Original Article

Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study

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Abstract

Background**:** Atypical respiratory pathogens such as *Mycoplasma pneumoniae*, *Legionella* species, and *Chlamydia pneumonia*e are isolated with increasing frequency from community-acquired pneumonia (CAP). This study highlights the importance of organisms responsible for CAP.

Methodology: One hundred consecutive patients with clinically and radiographically diagnosed CAP were evaluated from October 2005 to October 2006. Sputum, bronchoalveolar lavage, and blood samples were collected for microbiological culture. Determination was performed for specific immunoglobulin M (IgM) for *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Coxiella burnettii*, *adenovirus*, and *influenza virus.*

Results: The most common isolated bacteria was *Streptococcus pneumoniae* (22%) followed by *Haemophilus influenzae* (18%*). Mycoplasma pneumoniae* was isolated from 5% and *Legionella pneumophila* was isolated from 5% of patients. The most common positive serological reaction was for *Chlamydia pneumoniae* (30%) and *Adenovirus (*30%). In the study of accuracy of determination of specific IgM for *Mycoplasma pneumoniae* and *Legionella pneumophila* compared to culture, the sensitivity was 60% and 80% respectively, specificity was 93.7 %, and 98.9 % respectively, and accuracy was 92 % and 97 % respectively.

Conclusion: This study highlights the prominence of mixed bacterial/viral infections in lower respiratory tract infection diagnosis. Our data showed that at least 30% of our patients had concurrent infections. This observation raises two important questions: 1) whether sequential or concurrent viral and bacterial infections have a synergistic impact on the evolution of disease in children; and 2) should diagnostic batteries for any patient with CAP include methods for detecting both the typical and atypical bacterial or viral pathogens.

Key words: CAP, *Legionella*, *Mycoplasma pneumoniae*

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Introduction

Community-acquired pneumonia (CAP) is defined as pneumonia acquired outside the hospital setting [1]. It is an important infectious disease not only in developing countries but also in developed countries [2]. Distinguishing pneumonia from upper respiratory infections, particularly bronchitis, is difficult based on symptoms alone. Sustained high fever, chills and pleuritic chest pain suggest pneumonia, but these are not always present. Physical findings such as dullness, râles, and signs of consolidation strongly favor pneumonia, but their absence does not exclude diagnosis. Even when signs and symptoms are indicative of pneumonia, chest radiography should be performed to confirm diagnosis [3]. Chest radiograph is considered the reference standard for diagnosing the presence of pneumonia. A commonly held view is that alveolar densities and patchy or interstitial densities seen in chest X rays are indicators of pneumonia; however, these indications lack specificity in diagnosing the microbial cause in CAP [4].

Although *Streptococcus pneumonia*e remains the bacterium most commonly implicated in CAP, the atypical respiratory pathogens *Mycoplasma pneumoniae*, *Legionella species* and *Chlamydia pneumoniae* are being isolated with increasing frequency [5]. Viruses such as influenza, adenovirus, and respiratory syncytial virus may also be included as a cause of atypical pneumonia [6].

Studies on pneumonia usually focus on the most successful of the commonly suspected pathogens. The most common diagnostic specimens are blood and sputum for detecting bacterial pathogens such as *Pneumococci*, *Haemophilus influenzae* and *Staphylococcus aureus*, and serum for detecting

specific antibodies to *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* [7]. Depending on clinical presentation, other tests, such as direct antigen detection in urine for *Legionella* and sometimes antibodies detection for rare pathogens including *Coxiella burnetii* and viruses, have to be considered [8].

Molecular techniques continue to gain importance for the diagnosis of *Chlamydia pneumonia*e, *Mycoplasma pneumonia*e, *Legionella*, and viral respiratory infections. However, their availability at present is mainly restricted to certain laboratories [9].

We undertook this study to determine whether commonly available culture, phenotypic identification kits, and serodiagnostic tests can be used to 1) predict *Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae, Coxiella burnettii* , adenovirus and influenza virus in CAP; and 2) study the value of serological diagnosis of *Legionella pneumophila* and *Mycoplasma pneumonia*e in comparison to or in conjunction with culture.

