



Most common bacterial agents in hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease

Najčešći bakterijski uzročnici kod hospitalizovanih bolesnika sa akutnim pogoršanjem hronične opstruktivne bolesti pluća

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Abstract

Background/Aim. Infection is the major cause of acute exacerbation of chronic obstructive pulmonary disease (AE-COPD). The aim of the study was to establish the most common bacterial agents of AE-COPD in patients hospitalized in a tertiary medical care institution. **Methods.** This retrospective study included the patients hospitalized due to infective AE-COPD in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica in a 12-month period. We evaluated clinical data, spirometry, pathogen etiology in the patients with positive sputum findings and disease outcome. **Results.** The study included a total of 81 patients, 47 (58.02%) males and 34 (41.97%) females of the mean age of 65.7 years. The mean history of the disease was 14.5 years. The median forced expiratory volume in one second (FEV₁) was 30.12%. The most common isolated pathogens were *Pseudomonas aeruginosa* (n = 36; 38.29%), followed by *Haemophilus influenzae* (n = 25; 26.59%) and *Streptococcus pneumoniae* (n = 16; 17.02%). Coinfections were present in 9 (9.57%) of the patients. The median FEV₁ values of 28.67%, 37.23%, and 42.26% were registered in the patients with *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* induced infection, respectively. The case fatality rate (CFR) was 6.2%. Of the deceased, 100% had *Pseudomonas aeruginosa*-induced infection. A statistically significant difference in FEV₁ values was registered between the patients with and without *Pseudomonas aeruginosa* in their sputum finding ($p = 0.016$). **Conclusion.** The most common pathogens in patients with AE-COPD were *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. The CFR was 6.2%. All (100%) deceased patients had the infection induced by *Pseudomonas aeruginosa*.

Key words:

pulmonary disease, chronic obstructive; acute disease; diagnostic techniques and procedures; sputum; bacteriological techniques; *pseudomonas aeruginosa*; *haemophilus influenzae*; *streptococcus pneumoniae*; mortality.

Apstrakt

Uvod/Cilj. Infekcija je glavni uzročnik akutnog pogoršanja hronične opstruktivne bolesti pluća (AEHOBP). Cilj rada bio je da se odrede najčešći bakterijski uzročnici AEHOBP kod bolesnika koji su hospitalizovani u ustanovi tercijernog ranga. **Metode.** Ova retrospektivna studija obuhvatila je bolesnike hospitalizovane na Institutu za plućne bolesti Vojvodine zbog infektivne AEHOBP u periodu od jedne godine. Analizirali smo kliničke podatke, spirometriju, bakterteriološki nalaz sputuma i ishod bolesti. **Rezultati.** U studiju je bio uključen ukupno 81 bolesnik, 47 muškaraca (58,02%) i 34 žene (41,97%), prosečne starosti 65,7 godina, prosečnog trajanja bolesti 14,5 godina. Prosečni forsirani ekspiratorni volumen u jednoj sekundi (FEV₁) bio je 30,12%. Najčešći izolovani patogen bio je *Pseudomonas aeruginosa* (n = 36, 38,29%), potom *Haemophilus influenzae* (n = 25, 26,59%) i *Streptococcus pneumoniae* (n = 16, 17,02%). Mešana infekcija bila je prisutna kod 9 (9,57%) bolesnika. Prosečni (FEV₁) kod bolesnika sa izolovanim *Pseudomonas aeruginosa* iznosio je 28,67%, sa izolovanim *Haemophilus influenzae* 37,23% i sa izolovanim *Streptococcus pneumoniae* 42,26%. Ukupan letalitet iznosio je 6,2%. Svi (100%) preminuli imali su infekciju uzrokovanu *Pseudomonas aeruginosa*-om. Postojala je statistički značajna razlika u vrednosti FEV₁ između grupa bolesnika sa ili bez izolovanog *Pseudomonas aeruginosa* u sputumu ($p = 0.016$). **Zaključak.** Najčešći izolovani uzročnici AEHOBP bili su *Pseudomonas aeruginosa*, *Haemophilus influenzae* i *Streptococcus pneumoniae*. Ukupan letalitet iznosio je 6,2%. Svi (100%) preminuli imali su infekciju uzrokovanu *Pseudomonas aeruginosa*-om.

