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Microbial etiologies in community acquired pneumonia (CAP)

Community-acquired pneumonia (CAP) remains a common and persistent cause of morbidity and mortality. CAP is commonly defined as an acute infection of the pulmonary parenchyma associated with at least some symptoms of lower respiratory tract infection. It is accompanied by the presence of acute infiltrates on chest radiograph and auscultatory findings in patients not hospitalized or residing in a long-term-care facility for more that 14 days before the onset of the symptoms (2).

Many studies evaluating the causes of CAP in adults have failed to identify the cause of 40%–60% of cases of CAP and have detected more than two etiologies in 2%–5% (6). The most common etiologic agent identified in CAP is *Streptococcus pneumoniae* and *Haemophilus influenzae* but atypical pathogens, mainly *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, also play an important role as causative agents. Other atypical pathogens implicated in CAP are respiratory viruses (influenza A and B, parainfluenza viruses, and respiratory syncytial virus), *Chlamydia psitaci*, and *Coxiella burnetii* (1, 10, 13, 14, 18). These agents can cause severe as well as mild-to-moderate illnesses and they are implicated in up to 40% of CAP cases and occur commonly as co-pathogens in mixed-infection CAP (mortality up to 25%). Other less frequently found pathogens include: *Staphylococcus aureus, Streptococcus pyogenes, Neisseria meningitidis, Moraxella catarrhalis, Klebsiella pneumoniae*, other Gram-negative rods and microbes. Nowadays, it is known that potentially unpathogenic agents existing as elements of physiological flora in upper and lower respiratory tract may become opportunistic causing CAP. However, these infections affect both patients with diagnosed hypoimmunity and immunocompetent subjects. Among them, *Corynebacterium pseudodiphtheriticum* pneumonia has been described in medical literature concerning cases of pneumonia in immunocompetent patients with *C. pseudodophtheriticum* as the only isolated microorganism, for example (12). Some pathogens cause pneumonia more often in subjects exposed to specific risk factors. For instance, pneumococcal pneumonia is especially likely to occur in the elderly and patients with a variety of medical conditions, including alcoholism, chronic obstructive pulmonary disease, chronic cardiovascular disease, HIV infection, immunoglobulin deficiency, and hematologic malignancy. *Pneumocystis carinii* is the most commonly identified cause of acute pneumonia in patients with AIDS. Legionella is an important cause of pneumonia in organ transplant recipients and in patients with renal failure, chronic lung disease, lung cancer, AIDS, and in smokers. There are seasonal differences in the incidence of many of CAP causes. Pneumonia due to *S. pneumoniae, H. influenzae*, and influenza occurs mainly in winter, whereas there is a summer prevalence of legionnaires' disease outbreaks. The incidence of CAP sharply increases among the elderly and the main risk factor for that infection in this group is chronic comorbidity.

A wide variety of diagnostic and therapeutic approaches have been suggested in evaluating patients with CAP. However, etiology of CAP cannot be determined on the basis of clinical manifestations, radiologic imaging or routine laboratory test results. Therefore, initial antibiotic therapy for adults with CAP should be empirical, based on accepted guidelines. In many cases, it is difficult to obtain bacteriology results before treatment decisions, so information concerning local etiologic agents in CAP and their sensitivity are very helpful.

The objective of the study was to determine bacterial causative pathogens of CAP with their antibiotic sensitivity focusing on atypical pathogens and oportunistic species.

MATERIAL AND METHODS

The examined population sample consisted of 50 patients treated in the Pulmonary Deptartment, age 31–85 (25 women and 25 men) with diagnosed community-aquired pneumonia (CAP). The patients complained of some of the following symptoms: cough, sputum production, dyspnoea, pleuritic chest pain, chills and rigors, malaise and myalgias. Many of the examined patients had fever >38°C and leukocytosis >10 000/mm^3. All patients had new infiltrates or consolidations on chest X-rays. In auscultatory findings, altered breath sounds and rales were reported.

