%, 10% of them were typical bacteria.

The difference in the results of bacterial yields could be explained by the fact that different laboratory and bacteriological techniques were used in different studies. Moreover, in our study we didn't investigated the viral causes of CAP.

Results of the current work revealed that atypical organisms were detected in 40% of our patients by using the most specific investigation PCR test, of them 26% were *Chlamydia pneumoniae* and 14 % were *Mycoplasma pneumoniae* (Chlamydia species incidence was twice the incidence of Mycoplama species).

These results were in accordance with Principi et al.[12] found that *Chlamydia* and *Mycoplasma pneumoniae* accounts for 32% of CAP.

However in a study conducted by Nagalingam et al.[16] who examined the frequency of infection with *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila* in Trinidad children presented with signs and symptoms of CAP reported atypical respiratory pathogens in 23.5% of cases only. This may highlight community differences of incidence.

Children with typical bacterial pneumonia usually run a severe disease course than those with atypical bacterial cause, and this is in agreement with Shoham et al.[17].

Comparing the clinical manifestations between PCR +ve group for atypical pneumonia and culture +ve group for typical pneumonia, cough and expectoration were significantly higher in the atypical pneumonia group (P = 0.04); while dyspnea was significantly higher in the typical pneumonia group (P = 0.04). These results were in concordance with Principi et al.[12] who concluded that absence of dyspnea in children more than 5 years was a suggestive finding for atypical pneumonia.

In the current study *Legionella pneumophilla* was not isolated in any case of pediatric pneumonia

and this was in concordance with Al-Ali et al.[18] who noted that that this organism is seen most frequently in adults rather than in children.

Whereas, Shoham et al.[17] found that 46% of the diagnosed legionellosis were community-acquired infections, but of them 78% had an underlying condition such as malignancy, immunosuppressed children and in children younger than the age of 1 year. This may explain why we didn't find any case of legionella infection as patients with underlying disease were excluded from the study.

In the present study, 26% (13/50) of the studied children have evidence of Chlamydial pneumoniae infection confirmed by PCR; (among them, 1 was aged 10 months and 12 aged more than 5 years old).

In agreement to our results, Stille et al.[14] detected Chamydia pneumoniae antibodies in 33% of their studied children cohort.

Other studies done by Al-Ali et al.[18] and Marchetti et al.[5] were in contrary with the current study as they found that *Chlamydia pneumoniae* was detected in only (3% and 6% respectively) of their studied children.

Lower percentage of *Chlamydia pneumoniae* found in other studies could be explained by the fact that other studies depend on the presence of antibodies against the organisms, and that most of the youngest children do not develop specific antibodies. This is attributed to the fact that serological test is not an accurate way to diagnose chlamydia infection and the only accurate way is by identification of the organism by culture or polymerase chain reaction.

Seven (14%) of the studied children have *Mycoplasmal pneumoniae* infection identified by PCR; 3 (42%) were less than 5 years and 4 (57%) were above 5 years old. PCR (the method used in our study) can identify *Mycoplasma pneumoniae* rapidly and fulfills the need for rapid identification with high sensitivity and high specificity[13].

In contrast to our results, other studies[12,14] found that *M. pneumoniae* infection is evident in (28%) of their studied children using serological tests. The difference in the percentage of Mycoplasma incidence in different studies was explained by the fact that the great majority of the Mycoplasma patients had milder form of infection and need no hospital admission and so they are treated as outpatients[17] and the current study was focused on hospitalized patients.

Asymptomatic Mycoplasma infection in a study done by Stille et al.[14] was 6%. Moreover; seasonal variations in prevalence and epidemic-like occurrence are also reported in Mycoplasma infections. In concordance to these results, several studies have found Mycoplasma infection rate of 10-14% in hospitalised pediatric patients and 20- 40% in ambulatory pediatric patients with pneumonia[1,13,19].

This fact may reflect a possible true higher frequency of atypical pathogens accused for childhood CAP than those detected in severe hospitalized children.

On comparing the clinical manifestations between Chlamidia patients and Mycoplasma patients, no significant difference was noted except for respiratory distress and tachypnea which were significantly higher in C. pn group ($P = 0.01$). Also, in this study no extra pulmonary manifestations could be detected in any of children with typical pneumonia.

In conclusion, no specific radiological feature or laboratory parameter allowed us to distinguish patients with *Mycoplasma pneumoniae* from those with *Chlamydia pneumoniae* infection. However, respiratory distress and tachypnea were significantly higher in C. pn. group. The microbiological results in this study showed obvious variation in causative pathogens in children with CAP. PCR technique proved to be helpful in this situation as it has become increasingly important for the diagnosis of atypical organisms causing atypical pneumonia. In addition it gives a rapid diagnosis than conventional methods used. Typical bacteria were isolated from 30% of the studied children and 30% were of unknown origin. Atypical bacteria were detected using PCR in 40% of the studied children, and *Chlamydia pneumoniae* was more frequently isolated than *Mycoplasma pneumoniae* in severe hospitalized CAP children.

ACKNOWLEDGEMENT

The authors thank all the participating children and their guardians for their understanding and coopera-

tion.

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