Ključne reči:

pluća, opstruktivne bolesti, hronične; akutna bolest; dijagnostičke tehnike i procedure; ispljuvak; bakteriološke tehnike; *pseudomonas aeruginosa*; *haemophilus influenzae*; *streptococcus pneumoniae*; mortalitet.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic progressive pulmonary disease which has a substantial morbidity and mortality^{1,2}. Over three million people died of this disease in 2005. COPD was the sixth most frequent mortality cause in 1990. Furthermore, it was predicted to take the third top position among death causes by 2020¹. With the disease progression, exacerbation occurs more frequently, inducing a faster lung function and life quality deterioration³⁻⁶.

The role of microorganisms, particularly bacteria, in COPD exacerbations has been in the focus of research for several decades. Over 70% of COPD exacerbations are due to respiratory infections, and bacterial infections are registered in more than 50% of the cases^{2,4,7-9}. Other exacerbation causes, including air pollution and temperature oscillations, are registered in 30% of the cases,^{2,10} while the causes of exacerbations remain obscure in even one third of the cases⁴.

The objective of this study was to identify the infectious agent of COPD exacerbations among the patients hospitalized in a tertiary medical care institution in one-year period. We analyzed if there existed a correlation between the bacterial agent and lung function (forced expiratory volume in one second – FEV₁), or the correlation between the bacterial agent and the treatment outcome, that is a fatal outcome of the disease.

Methods

This retrospective study, included the patients with acute exacerbation of COPD (AE-COPD) hospitalized in the Institute for Pulmonary Diseases of Vojvodina in 2012. The diagnosis was established in compliance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, with post-bronchodilation FEV₁/FVC (FVC – forced vital capacity) of < 70%. Acute exacerbation was diagnosed if the usual daily symptoms changed in terms of deteriorated dyspnea, cough and sputum production, requiring the treatment change.

The following characteristics of all the patients included in the study were reviewed: demographic features, symptoms, smoking habits, disease outcome; they were all submitted to physical examination, spirometry, staging of the disease in compliance with the GOLD criteria, arterial blood gas analyses, complete blood count, fibrinogen measurement and chest radiography. It was also analyzed which patients required the non-invasive or invasive mechanical ventilation and who had a fatal outcome of their disease. The fatal outcome of the disease was analyzed in correlation to the FEV₁ value and the presence of *Pseudomonas aeruginosa* in the sputum.

The patients with radiographically verified pneumonia were excluded from the study.

Microbiology

Expectorated sputum specimens were collected into sterile containers and processed according to the standard procedures¹¹. The microscopic sputum preparations were Gram stained, viewed under low magnification (100×), and used to establish leucocyte and epithelial cell counts. The presence

of ≥ 25 leucocytes and ≤ 10 epithelial cells in a single field of vision determined the specimen suitable for cultivation¹². Sputum samples were sputolysine-homogenized, and then, using the calibrated 1 μ L loop, cultivated in culture media: blood agar, chocolate agar, and MacConkey agar. Culture plates were then incubated at 37°C, the blood and chocolate agar in the presence of 5% CO₂, and the MacConkey agar in aerobic conditions. The culture plates were examined 24 hours later in order to establish the clinically relevant pathogens. To isolate *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, blood and chocolate agar were used. MacConkey agar was used to isolate *Pseudomonas species*, *Acinetobacter species* and *Enterobacteriaceae* spp. Bacterial agents were identified by the conventional methods, in compliance with the standard procedures¹³. The bacterial growth was interpreted semiquantitatively, determining the number of Colony Forming Unit (CFU) in one milliliter of the sample. The growth of the isolate with $\geq 10^5$ CFU/mL was considered diagnostically relevant¹¹.

Statistical analysis

Statistical analysis was performed using descriptive statistics, absolute and relative frequencies, mean \pm standard deviation (SD) values, χ^2 test, Student *t*-test, and nonparametric test (Mann-Whitney test).

