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**EVOLUTION OF BRONCHITIS IN YOUNG PATIENTS FROM THE POINT OF VIEW OF INSTRUMENTAL AND BACTERIAL EXAMINATION METHODS**

ORIJANA TYSH, LARYSA MATIUKHA, ANATOLY SERGIENKO*
Department of Family Medicine and Outpatient Care Shupyk National Medical Academy of Postgraduate Education;
*Kyiv City Clinical Hospital No. 17

**ИНСТРУМЕНТАЛ ВА БАКТЕРИАЛ ТЕКШИРИШ НУҚТАИ НАЗАРИДАН ЁШЛАРДА БРОНХИТ ЭВОЛЮЦИЯСИ**

ОРИДЖАНА ТИШ, ЛАРИСА МАТИУХА, АНАТОЛИЙ СЕРГИЕНКО*
Шупика номидаги дипломдан кейинги таълим Миллий тиббиёт академияси оилавий тиббиёт ва амбулатор даволаш бўлими;
*Киев шаҳар 17 - клиник шифохонаси

**ЭВОЛЮЦИЯ БРОНХИТА У МОЛОДЫХ ПАЦИЕНТОВ С ТОЧКИ ЗРЕНИЯ МЕТОДОВ ИНСТРУМЕНТАЛЬНОГО И БАКТЕРИАЛЬНОГО ИССЛЕДОВАНИЯ**

ОРИДЖАНА ТЫШ, ЛАРИСА МАТИУХА, АНАТОЛИЙ СЕРГИЕНКО*
Отделение семейной медицины и амбулаторного лечения Национальной медицинской академии последипломного образования имени Шупика;
*Киевская городская клиническая больница № 17

*Биз 18 ёшдан 35 ёшгача бўлган 128 нафр беморни текширишдан ўтказдик. Натижаларни таққослаш учун алоҳида гуруҳ сифатида пастки нафас йўлларининг касалликлари бўлмаган 20 нафар кўнгиллилар (валантер) танланди. Уларда нафас йўларининг ёт жисми бор эди ва уларга бактериологик текшириш учун бронхоальвеоляр лаваж билан бронхоскопия ўтказилди. 108 нафар беморда рецидивланувчи бронхит (ICD-10 J.40) диагностика қилинди. Бу беморлар бронхоальвеоляр лаважда лактобацилл бактериялар ва бифидобактериялар тўлиқ ёки нотўлиқ борлиги ёки йўқлигига боғлиқ равишда қўшимча 3 гуруҳга бўлинди. Антибактериал терапия таъсирида ичакларда биоценоз ўзгаришини бронхлардаги микробиоценоз ўзгариши билан таққослаш ва улар ўртасидаги боғлиқликни аниқлаш мақсадида ахлат ҳам қўшимча бактериологик текширишдан ўтказилди. Статистик микробиоценоз бўйича параметрлари ҳар хил бўлган (p<0,05) антибиотиклар 47,5% қабул қилган I гуруҳдаги беморларга нисбатан антибиотиклар қабул қилган III гуруҳдаги 80% беморларда бронхиал микробиоценоз энг юқори кўрсаткичларга эришди. 72,2% беморлага антибиотиклар қўлланилган II гуруҳдаги беморларда лакто- ёки бифидобактериялар аниқланди. Аммо бу ўзгаришлар I гуруҳдаги параметлар бўйича деярли фарқ қилмади (p<0,06), бу бронхиал микробиоценозда манфий ўзгаришлар тўлиқ бўлмаганлигидан дарак беради. Олинган маълумотларга асосланган ҳолда, пастки нафас йўлларида қўшимча касалликлари бўлмаган 100% текширилган беморларда бронхоальвеоляр лаважда лактобацилл ва бифидобактериялар аниқланди.*