Materials and Methods

The study was conducted on one hundred consecutive patients presenting with community acquired pneumonia. Patients were evaluated prospectively from October 2005 to October 2006. The study population consisted of a) all adult (> 15 years old) patients presenting to the Emergency Department at Mansoura hospital who were deemed by the Emergency Physician to have pneumonia, and b) all adult patients admitted to the hospital whose diagnostic code included pneumonia and where non-aspiration pneumonia was the working diagnosis at first consultant contact following admission. Patients were excluded if, on review of the case notes, insufficient data was present to calculate CURB-65 score (CURB-65 score measured severity of pneumonia according to presence of confusion, measurements of urea nitrogen, respiratory rate, blood pressure, 65 years of age and older [10]), or if it was apparent from the case notes that the pneumonia was hospital-acquired, or that the reason for admission was another diagnosis. The clinical diagnosis was based on clinical assessment, initial pathology results, and both posteroanterior and lateral views of chest radiographs. Patients' assessments were performed according to CURB 65 scores [10].

The CURB index was derived from the original British thoracic society (BTS) study and uses four core clinical features: confusion at new onset (or worsening of existing state for those with a background of cognitive impairment); serum urea > 20 mg/dl; respiratory rate ≥ 30/min; and blood pressure (systolic blood pressure < 90 mm Hg or diastolic blood pressure ≤ 60 mm Hg). The presence of two or more of these four criteria led to a "severe" classification. This tool has been validated independently in several recent studies [10]. The CURB-65 index is a further modification of the BTS prediction rules. Age ≥ 65 years is added as a fifth variable to the four core variables mentioned above. To be classed as severe, a patient needed to meet three or more of the five variables. Informed written consents were obtained from all participants and the study was approved by the Ethics Committee of Mansoura University. All patients were subjected to full clinical and radiological examinations.

- *Samples:* One bronchoalveolar lavage (BAL) was taken from each patient under anesthesia. BAL was obtained from those patients to diagnose if there was other hidden pathology. We would not normally recommend this as a routine investigation for culture in CAP.
- One morning sputum sample was obtained from each patient.
- Three sterile blood samples were collected for blood culture both aerobically and anaerobically by BACTEC 9050 blood culture system.
- Two blood samples were obtained from each patient at admission and after 10 days for rising IgM titer for specific IgM for *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydia pneumoniae, Coxiella burnettii*, Influenza virus, and adenovirus by ELISA kit (Virotech kit-GmbH-Lowenplatz 5Russelsheim D-65428-GERM). Samples were considered positive when 1) a four-fold or greater titer increase was observed, or 2) seroconversion from negative titer to positive occurred. Laboratory results were available for the clinical management of the patients.

BAL and Sputum culture

Quantitative culture of sputum and BAL were carried out by 1:2 dilution of homogenized sputum that was diluted to a final dilution of 1:100 in sterile broth. BAL was diluted 1:2 then 0.005 ml was

inoculated onto a blood agar plate. Inocula were spread over one-half of the plate and then streaked over the other half so that 25 or more colonies of the same organism would then indicate that 10^6 or more of that pathogen were present in each milliliter of the original sputum or 10^4 for BAL, and would be considered clinically significant. Plates were incubated aerobically at 37°C with 5% CO_2 for 24-48 hours. Bacterial isolates were identified by their biochemical characteristics via the Microscan system (Siemens-France) [11]. *Candida* isolated alone from sputum, *E.coli, Serratia, Proteus and Pseudomonas* were considered as oropharyngeal overgrowth secondary to antibiotic therapy. However, the culture results were interpreted in the light of prior antibiotic therapy and response to therapy.

In addition, culture of sputum was done on specific media for *Legionella pneumophilia* and *Mycoplasma pneumonia*e where any colony would be considered relevant.

Legionella culture

Legionella agar base and its enrichment were used in the preparation of legionella agar. The complete medium is based on the charcoal yeast extract formula with L-cysteine HCI and ferric pyrophosphate supplement. The agar and supplement were supplied from Becton Dickinson Microbology Systems, USA.

