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The article was received
2020.04.09

UDC 616.233-007.64-008.8-076-053.8(477.63) https://doi.org/10.26641/2307-0404.2020.3.214823

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MICROBIOLOGICAL PROFILE OF SPUTUM IN STABLE ADULT PATIENTS WITH BRONCHIECTASIS IN THE DNIPRO REGION OF UKRAINE

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Цитування: Медичні перспективи. 2020. Т. 25, № 3. С. 104-110
Cited: Medicni perspektivi. 2020;25(3):104-110

Key words: *bronchiectasis, sputum, drug resistance, Pseudomonas aeruginosa*
Ключові слова: *бронхоектазія, бронхоектази, мокротиння, антибіотикорезистентність, Pseudomonas aeruginosa*
Ключевые слова: *бронхоэктазия, бронхоэктазы, мокрота, антибиотикорезистентность, Pseudomonas aeruginosa*

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Abstract. Microbiological profile of sputum in stable adult patients with bronchiectasis in the Dnipro region of Ukraine. Gashynova K.Yu., Suska K.S., Dmytrychenko V.V. *Chronic respiratory tract infection and relapsing exacerbations worsen the quality and reduce the life expectancy of patients with bronchiectasis. This work aimed to identify the spectrum of pathogens and to determine their profile of antibiotic resistance in the sputum of patients with bronchiectasis in the Dnipro region. Sputum of 60 patients in a stable phase with confirmed bronchiectasis was a subject to microbiological examination and determination of antibiotic sensitivity according to generally accepted CLSI recommendations. According to the results of the study, it was found that 70% of patients have sputum colonization by pathogens in the stable phase of the disease, and the most common pathogens are Pseudomonas aeruginosa and Haemophilus influenzae, which is in line with the global trend. Haemophilus influenzae was sensitive to ampicillin, amoxicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, ceftriaxone, cefotaxime, cefepime, ciprofloxacin, levofloxacin and moxifloxacin in a hundred percent of cases. However, more than half of the strains of Pseudomonas aeruginosa were resistant to one or more drugs with anti-Pseudomonas activity. In particular, the highest level of resistance was identified to such drugs as imipenem, aztreonam, ceftazidime. The problem of antibiotic resistance is alarming and once again indicates the need for the regular microbiological examination of the sputum of patients with bronchiectasis even in a stable phase for subsequent rational administration of antibacterial therapy.*

Реферат. Микробиологический профиль мокроты у стабильных взрослых больных бронхоэктазией в Днепровском регионе Украины. Гашинова К.Ю., Суська К.С., Дмитриченко В.В. *Хроническая инфекция дыхательных путей и рецидивирующие обострения ухудшают качество и снижают продолжительность жизни пациентов с бронхоэктазией. Целью данной работы было выявление спектра патогенов и определение их профиля антибиотикорезистентности в мокроте пациентов с бронхоэктазией Днепровского региона. Мокрота 60 пациентов в стабильной фазе с подтвержденной бронхоэктазией подлежала микробиологическому исследованию и определению антибиотикочувствительности согласно общепринятым рекомендациям CLSI. По результатам исследования установлено, что 70% пациентов имеют колонизацию мокроты патогенными возбудителями в стабильной фазе заболевания, а наиболее частыми возбудителями являются Pseudomonas aeruginosa и Haemophilus influenzae, что соответствует мировой тенденции. Haemophilus influenzae была чувствительна к препаратам ампициллин, амоксициллин, амоксициллин/клавуланат, пиперациллин/тазобактам, цефуроксим, цефтриаксон, цефотаксим, цефепим, ципрофлоксацин, левофлоксацин и моксифлоксацин в ста процентах случаев. Однако более половины штаммов Pseudomonas aeruginosa оказались резистентными к одному и более препаратам с антисинегнойной активностью, в частности, самый высокий уровень резистентности выявлен к препаратам имипенем, азтреонам, цефтазидим. Проблема антибиотикорезистентности настораживает и в очередной раз указывает на необходимость регулярного микробиологического исследования мокроты пациентов с бронхоэктазией даже в стабильной фазе с целью последующего рационального назначения антибактериальной терапии.*

