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## **ENGLISH VERSION: THE EFFECTIVENESS AND NECESSITY OF CARRYING OUT ETIOLOGICAL DIAGNOSTICS IN PATIENTS WITH LOWER RESPIRATORY TRACT INFECTIONS\***

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*ACCORDING to our data, the effectiveness of microbiological diagnostics in patients with lower respiratory tract infections with the subsequent establishing etiologic diagnosis was 46%. Etiologic agent was identified in 35% of cases in patients with community-acquired pneumonia (CAP) and 58% of cases in patients with acute exacerbation of chronic obstructive pulmonary disease (AE COPD). H. influenzae, S. pneumoniae, H. parainfluenzae were the most common microorganisms detectable in patients with CAP and AE COPD 83,4% and 52,3% respectively. K. pneumoniae and P. aeruginosa (28.5%) accounted a considerable part of cases in patients with AE COPD. M. catarrhalis strains were determined in 14,4% of cases. S. pneumoniae strains resistance to penicillin was 22%, H. parainfluenzae and K. pneumoniae to aminopenicillins - 33% and 67%, respectively, S. aureus to aminoglycosides and fluoroquinolones - 33%. Multiple drug resistance was 3%. Etiologic diagnosis makes it possible to adjust and improve the efficiency of empirical antibiotic therapy based on the level of resistance of the pathogens despite the relatively low level of efficiency and the variety of identified pathogens.*

**Key words:** infectious agents, microbiological diagnostics, community-acquired pneumonia, chronic obstructive pulmonary disease, antibacterial drugs resistance.

In the treatment of infections of lower respiratory tract (ILRT) a key role belongs to anti-bacterial therapy (ABT), administration of which in the majority of cases is of empiric character, caused by necessity to start treatment in the shortest terms and is based on the data of world researches [9, 13,16].

The necessity of etiologic diagnostics is due to the need of ABT correcting in a concrete patient in the absence of response to initially administered empiric ABT therapy; this may be linked with atypical pulmonary pathogen or with the change of profile of resistance of main respiratory causative agents to anti-bacterial agents (ABA). Establishing etiologic diagnosis gives possibility, if necessary, to pass from ABA of a wide spectrum (or combined therapy) to agent of a narrow spectrum (to mono-therapy), favoring reduction of expenditures, decrease of risk of development of unfavorable reactions to medicinal agents and selection of antibacterial resistance [2,4,9].

In patients with community acquired pneumonia (CAP) undergoing treatment in out-patient unit, in accordance with confirmed treatment standards it is not recommended to carry out microbiologic sputum examination. This examination is being carried out under conditions of inefficiency of started empirical ABT or due to frequent relapses of respiratory episodes, taking into ac-

count short period of time, which may testify to pathogen presence, the activity of which was not stopped while using initially administered ABT [10].

Microbiologic examination of sputum in patients with infectious exacerbation of COPD is important both in the period of exacerbation with the aim to define etiologic causative agent, and in remission stage with the aim of revealing microorganisms, carriage of latter is characteristic for a patient during chronic course of the disease [7,11]. Due to the fact that carrying out a complete eradication of these microorganisms from respiratory ways is not possible, this information gives possibility to prognosticate increase of which causative agent's virulence may have etiologic significance in case exacerbation of the process. In the same manner it is important to pay attention to possibility of achieving positive result, linked with contamination of pathologic material with microorganisms of upper respiratory ways [8, 9].

In both cases amount of microorganisms concentration in the material examined in accordance with confirmed procedures should be considered, the higher the amount the more probable is etiologic significance of identified microorganism in the inflammatory process in a concrete nosology.

Carrying out microbiologic diagnostics in patients hospitalized to in-patient units is a necessary prerequisite

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for establishing etiologic diagnosis and is caused by a more severe course of the process as compared with out-patients and by presence of epidemiologic risk factors [2, 10]. However, even on condition of hospitalization, considering extended possibilities of laboratory diagnostics in revealing etiologically appreciable causative agents in patients with CAP and infectious exacerbation of COPD, this is possible only in 30-50% of cases, while carrying out microbiologic examination. [2, 9, 10].

Obtained negative result in the course of examination may be linked with a lot of factors, such as: insufficient awareness of patients on correct taking of clinical material, extensive territorial disunity of microbiologic laboratories and medical treatment facilities; this may have impact on time and quality of provision and transport of samples of pathologic material [4].

Taking into account the abovementioned, the aim of our work was assessment of carrying out microbiologic examination of pathologic material obtained from CAP patients and infectious exacerbation of COPD with the further revealing of structure of etiologically appreciable causative agents and level of their resistance to the main ABA, by means of performing investigations at clinical hospitals of Dnipropetrovsk.

