

## Investigation of the causative agents for community-acquired pneumonia in adult patients

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**Aim:** Community-acquired pneumonia (CAP) is a common condition in healthy people, causing morbidity and mortality worldwide despite latest advances in therapy and immunization procedures. Causative agents cannot be detected in approximately 50% of CAP episodes and therapy is initiated empirically. We aimed to determine the spectrum and frequency of the causative agents in patients with CAP in a university hospital.

**Materials and methods:** Seventy seven adult patients hospitalized with CAP from November 2007 to March 2008 were included. CAP was diagnosed with clinical, radiological, and laboratory signs.

**Results:** Sputum and blood cultures grew *Streptococcus pneumoniae* in 15 specimens; *Haemophilus influenzae* in 4, *Klebsiella pneumoniae* in 4, *Staphylococcus aureus* in 3, and *Escherichia coli* in 2. *Mycoplasma pneumoniae* DNA was detected in serum from 10 patients with RT-PCR. *Legionella pneumophila* urinary antigen was detected in 5 patients. Serological IgM antibodies to *Chlamydia pneumoniae* in 7 patients and *Respiratory Syncytial Virus* in 2 patients were observed. Etiology was not determined in 32.5% of patients. The most frequently identified pathogens causing CAP were *S. pneumoniae*, *M. pneumoniae*, and *C. pneumoniae* in descending order in our hospital.

**Conclusion:** Although determination of causative agents in all CAP patients has not been accomplished, knowledge of the spectrum and frequency of local causative agents are valuable for targeted therapy.

**Key words:** Community-acquired pneumonia, sputum cultures, *S. pneumoniae*, *M. pneumoniae*

### Erişkin hastalarda toplum kaynaklı pnömoni etkenlerinin araştırılması

**Amaç:** Toplum kaynaklı pnömoni (TKP), normal kişilerde, günlük yaşamı sırasında gelişen pnömonidir. Yeni antibiyotiklerin de kullanımı ile tedavi ve immünizasyon yöntemlerindeki gelişmelere rağmen tüm dünyada en önemli mortalite ve morbidite nedenlerinden biri olmaya devam etmektedir. TKP olgularının yaklaşık yarısında spesifik etken saptanamamakta ve empirik tedavi uygulanmaktadır. Bu çalışma bir üniversite hastanesinde TKP'ye neden olan etkenlerinin saptanması amacı ile yapılmıştır.

**Yöntem ve gereç:** Kasım 2007-Mart 2008 tarihleri arasında TKP tanısı ile hastaneye yatırılan 77 erişkin hasta çalışmaya dahil edilmiştir. TKP tanısı, klinik, radyolojik ve laboratuvar bulgularına göre konmuştur.

**Bulgular:** Balgam ve kan kültürü örneklerinden 15'inde *Streptococcus pneumoniae*, 4'ünde *Haemophilus influenzae*, 4'ünde *Klebsiella pneumoniae*, 3'ünde *Staphylococcus aureus*, 2'sinde *Escherichia coli* izole edilmiştir. Real time PCR ile 10 hastanın serumunda *Mycoplasma pneumoniae* DNA'sı saptanmıştır. Hastaların 5'inin idrarında *Legionella pneumophila* antijeni bulunmuştur. Serolojik olarak 7 hastada *Chlamydia pneumoniae* ve 2 hastada *Respiratory syncytial virus* IgM antikorları saptanmıştır. Hastaların % 32,5'inde etyolojik bir patojen saptanmamıştır. Hastanemizdeki en sık rastlanan CAP etkenleri sırası ile, *S. pneumoniae*, *M. pneumoniae* ve *C. pneumoniae* olarak bulunmuştur.

**Sonuç:** TKP'li olgularda tamamında etkenlerin belirlenmesi mümkün değildir. Uygun tedavi protokollerinin oluşturulmasında o bölgede en sık rastlanan patojenlerin bilinmesinin önemli olduğu sonucuna varılmıştır.

**Anahtar sözcükler:** Toplum kaynaklı pnömoni, balgam kültürü, *S. pneumoniae*, *M. pneumoniae*

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## Introduction

Pneumonia is an acute infectious disease of the lung parenchyma distal to the terminal bronchioles. It is a condition characterized by inflammation in airspaces, resulting in consolidation of one or more lobes of the lung (1,2). Most community-acquired pneumonia (CAP) incidents are managed outside the hospital in otherwise healthy people (3). In the USA, CAP ranks 6<sup>th</sup> among reasons of death and is the leading cause of mortality from an infectious disease. Although latest advances in therapy, including new antibiotics and immunization techniques, CAP is still one of the leading causes of morbidity and mortality worldwide (3, 4).