Bronchiectasis (BE) is a chronic respiratory disease characterized by clinical cough syndrome, sputum production, chronic respiratory infection and radiologically abnormal permanent bronchodilation [11]. The prevalence of BE in Europe and North America ranges from 67 to 566 per 100,000 population, while in China it is 1,200 per 100,000. Chronic respiratory infection, inflammation and exacerbation are the main components of the alteration vicious circle which leads to damage to the bronchial walls and lung tissue [12]. Neutrophilic inflammation occupies a key position in the body's immune response to infection; in turn, neutrophil proteases lead to further damage to the tissues of the respiratory tract and trigger a cascade of pathophysiological reactions [11]. One of the main challenges for scientists and clinicians is the identification of etiological factors in the development of BE which is associated with the heterogeneity of various aspects of the disease [10]. From a clinical point of view, the classic patient with BE suffers from daily productive cough with periodic exacerbations, but there is a population of patients in whom BE can be detected accidentally during the examination for pneumonia or hemoptysis. In turn, some patients may have an almost asymptomatic course of the disease. From a functional point of view, patients can have both normal external respiratiry function and obstructive or restrictive disorders [8].

Exacerbations of BE is a key target for therapy as they are associated with increased severity of local and systemic inflammation and progressive lung damage [16]. Limited data indicate that during exacerbations patients with BE often secrete those bacterial species that usually colonize sputum in a stable phase [4]. Detection of the pathogen during exacerbation may be a consequence of bacterial growth of a pre-existing microorganism, and may be associated with gaining of new strains [15]. Existing recommendations for the management of BE patients suggest to study the culture of sputum at least once a year in a stable phase 11].

Pseudomonas aeruginosa and *Haemophilus influenzae* are now the most common bacteria found in the sputum and broncho-alveolar lavage of patients with BE, but other pathogens, including fungi, mycobacteria and viruses can also colonize the airways of patients [12]. It is known that the microbiological profile of sputum and the sensitivity of the detected pathogens to antibiotics in BE patients depends on many factors, including geographical location [12]. At the same time, there are only isolated data on the state of this problem in Ukraine [2, 3, 14].

The aim of the work is to study the microbiological profile of sputum of BE patients in the stable phase and the profile of antibiotic resistance in the Dnipro region of Ukraine.

MATERIALS AND METHODS OF RESEARCH

Patients with BE (residents of Dnipropetrovsk region) were prospectively included in the study on the basis of the Department of Occupational Diseases and Clinical Immunology of the State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine" from October 2018 to October 2019. Inclusion criteria: adult men and women with BE confirmed by high-resolution computed tomography on the basis of the following radiographic criteria: 1) no bronchial narrowing in the direction from the center to the periphery; 2) the inner diameter of the bronchi is larger than the diameter of the corresponding pulmonary artery or 3) visualization of the peripheral bronchi with an interval of one centimeter from the surface of the visceral pleura [6]. The stable phase of BE (absence of change of symptoms and a therapeutic mode within at least 8 weeks) was obligatory. Exclusion criteria: cystic fibrosis, active tuberculosis, history of malignancy, pregnancy and lactation.

Sputum samples were considered suitable for evaluation if they contained <10 squamous epithelial cells in the field of view under microscopy. Microbiological examination of sputum samples was performed by conventional bacteriological methods of growth on nutrient media [9]. Sputum was obtained by the method of spontaneous expectoration, in patients with unproductive cough it was planned to study the induced sputum. Susceptibility to antibacterial drugs was determined using the disco-diffusion method according to standards [7, 13].

Statistical analysis was performed using STATISTICA 6.1 (StatSoft Inc., USA, N AGAR909 E415822FA). Data were presented as mean (standard deviation, SD) in the case of quantitative variables, or as absolute numbers and percentage (n,%) in the case of qualitative variables. The distribution of variables was analyzed using the Shapiro-Wilk test. The chi-square criterion was used to compare two independent binary samples. The 95% confidence interval was calculated for independent variables, with $p \leq 0.05$ being considered significant [1].

