INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most common infectious diseases treated in the hospital setting, and is associated with significant morbidity and mortality (Bartlett et al, 1995; Ministry of Public Health, 1998). The selection and timing of initial antimicrobial treatment is an important clinical decision (Meehan et al, 1997). This decision is usually made before the results of specific microbial tests are available. Antibiotic treatment for CAP is, therefore, initially empirical; relying on epidemiological data of the causative pathogens in a particular geographic area. Although Streptococcus pneumoniae remains the most prevalent isolated etiologic agent, other organisms such as Haemophilus influenzae and Moraxella catarrhalis, as well as atypical pathogens, including Chlamydia pneumoniae and Mycoplasma pneumoniae, are now being reported more frequently than in the past (Mandell, 1995). Serological tests for atypical pathogens are often not available, and can not be performed in a routine laboratory. Some specific pathogens, such as Burkholderia pseudomallei; are found in endemic areas of the world (Boonsawat et al, 1990). Local epidemiological data are needed to develop practice guidelines for each country. We investigated the etiologies of CAP in patients requiring hospitalization and evaluated treatment outcomes in the hospital setting.

MATERIALS AND METHODS

This prospective study was carried out between January 2001 and December 2002 at Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand. Patients, 15 years or older,
who were admitted with CAP were included in this study. The diagnosis of CAP was based on 1) acute onset ≤ 2 weeks; 2) presenting with three of five of the following signs and symptoms: fever, cough with or without sputum production, dyspnea, pleuritic chest pain, and consolidation or crackles on physical examination; and 3) new infiltration on chest radiographs. We excluded patients who were 1) HIV positive; 2) transferred from another hospital; or 3) hospitalized within 3 weeks before admission.

The demographic data collected for the patients included: age, sex, occupation, and underlying diseases. The clinical symptoms and signs of each patient, and their onset before admission, were also documented. The initial laboratory investigations comprised: a complete blood count (CBC), chest radiograph, sputum Gram's stain and culture, blood culture, and a 5-ml clotted blood sample for serologic testing for C. pneumoniae and M. pneumoniae. Convalescent serum samples were obtained 2-3 weeks later. All serum samples were separated immediately and stored at -20ºC until the serology tests were done.

The etiology of the pneumonia was achieved by isolation of the organisms from samples of blood, sputum, pleural fluid or other sterile sites. A current infection with C. pneumoniae was defined by the detection of IgM antibody or a rising IgG antibody titer in paired sera. A current infection with M. pneumoniae was defined as a four-fold rise in the titer of paired sera by the particle agglutination test. The methodologies for the serology tests for C. pneumoniae and M. pneumoniae are described below.

The management of each patient depended on the individual physician. The results of the serology tests were not used for treatment decisions, because the sera were kept and analyzed at the end of the study. The outcomes of treatment, complications, and length of hospitalizations of all the CAP patients were also evaluated.

Serology for C. pneumoniae antibody detection

The Sero CP™-IgM (or IgG) Test is an enzyme immunoassay used for detecting IgM (or IgG) antibodies against C. pneumoniae in serum samples. The procedure was performed as described in the manufacturer's instructions. In brief, all the components of the reagent kit and specimens were brought to room temperature, and mixed before use. Fifty microliters of the two negative controls, one positive control and the diluted serum sample (dilute serum 1:105 with diluent for IgM or IgG) each were dispensed into separated wells on the test strip and incubated for 1 hour at 37ºC in a moist chamber. After incubation, the strips were washed with washing buffer 3 times, then tapped dry. Fifty microliters of horseradish peroxidase (HRP) conjugated antihuman IgM (or IgG) at a dilution of 1:300 was added into each well. The strips were further incubated for 1 hour at 37ºC in a moist chamber. After washing with buffer 3 times, 100 µl of tetramethylbenzidine (TMB) substrate was dispensed into each well and incubated at room temperature for 15 minutes. The reaction was stopped by adding 100 µl of 1 M H₂SO₄. The absorbance at 450 nm wavelength was determined within 30 minutes.

For the test to be valid, it had to meet the following criteria: 1) the absorbance of the positive control should be ≥0.8; and 2) the average absorbance of the negative control should be >0.1 and ≤0.4.

Calculation of cut-off value (COV) and cut-off index (COI)

The COV and COI were calculated according to the following formula;

\[ \text{COV} = \text{NC} \times 2 \]
\[ \text{COI} = \frac{\text{Absorbance of the serum sample}}{\text{COV}} \]

Interpretation of results

- If the OD was <COV and the COI was <1.0, the result was negative (ie no IgM or IgG antibody was detected). If the COV≤OD≤1.1 x COV, and the COI was between 1 and 1.1, the result was borderline (ie a low level of IgM or IgG antibody). If the OD >1.1 x COV and the COI was >1.1, the result was positive (ie a relevant level of IgM or IgG antibody). Then a 'current' C. pneumoniae infection was indicated when testing for serum IgM and a 'current or past' infection with C. pneumoniae was diagnosed when testing for IgG.

