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*The role of opportunistic species of *Corynebacterium*  
*pseudodiphtheriticum* in pathogenesis of CAP  
(Community Acquired Pneumonia)*

The contribution of opportunistic species of the *Corynebacterium* type-*C. pseudodiphtheriticum* was signalled in many works among the described cases of inflammatory lower airways (1-5, 7-9,13). This potentially unpathogenic micro-organism occurring as an element of physiological flora on mucous membranes of nosopharyngeal cavity in favourable conditions may become a typical opportunist causing serious infections, especially in patients with hypoimmunity (2). Among the described cases besides respiratory tract infections (including pharyngitis, necrotising tracheitis, tracheobronchitis or bronchitis and pneumonia) (5,6,9,10,12) the following were found: endocarditis (4,11), urinary system infection, infection of wounds, infections of skin, and which is very important, the infections were related not only to patients with discovered hypoimmunity but also immunocompetent ones, in whom symptoms of hypoimmunity were not observed (3,7). This information has also been proved by our observations about the contribution of *C. pseudodiphtheriticum* in aetiology of CAP (Community Acquired Pneumonia) in immunocompetent patients.

The objective of the research was to determine the contribution of opportunistic species of the *Corynebacterium* type CAP in patients hospitalized in the Pulmonary Department of Medical University of Lublin.

MATERIAL AND METHODS

A group of patients subjected to a detailed analysis in relation to species of the isolated microorganisms, in which we were interested included 8 patients from whom *C. pseudodiphtheriticum* was cultured out of sputum, aged 27-67 with a medical history and clinical and radiological findings consistent with community acquired bronchopneumonia or lobar pneumonia. Patients had new infiltrates or consolidations on chest X-rays that cannot be attributed to some other aetiology. The patients complained of some of the following signs and symptoms of pneumonia: cough, sputum production, dyspnoea, pleuritic chest pain, chills or rigors, malaise and myalgias. Auscultatory findings such as rales or evidence of pulmonary consolidation were reported. Most of the observed patients had fever >38°C and leucocytosis > 10.000/mm<sup>3</sup>. There were not any people with an accompanying disease like diabetes, cancer or immune deficits among the examined patients.

Expectorated purulent samples were examined microscopically to determine the suitability for culture (presence of >25 polymorphonuclear leukocytes and <10 squamous cells per low power

field 100 x of a Gram stained specimen defined as an "adequate sputum"), in the visual area around 10 bacterial cells were found (club-like, Gram-positive). Adequate specimens were cultured according to standard microbiological methods. Identification of isolates has been made according to biochemical features by using API Coryne. In order to examine additional biochemical characteristics a set API ZYM was applied (bioMérieux). Identification of other species also isolated from sputum was made using API E and API Staph tests. Determination of antibiotic sensitivity of the isolated strains of *C. pseudodiphtheriticum* was performed by means of the disc diffusion method (according to Birby-Bauer) on the Muller-Hinton medium with addition of 5% sheep blood using the following discs with antibiotics for the determination: Penicillin (10 units), Ampicillin (10 µg), Piperacillin (100), Cepazolin (30), Cefuroxime (30), Cefotaxime (300), Imipenem (10), Gentamicin (10), Amikacin (30), Tetracycline (30), Erythromycin (15), Doxycycline (30), Vancomycin (30), Teicoplanin (30), Lincomycin (15), Clindamycin (2), Trimethoprim/ Sulfamethaxazol (23,75/1,25), Ciprofloxacin (5), Ofloxacin (5), Chloramphenicol (30) (Becton Dickinson). The zone of growth inhibition around discs was read out according to NCCLS recommendations.

In each patient blood culture was also made, their blood being collected on the day of sputum submission for examination. For microorganism cultures from blood the BBL Septi-Chek (TSB) medium was used (Becton Dickinson).