***Калит сўзлар:*** *микробиоценоз, бронхит, лактобацилл бактериялар, бифидобактериялар, бронхоальвеоляр лаваж.*

*We examined 128 patients with the age from 18 to 35 years. 20 individuals without preexisting lower respiratory tract diseases were chosen for comparison as a seperate group. They were clinically and endoscopically dignosed with a foreign body of the respiratory tract and underwent bronchoscopy with bronchoalveolar lavage for bacteriological analysis. 108 patients were diagnosed with recurrent bronchitis (ICD-10 J.40). These patients were further divided into 3 groups. We also performed the additional bacteriologic analysis of feces in order to determine the relationship between changes in the bronchial microbiocenosis under the influence of the antibiotic therapy in comparison with the changes in the intestinal biocenosis. 80% of patients from the group III (no lactobacilli bacteria and bifidobacteria), who received antibiotics, demonstrated chages in bronchial microbiocenosis, which were statistically different (p<0,05) from the parameters of the microbiocenosis in the group I (control), where antibiotics were administered in 47,5%. In the patients from the group II, where antibiotic therapy was used in 72,2% of patients, either lacto- or bifidobacteria were found. However these changes were not significantly different (p<0,06) from the parameters of the group I, which may indicate the incompleteness of negative changes in the bronchial microbiocenosis. Based on the acquired data we found lactobacilli bacteria and bifidobacteria in bronchoalveolar lavage in 100% of examined individuals, who had no prior lower respiratory tract diseases.*

***Key words:*** *microbiocenosis, bronchitis, lactobacilli bacteria, bifidobacteria, bronchoalveolar lavage.*

**Introduction.** The colonization of the human body by microorganisms starts immediately after the birth from the surrounding environment, which plays a major role in the development of a healthy microbiome. After the complete development the microbiome becomes an independent body that provides vital functions for its host [3,9,6]. According to the latest knowledge, the normal human microflora is considered to be a system consisting of many microbiocenoses. They are characterized by a specific composition of microflora species and occupy the specific biotope in the human body.

Until recently, the whole number of microorganisms living in the healthy human body, remained unexplored. Today due to the large international research trials within the Human Microbiome Project Meta HIT project (2008-2016), it became possible to provide a better understanding of the role of the microflora. The human body contains trillions of microorganisms. Their number exceeds the number of somatic cells in 10 times, and their total mass comprises 1-3% of the human body weight (Human Microbiome Jumpstart Reference Strains Consortiumet al., 2010; National Human Genome Research Institute 2012; Meta HIT Consortium, 2016). Microorganisms colonize not only all surfaces, but also tissues and organs that were once considered sterile – breast milk, placenta, bronchi (Martin R.et al., 2010; Gerritsen J.et al, 2011; Aagaard K. et al., 2014) [7, 11].

The role of normal respiratory microflora in maintaining the homeostasis of an organism is well established. The divisions of the upper respiratory tract have a high microbial burden, since their mucous membrane is the first to resist the actions of various environmental factors. At the same time the microflora itself protects the body from pathogenic microorganisms, providing "colonization immunity", that is, the resistance of the mucous membranes to more aggressive microbial colonization prevents the fixation of bacteria and all other pathogens to their surface [1,3,5].

The human organism and its normal microflora is the only interrelated, interdependent natural complex, which state largely determines the state of human health. Under the influence of various adverse factors on the human body, or its normal microflora, changes occur in the internal environment of the organism, and the state of its normal microflora, first of all in the intestinal microflora. In the different parts of the gastrointestinal tract, the composition of normal microflora significantly differs. Normal microflora of the oral cavity and pharynx is characterized by a large variety of known species. It consists of streptococci, staphylococci, lactobacilli, corinebacteria and a large number of anaerobes, especially bacteroids [8,10].

It has been established that the human intestine contains more than $10^{14}$ microorganisms, that belong to more than 1000 species and form an intestinal microbiote. The predominance of a certain type of microflora depends on genetic, geographical, ethnic and other factors. Each person has a unique inherent microflora that is associated with the diet, family history, the presence of diseases, the region of residence and other factors.

There are 3 levels of natural barrier protection that is performed by the normal intestinal microflora. The first level (microbial-microbial), when the normal flora interferes with colonization by pathogens and provides colonization resistance by competition for substrates. The second level (microbial-epithelium), when the normal microflora maintains and enhances the barrier function of the biotope cells by increasing mucus production, thickening of the connections, regeneration of the epithelium. The third level (microorganism-immune system) is associated with the fact that a healthy intestinal microflora has immunomodulatory function and is able to enhance the immune response. [2,4,12].