Sputum samples and BAL were plated as described above, incubated at 2.5% CO_2 at 35°C for seven days, and examined daily for evidence of growth. Colonies were identified by gram stain and by indirect immunoflourescence stain for detection of *Legionella pneumophila* serogroup I (Novo castra, United Kingdom).

Culture for Mycoplasma

Mycoplasma pneumoniae culture was conducted with the *Pneumofast* (International Mycoplasma, Signes, France) kit according to the manufacturer's recommendations. The kit contains both reagents for the preparation of solid agar plates and Pneumofast trays for broth culture. The trays contain 10 separate wells, allowing semi-quantitative determination of colony counts, biochemical identification of growing organisms, and antimicrobial resistance testing. The plates and trays were cultured at 37°C for 12 days and were examined daily for the presence of colonies with a granular and/or a fried egg appearance or a color change in the tray wells. Positive cultures resistant to ampicillin (40 µg/ml), sulfa-trimethoprim (4 µg/ml), and lincomycin (1 µg/ml) but sensitive to erythromycin (8 µg/ml) were identified as *M. pneumonia*e. ELISAs were used for specific IgM measurement for *Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae, Coxiella burnettii*, and adenovirus influenza virus (Genzyme Virotech, Rüsselsheim, Germany).

The antibody for each tested pathogen was detected separately in serum samples by specific ELISA kits. The specific antigen solution was used to coat the microplate. The antibodies from patients' sera form an immune complex with the antigen coated on test strip and the enzyme conjugate attaches to this complex. We added TMB solution that turns yellow after adding a stopping solution and then the absorbance was read at 450 nm.

Statistical Methods

The sensitivity, specificity, positive predictive value and negative predictive value for *Mycoplasma pneumoniae* and *Legionella pneumophila* serological tests were calculated using the culture as the gold standard. Overall accuracy was the number of correct results over the total number of the results.

Results

This study was conducted on 100 consecutive patients presenting with CAP. Patients ranged in age from 18-70 years. Sixty-five percent of the subjects were male and 35% were female. Twenty-five patients (25%) had received oral antibiotic therapy within the preceding two-week period. Many patients had co-morbidities: 20 patients (20%) had chronic obstructive lung disease; 15 patients (15%) had chronic liver disease; and 10 patients (10%) had congestive heart failure. The median duration of symptoms before admission was five days ± (Standard deviation) SD 2.0. The median duration of hospitalization was 5 days ± SD3.0. There was no relation between the CURB 65 score and the pathogen detected. All patients had radiological abnormalities in the form of alveolar densities (Table 1).

At least 1 respiratory pathogen was identified in 86 patients (86%). Bacteria with or without co-infecting pathogens were identified in 76 (76%) patients (data not shown).

Adenovirus and *Chlamydia pneumoniae* were the most prevalent pathogens among patients with a ratio of 30% for each, followed by *Pneumococci* (22%), *Haemophilus influenzae* (18%), *Staphylococcus*

Table 1. Patient characteristics, number in each severity score group, management and outcomes.

Age (range, years)	(18–70)
18–38	14 (14.%)
39–58	34 (34.%)
59–65	34(34 %)
>65	18(18%)
Gender (male)	65 (65%)
Median duration before admission	5 days
Current smokers	83 (21.1)
Antibiotics prior to presentation	25 (25 %)
Co-morbidities	
Congestive cardiac failure	10(10 %)
Chronic liver disease	15(!5%)
COPD	20(20%)
Diabetes	22(22%)
CURB-65 severe	20(25%)
Relation of severity score to pathogen	No.
Admitted to ICU	8(8%)
Need for aggressive treatment	10 (10%)
Median duration of hospitalization	5 days
Discharge diagnosis of pneumonia	88 (88 %)
Readmitted within 2 weeks	12(12%)
Abnormal radiological findings	100 (100%)
Values in parentheses are percentages.	
COPD, chronic obstructive pulmonary disease; LOS, length of stay.	

