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Microbiota of the lower respiratory tract in community-acquired pneumonia, including cases associated with SARS-CoV-2

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Abstract

Introduction. Many aspects of the pathogenesis and pathomorphology of pneumonia associated with novel coronavirus require a comprehensive study using modern diagnostic methods.

The **aim** of the study was to study the microbiota of the lower respiratory tract in community-acquired pneumonia associated with SARS-CoV-2, to assess the antibiotic and phage resistance of circulating strains of microorganisms.

Materials and methods. The analysis of biosamples from 486 patients undergoing inpatient treatment in five mono-hospitals in Tyumen and Tyumen region with a diagnosis of moderate and severe community-acquired pneumonia was carried out. In almost 90% of cases patients received oxygen therapy, about 8% of patients were connected to ventilators. The inoculation of the cultures with clinical samples was carried out for six months (from April to October 2020). The isolated bacterial strains were identified by mass spectrometry. The resistance to antimicrobial drugs and bacteriophages was assessed for identified isolated.

Results. Gram-positive cocci, mainly opportunistic microorganisms of the genus *Streptococcus* and *Candida fungi* predominated in the microbiota of the lower respiratory tract of patients diagnosed with community-acquired pneumonia associated with SARS-CoV-2. At the same time, bacteria of the *Enterobacteriaceae* family and non-fermenting gram-negative bacteria were less common compared to patients without coronavirus infection. In the structure of pathogens, the leading position was occupied by the bacteria *K. pneumoniae* and *Acinetobacter* spp. The analysis of the sensitivity of microorganisms to antimicrobial drugs showed the highest resistance rates in strains of *Acinetobacter* spp., *Enterococcus* spp., *Coagulase-negative Staphylococcus*. It has been established that in the group of patients with community-acquired pneumonia associated with SARS-CoV-2, the risk of infection with *Streptococcus* spp. with high level of antibiotic resistance was 1.5 times higher, and taking into account the 95% confidence interval, the value of this indicator ranged from 1.1 to 2.1 times.

Conclusion. The data obtained indicate that the microbiota of the lower respiratory tract in community-acquired pneumonia associated with SARS-CoV-2 is represented mainly by bacteria of the genus *Streptococcus*, which have a high level of resistance to antimicrobial drugs.

Keywords: *antibiotic resistance, community-acquired pneumonia, SARS-CoV-2, bacterial strains, sputum, bronchial lavage water, bronchoalveolar lavage*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committe of the Tyumen Region Infection Pathology Research Institute (protocol No. 2, 20.03.2020).

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Научная статья
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Микробиота нижних дыхательных путей при внебольничных пневмониях, в том числе ассоциированных с SARS-CoV-2

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Аннотация

Введение. Многие аспекты патогенеза и патоморфологии коронавирусной пневмонии нуждаются во всестороннем комплексном изучении с использованием современных методов диагностики.

Цель исследования — изучение микробиоты нижних дыхательных путей при внебольничных пневмониях (ВБП), ассоциированных с SARS-CoV-2, оценка антибиотико- и фагорезистентности циркулирующих штаммов микроорганизмов.

Материалы и методы. Проведён анализ биопроб от 486 пациентов, находящихся на стационарном лечении в 5 многоспиталях Тюмени и Тюменской области с диагнозом ВБП средней и тяжёлой степени. Почти в 90% случаев пациенты получали оксигенотерапию, около 8% больных были подключены к аппаратам искусственной вентиляции лёгких. Посев клинического материала осуществлялся на протяжении 6 мес (с апреля по октябрь 2020 г.). Идентификацию выделенных штаммов бактерий выполняли методом масс-спектрометрии. У обнаруженных изолятов определяли резистентность к антимикробным препаратам и бактериофагам.