Based on the above mentioned data, we hypothesized the possible presence of lactobacteria and bifidobacteria as the microflora, which provides "colonization immunity" in bronchi. Violation of this balance may occur in cases of the lower respiratory tract diseases (recurring bronchitis, J40).

**Purpose.** To study the evolution of bronchitis using radiological methods, bronchoscopy and bacteriological studies in young patients with lower respiratory tract infection under the influence of treatment.

**Materials and methods of the study.** We performed a prospective study of 108 patients with the age from 18 to 35 years, who were examined at the Department of Family Medicine and Outpatient Care, Shupyk National Medical Academy of Postgraduate Education (NMAPE) from November, 2018 to May, 2017. 61 were men (56.5 ± 4.8%), and 47 – women (43.5 ± 4.8%). A control group included 20 individuals without lower respiratory diseases (men – 13 (65 ± 10.7%), women – 7 (35 ± 10.7%). All patients in the study group had recurrent bronchitis (ICD-10: J.40 - bronchitis is not specified as acute or chronic), with 3-4 episodes within the previous 6 months. The duration of the disease was 0.5 to 2 years, which was manifested by repeated cough, sometimes with sputum, increase in body temperature 37.5 ºC - 39.2 ºC, difficulty breathing. Chest X-ray was performed in all patients for differential diagnosis. In all patients, bronchoalveolar lavage was taken during the bronchoscopy procedure at the endoscopic department of the Kyiv City Clinical Hospital No. 17 and the Kyiv Regional Clinical Hospital.

The material was taken from patients during antibiotic therapy, which took into account a number of antibiotics and the timing of administration. The obtained material was placed in a sterile container and was transported to the bacteriological laboratory

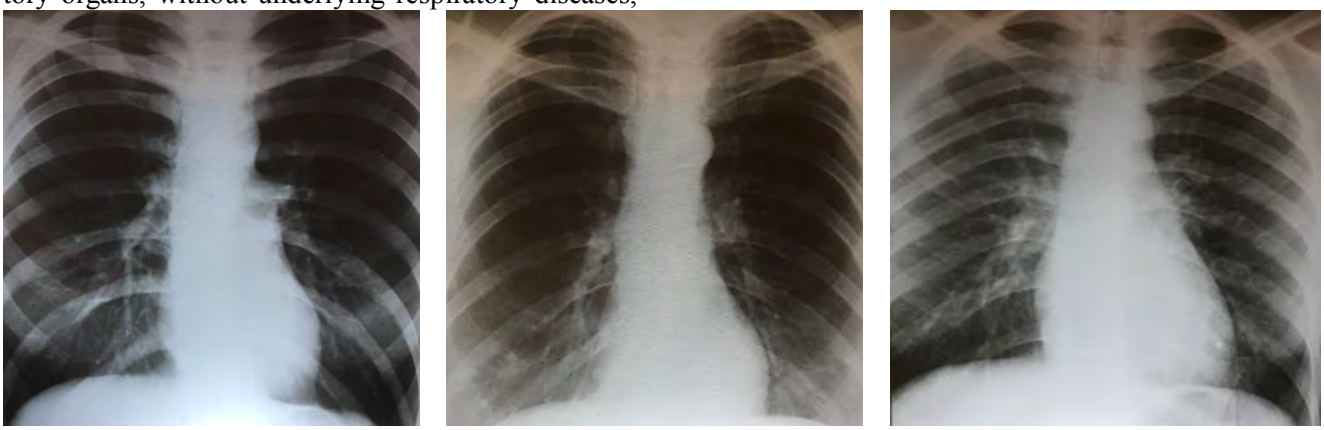
of the "Ukrainian Center for Control and Monitoring of the Ministry of Health of Ukraine" for up to 2 hours. After the material was delivered to the laboratory, a microbiological study was started. Bronchoalveolar lavage (BAL) 0.1 ml was placed on a blood agar (BA), chocolate agar (CHOC), Endo medium, yellow-salt agar (YSA), Enterococcus Selective Agar, Saburo agar. In addition, the BAL was diluted from $10^{-1}$ to $10^{-8}$, and 1 ml of this solution was seeded in a medium for bifidobacteria and lactobacilli.