### Materials and research methods

Patients with diagnosed CAP and infectious exacerbation of COPD undergoing in-patient treatment in clinics of Dnipropetrovsk were included in the investigation. Diagnosis of pneumonia was confirmed by X-ray and established according to the Order N128 of HM of Ukraine from 19.03.2007. Diagnosis of COPD was established in accordance with GOLD criteria (2008) and with the Order N128 of HM of Ukraine from 19.03.2007. Patients with administered anti-biotic therapy lasting less than 24 hours were not included in the study.

All patients underwent general clinical methods of examination, X-ray examination of chest organs in two projections, external respiration function (ERF) was assessed by means of spirograph MasterLab (Viasis, Germany).

Microbiologic examination was carried out at the laboratory of diagnostic center of LLP "Pharmacies of medical academy" of Dnipropetrovsk

Sputum discharge was used as material for investigation in microbiologic diagnostics. Identification of microorganisms was performed by means of conventional cultural methods [4]. Study of sensitivity to anti-bacterial agents was carried out by disc-diffusion method and by defining minimal inhibiting concentration (MIC) of antibacterial agent. Test of disc sensitivity was performed according to CLSI (earlier NCCLS guidelines) [18], as all methods of sensitivity defining.

Sampling was carried out in accordance with confirmed procedures [4]. Delivery of samples into laboratory

lasted for 1,5 -2 hours from the moment of taking, with observance of generally accepted rules of transportation.

Statistical processing of the data was done by means of "Microsoft Office Excel" and "Statistica 6" using non-parameter statistical methods.

### Research results

Investigation was divided into two stages. At the first stage etiologically appreciable microorganisms in patients with CAP and infectious exacerbation of COPD were defined, at the second stage - resistance of the revealed causative agents to ABA was analyzed.

In the process of investigation 102 patients were examined. Of all patients, 23 (22,6%) persons with CAP did not experience productive cough, even in stimulating of sputum production, they did not have indications for fiberoptic bronchoscopy, which would make it possible to obtain pathologic material directly from respiratory ways. These patients were excluded from further investigation. Data on investigation design are presented at figure 1.

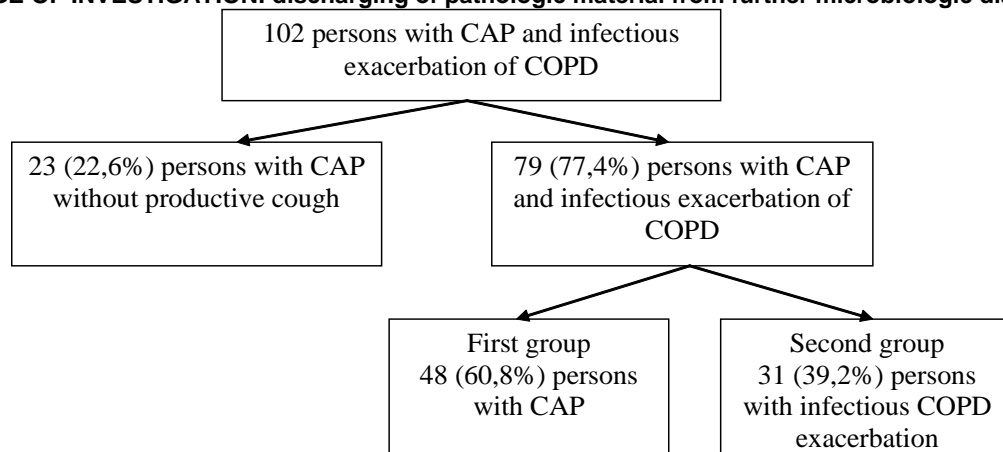
At the first stage 79 persons (77,4%) with productive cough were included in the investigation. They were divided into two groups according to the established diagnosis. First group included 48 persons with established diagnosis of CAP, median age was 58,6 (48,0-62,0) years; there were 27 men and 21 women, 56% and 44% correspondingly. Among them 12 (25%) patients were hospitalized on the first or second day from the onset of disease. The basic group of patients – 27 (56%) were hospitalized on the third and fourth day, 9 (19%) patients were hospitalized on the fifth and sixth day and longer period of time.

Second group included 31 persons with established diagnosis of COPD in the stage of infectious exacerbation, median age was 64,7 (53,0-67,0) years; there were 19 men and 12 women, 61% and 39% correspondingly. Herewith, 16 (52%) persons were hospitalized on the third – sixth day from the disease onset, in all other cases 15 (48%) – time period was more than one week.

Among two groups when carrying out microbiologic investigation of pathologic material from lower respiratory ways, namely sputum discharge, we managed to reveal infectious agents only in 46% of cases.