Between 22% to 42% of CAP episodes are admitted to hospital and between 5% to 10% are hospitalized in intensive care units (ICU) (5). Mortality rates of CAP episodes vary from a low percentage of 1%-5% for ambulatory patients, and 12% for hospitalized patients to a high percentage of 36%-40% for patients requiring ICU (3, 4, 6). Annual cost of CAP is estimated as \$20 billion in the USA and £400 million in the UK (7). Mean cost of a patient hospitalized with pneumonia was calculated as 1479 TL (1\$= 1.34 TL) in a single report on cost analysis of pneumonia in Turkey (8). Increasing resistance patterns due to inappropriate antibiotic treatment also contributes to morbidity, mortality, and management costs. Early appropriate treatment has been shown to decrease to 30 days mortality rate (9). Therefore, the knowledge of likely causative pathogens is vital.

This study was aimed to determine causative agents of CAP and provide guidance for the management of CAP episodes in our hospital.

## Materials and methods

Patients with CAP hospitalized in the Chest Disease Clinics, Faculty of Medicine, Gaziantep University from November 2007 to March 2008 were prospectively included. CAP was defined as new infiltrate(s) on chest radiography performed prior to enrollment, and at least 1 major (fever, cough, or sputum) or 2 minor (dyspnea, chest pain, pulmonary consolidation on physical examination, or leukocytosis) signs and symptoms consistent with pneumonia. Patients who had been hospitalized

within 10 days, patients who had been on immunosuppressive treatment with malignancy diagnosis, and patients with known immunodeficiency syndrome were excluded.

We collected information about risk factors such as cigarette smoking and alcoholism and chronic diseases (chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), etc.). All patients with suspected pneumonia had their chest X-ray (CXR) taken following physical examination.

**Sputum specimens:** Suitable sputum samples were selected by Gram staining. Purulence was measured by microscopy and the specimen was accepted if there were >25 neutrophils and ≤10 epithelial cells in the ×10 magnifying area (4). Otherwise new specimen was requested. Sputum specimens from 51 patients were found to be proper for evaluation.

Sputum samples were inoculated on 5% blood agar, eosin methylene blue agar, and chocolate agar. Cultures were incubated at 37 °C for 24-48 h. Isolated bacteria was considered pathogenic if the Gram stain and cultured bacteria were in accordance and were identified by classical identification techniques and Vitek 2 (Biomerieux, France) full automatic identification system. For fungal pathogens, sputum samples were inoculated on Sabouraud dextrose agar (SDA).

**Blood cultures:** Pretreatment, 2 blood culture samples were collected from all patients following hospitalization and were incubated in a Bact-T Alert 3D (Biomerieux, France) full automatic blood culture system. Samples were identified by classical techniques with a Vitek 2 full automatic bacteria identification system.

**Blood samples:** Serum samples were divided to 3-4 aliquots after centrifugation.

CRP tests were performed using a BN II (Dade Behring, USA) device nephelometrically according to test instructions. Reference interval was 0-5 mg/dL.

White blood cell count was performed by SYSMEX XT 2000i (Roche, Japan) device according to test instructions. Reference interval was 4000-10,000/mm<sup>3</sup>.

**Real time PCR (RT-PCR):** Detection of *M. pneumoniae* DNA in sera collected from patients was

performed by RT-PCR. ABI Prism 7000 (Applied biosystem) phlorometric RT-PCR system and Roboscreen (Germany) PCR kits were used. Procedures of mix preparation and thermal cycling were performed according to the manufacturers' instructions. These procedures detected *M. Pneumoniae* DNA from samples with FAM labeled target area.

**Serological evaluation:** Detection of IgM antibodies to *L. pneumophila*, *Coxiella burnetti*, *Adenovirus*, RSV, *Influenza virus*, *Parainfluenza virus* in sera collected from patients was performed using Pneumoslide IgM (Vircell, Spain) slides and the indirect immunofluorescent antibody (IFA) technique. Evaluation was performed on an Olympus BX 50 fluorescent microscopy by 2 authors separately.

**Urine samples:** *L. pneumophila* serogroup 1 antibodies in urine samples were detected by DRD Diagnostics (Germany) kits according to manufacturers' instructions, using enzyme immunosorbent linked assay (ELISA).

## Results

A total of 77 adult patients, 54 male (70.1%), with pneumonia were enrolled in the study. Mean age was  $54.67 \pm 16.99$  years (range 20-83). Thirty five (45.5%) patients were older than 60.