Cultures on blood agar, chocolate agar and Endo medium were incubated at t $^0$ 37 $^0$C during 24 hours, at yellow-salt agar - at 37 $^0$C during 48 hours, on Saburo agar - at 24 $^0$C during 5 days, and on the medium for bifidobacteria and lactobacilli - at 37 $^0$C during 72 hours. We also made a series of dilutions of feces from $10^{-1}$ to $10^{-8}$. The resulted suspensions were sown on nutrient medium in the following order: 0.1 ml at $10^{-1}$- Endo medium, Ploskirev and Selenite broth, $10^{-3}$ - Endo medium, yellow-salt agar, Enterococcus Selective Agar, Saburo agar, Simon's medium, blood agar 1 ml at $10^{-6}$, $10^{-7}$, $10^{-8}$ - medium for bifido- and lactobacillus. Sowings from Endo medium, Ploskirev, Selenite broth and bifidobacterium and lactobacillus medium were incubated at 37 $^0$C during 24 h on yellow-salt agar, Enterococcus Selective Agar, Simon medium at 37 $^0$C during 48 hours, in medium for bifidobacteria and lactobacilli at 37°C during 72 h, Saburo agar - at 24 $^0$C during 5 days. Sowing from Selenite broth was placed on bismuth-sulfite agar and incubated at 37 $^0$C during 48 hours.

All sowings from bronchoalveolar lavage and feces were reviewed, colonies of every kind of species were counted and identified by studying their morphological, cultural, tincture and biochemical properties. All nutrient mediums, both for primary sowing and for the identification of microorganisms, were developed by "Pharmactiv" Ltd. (Kyiv, Ukraine). 20 young patients with clinical and bronchoscopic diagnosis of foreign bodies of the respiratory organs, without underlying respiratory diseases, who had not received antibiotic therapy within the previous 12 months, were selected as a control group. We considered these individuals having a healthy microflora of the respiratory tract. Depending on the presence or absence of lactobacilli bacteria and bifidobacteria, all examined patients with recurrent bronchitis were divided into 3 groups.

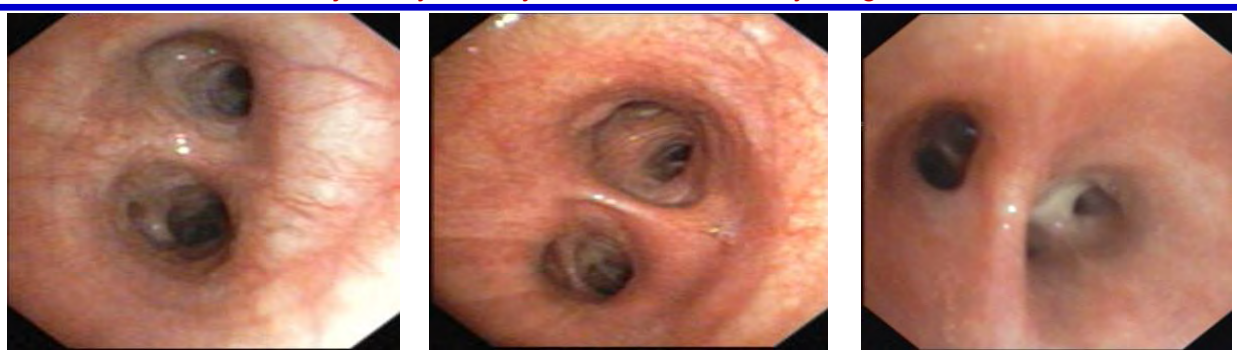
The results were processed using the Microsoft Exel® and STATISTICA for Windows 7.0. For each parameter, the mean (M) and its standard deviation (SD) were calculated and the results were expressed as M ± SD. To determine the presence of a statistically significant relationship with the risk factor, the Pearson's chi-squared test ($\chi 2$) and the odds ratio (OR) were used when the groups of the subjects were compared by the frequency of the risk factor.