Purulent sputum was examined microscopically to determine culture suitability (presence of >25 polimorphonuclear leukocytes and <10 squamous cells per low power field 100 x of a Gram-stained specimen defined as an adequate sputum), revealing in the visual area approximately 10 bacterial cells (club-like, Gram-positive). All adequate specimens were cultured according to standard microbiological methods (medium: Columbia agar with 5% sheep's blood, Chapman, McConkey's Sabouraud and Haemophilus agar [bioMèrieux]). Isolates identification was made according to biochemical features using API Strep, API NH, API Staph, API E, API NE, API Coryne (bioMèrieux). Antibiotic sensitivity determination of isolated strains was performed by means of the disc diffusion method (according to Birby-Bauer). During the first day of hospitalization, blood culture was made; the BBL Septi-Chec (TSB) medium was used for that examination (Becton Dickinson). In the assessment of atypical pathogen contribution in etiology of CAP, the routine serological diagnostic approach was performed. Serological examination towards *Legionella pneumophila, Chlamydia pneumoniae* with the micro-immunofluorescence method (MIF – Pointe Sciencific) application, and *Mycoplasma pneumoniae* (ELISA – Pointe Scientific) based on the determination of specific IgG, IgM antibody, and for *C. pneumoniae* – also IgA, was conducted. *Legionella pneumophila* urine antigen tests were also performed.

RESULTS

In the group of 50 examined patients, CAP mostly affected 60-69-year-old subjects. Seventy-five per cent cases of CAP occurred in patients above 50 (Tab. 1). No microorganism was isolated from serum of any examined subject. *Staphylococcus aureus* appeared to be the dominant causative bacterial pathogen isolated from sputum (17.9% of all 56 isolated strains). Moreover, all isolated *Staphylococcus aureus* were metycylin-sensitive. The second isolated strain was *Haemophilus parainfluenzae* – 12.5%, the third one – *Haemophilus influenzae* – 8.9%. Special attention should be paid to *Corynebacterium pseudodiphteriticum* (8.9% of all isolated pathogens), which is not a typical human pathogen. However it has recently been recognized as a microorganism causing pneumonia both in healthy and immunodeficient patients. In the case of three patients, *C. pseudodiphtheriticum* was the only isolated strain

showing high antibiotic sensitivity. In other five patients, *C. pseudo diphteriticum* was identified together with other agents: *K. pneumoniae* and *S. aureus*. Gram-negative bacteria made up a high percentage of 11.8% of all isolated bacterial strains (*Klebsiella pneumoniae* –3.6%, *Pseudomonas aeruginosa* – 3.6%, *Klebsiella oxytoca* – 1.8%, *Escherichia coli* – 1.8%).

Table 1. Age of examined patients

Age (years)	Percentage of examined patients (n=50)
20 – 29	4.8
30 – 39	9.5
40 – 49	11.5
50 – 59	19.5
60 – 69	35.7
70 – 79	16.7
80 – 89	2.4

Microbiologic cultures of sputum yielded potentially pathogenic microorganisms in 69.6% of all patients; only physiological flora (*Streptococcus viridans, Neisseria* spp.) was isolated from sputa of the rest of 30.4% of subjects. *Streptococcus pneumoniae* was not isolated in our study (Tab. 2).

Table 2. Pathogens isolated from sputa of patients with CAP

Species	Percentage of isolated strains 56 strains – 50 patients
1. Staphylococcus aureus	17.9
2. Haemophilus parainfluenzae	12.5
3. H. infuenzae	8.9
4. Corynebacterium pseudodiphtheriticum	8.9
5. Moraxella catarrhalis	5.3
6. Candida albicans	5.3
7.Klebsiella pneumoniae	3.6
8. Pseudomonas aeruginosa	3.6
9. K.oxytoca	1.8
10. Escherichia coli	1.8
11. Streptococcus viridans, Neisseria spp.	30.4