Результаты. В микробиоте нижних дыхательных путей пациентов с диагнозом «ВБП, ассоциированная с SARS-CoV-2» превалировали грамположительные кокки, преимущественно условно-патогенные микроорганизмы рода *Streptococcus* и грибы рода *Candida*. При этом бактерии семейства *Enterobacteriaceae* и неферментирующие грамотрицательные бактерии встречались реже, чем у пациентов без COVID-19. В структуре патогенов лидирующее положение занимали бактерии *Klebsiella pneumoniae* и *Acinetobacter* spp.Анализ чувствительности микроорганизмов к антимикробным препаратам показал наиболее высокую резистентность у штаммов *Acinetobacter* spp., *Enterococcus* spp., коагулазонегативных *Staphylococcus* spp. Установлено, что в группе пациентов с ВБП, ассоциированной с SARS-CoV-2, шансы встретить штаммы *Streptococcus* spp. с высокой устойчивостью к антибиотикам в 1,5 раза выше, а с учётом 95% доверительного интервала величина этого показателя колебалась в пределах 1,1–2,1 раза.

Вывод. Полученные данные свидетельствуют о том, что микробиота нижних дыхательных путей при ВБП, ассоциированных с SARS-CoV-2, представлена преимущественно бактериями рода *Streptococcus*, обладающими высоким уровнем резистентности к антимикробным препаратам.

Ключевые слова: *антибиотикорезистентность, внебольничная пневмония, COVID-19, SARS-CoV-2, штаммы бактерий, мокрота, промывные воды бронхов, бронхоальвеолярный лаваж*

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The lung microbiota functions within healthy lung tissue and influences immune responses under physiological and pathological conditions. The relatively low abundance of lung microbiota makes it difficult to isolate, culture and identify functional microbes. However, the low biomass does not undermine its potential role in shaping local lung immunity [1].

The composition of the respiratory microbiome is determined by the balance of 3 factors: microbial immigration to respiratory tracts through inhalation; microbial elimination from respiratory tracts through cough; microbial colonization rate. The presence of lung disease alters the local conditions, propelling the mechanism of selective bacterial growth. The well-known phenomenon of bacterial colonization during advanced lung diseases can explain a significant increase in bacteria species adapted to specific conditions. The ubiquity of subclinical microaspiration of pharyngeal secretions among healthy people is an experimentally verified

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observation. It was found that the microbiome of lungs is more similar to that of the oropharynx than that of the nasopharynx. There is evidence suggesting that bacterial communities of the lower respiratory tract (LRT) can play a certain role in the pathogenesis and progression of interstitial lung diseases.

It has been found that the microbiome in healthy lungs is dominated by *Bacteroidetes* and *Firmicutes*. The bronchial tree is most often colonized by bacteria of genera *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Veillonella, Megasphaera* and *Sphingomonas*. The research of viral-bacterial interaction in lungs on community-level is still in its early years [2].

Community-acquired pneumonia (CAP) is a serious infectious disease and a leading cause of death among humans [3]. According to the literature data, the CAP etiology was identified by PCR in 88.5% of cases. The leading pathogens are *S. pneumoniae* and *Haemophilus influenzae*, which were detected in 86.3% and 41.2% of cases, respectively. The major CAP pathogens include *Mycoplasma pneumoniae* (23.6%), *Adenovirus* (14.9%). *Chlamydophila pneumoniae* has been detected much more rarely (6.7%) [4]. Although 1–6% of the population are carriers of *Klebsiella pneumoniae* living in the nasopharynx, and 5–38% have it in their intestines, *K. pneumoniae* carriers account for 23% among hospitalized patients [5]. It has been shown that representatives of the family *Enterobacteriaceae* (*K. pneumoniae*) are indicative of unfavorable prognosis for the process in the lungs [6]. The information about pathogens of bacterial pneumonia bears evidence to polymicrobial etiology of multiple microbial and viral communities [4, 7].

Presently, the COVID-19 pandemic has been seen as the most serious global health emergency in the last one hundred years. Many patients develop pneumonia and need to be hospitalized or can have pneumonia progressing into respiratory complications [8]. SARS-CoV-2 can become exceptionally dangerous when secondary bacterial pneumonia is developed as a complication in a patient with COVID-19. A sizeable proportion of severe cases and deaths caused by the coronavirus in Russia in March-May 2020 is associated with secondary bacterial pneumonia and, to a far lesser extent, with concurrent viral infections [9].