**Results and discussion.** To achieve this goal for differential diagnosis, the difference between the radiographic imaging in the control group (virtually healthy people) and in patients with recurrent bronchitis (3-4 episodes within the previous 6 months) was estimated using the chest X-ray. Acute bronchitis and recurrent bronchitis, which is not repeated once, cannot be identified using direct radiographic signs, because X-rays pass through the structure of the bronchial tree. Indirect signs, such as "air bronchogram" may be more helpful for this purpose. This sign depicts the indistinct contours of the lung roots due to the tissue swelling, which makes it impossible to confirm the progression of the disease. Examples of the corresponding X-rays are shown in Fig.1 for comparison. The moment of the bronchitis evolution cannot be visualized on the X-rays. The difference between a normal chest X-ray and an X-ray with recurrent bronchitis is absent in contrast to chronic bronchitis, where the X-ray shows the enlarged pulmonary texture, accompanied by the "pair strips" and "air bronchogram" signs. At that time, during the endoscopic examination, the moment of bronchitis evolution was evident (Fig. 2).



a) Normal b) Recurrent bronchitis c) Chronic bronchitis

**Fig. 1.** Examples of X-rays



a) Normal image b) Recurrent bronchitis c) Chronic bronchitis

**Fig. 2.** Examples of bronchoscopic images

Here the difference between the normal picture and recurrent bronchitis is sufficient to be visualized and for the diagnosis. In a case of recurrent bronchitis, a vascular picture is enlarged, a small amount of mucus is present, unlike the healthy bronchial mucosa, and in contrast to the chronic bronchitis, mucosal edema and the overproduction of thick bronchial secret. We did not find literature data on the qualitative or quantitative content of lactobacilli bacteria and bifidobacteria in the bronchi of helathy indidviduals. Therefore, we created the control group of young people who did not have lower respiratory tract diseases and did not take antibiotic therapy for any reason within 1 last year, but who have been clinically and endoscopically diagnosed with a foreign body of the respiratory tract. We considered this group healthy in terms of the presence of the bronchial biotope which is characterized by the physiological parameters of normal flora. We have determined that lactobacilli and bifidobacteria were found in 100% of cases in dilutions of $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$. The fact that normal flora is present in all examined indidviduals from the group of comparison makes it possible to suggest its importance in the formation of the homeostasis of the organism. This group can not be regarded as a control group, because the subjects did not take antibiotics as a risk factor. In exsperts opinion, there is no practical possibility of determining the single standard for the composition of microbiota [7]. Therefore, we focused our attention on the generalization of features for the studied groups rather than the individual content of the biotope. Considering this, we pointed out the fact that in groups where lactobacilli or bifidobacteria were present apart or together, the diversity of the bronchial biota was wider. In addition to lactobacilli bacteria and bifidobacteria, one to three different bacterias, mushrooms (S. salivarius, S. mitis, S.virida, S. faecalis, S. pneumoniae, S.haemolyticus, S.aureus, S. aeruginosa, Penicillium spp, H. parainfluenzae, Escherichia coli) in various chaotic combinations in terms of qualitative and quantitative composition. In the absence of lactobacilli bacteria and bifidobacteria, only one or two types of bacteria (S. salivarius, S. mitis, Escherichia coli) were present, indicating the "poverty" of the bronchial biota and a favorable basis for the progression of the lower respiratory tract infection in the future, chronicity the process. In faeces in this group lactobacilli bacteria and bifidobacteria from $10^{-3}$ до $10^{-7}$ were found, pathogenic enterobacteria were not detected. Total number of E. coli from $10^{-4}$ до $10^{-8}$, E. coli with poorly expressed enzymatic properties to 11%, hemolytic E. coli to $10^{-4}$, conditionally pathogenic enterobacteria to $10^{-3}$, pathogenic Staphylococcus aureus to $10^{-3}$, Candida mushrooms to $10^{-3}$. These data reflect the higher stability of the intestinal normal flora than bronchial tubes to antibiotic therapy. With the anamnestic and clinical data we have identified a group of patients with bronchitis, which was repeated within the previous 6 months 3-4 times. These patients were further divided into 3 groups, depending on the complete or incomplete presence or absence of lactobacilli bacteria and bifidobacteria in the bronchoalveolar lavage. (See Fig. 3).