In the examined group of patients, all isolated strains showed high antibiotic sensitivity. The lowest sensitivity was found for *Staphylococcus aureus*; all isolated strains of that pathogen produced beta-lactamase and were Methicillin-resistant (Tab. 3). Determining the level of specific antibodies of IgM, IgG, and IgA classes proved the contribution of atypical pathogens to CAP etiology. Serological criteria of chlamydial acute infection: titer; IgM>1:16, IgG>1:512, IgA>1:512, and of chronic or past infection: titer; IgG 1:64-512, IgA 1:16-512 were adopted. The majority of the analyzed patients – 26 (52%) – had antibodies' titers characteristic of chronic or past infections; in 20 (40%) of these cases, other pathogens were isolated from sputa, whereas for the rest 6 patients, negative sputum cultures were obtained. In 10 (20%) patients, high stable IgG antibody titers, characteristic of acute infection were observed; in seven of these

Table 3. Antibiotic sensitivity of strains isolated from sputa of patients with CAP. Sensitivity expressed as % (n=56)

	S.aureus	*H.parainf.*	*C.pseud.*	*P.aerugin.*	*H.influen.*	*M.catarrh.*	*E.coli*	*K.oxytoca*	*K.pneum.*
Oxacillin	100	------	------	-------	-------	-------	---------	---------	-------
Cefuroxime	100	100	100	0	100	100	50	100	100
Erythromycin	100	-----	100	0	------	100	0	0	0
Ampicillin	25	100	100	50	100	100	100	100	50
Amox/Clav.	100	100	100	100	100	100	50	100	100
Cefaclor	100	100	100	0	100	100	100	100	100
Cefixime	2.5	100	100	0	60	100	100	100	100
Cefpodoxime	2.5	100	100	0	100	100	100	100	100
Ceftriaxone	100	100	100	100	100	100	100	100	100
Ciprofloxacin	100	100	100	100	100	100	100	100	100
Clarithromycin	100	50	100	0	100	100	0	0	0
Levofloxacin	-----	------	-----	100	-------	-------	-------	-------	-------
Trovafloxacin	-----	------	-----	100	-------	-------	-------	-------	-------
Ofloxacin	-----	------	-----	100	-------	-------	-------	-------	-------
Nalidixic Acid	-----	------	-----	0	-------	-------	-------	-------	-------
Gentamicin	-----	------	-----	100	-------	-------	-------	-------	-------
Trimeth/Sulfa.	-----	------	-----	100	-------	-------	-------	-------	-------

Table 4. Levels of antibodies for *Chlamydia pneumoniae* in sera of patients with CAP

	Level of antibodies-Positive antibodies titers	Number and percentage of seropositive study patients [titers] (n=50)	Number and percentage of seropositive study patients having other pathogens isolated from sputum (n=50)	Number and percentage of seropositive study patients with negative culture from sputum (n=50)
Ch.pneumoniae IgM ↑ IgG ↑ IgA ↑ (acute infection suggested)	IgM>1:16 IgG>1:512 IgA>1:512	10 (20%)	7 (14%)	3 (6%)
Ch.pneumoniae IgG ↑ IgA ↑ (chronic or past infection suggested)	IgG 1:64-512 IgA 1:16-512	26 (52%)	20 (40%)	6 (12%)

patients, other pathogen were also isolated (Tab. 4). In the examined group of subjects, no case of *Legionella pneumophila* infection was identified in urine antigen test and immunofluorescence test. In four (8%) patients, increased levels of specific antibodies against *Mycoplasma pneumoniae* were observed (IgG>1:128, IgM>1:16); in 2 of these patients, other pathogens were isolated from sputa (Tab. 5).

Table 5. Levels of antibodies for *Mycoplasma pneumoniae* in serum of patients with CAP

	Level of antibodies - Positive antibodies titers	Number and percentage of seropositive study patients [titers] (n=50)	Number and percentage of seropositive study patients having other pathogens isolated from sputum (n=50)	Number and percentage of seropositive study patients with negative culture from sputum (n=50)
M. pneumoniae	IgM>1:16 IgG>1:512 IgA>1:512	4 (8%)	2 (4%)	2 (4%)

In some cases, two pathogens were isolated at the same time. Mixed pathogen isolation: *Klebsiella pneumoniae* + *Corynebacterium pseudidiphtericum*, *Staphylococcus aureus* + *Corynebacterium pseudodiphtericum*, *Haemophilus influenzae* + *Moraxella catharalis* was accompanied by the presence of high titer of *Chlamydia pneumoniae* (serological criteria of acute chlamydial infection). In 26 cases, only one pathogen was isolated, and was accompanied by serological markers of chronic or past chlamydial infection (Tab. 6). In the case of 4% of all patients with the pathogen isolated from sputum, high titer of IgG antibodies for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were observed.

