



# SPUTUM ISOLATES FROM AMBULATORY PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE – FREQUENT PATHOGENS AND ANTIBIOTIC RESISTANCE

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## ABSTRACT:

**Purpose:** The clinical aspects of antibiotic resistance (ABR) of patients in ambulatory practice with chronic obstructive pulmonary disease (COPD) are not investigated thoroughly. The aim of the present study is to find the most frequent respiratory pathogens of ambulatory COPD patients and to evaluate the resistance of their isolates to tested antibiotics.

**Material&Methods:** For 3 year period 249 COPD patients from Pulmonary Ambulatory Practice in Pleven were retrospectively studied. Data were collected from medical documents. The sputum samples from the ambulatory COPD patients were tested via direct microscopy and on culture medium. The ABR of the isolates was tested.

**Results:** Pathogens were isolated from 71 (28.5%) out of 249 sputum samples from COPD ambulatory patients. Most frequent isolates, we found were: *Pseudomonas aeruginosa*- 5.22%, *Hemophilus influenzae* - 4.82%, *Klebsiella pneumoniae* - 4.42% and *Escherichia coli* - 3.61%. A significant correlation was found between ABR and the results from lung function testing. In patients with forced expiratory volume per 1 second - FEV1 < 50%, antibiotic resistant isolates were found more frequently ( $\chi^2 = 7.59$ ,  $p=0.0059$ ). Similar correlation was found among smokers. Resistant isolates were found more often among smoker than in non-smokers ( $\chi^2 = 5.01$ ,  $p= 0.0252$ ).

**Conclusions:** The regular microbiological testing of sputum samples, including in ambulatory practice, with determination of most frequent isolates and their ABR would be helpful for the good clinical practice.

**Key words:** antibiotic resistance, ambulatory, COPD, sputum, isolates

## INTRODUCTION:

Respiratory infections play important role in the progression of the chronic obstructive pulmonary disease (COPD). Viruses, bacteria or irritants could cause COPD exacerbations. Around 70% of exacerbations are infectious in nature, either bacterial, viral or mixed. The theme about connection between infectious exacerbations of COPD and lower respiratory tract infections is still debatable. [1]. It is

fact that there is correlation between the bacterial colonization of the respiratory tract of patients with exacerbations of COPD or bronchiectasis as well as the development of antibiotic resistance by mutation [2, 3]. The clinical aspects of antibiotic resistance (ABR) of COPD patients in ambulatory practice are not investigated thoroughly.

The aim of the present study is to make clinical estimation of signs of antibiotic resistance in ambulatory patients with COPD.

## MATERIALS AND METHODS:

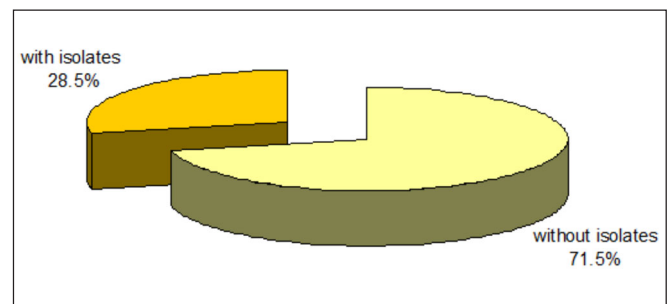
For 3 year period 249 COPD patients from Pulmonary Ambulatory Practice in Pleven were retrospectively studied. Data were collected from medical documents (documents from ambulatory examinations of the patients, results from microbiological sputum samples testing and results from lung function tests).

The sputum samples from the ambulatory COPD patients were evaluated via direct microscopy and on culture medium. The ABR of the isolates was tested. Patients were assessed by the following indices: sex, age, smoking status and lung function test results. The data were statistically processed by Statgraphics plus (for Windows version 2.1 6/20/96). The correlation between the mentioned above criteria and ABR was investigated.

## RESULTS:

Pathogenic isolates were found in 71 (28.5%) sputum samples of ambulatory COPD patients (n= 249) (fig.1).