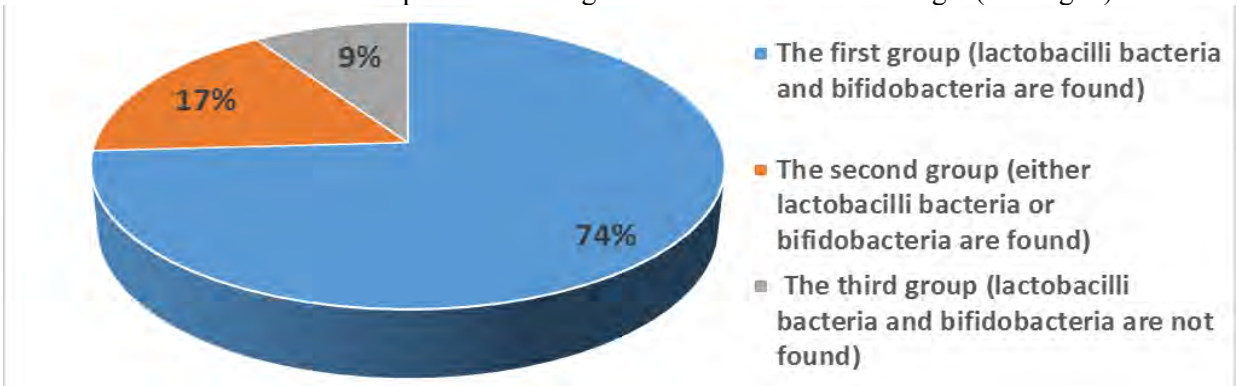



**Fig. 3.**

Table 1.

Distribution of patients depending on antibiotic therapy. Patients received semi-synthetic penicillins, cephalosporins III generation, macrolides from 5 days to 19 days in different combinations

| ***Antibiotic therapy within the last 6 months*** | ***The first group (control) (lactobacilli bacteria and bifidobacteria are found)*** | | ***The second group (either lactobacilli bacteria or bifidobacteria are found)*** | | ***The third group (lactobacilli bacteria and bifidobacteria are not found)*** | |
|---|---|---|---|---|---|---|
| | abs. | %±Δ% | abs. | %±Δ% | abs. | %±Δ% |
| *Absent* | 42 | 52,5±5,6 | 5 | 27,8±10,6° | 2 | 20,0± 12,6* |
| *Present* | 38 | 47,5±5,6 | 13 | 72,2±10,6° | 8 | 80,0± 12,6* |

Notes: * - statistically significant difference between the indicator in group 3 compared with the control group 1 (p<0,05).

- not statistically significant difference between the indicators in group 2 compared with the control group 1 (p<0,06).

The highest proportion of patients was included in the group I (control, because it was this group was close to the indicators of the comparative healthy group), where in bronchoalveolar lavage lactobacilli bacteria and bifidobacteria were detected - 80 ((74,1 ± 4,2)%) persons. The group II 18 ((16,6 ± 3,6)%) was inspected, in which only lactobacilli bacteria were detected in bronchoalveolar lavage, or bifidobacteria. The group III - those in which in bronchoalveolar lavage wasn't detected no lactobacilli bacteria no bifidobacteria - 10 ((9,3 ± 2,8)%) of the examined. Subsequently, the data of the groups were analyzed depending on the presence or absence of antibiotic therapy during the previous 6 months. The data is given in Table 1.

According to the obtained data, the group I patients who did not use antibiotic therapy within the previous 6 months was 42 ((52,5 ± 5,6)%) individuals and 38 ((47,5 ± 5,6)%) individuals, where bronchoscopy was performed after or during antibiotic therapy. Among the surveyed group II, it was found that they did not use antibiotic therapy during the previous 6 months in 5 ((27.8 ± 10.6)%) patients, and 13 ((72.2 ± 10.6)%) - received antibiotic therapy during the previous 6 months. In group III it was found that they did not take antibiotic therapy 2 ((20,0 ± 12,6)%) patients and 8 ((80,0 ± 12,6)%) - patients took it within the previous 6 months.