Combining of two or more etiologically appreciable microorganisms occurred in 7% of cases of revealing of causative agents among two groups. So, in the patient with CAP association of *H. influenzae* and *M. catarrhalis* was revealed, and in the patient with infectious exacerbation of COPD – combination of three main respiratory pathogens: *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*. Namely association of some causative agents may create conditions for increasing treatment period and lingering character of inflammatory process course in the lungs, due to specific features of each microorganism taken separately.

**I-ST STAGE OF INVESTIGATION: discharging of pathologic material from further microbiologic diagnostics**



**II-nd STAGE OF INVESTIGATION: study of resistance of identified respiratory pathogens**

36 (46%) of persons with identified etiologically appreciable microorganism

Fig. 1 Design of the investigation

Distribution of microorganisms in each group taken separately is presented in the table 1.

It should also be mentioned, that in the first group we managed to reveal etiologically appreciable causative agent in 17 (35%) of cases, herewith lesion of pulmonary tissue in these patients was more severe than in cases of negative research result, whereas in the second one etiologic causative agent was revealed in 19 (58%) of cases.

Table 1  
Distribution of infectious agents of both groups (abs (%))

Infectious agents	Amount of the isolated microorganisms	
	CAP	COPD
<i>H. influenzae</i>	7 (39%)	4 (19%)
<i>S. pneumoniae</i>	4 (22,2%)	5 (23,8%)
<i>H. parainfluenzae</i>	4 (22,2%)	2 (9,5%)
<i>M. catarrhalis</i>	1 (5,5%)	3 (14,4%)
<i>S. aureus</i>	2 (11,1%)	1 (4,8%)
<i>K. pneumoniae</i>	-	4 (19%)
<i>P. aeruginosa</i>	-	2 (9,5%)

Analyzing the data obtained, it should be mentioned that in patients of the first group the most part of identification accounts for *H. influenzae*, *S. pneumoniae*, *H. parainfluenzae* 15 (83,4%), whereas in patients of the second group together with the main respiratory causative agents such as *H. influenzae*, *S. pneumoniae*, *H. parainfluenzae* 11 (52,3%), a significant share of identifications of such gram-negative microorganisms as *K. pneumoniae*, *P. aeruginosa* 6 (28,5%) was defined; this is characterized by a severe and long-termed disease course and complications in providing efficient empirical ABT. In the patients of the second group strains of *M. catarrhalis* occurred the most often, than in the patients of the first group.

Strains *H. influenzae* and *S. pneumoniae* were related to the main respiratory pathogens in the both groups, level of their identification may be impacted by a rapid death of these microorganisms, due to their "nicety" yet before the beginning of investigation.

So, *H. influenzae* requires X and V (coenzyme ferment of dehydrase) blood factors in nutrient medium, nutrient medium of *S. pneumoniae* must be enriched with

defibrinated blood of animals in 5% concentration, they are in need of incubation in the atmosphere with the increased level of CO<sub>2</sub> [4,8].

It should be also mentioned that in 7(64%) of cases among 11 patients with CAP there were identified *H. influenzae* and *H. parainfluenzae*, in case-history presence of acute respiratory episode was noted. Infectious processes, caused by *H. influenzae* and *H. parainfluenzae* were characterized by a milder disease course and affected young and median age patients, whereas strain *S. pneumoniae* became etiologic cause both of severe disease course and moderate one and affected patients of all age statuses.

Strains of *S. aureus* were revealed in two cases in patients with CAP and in one case – in infectious exacerbation of COPD. Of them two patients from the first and second group were older than 60 years, and the third patient, aged 46 had acute respiratory episode in case-history. Though *S. aureus* is not related to the main etiologically appreciable causative agents in these nosologies, elderly age of a patient, alcohol abuse and presence of acute respiratory episode in case-history may be prerequisite of etiologic role of this causative agent in development of the disease. In these cases possibility of sputum colonization, which may mask pneumococcus or aspiration pneumonia should be considered too [8, 9].

Strains *P. aeruginosa* and *K. pneumoniae* were revealed in patients with infectious exacerbation of COPD 2/4 (28,5%) of cases, they are more typical for the diseases running chronic course. Among patients with CAP these causative agents were not revealed. They are able to cause a more severe course and are characterized by substantial levels of resistance, this in its turn, points to the necessity of establishing etiologic diagnosis in gram-negative infections [6, 7, 8].

Yet another problem, causing necessity of carrying out microbiologic diagnostics in patients with ILRT is a global slant of growth of microorganism resistance to ABA in the world. Defining of their sensitivity to ABA remains to be an important chain of diagnostic program.

For this reason at the second stage of our investigation there was carried out analysis of resistance of the revealed microorganisms to ABA. Distribution of these etiologically appreciable microorganisms is presented at the fig. 2.