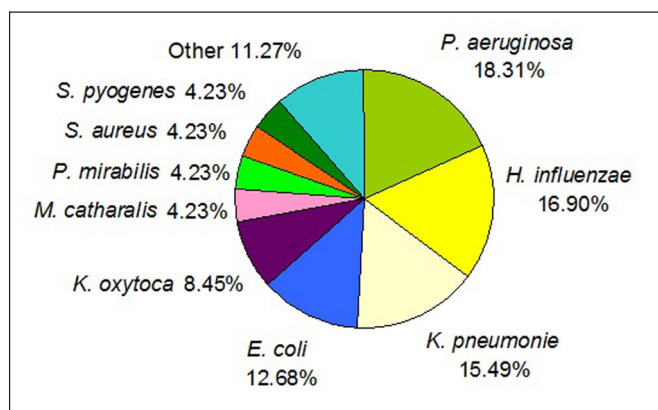
**Fig. 1.** Isolates were found in 28.5% of the sputum samples of ambulatory COPD patients (n= 249).



The average age was 68 years (SD ± 9.10). Male were 60 (84.5%) and female were 11 (15.5%). Non-smokers were 17 (25%) and ex-smokers and current smokers were 54 (75%), with 29.94 (SD ± 24.24) pack-years of smoking.

The most frequently isolated pathogens were: *Pseudomonas aeruginosa* - in 13 cases (18.31%), *Hemophilus influenzae* - in 12 cases (16.9%), *Klebsiella pneumoniae* - 11 (15.59%) and *Escherichia coli* - 9 (12.68%) (calculated as a percent of all cases with isolates) (fig. 2).

**Fig. 2.** Most frequent isolates from ambulatory COPD patients' sputum samples (presented as a percent of all cases with isolates).



All sputum samples with *P. aeruginosa* were found in smokers with COPD. Resistant pathogens to the tested antibiotics were isolated from 29 of 71 cases (40.9%). A significant correlation was found between ABR and the results from lung function testing. In patients with forced expiratory volume per 1 second - FEV1 < 50%, resistant isolates were found more frequently ( $\chi^2 = 7.59$ ,  $p = 0.0059$ ). Similar correlation was found among smokers. Antibiotic resistant isolates were found more often among smoker than in non-smokers ( $\chi^2 = 5.01$ ,  $p = 0.0252$ ). These results are presented in Table 1.

**Table 1.** Correlation between antibiotic resistance of the isolates, FEV1 and smoking status of the patients.

	Resistant isolates (n=29)	Sensitive isolates (n=42)	
Patients with FEV1 > 50%	9 (12.7%)	27 (38%)	
Patients with FEV1 < 50%	20 (28.2%)	15 (21.1%)	$\chi^2=7.59$ , $p<0.05$
Non-smokers	3(4.23%)	14 (19.72%)	
Smokers	26 (36.62%)	28 (39.44%)	$\chi^2=5.01$ , $p<0.05$

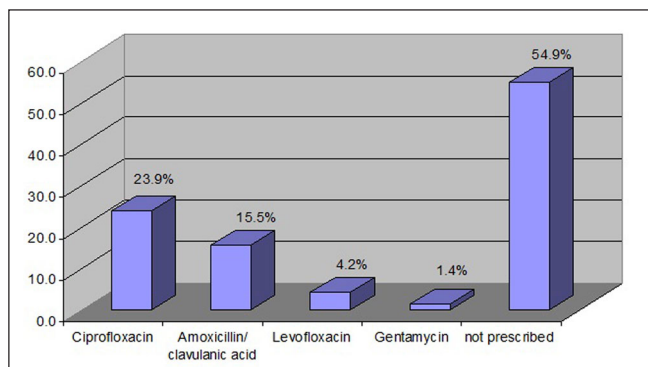
**DISCUSSION:**

There is a significant correlation between rates of antibiotic resistance and lung function in investigated patients. Patients with bad lung function (FEV1 < 50%) had more "resistant" pathogenic isolates ( $\chi^2=7.59$ ,  $p<0.05$ ). There was also a significant correlation between the ABR and smoking - antibiotic resistant pathogens were isolated more often from sputum samples from smokers than from

Patients with *H. influenzae* were predominantly elderly - half of them were at the age between 70-79 yrs (6/12 cases).