Results

During the examined 12-month period, the positive bacteriological sputum finding was registered in 81 patients treated in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, with the total of 94 (27.6%) positive isolates of 340 examined sputum samples in total. Of these patients, there were 47 (58.02 %) males and 34 (41.97%) females. The mean age of the patients was 65.7 ± 8.48 . The disease had a 14.5-year long mean history (SD = ± 8.75). Among the examined patients, 56 (69.1%) were active smokers, 17 (21.0%) were ex-smokers, and 8 (9.9%) were non-smokers. The patients' mean FEV₁ amounted to $30.12 \pm 12.35\%$. Demographic features of the examined patients are presented in Table 1.

The patients' clinical features are presented in Table 2. Most patients (69; 85.2 %) had concurrent symptoms of dyspnea and productive cough, while in 12 (14.8%) patients the latter symptoms were accompanied with fever. The mean white blood count was $11.86 \pm 5.02 \times 10^9/L$. Four (4.9%) of the patients required non-invasive mechanical ventilation (NIV). Two (2.5%) of the patients were treated in the Semi-Intensive Care Unit and none in the Intensive Care Unit. The overall CFR amounted to 6.2%.

The most common pathogen was *Pseudomonas aeruginosa* isolated in 36 (38.29%) patients, followed by *Haemophilus influenzae* isolated in 25 (26.59%) of the patients, and *Streptococcus pneumoniae* isolated in 16 (17.02%) patients. The mixed bacterial infection type was registered in 9 (9.57%) of the patients. The sputum bacteriology is presented in Table 3.

Table 1
Demographic features of the patients with positive sputum bacteriology hospitalized in the Institute for Pulmonary Diseases of Vojvodina, n = 81

Parameter	Values
Age (years), $\bar{x} \pm SD$	65.7 \pm 8.48
Sex, n (%)	
males	47 (58.02)
females	34 (41.97)
Disease history (years), $\bar{x} \pm SD$	14.5 \pm 8.75
Smoking habits, n (%)	
active smokers	56 (69.1)
ex-smokers	17 (21.0)
non-smokers	8 (9.9)
Home oxygen concentrator, n (%)	
available	14 (17.3)
unavailable	67 (82.7)
Mean FEV ₁ (%), $\bar{x} \pm SD$	30.12 \pm 12.35

FEV₁ – forced expiratory volume in one second;
n – number of patients.

Table 2
Clinical features of the patients with positive sputum bacteriology hospitalized for AE-COPD, n = 81

Parameter	Values
Hospitalization length in days	14
Dyspnea, cough, sputum production, n (%)	69 (85.2)
Fever, n (%)	12 (14.8)
White blood count, $\bar{x} \pm SD$	11.86 \pm 5.02 $\times 10^9/L$
NIV, n (%)	4 (4.9)
Stay in the semi-ICU, n (%)	2 (2.46)
Overall mortality (%)	6.2

AE–COPD – acute exacerbation of chronic obstructive pulmonary disease; ICU – intensive care unit;
NIV – non-invasive mechanical ventilation;
n – number of patients.

Table 3
Sputum bacteriology in AE-COPD patients, n = 81

Parameter	n (%)
Total number of isolates	94
<i>Pseudomonas aeruginosa</i>	36 (38.29)
<i>Haemophilus influenzae</i>	25 (26.59)
<i>Streptococcus pneumoniae</i>	16 (17.02)
<i>Acinetobacter spp.</i>	7 (7.45)
<i>Moraxelacatarrhalis</i>	3 (3.19)
<i>Serratia spp.</i>	3 (3.19)
<i>Proteus spp.</i>	2 (2.13)
<i>Stenotrophomonasmaltophilia</i>	2 (2.13)
Mixed infection	9 (9.57)

AE–COPD – acute exacerbation of chronic obstructive pulmonary disease; n – number of patients.

FEV₁ findings were analyzed in correlation to the sputum bacteriology. The patients with isolated *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae* had the mean FEV₁ values of 28.67%, 37.23%, and

42.26%, respectively. The correlation of sputum bacteriology and FEV₁ values are reviewed in Table 4.