Based on the acquired data 80% of patients from the group III (no lactobacilli bacteria and bifidobacteria), who received antibiotics, demonstrated chages in bronchial microbiocenosis, which were statistically different (p<0,05) from the parameters of the microbiocenosis in the group I (control), where antibiotics were administered in 47,5%. In the patients from the group II, where antibiotic therapy was used in 72,2% of patients, either lacto- or bifidobacteria were found. However these changes were not significantly different (p<0,06) from the parameters of the group I, which may indicate the incompleteness of negative changes in the bronchial microbiocenosis.

These data were considered by us when calculating the odds ratio (OR) - for qualitative indicators.

Relative Risk (RR), according to our data, was 2.49 with a 95% confidence interval of 1.16 to 5.37. The fact that the relative risk value obtained is not equal to 1 (one) proves that this is a real property of the population under study, and not an accidental fluctuation due to our sample. Since 1 is not included in the confidence interval and the relative risk value is statistically significantly different from 1, we can assert (with a probability of error less than 5%) that the use of antibiotic therapy statistically significantly increases the frequency of changes in the microflora of the bronchial tubes in recurrent bronchitis by 2.49 times.

Comparing the groups with the frequency of detection of risk factors by two methods, the criterion $\chi 2$ (Pearson's chi-squared test) and the odds ratio (OR), confirmed that with the adoption of antibiotic therapy, there was a significant decrease in the presence of lactobacilli bacteria and bifidobacteria in bronchoalveolar lavage. These data suggest that we consider the prevention normal microbiocenosis of bronchial tubes and may restore or preserve it by using probiotics in the complex treatment of recurrent bronchitis.

**Conclusions.** 1. Based on the acquired data we found lactobacilli bacteria and bifidobacteria in bronchoalveolar lavage in 100% of examined individuals, who had no prior lower respiratory tract diseases. 2. Antibiotic therapy significantly increases the frequency of microflora changes in the case of recurrent bronchitis 2,49 times. 3. Broncial microbiocenosis is more sensitive to antibiotic therapy than the intestinal microbiocenosis.

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## ЭВОЛЮЦИЯ БРОНХИТА У МОЛОДЫХ ПАЦИЕНТОВ С ТОЧКИ ЗРЕНИЯ МЕТОДОВ ИНСТРУМЕНТАЛЬНОГО И БАКТЕРИАЛЬНОГО ИССЛЕДОВАНИЯ

ОРИДЖАНА ТЫШ, ЛАРИСА МАТИУХА, АНАТОЛИЙ СЕРГИЕНКО*

Отделение семейной медицины и амбулаторного лечения Национальной медицинской академии последипломного образования имени Шупика;
*Киевская городская клиническая больница № 17

Мы исследовали 128 пациентов в возрасте от 18 до 35 лет. Для сравнения в качестве отдельной группы были выбраны 20 валантеров без предшествующих заболеваний нижних дыхательных путей. У них было инородное тело дыхательных путей и они подверглись бронхоскопии с бронхоальвеолярным лаважем для бактериологического анализа. У 108 пациентов был диагностирован рецидивирующий бронхит (ICD-10 J.40). Эти пациенты были дополнительно разделены на 3 группы. Мы также провели дополнительный бактериологический анализ фекалий, чтобы определить взаимосвязь между изменениями бронхиального микробиоценоза под влиянием антибактериальной терапии по сравнению с изменениями в биоценозе кишечника. 80% пациентов из III группы (без бактерий лактобацилл и бифидобактерий), которые получали антибиотики, продемонстрировали главное в бронхиальном микробиоценозе, которые были статистически различны ($p<0,05$) по параметрам микробиоценоза в группе I (контроль), где антибиотики вводились в 47,5%. У пациентов из II группы, где антибиотикотерапия использовалась у 72,2% пациентов, были обнаружены либо лакто-, либо бифидобактерии. Однако эти изменения не были существенно различны ($p<0,06$) по параметрам I группы, что может указывать на неполноту отрицательных изменений в бронхиальном микробиоценозе. Основываясь на полученных данных, мы обнаружили бактерии лактобацилл и бифидобактерии в бронхоальвеолярном лаваже у 100% обследованных лиц, у которых не было предшествующих заболеваний нижних дыхательных путей.

***Ключевые слова:*** *микробиоценоз, бронхит, бактерии лактобацилл, бифидобактерии, бронхоальвеолярный лаваж.*