Antibiotic treatment was administrated in 32 (45.1%) of all patient with isolates (fig.3). Ciprofloxacin was prescribed in most of the cases with isolates (in 23.9% of the cases).

**Fig. 3.** The antibiotic treatment of the cases with sputum isolates was presented. In 54.9% of the cases antibiotic was not prescribed. The most frequently used antibiotics were Ciprofloxacin and Amoxicillin/clavulanic acid.



non-smokers ( $\chi^2=5.01$ ,  $p<0.05$ ) (Table 1).  
Viral and bacterial infections, alone or in combination, are important part of the COPD progression. They could be initial triggers for the disease or could provoke severe exacerbations. In the present study the most frequently isolated pathogen was *Pseudomonas aeruginosa* that was found only in smokers' sputum samples. This microorgan-

ism usually is found in patients in severe COPD stage, with severe exacerbation and on mechanical ventilation [4, 5]. According to Sethi the chronic infection with this pathogen leads to rapid progression of the disease [6]. The production and the presence of specific antibodies in the serum could not help to overcome the infection with *Pseudomonas aeruginosa* [4]. The frequent treatment of the severe exacerbations of patients in advanced stage of the disease with antibiotics is associated with developing an antibiotic resistance of *Pseudomonas aeruginosa* [5].

According to literature *Haemophilus influenzae* is one of the most frequently isolated pathogens in patients with COPD [4, 6]. In this study it was found in 16.9% of the cases with isolates. Older patients, between 70-79 yrs, were predominantly infected (6/12 of the cases with *H. influenzae*). This microorganism is easily adaptable to variable conditions by changes in its genetic expression, which can modify its virulence [4]. Permanent or repeatable exposition of the respiratory airways to the negative influence of metabolic substances from *Haemophilus influenzae* and the invasion of new serotypes may contribute for the infectious COPD exacerbation. The developed immune response is type-specific and do not prevent from re-infections, caused by antigen different strains [4]. Simultaneous presence of two of the most common respiratory pathogens - *H. influenzae* and *S. pneumoniae* in the upper airways leads to synergic inflammatory response with neutrophil infiltration in the respiratory mucosa [7]. Invasion with new *H. influenzae*, *S.s pneumoniae* or *M. catarrhalis* serotype doubled the risk for exacerbation in ambulatory COPD patients [8, 9].

*Klebsiella pneumoniae* is usually isolated from sputum samples of COPD patients. It was found in 15.6% of the cases with isolates in our study. Some authors claim that this pathogen is more typical for hospitalized patients (about 20% of the isolates) [10].

In 12.7% of the cases with isolates an *Echerihia coli* was found. This pathogen doesn't usually cause respiratory infections, but it could be found in elderly patients with a lot of concomitant diseases. *E. coli* is associated with severe pneumonias that necessitate hospitalization and are with higher lethal outcome rate [11].

In patients without isolates (178 cases) the exacerbation could be due to atypical bacteria, viruses or fungi. Some authors point out the importance of some viral respiratory pathogens (*RSV*, *Influenza A* and *B*, *Picornaviruses*) that could be found in up to 64% of the cases with COPD exacerbation [12, 13]. More specific serologic, microbiologic and immunologic, molecular techniques, tests and mediums are needed to find these pathogens. However current diagnostic methods increase the chance to isolate the pathogen and an etiological treatment could be conducted. *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, viruses (*Influenza*, *Parainfluenza*, *Rhinovirus*, *Adenovirus*, *RSV*) and fungi (*Pneumocystis jirovecii*) are though as frequent triggers for COPD exacerbation [6, 14]. The adequate interpretation of the results from the diagnostic tests is very important.

The more frequent isolation of resistant pathogens in patients with worse FEV<sub>1</sub> (<50%) could be explained with the patients main disease - COPD. FEV<sub>1</sub> decreasing in these patients is gradual and irreversible and the frequent exacerbations accelerate rapidly the worsening of the lung functions [8]. Many studies proved that frequent exacerbation lead to rapid progression of the COPD [4, 15, 16]. Patients pointed that their quality of life was worse after the exacerbation (assed by standardized questionnaires) [17]. In patients with lower lung functions a longer duration of the disease is supposed. It is associated with more frequent exacerbations [6] and bacterial infections. Constantly treatment of these infections with antibacterial drugs predisposes for development of antibiotic resistant pathogens [18].

