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RETROSPECTIVE ANALYSIS OF ANTIBIOTIC THERAPY AND SENSITIVITY TO ANTIFUNGALS OF FUNGI OF THE GENUS *CANDIDA* SECRETED FROM PATIENTS WITH VIRAL-BACTERIAL PNEUMONIA ASSOCIATED WITH COVID-19

The appointment of antibacterial agents for the treatment of pneumonia that develops with COVID-19 is one of the treatment regimens. Antibacterial agents are prescribed only in the case of the presence of confirmed bacterial co-infection but can be appointed empirically. This approach promotes the development of antibiotic resistance of opportunistic and saprophytic microflora of almost all habitats, including the oropharynx, which can lead to dysbiosis with activation of fungal flora. The aim of the study was to analyze the composition of the oropharyngeal microbiome of patients with viral and bacterial pneumonia who took antibiotics, as well as the sensitivity of fungi of the genus Candida to antifungal drugs for effective treatment of the underlying disease. Methods. The results of the bacteriological examination of 113 patients treated with a diagnosis of COVID-19 were analyzed. Microbiological examination of oropharyngeal swabs was performed by the classical bacteriological method with dosed seeding of suspended material on differential diagnostic media (in particular Sabouraud agar was used for selection of fungi of the genus Candida) and genus identification by morphological, cultural, biochemical properties. Results. PCR tests were performed for all patients in the clinical trial to confirm the diagnosis of viral and bacterial pneumonia. According to the results of the bacteriological examination, fungi of the genus Candida were found in 52 (46.0%) patients with pneumonia associated with COVID-19. The analysis of prescriptions showed that only 14 (26.9%) patients were treated with one antibiotic, two antibiotics were prescribed to 31 (59.6%) patients, and three — to 7 (13.5%). In the structure of antibiotic therapy, the lion's share were cephalosporins

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of the third generation (ceftriaxone, hepacef) — 33 (63.5%), and macrolides (azithromycin) — 16 (30.8%) patients. In the structure of the oropharyngeal microbiome, according to the results of the bacteriological research, fungi of the genus *Candida* significantly prevailed, which were found in 52 (46.0%), and in 29 patients (25.7%) *S. pneumonia* was found. The sensitivity of fungi of the genus *Candida* to antifungal agents was analyzed, and the maximum number of resistant strains was detected to nystatin and amphotericin — 38.5% and 26.9%, with only 8 (15.3%) fungi of the genus *Candida* sensitive to nystatin. **Conclusions.** All patients with viral-bacterial pneumonia associated with COVID-19 received antibiotic therapy with the lion's share of third-generation cephalosporins (63.5%) and macrolides (30.8%). According to the results of the bacteriological examination of the oropharyngeal microbiome after antibiotic therapy, fungi of the genus *Candida* predominated (46.0%), followed by *S. pneumoniae* (25.7%). Isolated strains of fungi of the genus *Candida* showed resistance to nystatin (38.5%) and amphotericin (26.9%). Antifungal agents of the imidazole subgroup have shown high efficiency and a low percentage of resistant strains, which allows us to recommend them for the treatment of complications of COVID-19 caused by fungi of the genus *Candida*.

Keywords: antifungal agents, sensitivity, resistance, *Candida* spp., oropharynx, microbiome, patients diagnosed with COVID-19.

The COVID-19 pandemic was declared by the WHO on March 11, 2020, and since then Ukraine, like the rest of the world, has been living with the slogan of fighting coronavirus infection. At the time of the pandemic announcement, cases of current infection were registered in 114 countries, and their number exceeded 118 thousand cases, including more than 4 thousand of deaths. On February 18, 2021, the pandemic spread to 218 countries, and the number of cases was 110, 526,961, of which fatal ones were 2,442,989 [1].

The number of patients with a confirmed diagnosis of COVID-19 amounts to 1.4% of the total number of people on the planet, and in Ukraine — 3%. COVID-19 has a very broad clinical spectrum, ranging from asymptomatic cases that do not need inpatient care, and ending with the development of bilateral pneumonia, which can progress to respiratory failure, and in some cases — to multiple organ failure and death [2]. Clinically manifestation of COVID-19 is accompanied by polymorphic lesions of organs and systems throughout the body, but in the first place is the defeat of the immune and respiratory systems [3]. The newness of this disease is the rapid increase in symptoms and the great number of deaths, which prompted physicians to urgently develop treatment regimens for inpatients, which were not always justified.