- If the initial and convalescent titers were both borderline the specimen was considered negative.

- To differentiate between a past and current infection when testing for serum IgG, a rise
in COI in the second sample by at least 40% was considered a current infection.

Serology test for *M. pneumoniae* antibody detection

SERODIA-MYCO II is a particle agglutination test kit for the detection of anti-*M. pneumoniae* antibody in human serum. The procedure was performed as described in the instruction manual. The U-shaped microtray was used for a gelatin particle agglutination test as follows. One hundred microliters of the serum diluent was placed into well 1 and 25 µl of diluent into wells 2 through 8 (or more). Twenty-five microliters of specimen was added to well 1 (serum dilution=1:5), mixed; and 25 µl of the mixture was added to well 3. A serial 2-fold dilution was prepared sequentially up to well 8 (or more). Twenty-five microliters of unsensitized particles (tanned gelatin particles) was added to well 2 (final dilution=1:20). Twenty-five microliters of sensitized particles (gelatin particles sensitized with *M. pneumoniae* antigen) was added to wells 3 (final dilution=1:40) through 8 (final dilution=1:1,280) or more. Twenty-five microliters of unsensitized particles was added to well 2 and 25 µl of sensitized particles was added to wells 3 through 8, mixed and incubated for 3 hours. For the reagent control, a mixture of serum diluent was prepared with both sensitized and unsensitized particles.

Reading the agglutination pattern according to the criteria in the instruction manual confirmed that: 1) the reaction of each specimen and unsensitized particle was negative; 2) the reagent control was negative; and 3) the titer of the positive control was 1:320 on final dilution.

Interpretation of results

- A specimen showing negative with unsensitized particles (1:20 final dilution, well 2) but positive with sensitized particles (1:40 final dilution, well 4 or more) was interpreted as positive. The end antibody titer was determined as the final dilution giving a positive result.
- A specimen showing negative with sensitized particles (1:40 final dilution, well 4) was interpreted as negative.
- A specimen showing negative with unsensitized particles (1:20 final dilution, well 2) and demonstrating positive and negative with sensitized particles (1:40 final dilution, well 3) was interpreted as indeterminate.

A current infection with *M. pneumoniae* was diagnosed if there was a four-fold rise in titer in the paired sera.

Ethics

The Ethics Committee of the Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, approved the research protocol.

Statistical analysis

Descriptive statistics were used. The means and standard deviations were calculated for the continuous data; the numbers and percentages were calculated for the categorical data.

RESULTS

During the 2-year study, 254 patients were diagnosed with CAP and admitted to our hospital. Eighty-six of them (33.8%) presented with severe CAP on initial presentation. A diagnosis of severe CAP, defined by American Thoracic Society (ATS) criteria (Niederman et al., 2001), requires one of two major criteria or two of three minor criteria. The major criteria are: 1) need of mechanical ventilation; and 2) septic shock. The minor criteria are: 1) systolic blood pressure <90 mmHg; 2) multilobar involvement; and 3) PaO2/FiO2 <250.

The average age, of the 124 male and 130 female patients, was 56.4 (SD 19.8) years. Twenty-four percent were farmers and 14% were in government service. The mean incubation period was 5.6 (SD 4.2) days. Most (87%, 221 of 254) patients had one or more co-morbidities. Underlying diseases included cardiovascular disease (60 cases), diabetes mellitus (48), autoimmune disease (34), renal disease (29), neurological disease (24), hematological disease (21), chronic obstructive lung disease (14), asthma (8), and cirrhosis (6) (Table 1).

The causative organisms were identified in 145 patients (57.1%). *S. pneumoniae* was found in 11.4% of the isolates and was the predomi-
nant pathogen among the hospitalized CAP patients (Table 2). B. pseudomallei was the second most frequently observed pathogen (11.0%), followed by K. pneumoniae (10.2%). C. pneumoniae was found in 8.7% of patients; and M. pneumoniae, another atypical pathogen, was found in 3.9%. Other known pathogens were H. influenzae (4.3%), S. aureus (3.5%), E. coli (3.1%), Streptococcus spp (3.1%), P. aeruginosa (2.4%), M. catarrhalis (0.8%), P. fluorescence (0.4%), and P. cepacia (0.4%).

Dual infections were found in 16 patients (6.3%) (Table 3). C. pneumoniae was the most common co-infecting pathogen. The most common dual pathogens were C. pneumoniae co-infected with Streptococcus spp, found in 4 cases. Other dual infections were: E. coli + K. pneumoniae (3 cases); C. pneumoniae + K. pneumoniae (2); C. pneumoniae + B. pseudomallei (2); M. pneumoniae + Streptococcus spp (1); M. catarrhalis + S. pneumoniae (1); C. pneumoniae + M. pneumoniae (1); C. pneumoniae + H. influenzae (1); and C. pneumoniae + E. coli (1).