ICU: Intensive care unit
CURB-65 = Confusion, Urea nitrogen, Respiratory rate, Blood pressure, 65 years of age and older.

Table 2. Prevalence of pathogens among patients.

Pathogen	**Positive cases (%)**		**Culture of sputum Positive cases(%)**		**Serology Positive cases (%)**		**Blood culture Positive cases(%)**	
Adenovirus	30	(30)	-	-	30	(30)	-	-
Chlamydia	30	(30)	-	-	30	(30)	-	-
Pneumococci	22	(22)	22	(22)	-	-	10	(10)
Haemophilus	18	(18)	18	(18)	-	-	-	-
influenzae	-	-	-	-	-	-	-	-
Staph.aureu	10	(10)	10	(10)	-	-	10	(10)
Mycoplasma	9	(9)	5	(5)	9	(9)	-	-
pneumoniae	-	-	-	-	-	-	-	-
Legionella	6	(6)	5	(5)	6	(6)	-	-
pneumophilia	-	-	-	-	-	-	-	-
Coxiella	2	(2)	-	-	2	(2)	-	-

Table 3. Mixed Infections in CAP.

	Number	Percentage
Chlamydia pneumoniae& Pneumocci	15	50
Mycoplasma pneumoniae& Pneumoccoci	5	16.7
Adenovirus & *Staph.aureus*	10	33.3
Total	30	100

aureus (10%), *Mycoplasma pneumoniae* (9%), *Legionella* (6%), *Coxiella* (2%) and *Infleunza* (1%), table 2.

Mixed infections as determined by colony counts and serological study were detected in thirty patients (30%). The most common associated pathogens were positive serology for *Chlamydia pneumoniae* and Pneumoccoci followed by *Mycoplasma pneumoniae* and Pneumoccoci (Table 3).

A review of the blood culture results showed 20 cases (20%) with positive sputum cultures; of these, 10% were positive for *Pneumococci* and 10% for *Staph. aureus* (Table 2).

In comparing serology for *M. pneumoniae* and *Legionella pneumophila* with specific culture, the sensitivity was 60% and 80% respectively, while specificity was 93.7% and 97.9%. The respective positive predictive value was 55.6% and 83.3% and the negative predictive values were 91.3% and 98.9% (Table 4).

Discussion

Atypical respiratory pathogens such as *Mycoplasma pneumoniae* and intracellular pathogens such as *Legionella* and *Chlamydia pneumoniae* are a significant cohort of the etiological agents underlying community-acquired pneumonia. The clinical signs or radiological features of atypical pneumonia are generally insufficient to predict accurately the pathogen involved and must be augmented by microbiological and serological tests [12].

In our study, culture from sputum and/or BAL revealed that typical bacteria were isolated from 88.38% of the recruited patients in the study. The most common microorganisms were *Pneumococci* (22%) followed by *Haemophilus influenza*e (18%). Similarly, Watari *et al*. [13] reported that typical bacteria accounted for up to 79.6% of the etiology of CAP from 74 patients in Tokushukai, Japan.

Other published studies have also reported that *S. pneumonia* was the most commonly isolated bacterium implicated in CAP, followed by *Haemophius influenzae* [5,14,15].

In evaluating the extent of atypical respiratory agents in our patients we found that specific sputum and BAL cultures for *L. pneumophilia* were positive in 5%, as were cultures for *M. pneumoniae*. Other studies give variable positivity rates for these organisms, ranging from 1% to 27 % [16,17]. Positive culture rates for these atypical pathogens more than likely depend on the patient population, socioeconomic factors, age, and possibility of exposure. In addition, detection of specific antigens in urine had been reported as a sensitive means of identifying the presence of *Legionella* [2]. Perhaps, by adding this test to those evaluated above, more cases of *Legionella*-caused atypical CAP could be diagnosed.

Clinically, *Mycoplasma pneumoniae* and *Legionella pneumophila* cannot be differentiated from pneumonia caused by other bacteria or viruses. Specific diagnosis is important because of the serious nature of these diseases and to allow appropriate therapeutic decisions to be made [18].