In particular, this applies to the appointment of antibacterial agents for the treatment of pneumonia that develops with COVID-19. On November 30, 2020, the Deputy Minister of Healthcare of that time, Chief State Sanitary Doctor Viktor Liashko claimed: “Against a background of the coronavirus epidemic, we are significantly increasing sales of antibiotics in pharmacies and their usage in health care facilities, as well, including those, which provide health care to patients with coronavirus disease”. Nowadays, the Ministry of Healthcare is redistributing 400,000 vials of meropenem and 700,000 vials of amoxicillin to the reserve for hospitals. These antibiotics will be in reserve in order to treat patients with coronavirus disease if necessary. According to the Protocol “Provision of medical care for the treatment of coronavirus disease (COVID-19)” approved by the order of the Ministry of Healthcare of Ukraine dated April 2, 2020 No. 762, antibacterial drugs are prescribed only in the presence of confirmed bacterial co-infection (namely after receiving positive bacteriological results: blood and/or sputum analyses). But given the fact that to obtain analysis results, we need to wait for a few days, antibiotics, under certain indications, can be also prescribed empirically [4].

This approach actively promotes the development of antibiotic resistance of opportunistic and saprophytic microflora of almost all habi-

tats, including the oropharynx, and can lead to dysbiosis with the activation of fungal flora. This is due to the fact that some patients take antibiotics on an outpatient basis as self-medication.

There have been many reports of the direct correlation between the severity and duration of infections of different localization and the depth of dysbiosis by reducing the protective functions of the immune-microbiological system [5].

The **aim** of our study was to analyze the frequency of secretions from the oropharyngeal microbiome of patients with viral-bacterial pneumonia receiving antibiotics, fungi of the genus *Candida* and to determine their sensitivity to antifungal drugs for effective treatment of the underlying disease.

Materials and methods. The results of the bacteriological examination of 113 patients who were treated in Ternopil City Hospital No3 from September to December 2020 with a diagnosis of COVID-19 were analyzed for viral and bacterial pneumonia. The diagnosis was confirmed by PCR test. Microbiological examination of oropharyngeal swabs was performed by the classical bacteriological method with dose culture of suspended material on differential diagnostic medium (in particular Sabouraud agar was used for selection of fungi of the genus *Candida*) and genus identification by morphological, cultural, and biochemical properties. The Kirby-Bauer test was used to study the sensitivity of isolated pure cultures to antifungal agents. The results were evaluated by determining the diameter of the growth inhibition zone using the standard tables [6].

In addition, the medical histories of these patients were processed in order to determine the features of antibiotic therapy, which was carried out during inpatient treatment.

The results of the study were processed according to the general rules of variation statistics using a standard set of programs for a personal computer.

Results. All patients in the clinical trial to undergo a diagnosis of viral and bacterial pneumonia underwent PCR tests on an outpatient or

inpatient stage, radiography, or computed tomography of the chest.

According to the results of the bacteriological examination, fungi of the genus *Candida* were found in 52 (46.0%) patients with pneumonia associated with COVID-19. A retrospective analysis of prescriptions in patients' medical histories suggests that they all received antibiotic therapy, but only 14 (26.9%) patients were treated with one antibiotic, whereas the remaining patients took two or three antibacterial drugs in combination or sequentially. In particular, two antibiotics were prescribed to 31 (59.6%) patients and three antibiotics — to 7 (13.5%). In the structure of antibiotic therapy, the lion's share were cephalosporins of the third generation (ceftriaxone, heparcef) — 33 (63.5%), and macrolides (azithromycin) — 16 (30.8%) patients (Table 1).

Carbapenems were less frequently applied — 23.1% and fluoroquinolones — 19.2%. Beta-lac-

Table 1. Antibacterial therapy for patients with viral-bacterial pneumonia associated with COVID-19; carriers of fungi of the genus *Candida*

Antibiotic	Number of appointments	
	n	%
Cephalosporins:		
Ceftriaxone	30	57.7
Heparcef	3	5.8
Macrolides:		
Azithromycin	16	30.8
Carbapenems:		
Meropenem	9	17.3
Brenham	3	5.8
Beta-lactam antibiotics:		
Flemoxin	15	28.8
Abipim	1	1.9
Fluoroquinolones:		
Levofloxacin	9	17.3
Abiflox	1	1.9
Aminoglycosides:		
Gentamicin	1	1.9

tam antibiotics were prescribed almost as often as macrolides — 30.7%. Such potential antibiotic therapy could lead to the activation of the fungal flora, which resulted in the suppression of the indigenous oropharyngeal microbiota in patients with COVID-19. In the structure of the oropharyngeal microbiome, according to the results of bacteriological research, fungi of the genus *Candida* significantly prevailed, which were found in 52 (46.0%) of 113 patients who participated in the current study. In second place by the frequency of detections were *S. pneumoniae* — 29 (25.7%) (Table 2). *K. pneumoniae* (13 (11.5%)), *S. aureus* (11 (9.7%)), and *E. coli* (7 (6.2%)) were revealed less often in the oropharynx of patients. One patient was noted with a monoculture of enterococci.