Within the routine serological diagnostics aiming at determination of the contribution of atypical pathogens in aetiology of the examined cases of CAP a serological examination was done towards *Chlamydia pneumoniae* with the application of the micro-immunofluorescence method (MIF - Pointe Scientific), and *Mycoplasma pneumoniae* (ELISA-Pointe Scientific). The examination consisted in determination of the level of specific antibodies in classes IgM and IgG (for *C.pneumoniae* also in class IgA) in patients' blood serum collected on the day of patient's admission to the clinic and microbiological examination of sputum.

## RESULTS

Analysis of eight cases of patients hospitalized in the Pulmonary Department of Medical University of Lublin was made with clinically diagnosed CAP, in which *C. pseudodiphtheriticum* was cultured from the collected samples of expectorated sputum (Tab. 1).

Table 1. Contribution of *C. pseudodiphtheriticum* and atypical microorganism in aetiology of CAP in the group of examined patients

Patient No	Age/Sex	Species isolated from sputum	Level of antibodies for <i>C. pneumoniae</i>			Level of antibodies for <i>M. pneumoniae</i>	
			IgM	IgG	IgA	IgM	IgG
1	64/ M <sup>b</sup>	<i>C. pseudodiphtheriticum</i>	<1:16	1:256	NEG <sup>a</sup>	NEG	NEG
2	67/ M	<i>C. pseudodiphtheriticum</i>	<1:16	1:512	1:128	NEG	NEG
3	62/ M	<i>C. pseudodiphtheriticum</i>	<1:16	1:512	1:256	NEG	NEG
4	66/ M	<i>C. pseudodiphtheriticum</i>	NEG	NEG	NEG	NEG	NEG
5	50/ F <sup>c</sup>	<i>C. pseudodiphtheriticum</i>	NEG	NEG	NEG	NEG	NEG
6	27/ F	<i>C. pseudodiphtheriticum</i>	NEG	NEG	NEG	NEG	NEG
7	59/ M	<i>C. pseudodiphtheriticum</i> <i>K. pneumoniae</i>	<1:16	1:256	1:128	* NEG	NEG
8	46/ M	<i>C. pseudodiphtheriticum</i> <i>S. aureus</i>	<1:16	1:256	NEG	NEG	NEG

NEG<sup>a</sup> – negative result, M<sup>b</sup> – male, F<sup>c</sup> – female

In six cases *C. pseudodiphtheriticum* was the only microorganism isolated from sputum, whereas in the other two cases simultaneously two other species potentially pathogenic were cultured – *Klebsiella pneumoniae* and *Staphylococcus aureus*. *C. pseudodiphtheriticum* was the dominating microorganism in reference to the accompanying species in the quantitative estimation in the direct sputum preparation stained by means of the Gram method as well as in the quantitative estimation of the culture growth  $10(4) - 10(7)$  CFU/ml. In direct sputum preparations stained by means of the Gram method Gram-positive club-like bacteria were observed occurring individually or in cakes made of a few. In the visual area they occurred in the number of around 10 bacteria cells on average. They were often located on the surface of epithelial cells existing in the sputum preparation. Growth of *C. pseudodiphtheriticum* on the Columbia agar medium with 5% sheep blood (bioMèricux) was observed after 24h in the form of small white-and-grey colonies that were non-shiny; after 48h the colonies reached the size of 1–2 mm, they were whitish, round, of mat surface and did not produce hemolysis of beta-type. They were catalase-positive.