Smoking was reported by 26 (33.8%) of the patients, 2 of which also consumed alcohol. None of the patients over 60 smoked. Twenty six percent (20) of the patients were suffering from chronic illnesses. Leukocytosis ( $>10.000/\text{mm}^3$ ) was found in 49 patients

(63.6%) and leukopenia ( $<4000/\text{mm}^3$ ) in 3 (3.9%). CRP levels were elevated in 65 patients (84.4%). Only 51 (66.2%) of the cases provided sputum samples.

Sputum culture results were evaluated in view of the gram stain findings and the predominant semi-quantitatively determined microorganism was identified as the causative pathogen. Microorganisms isolated from cultures (sputum and blood) are shown in Table1.

Bacterial pathogens were isolated from 24 of 51 sputum samples and *S. pneumoniae* was the most common bacteria (25.5%). *Candida* spp was co-isolated from 6 sputum samples: 3 sputum samples with *S. pneumoniae*, 2 samples with *K. pneumoniae*, and 1 sample with *E. coli*. Pathogenic bacteria were isolated only from blood cultures in 4 patients: *S. pneumoniae* from 2 patients, *K. pneumoniae* from 1 patient, and *S. aureus* from 1 patient. In addition, *S. epidermidis* was isolated from blood culture of 1 patient whose sputum sample grew *S. pneumoniae* and of 2 patients whose sputum samples were negative for any pathogenic bacteria. Therefore 3 *S. epidermidis* isolates were considered contaminant bacteria because repetitious cultures were negative or inconsistent with results of sputum cultures. A bacteriological aetiology could be determined in 28 patients (36.4%) from blood and sputum culture samples.

*M. pneumoniae* DNA was detected in 10 samples (13.0%) by RT-PCR.

Five urine samples (6.5%) were positive for *L. pneumophila* serogroup 1 antibody.

Table 1. Distribution of microorganisms isolated from sputum and blood cultures.

Microorganism	Sputum culture (n)	Blood culture (n)	Total
<i>S. pneumoniae</i>	13	4	15
<i>H. influenzae</i>	4	-	4
<i>K. pneumoniae</i>	3	2	4
<i>S. aureus</i>	2	2	3
<i>E. coli</i>	2	1	2
<i>Candida</i> spp.	6	-	6
<i>S. epidermidis</i>	-	3	3

IgM antibodies were observed against *C. pneumoniae* in 7 (9.1%) patients, RSV in 2 (2.6%) patients, and *L. pneumophila* in 5 (6.5%) patients whose urine *Legionella* antigen was also positive. A microbiological aetiology could not be determined in 25 patients (32.5%). The frequency distribution of microbial agents according to age intervals is shown in Table 2.

A pathologic microorganism could be identified in 52 (67.5%) of 77 patients with CAP with various methods and none of the organisms searched for was present in the remaining 25 patients (32.5%). The identified microorganisms were *S. pneumoniae* (19.5%), *M. pneumoniae* (13%), *C. pneumoniae* (9.1%), *L. pneumophila* (6.5%), *H. influenzae* (5.2%), *K. pneumoniae* (5.2%), *S. aureus* (3.9%), *E. coli* (2.6%), and RSV (2.6%).

**Discussion**

CAP represents a particular public health concern owing to high mortality and morbidity incidence rates and management costs (10). CAP management guidelines are prepared in many countries including Turkey (3-5, 11). Guides assist health care providers in stratifying patients according to severity of illness, hospitalization and admission to ICU decision, and utilization of diagnostic and treatment alternatives. Treatments consistent with these guides decrease

morbidity and mortality rates (12). Local microbiological data are taken into account in preparation of guides.

Bacterial, fungal, viral, and protozoal agents can be detected as causative pathogens in CAP episodes. Causative pathogens are not determined in approximately half of CAP episodes even in developed countries (4, 10). We could not determine a causative pathogen for 32% of CAP episodes in our series.

Several diagnostic techniques are suggested for the diagnosis of CAP, such as physical examination, radiological investigation, and gram staining for sputum, blood and sputum cultures, and serological tests. CRP, leukocyte count, and biochemical tests are used to support the diagnosis and predict prognosis (4,13). Elevated CRP level supports the diagnosis in pneumonia and its sensitivity is reported as high as 100% (14). Although it does not help to detect a specific causative agent, increased levels are correlated with prognosis (15). CRP level was elevated in 84.4% of our patients. Leukocytosis is present in most patients with CAP and leukopenia is reported as a sign of poor prognosis (10,13). The leukocyte count was elevated in 63.6% of our patients. The relationship of leukocyte counts or CRP levels with prognosis was not evaluated in this study. CAP is frequent and severe in especially older patients and also patients with comorbid diseases, such as COPD, diabetes mellitus,

Table 2. Distribution of microbial agents according to age intervals.