The CFR was 6.2%. Of the deceased, 100% had the infection induced by *Pseudomonas aeruginosa*. No statistically

Table 4

Sputum bacteriology correlated to FEV ₁	
Sputum bacteriology	FEV ₁ (%)
<i>Pseudomonas aeruginosa</i>	28.67
<i>Haemophilus influenzae</i>	37.23
<i>Streptococcus pneumoniae</i>	42.26
<i>Acinetobacter spp.</i>	24.23
<i>Moraxella catarrhalis</i>	35.20
<i>Serratia spp.</i>	37.73
<i>Proteus spp.</i>	38.25
<i>Stenotrophomonas maltophilia</i>	18.9

FEV₁ – forced expiratory volume in one second.

significant difference in FEV₁ values was registered between the patients who died and those who recovered ($p = 0.307$). A statistically significant difference in FEV₁ values was, however, registered between the patients with and without *Pseudomonas aeruginosa* isolated in their sputum samples ($p = 0.016$).

Discussion

This retrospective one-year study, carried out in the Institute for Pulmonary Diseases of Vojvodina, analyzed demographic features, bacteriological sputum findings, clinical characteristics and disease outcome in the patients hospitalized for an acute exacerbation of the chronic obstructive pulmonary disease in 2012.

In our study, we found that 81 patients had positive sputum bacteriology, with the total of 94 positive isolates of 340 examined sputum samples in total, or 27.6% positive isolates of all the examined samples. In their report published in the Chest in 2007, Ko et al.¹⁴ reported 32.3% positive sputum isolates of all the examined ones. In their article published in 2015, Ko et al.¹⁵ also reported 37.8% of positive sputum findings, while Boixeda et al.² in their study in 2012 reported 24.1% of positive bacteriological sputum findings, correlating well to our results.

Pseudomonas aeruginosa was the most common bacterium isolated in our study, registered in 38.29% of the patients, followed by *Haemophilus influenzae*, isolated from 26.59% of the patients, and *Streptococcus pneumoniae*, registered in 17.02% of the patients. In the study carried out by Ko et al.¹⁵ in 2005, similar results were obtained for *Haemophilus influenzae*, which was isolated in 23.1% of their cases, but not for *Pseudomonas aeruginosa* isolated in 6.3%, and *Streptococcus pneumoniae* isolated in only 4.0% of the cases, unlike our study results. Furthermore, Groenewegen and Wouters¹⁶ reported *Haemophilus influenzae* for the most common bacterium isolated in their study (in 45% of the cases), followed by *Streptococcus pneumoniae* isolated in 27%, and *Pseudomonas aeruginosa* registered in 15% of their cases, deviating from the results obtained in our study. Our results also differ from those obtained in the study carried out in Taiwan by Lin et al.⁵ in 2007, who isolated *Klebsiella pneumoniae* most frequently (19.6%), then *Pseudomonas aeruginosa* (16.8%), and *Haemophilus influenzae* (7.5%).

In our study, *Haemophilus influenzae* was more frequently isolated in the patients with FEV₁ > 30%, while

Ko et al.¹⁵ in their study carried out in 2005 isolated this bacterium more frequently in the patients with FEV₁ > 50%, correlating to the results reported by Lin et al.⁵ In our study, *Pseudomonas aeruginosa* was more frequently isolated in the patients with FEV₁ < 30%, as it was the case in the study by Ko et al.¹⁴ performed in 2007. In their study carried out in 2009, Larsen et al.⁶ reported the patients with FEV₁ < 1.0 L had *Pseudomonas aeruginosa*-induced infections more frequently. Lin et al.⁵ also reported *Pseudomonas aeruginosa* was more frequently isolated in very severe COPD cases, while Groenewegen et al.¹⁶ reported the positive sputum bacteriology was more frequently registered in the patients with lower FEV₁ in their study, but with no significant difference in the isolated bacterial species related to the severity of the disease.

All the deceased patients in our study had *Pseudomonas aeruginosa*-induced infection. Lin et al.⁵, applying the multivariate regression analysis in their study, have established the finding of *Pseudomonas aeruginosa* for one of independent case fatality risk factors, quite unlike Larsen et al.⁶ who reported the presence of this bacterium in the sputum had no influence on the CFR, which was primarily affected by the patients' age and low FEV₁. In our study, we registered no statistically significant difference in FEV₁ levels between the groups of deceased and recuperated patients ($p = 0.307$). However, the statistically significant difference in FEV₁ was established between the patients with and without the sputum finding of *Pseudomonas aeruginosa* ($p = 0.016$).