Table 2. Biochemical characteristics of *C. pseudodiphtheriticum* strains isolated from sputum of patients with CAP

No	Reactions	Result positive % (n=8)	Result negative % (n=8)
1	Nitrate reduction	100	0
2	Pyrazinamidase	100	0
3	Pyrrolidonyl arylamidase	100	0
4	Alkaline phosphatase	62.5	37.5
5	Beta-glucuronidase	0	100
6	Beta-galactosidase	0	100
7	Alpha-glucosidase	0	100
8	N-acetyl-β-glucosaminidase	0	100
9	Esculin (β-glucosidase)	0	100
10	Urease	100	0
11	Gelatine (hydrolysis)	0	100
12	Glucose (f <sup>d</sup> )	0	100
13	Ribose (f)	0	100
14	Xylose (f)	0	100
15	Mannitol (f)	0	100
16	Maltose (f)	0	100
17	Lactose (f)	0	100
18	Sucrose (f)	0	100
19	Glycogen (f)	0	100
20	Catalase	100	0
21	Esterase (C4)	100	0
22	Esterase Lipase (C8)	100	0
23	Lipase (C14)	100	0
24	Leucine arylamidase	50	50
25	Valine arylamidase	0	100
26	Cystine arylamidase	100	0
27	Phosphatase acid	100	0
28	Naphthol-AS-BI-phosphohydrolase	100	0
29	α-galactosidase	0	100
30	α-glucosidase	0	100
31	α-mannosidase	0	100
32	α-fucosidase	0	100

f<sup>d</sup> - fermentation

Identification was made according to biochemical characteristics by means of API Coryne, the set for identification of Gram-positive bacteria. In the described cases the numerical identification of the observed profile for *C. pseudodiphtheriticum* was 7001004 - corresponding to 99.9 %Id or 7101004 - 99.6 %Id. Biochemical characteristics additionally determined by means of the API ZYM set proved uniform biochemical characteristics of the isolated stains within the species *C. pseudodiphtheriticum* (Tab.2). All strains showed reduction of nitrates, production of Pyrazinamidase, Pyrrolidonyl arylamidase, Urease, Catalase, Esterase, Esterase Lipase, Lipase, Cystine arylamidase, Phosphatase acid and Naphthol-AS-BI-phosphohydrolase. They did not have fermentation abilities in relation to the examined saccharides (glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, glycogen).

The isolated strains of *C. pseudodiphtheriticum* showed high antibiotic sensitivity (Tab. 3). They did not produce  $\beta$ -lactamases. Out of 20 antibiotics from different groups used for determination of strain susceptibility the highest resistance was discovered only towards 5 of them: Erythromycin (87.5%), Clindamycin (87.5%), Lincomycin (75.5%) as well as Trimeth./Sulfam.(37.5%) and Chloramphenicol (37.5%).

Table 3. Antibiotic sensitivity of *C. pseudodiphtheriticum* strains isolated from sputum of patients with CAP

No	Antibiotic	Sensitive % (n=8)	Intermediate % (n=8)	Resistance % (n=8)
1	Penicillin	100	0	0
2	Ampicillin	100	0	0
3	Piperacillin	100	0	0
4	Cefazolin	100	0	0
5	Cefuroxime	100	0	0
6	Cefotaxime	100	0	0
7	Imipenem	100	0	0
8	Gentamicin	100	0	0
9	Amikacin	100	0	0
10	Tetracycline	100	0	0
11	Erythromycin	12.5	0	87.5
12	Doxycycline	100	0	0
13	Vancomycin	100	0	0
14	Teicoplanin	100	0	0
15	Lincomycin	0	25.0	75.0
16	Clindamycin	0	12.5	87.5
17	Trimeth./Sulfam.	62.5	0	37.5
18	Ciprofloxacin	100	0	0
19	Ofloxacin	100	0	0
20	Chloramphenicol	37.5	25.0	37.5

In all patients in the examined group blood cultures were negative - no microorganisms were cultured. On the other hand, serological examination of blood serum (Tab. 1) collected at the same time (the first day of hospitalization when expectorated sputum was also examined) showed high titres in five patients of specific antibodies for *C. pneumoniae*, which in two patients were present only in class IgG (1:256), whereas three patients simultaneously had antibodies in two classes IgG (1:256 and 1:512) and IgA (1:128 and 1:256). The presence of specific antibodies in classes IgM and IgG for *M. pneumoniae* was not discovered.