Age (year)	<i>S. pneumoniae</i>	<i>H. influenzae</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>M. pneumoniae</i>	<i>L. pneumophila</i>	<i>C. pneumoniae</i>	RSV	Not-detected	TOTAL
20-29	2	1	-	1	-	-	-	1	-	1	6
30-39	3	-	-	-	-	1	2	-	-	5	11
40-49	2	1	-	-	-	2	-	1	1	5	12
50-59	1	-	1	1	-	4	1	2	-	3	13
60-70	3	1	1	1	1	1	2	2	1	5	18
>70	4	1	2	-	1	2	-	1	-	6	17
TOTAL	15	4	4	3	2	10	5	7	2	25	77

renal failure, CHF, and chronic liver disease (3, 16). In this study, 45% of the patients were  $\geq 60$  years old and 26% of the patients had at least one co-morbid disease and/or risk factor and 33.8% were smokers.

Examination of a high quality sputum sample collected from the respiratory tract by gram staining has a high diagnostic value. However, identification of gram stained members of the oropharyngeal flora is a major disadvantage of this technique (4,13). Furthermore, several studies reported that good quality sputum samples could be collected from 32%-39% of the patients with CAP (17, 18). The use of this technique can be limited to more severe cases of pneumonia for etiologic diagnosis and guiding treatment decisions (10). Gram staining is considered more valuable than sputum culture because of immediate results in this setting (4). In this study, the quality of sputum samples were determined by gram staining and then they were cultured and whether the bacteria isolated from sputum culture were identical with bacteria detected from corresponding sputum gram stain was noted. Several patients could not provide any sputum samples and also some samples were of poor quality. Appropriate sputum samples were collected from 66% of the patients. Obtaining 2 sets of blood culture samples from patients with CAP is recommended. Causative agents could be identified in 0%-30% of patients (mean 11%) by blood cultures (3, 13). In this study, a causative agent was identified with blood culture at a rate of 11.7%, moreover in 4 patients a causative agent was identified by blood culture only; the rest of the isolated bacteria were identical with bacteria from sputum culture.

Isolation of a causative agent from 36% of the patients was succeeded by sputum and blood culture. Küçükardalı et al. (19) reported this rate as 31%, Kömürçüoğlu et al. (20) reported 35%, and Fidan et al (21) reported 3.7%. The wide difference in the rates of isolation is thought to be the result of inclusion of ambulatory patients, previous antibiotic therapy, or the retrospective nature of some studies. We consider that it is impossible to prevent variability of antibiotic intake for Turkey where antibiotics can be purchased without prescription, which may indeed be the factor for some of the episodes in which no causative agent could be identified.

In our study, *S. pneumoniae* is the most frequent causal bacterium isolated, which is in correlation with published data (2,4,19).

*M. pneumoniae* is the second most frequent causal bacterium following *S. pneumoniae* in CAP episodes, may cause epidemics and is the most frequent agent for atypical pneumonia (10,22,23). For diagnosis, cold agglutination test, which is a non-specific test technique, ELISA, culture techniques, and PCR are used (4, 24). PCR is a rapid, sensitive, and specific technique to diagnose diseases caused by mycoplasma (22). Identification of mycoplasma as the cause of pneumonia is important because it is not sensitive to  $\beta$ -lactam antibiotics, which are commonly prescribed for treatment (25, 26). The second most frequent isolated bacterium was *M. pneumoniae* in this study and it was isolated from 13.0% of patients. *M. pneumoniae* was isolated from 17%-37% of reported CAP episodes (2, 11, 23, 27).

*L. pneumophila* and *C. pneumoniae* are isolated from 2%-8% and 5%-10% of patients with CAP, respectively (28-30). In this study, identification rates were 2.6% and 6.5%, respectively, and these results are correlated with the literature.

We determined *S. pneumoniae*, *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *H. influenzae*, *K. pneumoniae*, *S. aureus*, *E. coli*, and RSV in descending order as causative agents of CAP in our hospital.

In conclusion, this study was aimed to provide local epidemiologic data for causative agents in CAP episodes. Local data about the aetiology of CAP is needed because the identification of a causal agent in CAP episodes takes a long time and treatment decisions need to be taken quickly. Moreover, we consider that examination of high quality sputum samples by gram staining may guide clinical management decisions and also increases the diagnostic value of sputum cultures. Tests, such as ELISA and IFA, for the detection of IgM type antibodies against frequently encountered pathogens can be easily and efficiently performed in many laboratories. Utilization of these diagnostic techniques may be helpful in diagnosis and management of patients hospitalized for CAP.

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