Table 2. Frequency of detections of pathogenic and opportunistic microorganisms in the microbiota of the oropharynx of patients with viral-bacterial pneumonia associated with COVID-19

Microorganisms	Detection frequency	
	n	%
<i>Candida</i> spp.	52	46.0
<i>S. pneumoniae</i>	29	25.7
<i>K. pneumoniae</i>	13	11.5
<i>S. aureus</i>	11	9.7
<i>E. coli</i>	7	6.2
<i>Enterococcus</i> spp.	1	0.9

Due to the fact that fungi of the genus *Candida* were found in clinically significant concentrations frequently enough, it was necessary to determine their sensitivity to antifungal drugs with the purpose of effective further therapy and prevention of the development of generalized candidiasis (Table 3).

We analyzed the susceptibility of fungi of the genus *Candida* to synthetic antifungals, triazole derivatives (fluconazole and itraconazole), imidazole derivatives (clotrimazole and ketoconazole), and natural antibiotics of the polyene group that have antifungal activity (nystatin and amphotericin). The maximum number of resistant strains was detected to nystatin and amphotericin, 38.5% and 26.9% respectively, with only 8 (15.3%) fungi of the genus *Candida* being sensitive to nystatin.

This allows us to conclude about the low effectiveness of these drugs in relation to the fungal flora and not to recommend them for usage. Unlike natural antibiotics, synthetic antifungal agents, in particular the imidazole subgroup, have shown high efficiency and a low percentage of resistant strains, namely only 4 (7.8%) samples were resistant to ketoconazole and 11 (21.2%) to clotrimazole, which corresponds to the lowest values among the resistant fungi of the genus *Candida*. The maximum number of sensitive and moderately sensitive strains was found in these drugs, which allows them to be recommended

Table 3. Sensitivity to antifungals of fungi of the genus *Candida* obtained from patients with viral-bacterial pneumonia associated with COVID-19

Medicine	Number of strains					
	Resistant		Moderately Sensitive		Sensitive	
	Abs.	%	Abs.	%	Abs.	%
Fluconazole	12	23.1	24	46.2	16	30.8
Itraconazole	13	25.0	21	40.4	18	34.6
Clotrimazole	11	21.2	28	53.8	13	25.0
Nystatin	20	38.5	24	46.2	8	15.3
Ketoconazole	4	7.8	26	50.0	22	42.3
Amphotericin	14	26.9	25	48.1	13	25.0

for the correction of dysbiosis and prevention of systemic mycosis in patients with viral-bacterial pneumonia associated with COVID-19.

Discussion. *Candida* belongs to opportunistic pathogens, whose virulence for humans varies widely, and the ability to pathogenic depends on the condition of the macroorganism. *Candida* fungi are ubiquitous. As representatives of the normal microflora, they are found on the mucous membranes of the digestive tract and especially on the oral mucosa in 14–50% of almost healthy people. The incidence of candidiasis is increasing worldwide, and this is associated with the widespread use of antibacterial drugs, hormonal drugs, and cytostatics, as well as with an increase in the disease range, which creates a favorable background for the development of candidiasis [7]. In the case of pneumonia that develops against the background of infection caused by COVID-19, there are two contributing factors that lead to the activation of fungal flora such as the massive antibiotic therapy, which is confirmed by our study, and the actual effect on the body of SARS-CoV-2 virus, which causes a number of systemic immune disorders [3].

On the other hand, fungi of the genus *Candida* from the rank of pathogens, rarely secreted from patients, have become one of the main opportunistic microorganisms that can lead to nosocomial infections. In fact, the fungus *Candida* is the causative agent for about 15% of all nosocomial infections.

Their share in the overall structure of infections associated with the provision of medical care can reach 30% depending on the specialization of the department [8]. These data suggest that in patients with viral-bacterial pneumonia associated with COVID-19, such a high percentage of seeding of *Candida* fungi from the oro-

pharynx may be caused by parasitism within the hospital.

In addition to a sharp increase in the number of antibiotic-resistant bacteria, the COVID-19 pandemic can cause a mass flash of *Candida Auris* in hospitals, which is capable of rapid nosocomial spread and this is a common cause of the death of patients in intensive care units [2]. Therefore, the question of the usage of antifungal drugs in the treatment of such patients becomes extremely relevant, and currently, there is no relevant data in the literature.

Conclusions

1. All of the examined patients with viral-bacterial pneumonia associated with COVID-19 received antibiotic therapy, mainly with several drugs. Within the structure of antibiotic therapy, the lion's share were cephalosporins of the third generation (ceftriaxone, hepacef) — 33 (63.5%) and macrolides (azithromycin) — 16 (30.8%).

2. According to the results of the bacteriological research, in the structure of the oropharyngeal microbiome fungi of the genus *Candida* significantly prevailed, which were found in 52 (46.0%) of 113 patients, *S. pneumoniae* were in second place by the frequency of detections — 29 (25.7%) .