## DISCUSSION

Contribution of opportunistic species of the *Corynebacterium* type and related coryneforms in infections in people was described in numerous works (1–5,7,8,13). Undoubtedly, the most endangered group are patients with observed lowered efficiency of the immunological system resulting from either a basic disease (neoplasm, leukemia, transplants, surgical interventions, AIDS), or past numerous infections, senile age, and a long-lasting antibiotic therapy. A separate group of people are patients in whom symptoms of hypoiimmunity were not found and the material collected from the place of infection is unequivocal in interpretation of the etiological factor, which is little pathogenic opportunistic species of *Corynebacterium*.

The basis of our elaboration were eight cases of infections in patients hospitalized because of CAP, from whose sputum *C. pseudodiphtheriticum* was isolated. The group of the examined patients, except for one person (27 years of age) included elderly people, above 50 years old, two females and six males. None of them showed hypoiimmunity. However, the acute course of infection (CAP), and with high temperature, required hospital treatment.

Microbiological examination of sputum enabled us to determine the probable etiological factor of the infection. In all cases *C. pseudodiphtheriticum* was isolated in a significant amount. In two cases other potentially pathogenic species were also found: *K. pneumoniae* and *S. aureus*. In qualitative estimation of the culture and microscopic examination of sputum stained by means of the Gram method *C. pseudodiphtheriticum* was the dominating microorganism. Undoubtedly, the infections in two cases with the accompanying species *K. pneumoniae* and *S. aureus* were of mixed character.

The isolated species of *C. pseudodiphtheriticum* showed high antibiotic sensitivity. Like in reports of other authors we also observed high sensitivity in our research to  $\beta$ -lactamase antibiotics (1), quinolones, glycopeptides and aminoglycosides, whereas we found resistance to Clindamycin (7) as well as resistance to Erytromycin (7,14) and lincomycin (14). The applied empirical antibiotic treatment with antibiotics of the cephalosporine and quinolone groups resulted in the expected regression of infection. Good effects of treatment of infections caused by *C. pseudodiphtheriticum* by antibiotics of these two groups were confirmed by other authors (3). Generally, treatment with  $\beta$ -lactamase antibiotics is recommended in case of low-resistant strains (1). In spite of acute course of pneumoniae, microorganisms isolated from sputum did not reach blood, which was shown by negative blood cultures in all patients.

The contribution of atypical antigens of *C. pneumoniae* proved a very important element of diagnostics of the analysed cases of CAP. In examination of specific antibodies their high level was found in class IgG in five out of eight patients and simultaneously in three of them also a high level of IgA. The level of antibodies in class IgM was very low.

In case of the first acute infection caused by *C. pneumoniae* the first antibodies (after around 3 weeks from the onset of the infection) that occur in blood are antibodies of class IgM. On the other hand, antigens of class IgG are present in diagnostic titre only after around 6-8 weeks. In case of subsequent infections by *C. pneumoniae* IgG titres reach a high level above 1:512 very soon. If an increased level of IgA (1:16–1:64 and higher) is observed at a high titre of IgG, it proves a chronic character of the infection (15).

In case of our group of patients it can be stated that two of them (first and eighth) experienced the infection caused by *C. pneumoniae* before the infection with *C. pseudodiphtheriticum* (titres in class IgG of 1:256 prove this fact). In three other patients (second, third and seventh) an increased titre was also observed in class IgG (1:256 and 1:512) with simultaneous high level of IgA (1:128, 1:256), which indicates a chronic infection by *C. pneumoniae* accompanied by infection by *C. pseudodiphtheriticum*. Based on these data one can believe that in those patients the infection caused by *C. pneumoniae* was the factor possibly predestining to opportunistic infection by a little-pathogenic strain like *C. pseudodiphtheriticum*. In the examined group of eight patients in whom *C. pseudodiphtheriticum* occurred in significant amounts only in three patients (fourth, sixth and seventh) it was the only pathogen of the infection.