The analysis of the results of laboratory tests in patients with COVID-19-associated CAP, which were presented in scientific publications, shows that secondary infections co-join at different frequency rates (0–54%) [3, 10–13]. In the reported complications caused by co-infections, etiological agents included *M. pneumoniae, Legionella pneumophila, S. pneumoniae* and *K. pneumoniae.* M. V. Stulova *et al.* show that the most common bacterial pathogens include *S. pneumoniae* (70%), *S. agalactiae* (10%), *S. pneumoniae* in association with *Staphylococcus aureus* (6.6%) and *Pseudomonas aeruginosa* (13.3%) [8]. Sharifipour *et al.* found that *Acinetobacter baumannii* bacteria and *S. aureus* bacteria were detected in COVID-19 patients in 90% and 10% of cases, respectively; all *A. baumannii* strains were resistant to antibiotics [14]. The results of the microbiota analysis for patients in the Rostov Region showed that the most frequently detected etiological agents of bacterial CAP were bacteria belonging to the genus *Streptococcus.* Chen *et al.* found that only 4% of the hospitalized patients had concomitant fungal infections represented by *Candida albicans* and *C. glabrata* [15]. Note that identification of pathogens is a challenging task in low and middle-income countries, which may lack readily available and affordable clinical or biological markers that would be efficient in distinguishing bacterial and viral infections [10].

Some aspects remain unclear, including synergistic interactions between SARS-CoV-2 and some associated bacteria, the association of the disease severity with co-infections caused by antibiotic-resistant bacteria [16]. Among mechanisms of interaction of co-infecting agents, a special attention is given to the effect of viruses on toxin production by bacteria and to the effect of bacteria on infectivity of viruses. Co-infecting microorganisms help overcome the epithelial barrier; they can beneficially modify functions of the cells in the immune system and help pathogens evade the immune response. The diversity of viral-bacterial interactions in co-infection not only calls for new approaches to their timely detection and monitoring, but also brings forth new biotechnologies and co-infection strategies that are gaining priority attention throughout the world [17].

At present, the prevalence of antibiotic resistance has become a global concern [16, 18]. Most of the hospitalized patients with COVID-19 are administered antibacterial agents empirically [11, 12]. Such empirical therapy promotes emerging and spreading of antibiotic-resistant strains [18–21]. It should be remembered that rational antibiotic therapy is based on regional or local specific characteristics of bacterial agents' resistance [16, 18]. Patients with infections caused by resistant strains tend to need hospitalization more frequently and tend to stay longer in hospital; such infections may adversely affect the prognosis for the patient compared to diseases caused by susceptible microorganisms, and, consequently, may contribute to higher death rates [22, 23]. Rational antibacterial therapy is not conceivable without advanced knowledge of the etiological structure of the disease, antibiotic and phage resistance of the pathogen.

The aim of the study was to explore the microbial LRT consortium in SARS-CoV-2-associated CAP, to assess the antibiotic and phage resistance of the circulating strains of microorganisms.

Materials and methods

The materials used in the study included sputum, bronchial washings, bronchoalveolar lavage fluids from

486 patients of 5 monohospitals in Tyumen and Tyumen Region; all of them were diagnosed with moderate and severe CAP and all of them gave their informed and voluntary consent to participate in the study. Out of them, 282 patients were tested positive for SARS-CoV-2. Nearly 90% of the patients received oxygen therapy; around 8% of the patients were on a ventilator.

The biological materials for laboratory tests were collected and transported in compliance with the Russian laws and requirements applicable to pathogens of infectious human diseases belonging to Group 1 and 2 by their pathogenicity[1] (correspond to Group 3 and 4 according to international classification). The clinical samples were cultured for 6 months (from April to October 2020). Strains were isolated by using conventional techniques in compliance with Decree No. 535 "On Unification of Bacteriological (Microbiological) Research Methods Used in Clinical and Diagnostic Laboratories of Medical and Preventive Healthcare Facilities"[2]. The selected bacteria were identified by direct protein profiling, using time-of-flight mass spectrometry and Maldi BioTyper 3.0 software. The confidence level higher than 2.0 meant that the microorganism species was detected precisely. Each of the studied bacteria strains was provided with the reference to the National Center for Biotechnology Information.