3. Determination of the sensitivity of isolated strains to antifungals revealed their maximum resistance to nystatin and amphotericin, 38.5% and 26.9% respectively, and only 8 (15.3%) fungi of the genus *Candida* were sensitive to nystatin.

4. Antifungal drugs of the imidazole subgroup have shown high efficiency and a low percentage of resistant strains, namely only 4 (7.8%) samples were resistant to ketoconazole and 11 (21.2%) to clotrimazole, which makes it possible to recommend them for therapy of COVID-19 caused by fungi of the genus *Candida*.

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РЕТРОСПЕКТИВНИЙ АНАЛІЗ АНТИБІОТИКОТЕРАПІЇ ТА ЧУТЛИВІСТЬ ДО АНТИМІКОТИКІВ ГРИБІВ РОДУ *CANDIDA*, ВИДІЛЕНИХ ВІД ХВОРИХ НА ВІРУСНО-БАКТЕРІАЛЬНУ ПНЕВМОНІЮ, АСОЦІЙОВАНУ З COVID-19

Призначення антибактеріальних засобів для лікування пневмонії, що розвивається при COVID-19, є однією зі схем лікування. Антибактеріальні засоби призначаються лише за наявності підтвердженої бактеріальної ко-інфекції, проте можуть призначатися емпірично. Такий підхід сприяє розвитку антибіотикорезистентності умовно-патогенної та сапрофітної мікрофлори практично усіх біотопів, зокрема ротоглотки, та може призвести до дисбіотичних уражень з активізацією грибкової флори. **Мета роботи** — проаналізувати склад мікробіому ротоглотки пацієнтів з вірусно-бактеріальною пневмонією, що отримували антибіотики, а також чутливість грибів роду *Candida* до протигрибкових засобів з метою ефективної терапії основного захворювання. **Методи**. Проаналізовано результати бактеріологічного дослідження 113 стаціонарних пацієнтів, що перебували на лікуванні з діагнозом COVID-19. Мікробіологічне дослідження мазків із ротоглотки проводили класичним бактеріологічним методом з дозованим посівом суспензованого матеріалу на диференціально-діагностичні середовища (для виділення грибів роду *Candida* — середовище Сабуро) та родовою ідентифікацією за морфологічними, культуральними та біохімічними властивостями. Для дослідження чутливості до протигрибкових засобів виділених чистих культур використовували метод Кірбі-Бауера. **Результати**. Усім пацієнтам у комплексі клінічного дослідження для підтвердження діагнозу вірусно-бактеріальної пневмонії було проведено ПЛР тести. За результатами бактеріологічного дослідження гриби роду *Candida* виявлено у 52 (46.0 %) хворих на пневмонію, асоційовану з COVID-19. Аналіз листків призначень показав, що лише 14 (26.9 %) хворих лікували одним антибіотиком, два антибіотики було призначено 31 (59.6 %) пацієнту, три — 7 (13.5 %). У структурі антибіотикотерапії левову частку склали цефалоспорини III покоління (цефтріаксон, гепацеф) — 33 (63.5 %), та макроліди (азитроміцин) — 16 (30.8 %) пацієнтів. У структурі ротоглоткового мікробіому, за результатами бактеріологічного дослідження, вагомо переважали гриби роду *Candida*, які виявлено у 52 (46.0 %), у 29 пацієнтів (25.7 %) виявлено *S. pneumoniae*. В результаті аналізу чутливості грибів роду *Candida* до протигрибкових засобів

виявлена максимальна кількість резистентних штамів до ністатину та амфотерицину — 38.5 % та 26.9 % відповідно, причому до ністатину були чутливими лише 8 (15.3 %) грибів роду *Candida*. **Висновки.** Усі пацієнти з вірусно-бактеріальною пневмонією, асоційованою з COVID-19, отримували антибіотикотерапію, лівову частку якої склали цефалоспорини III покоління (63.5 %) та макроліди — 30.8 %. За результатами бактеріологічного дослідження ротоглоткового мікробіому, після антибіотикотерапії переважали гриби роду *Candida* (46.0 %), на другому місці за частотою виявлення — бактерія *S. pneumoniae* (25.7 %). Виділені штами грибів роду *Candida* проявили резистентність до ністатину (38.5 %) та амфотерицину (26.9 %). Протигрибкові засоби підгрупи імідазолів показали високу ефективність та низький відсоток резистентних штамів, що дозволяє рекомендувати їх для терапії ускладнень COVID-19, спричинених грибами роду *Candida*.

Ключові слова: протигрибкові засоби, чутливість, резистентність, *Candida* spp., ротоглотка, мікробіом, пацієнти з діагнозом COVID-19.