RESULTS AND DUSCUSSION

60 patients with BE in a stable phase were included in the study. The average age of patients was 52.9±14.3 years (from 23 to 74 years), 15 of them were men (25%). Patients mostly had no history of smoking – 46 patients (76.7%) never smoked. In 52 (86.7%) patients sputum was obtained during spontaneous expectoration. Pathogens were detected in the sputum of 43 patients (71.7%), eight of whom had a combination of pathogens (13.3%). *Pseudomonas aeruginosa* (n=15) was the most common, of which six were mucoid strains (40%) and *Haemophilus influenzae* (n=15). The results of isolation of pathogens are presented in Figures 1 and 2.

Among the combinations of pathogens there were combinations of Haemophilus influenzae with Candida albicans (n=2), with Escherichia coli (n=1), with Klebsiella pneumoniae (n=1) and with Aspergillus spp (n=1); combinations of Pseudomonas aeruginosa with Haemophilus influenzae (n=1), with Aspergillus niger (n=1); combination of Escherichia coli with Candida albicans (n=1).

Among 15 clinical isolates of *Pseudomonas aeruginosa*, seven (46.7%) were sensitive to such antibacterial drugs as ticaricillin/clavulanate, piperacillin/tazobactam, aztreonam, ceftazidime, cefepime, imipenem, meropenex, ciprofloxacin, levofloxacin gentamicin, amikacin, tobramycin, three (20%) were resistant to one of the drugs (levofloxacin, gentamicin, imipenem) and five isolates (33.3%) were polyresistant. Among those with resistance, non-mucoid clinical isolates predominated – ix of the nine non-mucoid (66.7%) and two of the six mucoid (33.3%) were resistant to one or more antibacterial drugs, but statistically the difference was insignificant (p=0.9 by the criterion of chi-square). According to the data presented in Figure 3, *Pseudomonas aeruginosa* in this patient population was the most resistant to imipenem (33.3%), aztreonam (26.7%) and ceftazidime (26.7%). In turn, most clinical isolates were sensitive to ticarcillin/clavulanate (93.3%), piperacillin/tazobactam (93.3%) and levofloxacin (93.3%). Resistance to cefepime, meropenem, gentamicin and tobramycin was found in 20% of *Pseudomonas aeruginosa isolates*, and to ciprofloxacin and amikacin in 13.3%.

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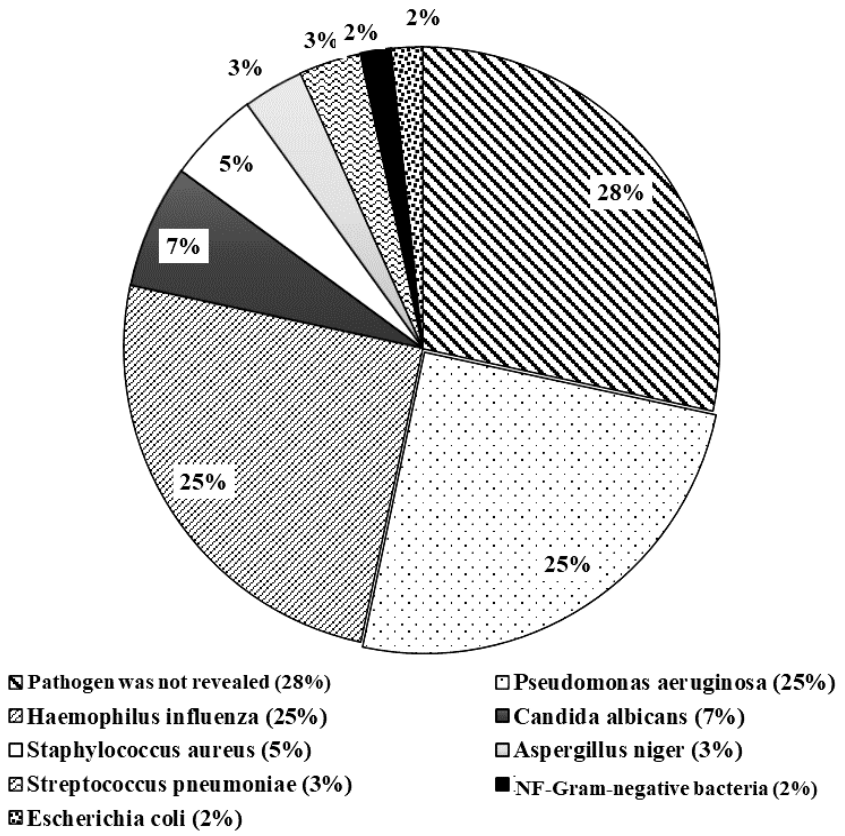