Chlamydia pneumniae is a recognized as a human respiratory pathogen with a unique biphasic life cycle characterized by an obligate intracellular (replicative) and an extracellular (infectious) form of the organism. It is widely distributed via the respiratory route and has proven itself to be a major contributor to respiratory disease among the world's populations. Infection ranges from an asymptomatic clinical picture to severe illness up to and including CAP [19]. Serological tests for Chlamydia infection are the most frequently used methods in the diagnosis of atypical respiratory infections [7], especially in laboratories which lack the facilities of culture or/and molecular techniques.

Positive specific IgM for *Chlamydia pneumoniae* and adenovirus had the highest positivity rate of all microorganisms detected, infecting 30% of our patients. This corresponds to previous observations where detection rates of *C. pneumoniae* in CAP ranged from 13% to 26% and detection of adenovirus was (26%) [20,21,22,23].

The high rate of occurrence of positive IgM for *Chlamydia pneumonia*e can be attributed to the presence of *Chlamydia trachomatis* in our locality

Table 4. Accuracy of *Mycoplasma* IgM and *Legionella* IgM compared to culture

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Accuracy**
Mycoplasma IgM	60%	93.7 %	55.6%	91.9%	92 %
Legionella IgM	80%	98.9 %	83.3%	98.9%	97 %

and the known cross-reactivity of their respective antigens [24], or it may represent a true response to the pathogen, which is common in hot, pet-breeding areas [25].

The lowest positive IgM rates were for *Coxiella burnetii* infections (2%) and influenza (1%). In other similar studies of CAP patients, Lee *et al*. [26] demonstrated that one case in 81 patients was positive for Coxiella and MacFarlane [27] reported that influenza virus was detected in 23 of 316 patients (7.2%) [26,27].

Since blood cultures are one of the most common cultures taken from these patients, we tried to determine their overall efficacy in aiding the diagnosis of CAP. We observed positive blood cultures in only 20% of our patients. These results parallel those of other investigators and lead one to question the value of blood cultures in the diagnosis of CAP [28,29].

Finally, there was no significant difference in clinical findings in determining the etiology of CAP. This was reported as the most common problem in differentiating legionnaire's disease from typical CAP as well as from *C. pneumoniae or M. pneumoniae* infections. The lack of clinical and radiologic specificity in CAP may also be compounded by a patient's recent antibiotic history, which can also ultimately affect bacterial culture results [30].

In the study of accuracy of IgM of *Mycoplasma pneumoniae* in diagnosis compared to culture, the sensitivity was 60%, specificity was 93.7% and accuracy was 92%. A previous study reported that the sensitivity of serological IgM testing when combined with polymerase chain reaction was 100% for detecting cases with *Mycoplasma pneumoniae* [31]. The reduced sensitivity of IgM may reflect the differences in the times that samples were acquired, as specific serological reaction to *Mycoplasma* is not found in early infection [12] and may be presented late as only a sign of a previous infection [32].

When time of sampling is properly managed, we can use specific IgM for *Mycoplasma pneumonia* detection early in CAP as a rapid surrogate marker of infection.

In the study of accuracy of *Legionella* IgM compared to culture, the sensitivity, specificity and accuracy were 80%, 98.9% and 92% respectively. The serological study for *Legionella* was a specific and accurate method which could be used to screen CAP patients in the absence of a culture facility [16]. This study highlights the importance of identifying mixed bacterial/viral when diagnosing lower respiratory tract infections. Our data showed that at least 30% of our patients had concurrent infections. Collectively, our findings and those of other investigators suggest that mixed bacterial/viral infections in the lower respiratory tract may be more common than previously supposed [33,34]. This observation raises two important questions: 1) whether sequential or concurrent viral and bacterial infections have a synergistic impact on the evolution of disease in children; and 2) should diagnostic batteries for any patient with CAP include methods for detecting both the typical and atypical bacterial or viral pathogens.

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