The major limitation of our investigation is that it included only bacteriological sputum findings, lacking the opportunity to perform virology analyses. Another limitation is that it provided no information on bacterial agents' sensitivity to antibiotics. In addition, our analysis included only hospital-treated exacerbations, lacking the insight into out-patient treated exacerbations. We will try to overcome these limitations in our additional future research work.

Conclusion

The most common bacterial agents isolated in our AE-COPD patients were *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. All the deceased patients had the infection induced by *Pseudomonas aeruginosa*. The patients with advanced disease stages had *Pseudomonas aeruginosa* sputum finding more frequently.

R E F E R E N C E S

- World Health Organisation. Chronic obstructive pulmonary disease (COPD). Available from: <http://www.who.int/respiratory/copd/en>
- Boixeda R, Rabella N, Sauca G, Delgado M, Martínez-Costa X, Mauri M, et al. Microbiological study of patients hospitalized for acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) and the usefulness of analytical and clinical parameters in its identification (VIRAE study). *Int J Chron Obstruct Pulmon Dis* 2012; 7: 327–35.
- Beasley V, Joshi PV, Singanayagam A, Molyneux PL, Johnston SL, Mallia P. Lung microbiology and exacerbation in COPD. *Int J Chron Obstruct Pulmon Dis* 2012; 7: 555–69.
- Global Strategy for the Diagnosis, Management and Prevention of chronic obstructive pulmonary disease. Available from: <http://www.goldcopd.org/>
- Lin S, Kuo P, Hsueh P, Yang P, Kuo S. Sputum bacteriology in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease in Taiwan with an emphasis on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Respirology* 2007; 12(1): 81–7.
- Larsen MV, Janner JH, Nielsen SD, Friis-Møller A, Ringbaek T, Lange P. Bacteriology in acute exacerbation of chronic obstructive pulmonary disease in patients admitted to hospital. *Scand J Infect Dis* 2009; 41(1): 26–32. 41(1): 26-32.
- Matkovic Z, Miravilles M. Chronic bronchial infection in COPD. Is there an infective phenotype. *Respir Med* 2013; 107(1): 10–22.
- Sethi S, Sethi R, Eschberger K, Lobbins P, Cai X, Grant BJ, et al. Airway Bacterial Concentrations and Exacerbations of Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care* 2007; 176(4): 356–61.
- Lode H, Allewelt M, Balk S, De Roux A, Mauch H, Niederman M, et al. A prediction model for bacterial etiology in acute exacerbations of COPD. *Infection* 2007; 35(3): 143–9.
- Sapey E, Stockley RA. COPD exacerbations. 2: aetiology. *Thorax* 2006; 61(3): 250–8.
- Health Protection Agency. Standard Unit. Evaluations and Standards Laboratory. Investigation of bronchoalveolar lavage, sputum and associated specimens: BSOP5711. London, UK: Health Protection Agency, Technical Services; 2005.
- Isenberg HD. Specimen Collection, Transport, and Acceptability. In: Isenberg HD, editor. *Clinical Microbiology Procedures Handbook*. Washington, DC: American Society for Microbiology; 2004. p. 1.5.1–1.5.18.
- York MK. Aerobic Bacteriology. In: Isenberg HD, editor. *Clinical Microbiology Procedures Handbook*. Washington DC: American Society for Microbiology; 2004. p. 3.1.1.-3.18.2.1.
- Ko FW, Ip M, Chan PK, Fok JP, Chan MC, Ngai JC, et al. A 1-year prospective study of the infectious etiology in patients hospitalized with acute exacerbations of COPD. *Chest* 2007; 131(1): 44–52.
- Ko FW, Ng TK, Li TS, Fok JP, Chan MC, Wu AK, et al. Sputum bacteriology in patients with acute exacerbations of COPD in Hong Kong. *Respir Med* 2005; 99(4): 454–60.
- Groenenegen KH, Wouters EF. Bacterial infections in patients requiring admission for an acute exacerbation of COPD; a 1-year prospective study. *Respir Med* 2003;7(7): 770–7.

Received on December 19, 2014.

Revised on August 7, 2015.

Accepted on November 11, 2015.

Online First July, 2016.