Fig. 1. Profile of microbiological pathogens revealed in sputum of patients with BE in a stable phase

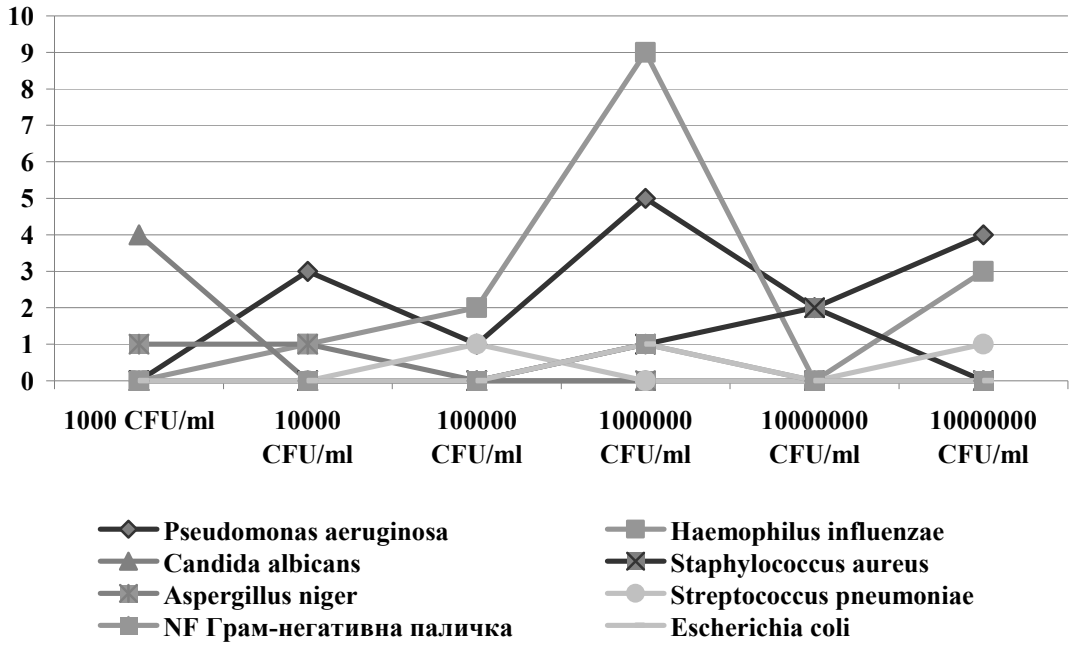

Fig. 2. Qualitative findings of isolated microorganisms in patients with BE in a stable phase