We found that more frequently antibiotic resistant pathogens were isolated from sputum samples of smokers (former and current smokers). Ineffective clearance of the solid particles, containing in the cigarette smoke from the airways, leads to smoldering inflammation, which is essential for the disease progression. The cigarette smoke initiates the development of chronic obstructive bronchitis with fibrosis, obstruction of the small airways and losing lung elasticity [7], hyperplasia of the goblet cells with mucus hypersecretion and forming a mucus plugs, airways cells apoptosis. These processes result in progressive cells lost and developing emphysema. The airways epithelium is very susceptible to the effects of the oxidative stress, produced by the cigarette smoke during their direct contact [19]. An inflammation and destructive changes are induced by the reactive oxygen species in the cigarette smoke and those, produced by the alveolar macrophages (triggered by the cigarette smoke). The systemic immunity is affected: decreasing of serum immunoglobulins, which is a predisposition for frequent severe bacterial infections [7].

Antibiotic treatment was administrated in 45.1% (32 patients) of the cases with isolates. In the rest 29 cases with isolates, but without antibiotic treatment were considered as colonization. The presence of bacteria in the lower respiratory airways in patients with COPD in stable condition is determined as colonization (the bacteria are without or with minimal pathological effect) [4, 6]. Colonization is found in 1/4-1/3 of the patients with COPD (secretion samples were taken with protected specimen brush) [20] and in the present study the colonizations were about 1/2 of the cases. In patients with frequent exacerbations and adverse stage of the disease, colonizations are observed more frequently and pathogens' type and bacterial count is assessed. In patients with COPD the bacterial growth is facilitated by the altered local immunity in the lower airways and their difficult clearance via the mucociliary mechanism [9].

The pathological changes in the lung are determined by the colonized pathogen. Colonization with *P. aeruginosa* is associated with structural changes in the respiratory mucosa and with advanced stage of the disease [4, 7]. *H. influenzae* is found in the lower respiratory airways in about 30% of the patients with COPD, independently of the severity of the disease. Colonized patients with this microorganism have more intense expectoration with purulent spu-

tum during their stable condition and more expressed common symptoms during exacerbation [4]. Marin et al. found a significant correlation between colonization with some specific pathogens (*H. influenzae*, *P. aeruginosa*, *Enterobacteriaceae*) and the neutrophilia in the bronchial secretion in patients in moderate COPD stage [21]. Multi bacterial colonization is rarely found [9]. Pathogens often present in the lower respiratory tract of COPD patients in stable condition and researchers are trying to determine if these pathogens provoke bacterial infection or trigger a smoldering inflammation [22].

Smokers are predisposed to be colonized with some specific pathogens - *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. pyogenes*, while in non-smokers more frequent isolates are  $\alpha$ - hemolytic *Streptococcus*, *Peptostreptococcus spp.* and *Prevotella spp.* After smoking cessation, smoker microflora acquires the same characteristics as the microflora of non-smokers [7].

### CONCLUSIONS:

Bacterial and viral infections have important place in COPD exacerbations and during stable condition. It was found that the respiratory infections result in different as-

pects that were determined by complex relationships between the macroorganism and the pathogen. The presence of pathogens during stable conditions is associated with more complicated and advanced stage of the disease that requires a reevaluation of the previous concept of "colonisation" in COPD patients [22].

Exacerbations are important factor for incidence and mortality in COPD. They reflect on patients' quality of life, working capacity and require costly treatment. On the other hand the problem with antibiotic resistance is still insufficiently studied in clinical aspect, but it reflects on exacerbation severity. Because of these reasons, physicians and patients should make efforts to prevent exacerbations. The regular microbiological testing of sputum samples, including the ambulatory practice, with determination of most frequent isolates and their antibiotic resistance, would be helpful for the good clinical practice.

### Abbreviation list:

**COPD**- Chronic obstructive pulmonary disease

**ABR**- antibiotic resistance

**FEV1**- forced expiratory volume in 1 second

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