DISCUSSION

Our knowledge concerning microbiological etiology of CAP is relatively limited, especially in the case of patients undergoing ambulatory treatment (15). Many studies conducted in order to evaluate causes of CAP in adults report that *Streptococcus pneumoniae* is the most common pathogen responsible for two-thirds of bacterial pneumonia cases (2). Other less frequently isolated typical etiologic agents are *Haemophilus influenzae, Moraxella catarrhalis,* and *Staphylococcus aureus.* Atypical pathogens: *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Legionella* species are common etiologic agents of CAP, associated with nearly half of all cases that result in hospitalization. In our study group (50 patients), unexpectedly no *Streptococcus pneumoniae* was isolated, however, more recent studies suggest a possible reduction in the etiologic role of this pathogen with the prevalence of atypical pathogens in CAP etiology (1.14). For example authors of one of the largest recent study (2 776 adult patients with CAP; USA) report that *S. pneumoniae* was implicated in only 12.6 % of CAP cases, comparing a low value with earlier CAP surveillance studies. They hypothesized that low rates of *S. pneumoniae* probably reflect the intensity of sputum Gram's stain and culture when these test were performed in the context of routine care (2,14). Other typical bacterial pathogens: *S. aureus, H. parainfluenzae, H. influenzae, M. catarrhalis,* similary to literature review data and other published studies, were isolated in 17.9%; 12.5%; 8.9%; 5.3% of cases, respectively.

One of the aims of the study was to determine the contribution of opportunistic species, for example *Corynebacterium pseudodiphteriticum*, in CAP etiology. It is known that despite being a well-known respiratory pathogen for immunocompromized patients, *C. pseudodiphtericum* has uncommonly been reported to occur in immunocompetent patients with CAP (4,7). In three of the examined patients, *C. pseudodiphtericum* was recognized as a single bacterial etiologic factor causing infection; in the case of other five patients, that agent was identified together with other typical or atypical bacterial pathogens. Apart from *C. pseudodiphteriticum* - *C. pneumoniae, K. pneumoniae* and *S. aureus* were detected as accompaning species, also acting in a pathogenic way.

Table 6. Concurrence of pathogens isolated from sputum and atypical microorganisms in mixed CAP etiology

Name of isolated strains	*S.aureus*	*H.parainfl.*	*C.albicans*	*C.pseud.*	*P.aerugin.*	*K.pneum.* *C.pseud.*	*H.influen.* *S.aureus*	*H.influen.*	*S.aureus* *C.pseud.*	*H.influen.* *M.catarrh.*	*M.catarrhal*	*E.coli*	*K.oxytoca*	*K.pneum.*	*S.viridans* *Neisseria spp.*
Number of isolated strains	7	7	3	3	2	1	2	2	1	1	2	1	1	1	16
Additionally *Chlamydia pneumoniae* IgM>16 IgG>512 IgA.512	1					1			1	1	1		1		1
Additionally *Chlamydia pneumoniae* IgG > 64< 512 IgA. >16 < 512	4	3		3	2		2	2			1			1	2
Mycoplasma pneumoniae IgM >,1:16 IgG > 1:128															2