Hospitalization averaged 12.9 (range, 1-115) days, and 71 patients (27.9%) stayed more than 2 weeks. Overall, 83.9% of patients improved with treatment, 10.2% did not improve and 5.9% died (Table 4). The most common complications were acute respiratory failure (31.1%) and septic shock (20.9%). Other complications prolonging hospital stay were parapneumonic effusions or empyema thoracis (13.0%), hospital acquired pneumonia (8.3%), acute renal failure (6.3%), extrapulmonary infection (3.5%), and pneumothorax (3.1%).

**DISCUSSION**

The importance of CAP has led numerous international organizations to publish guidelines to optimize care and improve outcomes. Several changes have occurred during the past decade that have impacted the management of CAP including: the increasing awareness of atypical pathogens (ie C. pneumoniae, M. pneumoniae, and L. pneumophila) and the emerging resistance of standard pathogens (most notably S. pneumoniae) (File et al, 1997; Bartlett et al, 1998). Therefore, local epidemiological data are required for developing guidelines for clinical practice.

Our study found pathogens in 57.1% of patients requiring admission to hospital for CAP, despite extensive laboratory investigations. This percentage is similar to that found in previous studies (Bates et al, 1992; Bohte et al, 1995; Lieberman et al, 1996). S. pneumoniae remained
the most frequently isolated etiologic agent, although its incidence appears to be decreasing. Gram-negative pathogens, such as *B. pseudomallei* and *K. pneumoniae*; were found more often in patients hospitalized for CAP. In our study, about 30% of hospitalized CAP patients presented initially with severe CAP and needed intensive care treatment. Among these, *B. pseudomallei* was the most common pathogen isolated in severe CAP, followed by *K. pneumoniae*. A report on the etiology of CAP hospitalized patients in Malaysia found *K. pneumoniae* (10.2%) was the most prevalent followed by *S. pneumoniae* (5.5%) (Liam et al, 2001). For severe CAP, *B. pseudomallei* was the most common pathogen reported in Singapore (Tan et al, 1998). These findings differ from western countries (Ewig et al, 1999). The reasons for this may be: diabetes mellitus was a common underlying disease and *B. pseudomallei* was endemic in these areas.

*C. pneumoniae* was the fourth most common isolated pathogen, accounting for 8.7% of CAP patients. This pathogen is now associated in approximately 10% of all cases of pneumonia worldwide (Kauppinen et al, 1995). When combined with *M. pneumoniae*, the prevalence of atypical pathogens for hospitalized CAP was 12.6% (8.7% *C. pneumoniae* and 3.9% *M. pneumoniae*). Moreover, *C. pneumoniae* was the most common co-infecting pathogen found in cases of dual infection. These findings are similar to those of Miyashita et al (2002) and Wattanathum et al (2003). Therefore, empiric coverage of these pathogens with doxycycline, macrolides, or the new fluoroquinolones should be considered (Plouffe, 2000; Salkind et al, 2002; File et al, 2003), since serologic diagnosis is not available in every hospital, and takes time waiting for the paired sera results.

The morbidity and mortality rates in our hospitalized CAP patients were approximately 16%, with 5.9% dying and 10.2% not improving. These figures are similar to a meta-analysis of CAP outcomes (Fine et al, 1995), where the overall mortality for hospital admitted patients was 13.7%; 17.6% for elderly patients and 19.6% for bacteremic patients. The length of hospitalization varied widely, ranging from 1 to 115 days. This may be due to the appropriateness of the initial antibiotic therapy and supportive care. The use of macrolides or new fluoroquinolones, as a part of an initial therapeutic regimen, was associated with lower mortality rates (Gleason et al, 1999; Brown et al, 2003) and reduced lengths of hospitalization (Stahl et al, 1999; Brown et al, 2003). Acute respiratory failure and septic shock were the two most common complications, which increased mortality, length of hospitalization, and cost of treatment. When the patients presented with either of these two complications, intensive care therapy was needed (Ewig et al, 1998).

In summary, the results of this study showed that *S. pneumoniae* was the most commonly isolated pathogen. Gram-negative bacilli, such as *B. pseudomallei* and *K. pneumoniae*, had a relatively
high prevalence in our region, especially in cases of severe CAP. The occurrence of atypical pathogens such as, C. pneumoniae and M. pneumoniae, was comparable to other studies. Co-infections were found in 6.3% of cases, and C. pneumoniae was the most common co-infecting microorganism. Ultimately, the reduction of pneumonia-related morbidity and mortality will depend on both appropriate initial antibiotic therapy and supportive care. The use of macrolides or new fluoroquinolones as a part of initial therapy for hospitalized CAP patients, and the use of antibiotics to cover B. pseudomallei for severe CAP, should be considered in our region.

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REFERENCES