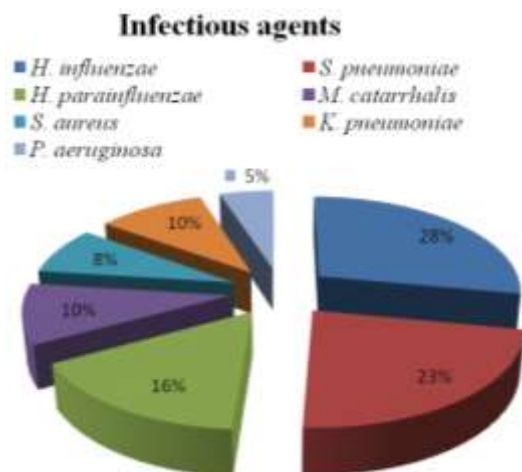


Fig. 2 Distribution of etiologically appreciable causative agents in patients with CAP and infectious exacerbation of COPD

Main microorganisms and ABA to which resistance was revealed are presented in table 2

By the data obtained in the course of investigation, *H. influenzae* was sensitive to the all ABA classes. Among *S. pneumoniae* 22% of strains were resistant to penicillins, 78% - sensitive ones, moderately sensitive were not revealed. 100% of *S. pneumoniae* strains were sensitive to macrolides, lincosamides, cephalosporins.

Obtained data as for resistance of *S. pneumoniae* to agents of penicillin row were similar with the data obtained in European countries, namely Romania and Bulgaria, Spain and Lithuania [13]. However, these data are higher than in other countries of Europe, where resistance to penicillin did not exceed 10% barrier. These data are confirmed by multi-center investigations, carried out in the world [12, 13, 14, 15, 16, 17].

Table 2  
Level of resistance of ILRW causative agents to ABA

ABA	<i>S. pneumoniae</i>	<i>H. parainfluenzae</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>
Penicillins	2 (22%)	-	-	-
Aminopenicillins	-	2 (33%)	-	2 (50%)
Defended aminopenicillins	-	1 (17%)	-	-
Aminoglycosides	-	-	1 (33%)	-
Fluoroquinolones	-	1 (17%)	-	-

Strains *M. catarrhalis* and *P. aeruginosa* were sensitive to the all classes of antibacterial agents. By the data of some multi-center investigations, *P. aeruginosa* is characterized by very sufficient levels of resistance to different classes of ABA, as distinct from the results obtained in our investigation [13, 15, 17].

Strains of *H. parainfluenzae* proved to be resistant to aminopenicillins in 33% of cases, to defended aminopenicillins and fluoroquinolones 83% of strains were sensitive, whereas 17% were resistant.

Strains *K. pneumoniae* in 59% of cases were also resistant to aminopenicillins. Whilst to defended aminopenicillins, cephalosporins, aminoglycosides and carbapenems strains *K. pneumoniae* were 100% sensitive. According to the world data, level of *K. pneumoniae* resistance to aminopenicillins is also sufficiently high and this causes necessity of a constant monitoring of this problem [13].

Considering strains of *S. aureus*, according to our data they were sensitive to caphalosporins and macrolids in 100% of cases, whereas resistance to fluoroquinolones and aminoglycosides was noted in 33% of cases. Moderately sensitive strains were not revealed.

Progressing of the disease severity, development of complications due to inefficiency of antibacterial therapy is also closely connected with microorganism resistance to two or more antibacterial agents. According to our data multi-resistance made up 3% and was sufficiently low.

Summing up it should be mentioned, that carrying out microbiologic diagnostics of pathologic material of patients with ILRT, taking into account accuracy of the performed investigation makes it possible to get answers arising in the process of etiologic diagnosis establishing, and in the following directs to rational correction, if necessary of empirically administered ABT; this increases efficacy of positive response to treatment program.

### Conclusions

1. We managed to reveal infectious agents of CAP and infectious exacerbation of COPD only in 46% of cases. According to our data, such microorganisms as *H. influenzae* (28%), *S. pneumoniae* (23%), *H. parainfluenzae* (16%), *M. catarrhalis* (10%) occurred the most often. Gram-negative infectious agents, such as *P. aeruginosa* and *K. pneumoniae* were defined in patients with chronic course of the process.

2. According to the obtained data, concerning sensitivity of infectious agents to ABA, there was determined resistance of *S. pneumoniae* strains to penicillins (22%), *H. parainfluenzae* and *K. pneumoniae* to aminopenicillins (33% and 50% correspondingly), *S. aureus* to aminoglycosides (33%), and *H. parainfluenzae* to defended aminopenicillins and fluoroquinolones (17%).

3. Microbiologic diagnostics must remain an integral chain of diagnostic program, despite sufficiently low level of effectiveness and taking into account a constant growth of resistance, whereas investigation itself requires more responsibility in the process of sampling and studying pathologic material to provide increase of its efficiency.

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