In the presented study, serology diagnostic methods were used to detect atypical pathogens. In many patients, high stable titer for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, characteristic of acute or past/chronic infections, were observed; however, no *Legionella pneumophila* was identified using the antigen urine test and serology diagnostic. In 16% of examined patients, together with high titer for *Chlamydia pneumoniae* (IgG, IgA) – other bacterial pathogens were isolated. In 4% of all study group, high titer for *Chlamydia pneumoniae* + *Mycoplasma pneumoniae* with typical pathogens' isolation was detected. These cases of CAP can be diagnosed as mixed etiology infections. Atypical pathogens are frequently involved in CAP etiology and between one-third and two-thirds of such infections are probably of a coinfection character (9). It is known that atypical infection may predispose to coinfection with typical pathogens, and such mixed-etiology pneumonia may be associated with high mortality (16). The proportion of CAP cases attributed to each of the various pathogens varies between studies, depending on geographical location as well as specificity and sensitivity of the test used. Comparisons of epidemiologic studies of CAP are difficult because their authors used different methods to confirm diagnosis and only thirteen large epidemiologic studies of CAP have been published since 1995 to 2002 (1,3,5,8,11,17–22). Laboratory methods for detecting atypical pathogens are slow, and there is significant overlap between atypical and typical CAP manifestations. The review of the literature revealed high variability in diagnostic methodologies. Standardization of serological techniques and development of uniform criteria for interpretation of serologic findings is necesarry to increase our knowlege of atypical infections, since accurate prediction of etiology cannot be made purely on clinical and radiologic grounds only. Unfortunately, it is in many cases difficult to perform bacterial tests before treatment, so local epidemiological and microbiological data are very helpful in making empirical treatment decisions. Consequently, empirical antimicrobial therapy, sufficient for both typical and atypical pathogens are the best choise (macrolides, fluoroquinolones, tetracyclines, or new ketolides) and can be recommended for the treatment of community-aquired pneumonia (2).

CONCLUSIONS

1. Many cases of community-aquired pneumonia (CAP) are infections of mixed etiology.
2. Local epidemiological and microbiological data can differ from information coming from literature so the results of local studies are helpful in making empirical treatment decisions.
3. Antimicrobials selected for empiric treatment of CAP should demonstrate good activity against atypical agents (because of high prevalence of atypical pathogens in mixed pneumonia).

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SUMMARY

The objective of the study was determination of bacterial etiologic factors, including atypical pathogens, of community acquired pneumonia (CAP) in adults and of antibiotic sensitivity of isolated strains. The examined group comprised 50 patients with clinical and x-ray

image of pneumonia. Patients' expectoration sputum was analyzed. Amongst all isolated bacteria, the most frequent were *Staphylococcus aureus* – 17.9%, *Haemophilus parainfluenzae* –12.5% and *H. influenzae* – 8.9%. Identification of *Corynebacterium pseudodiphtheriticum* in 8.9% of CAP cases drew our particular attention. *Staphylococcus aureus* was the least antibiotic sensitive microorganism. In the majority of patients (26; 52%), serologic markers of chlamydial infection were determined. Pneumonia often results from mixed typical and atypical flora infection. High percentage of atypical pathogens in the examined material suggests the necessity to administer intracellularly acting antibiotics.

Charakterystyka bakteryjnych czynników etiologicznych zewnątrzszpitalnego zapalenia płuc

Celem pracy było określenie bakteryjnych czynników etiologicznych zewnątrzszpitalnego zapalenia płuc u dorosłych, z uwzględnieniem udziału patogenów atypowych, oraz ocena wrażliwości na antybiotyki izolowanych szczepów. Grupa badana obejmowała 50 chorych z klinicznym i radiologicznym obrazem zapalenia płuc. Materiałem badanym była odkrztuszona przez pacjentów plwocina. Poddana analizie liczba izolowanych z plwociny gatunków drobnoustrojów wykazała największy udział *Staphylococcus aureus* – 17,9%, *Haemophilus parainfluenzae* –12,5%, *H. influenzae* – 8,9%. Na szczególną uwagę zasługuje udział w CAP *Corynebacterium pseudo-diphtheriticum* – 8,9%. Najniższą wrażliwość na antybiotyki stwierdzono u *Staphylococcus aureus*. Większość analizowanych pacjentów (26–52%) miała serologiczne wskaźniki przebytej infekcji chlamydiowej. Zapalenia płuc często wywoływane są florą mieszaną typową i atypową. Wysoki odsetek w badanym materiale atypowych patogenów sugeruje konieczność stosowania w leczeniu empirycznym antybiotyków działających wewnątrzkomórkowo.