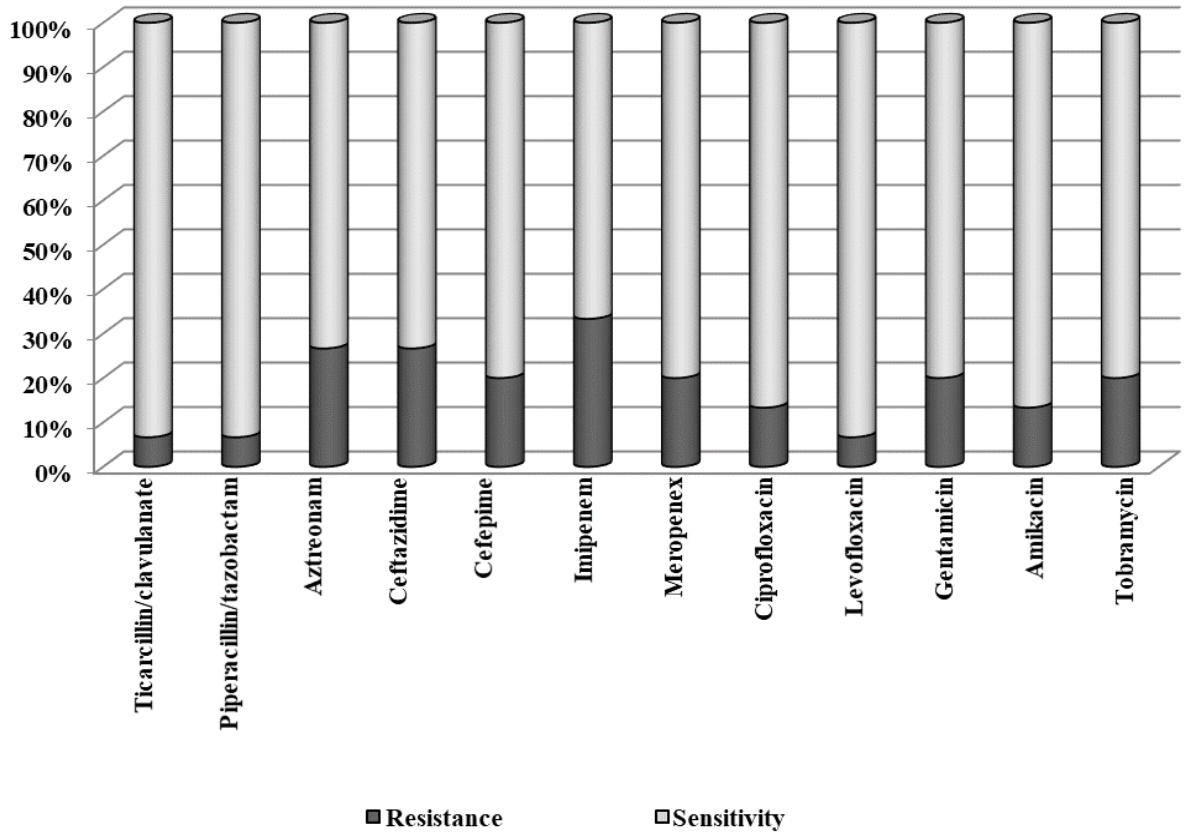

Fig. 3. Resistance phenotype of *Pseudomonas aeruginosa* to antibacterial drugs

All clinical isolates of *Haemophilus influenzae* (n=15) were sensitive to such drugs as ampicillin, amoxicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxin, ceftriaxone, cefotaxime, cefepime, ciprofloxacin, cyprofloxacin.

Among other pathogens resistance of two isolates of *Staphylococcus aureus* (66.7%) and one isolate of *Streptococcus pneumoniae* (50%) to drugs from the group of macrolides (erythromycin, azithromycin) and to clindamycin was revealed as well.

Among all clinical isolates that had resistance to at least one of the antibacterial drugs, only one isolate had a combination with another microorganism –polyresistant *Pseudomonas aeruginosa* (mucoid strain) with *Aspergillus niger* (9.1%).

CONCLUSIONS

1. Almost 87% of patients in the stable phase of the disease produce sputum and in 70% of patients pathological pathogens are detected using bacteriological methods of sputum examination. Pseudomonas aeruginosa and Haemophilus influenzae prevailed.
2. There is a problem of polyresistance of Pseudomonas aeruginosa to antibacterial drugs, in particular, more than a quarter of strains were resistant to imipenem, aztreonam and ceftazidime. At the same time, there was no statistically significant difference between the sensitivity to antibacterial drugs between mucoid and non-mucoid strains of Pseudomonas aeruginosa. The presence of an additional pathogenicity factor, such as the mucoid phenotype, requires further study.
3. Haemophilus influenzae in this population of patients was present in one hundred percent of cases sensitive to penicillins and protected penicillins, cephalosporins of the second, third and fourth generations, fluoroquinolones of the second and third generations. In turn, Staphylococcus aureus and Streptococcus pneumoniae have shown extremely high levels of resistance to macrolides and lincosamides, but the prevalence of these pathogens in this population is low, so further observations and studies are needed.
4. Based on the results obtained in BE patients in the Dnipro region, we consider it appropriate to monitor the microbiological profile of sputum of patients in a stable phase. And for the treatment of patients with frequent exacerbations, it is advisable to prescribe an antibiotic according to the resistance profile.

Conflict of interest. The authors declare no conflict of interest.

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The article was received
2020.01.08

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