In the other patients there was one of two or three contributing microorganisms in the infection. There are no doubts that *C. pseudodiphtheriticum* contributed as an opportunistic pathogen in community acquired pneumoniae (CAP) in the group of examined patients.

*C. pneumoniae* as a typical intracellular pathogen may result in long-lasting chronic infections with mild clinical symptoms. It cannot be ruled out that the response of the immunity system in case of chronic infections may predestine or promote infections caused by potentially unpathogenic - opportunistic microorganisms, including *C. pseudodiphtheriticum*. Since in cases described by other authors, where *C. pseudodiphtheriticum* was the cause of inflammatory lower airways, atypical pathogens such as *C. pneumoniae* were not found, their contribution should be also considered by the increase of serological diagnostics in this direction, in spite of the clinical symptoms not indicating the character of e.g. atypical pneumonia.

Three described cases, where *C. pseudodiphtheriticum* was an apparent etiological factor of pneumoniae proved the contribution of these microorganisms in aetiology of CAP.

## CONCLUSIONS

In the examined group of eight patients, suffering from CAP, on the basis of microbiological examination we recognised sputum as the etiological factor of infection by *C. pseudodiphtheriticum*. Only in three out of eight patients it was the only factor causing the infection. In the other patients, besides *C. pseudodiphtheriticum*, the microorganisms contributing to the infection include *C. pneumoniae* as a microorganism primarily causing the infection or predestining to infection, and *K. pneumoniae* and *S. aureus* as accompanying species, also acting in a pathogenic way. Application of cephalosporins and quinolones resulted in very good effects of treatment.

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#### SUMMARY

The analysis of eight cases of CAP (Community Acquired Pneumonia) was performed. The clinical samples of sputum were obtained from patients at which *C. pseudodiphtheriticum* strains were isolated in the quantity indicating the etiologic agent of infection. In two patients, *K. pneumoniae* and *S. aureus* were isolated simultaneously. They were considered as coexisting in the infection. *C. pseudodiphtheriticum* strains were highly susceptible to antibiotics. They were resistant to Erythromycin (87.5%), Clindamycin (87.5%), Lincomycin (75.5%), Trimeth./Sulfam.(37.5%), Chloramfenicol (37.5%). In the examined group of patients (five persons), the infection with *C. pneumoniae* was detected as recently passed or in progress with chronic character as the high level of specific antibodies (IgG or IgG and IgA) was present. That fact could predispose to infection with the opportunistic species of *C. pseudodiphtheriticum*. Of all the examined patients, three were infected with *C. pseudodiphtheriticum* as the only species responsible for infection (CAP).

#### Udział oportunistycznego gatunku *Corynebacterium pseudodiphtheriticum* w patogenezie zewnątrzszpitalnego zapalenia płuc

Przeprowadzono analizę ośmiu przypadków zewnątrzszpitalnego zapalenia płuc (CAP), w których z płwociny pacjentów izolowano *C. pseudodiphtheriticum* w ilości wskazującej, że jest to czynnik etiologiczny zakażenia. Od dwóch pacjentów izolowano równocześnie *K. pneumoniae* oraz *S. aureus*, które uznano za współuczestniczące w zakażeniu. Szczepy *C. pseudo-diphtheriticum* wykazywały wysoką wrażliwość na antybiotyki. Oporne były na: Erytromycynę (87,5%), Klindamycynę (87,5), Linkomycynę (75,5), Chloramfenikol (37,5) i Trimeth./Sulfam. (37,5). W badanej grupie chorych stwierdzono również u pięciu osób wysoki poziom przeciwciał anty - *C. pneumoniae* (tylko IgG lub IgG i IgA), co wskazuje na równoczesne zakażenie *C. pneumoniae* świeżo przebyte lub toczące się o charakterze przewlekłym, mogące predysponować do zakażeń oportunistycznych.