Antimicrobial resistance was assessed using the disk-diffusion test and the Mueller – Hinton agar (HiMedia); the results were analyzed in accordance with the applicable requirements and regulations[3].

The susceptibility of *Streptococcus spp.* (157 cultures) was assessed for ampicillin, amoxicillin/clavulanic acid, clindamycin, cefotaxime, levofloxacin, and azithromycin. Bacteria of the genus *Staphylococcus* (46 strains) were tested for resistance to inhibitor-protected ampicillin, ofloxacin, ciprofloxacin, levofloxacin, imipenem, meropenem, cefoxitin, clindamycin, azithromycin, and amikacin. Strains of bacteria from the genus *Enterococcus* (22 strains) were studied for the antibiotic susceptibility pattern by using disks with ampicillin, amoxicillin/sulbactam, amoxicillin/clavulanic acid, ciprofloxacin, levofloxacin, imipenem, meropenem, gentamicin, vancomycin. The inhibition zones were interpreted for isolates of non-fermenting gram-negative bacteria (74 strains) and *Enterobacteriaceae* bacteria (95 microorganism cultures) affected by ampicillin, amoxicillin/clavulanic acid, amikacin, ciprofloxacin, meropenem, imipenem, cefotaxime, cefepime, ceftazidime, cefoperazone/sulbactam, co-trimoxazole.

In accordance with the clinical recommendations[4], the assessment of susceptibility to two commercially available bacteriophages (Microgen SIC JSC): *Klebsiella pneumoniae*-specific bacteriophage, purified (P261, batch 1118) and sextaphage (P11, batch 0219) was performed for 30 *K. pneumoniae* strains.

The microorganism culture was applied to the dry surface of Mueller–Hinton agar in a 1.5×10^8 CFU/ml concentration and was uniformly distributed all over the surface of the medium. A few minutes after inoculated culture had dried up, the studied bacteriophages were applied in drops without touching the agar surface. The plates were incubated in the thermostat at 37 ºC for 24 hours.

The lytic activity of the phage was assessed by using a five-point scale (by the number of plus-signs):

- "–" — absent lytic activity;
- "+" — low activity;
- "++" — formation of a lysis zone with multiple colonies of secondary-growth bacteria;
- "+++" — the lysis zone with occasional secondary-growth colonies;
- "++++" — the clear lysis zone without secondary-growth colonies.

The study results were statistically processed using Statistica v.22 software (IBM SPSS) designed for research. If *p*-value criterion was below 0.05 and the confidence intervals for the difference in means did not contain "0", the assumption of their equality was rejected, it could be asserted with 95% confidence that the studied groups differed in the value of the assessed event.

Results and discussion

282 (58%) patients diagnosed with CAP were tested positive for SARS-CoV-2 with the help of PCR tests. Nearly in the tenth of the patients, regardless of SARS-CoV-2 presence, no potentially pathogenic bacteria were detected in the biomaterials (SARS-CoV-2 was detected in 10.6%, SARS-CoV-2 was not detected in 12.3%).

During the bacteriologic studies, a total of 430 strains of microorganisms were isolated from the bio-samples from the patients tested positive for SARC-CoV-2 RNA and 297 cultures were obtained from patients having negative results. Undoubtedly, the collection of sputum involves its contamination with bacteria of nasopharyngeal mucus (the upper respirato-

[1] SP 3.1.3597-20 Prevention of the Novel Coronavirus Infection (COVID-19). Moscow, 2020; MR 4.2.0114-16 Guidelines. Laboratory Diagnostics of Community-Acquired Pneumococcal Pneumonia. Moscow, 2016; MUK 4.2.3115-13 Instructional Guidelines. Laboratory Diagnostics of Community-Acquired Pneumonia. Moscow, 2014.

[2] Decree of the Health Ministry of the USSR, 22.04.1985, No. 535, On Unification of Bacteriological (Microbiological) Research Methods Used in Clinical and Diagnostic Laboratories of Medical and Preventive Healthcare Facilities.

[3] Clinical Recommendations, Assessment of Microorganisms' Susceptibility to Antimicrobial Agents. Moscow, 2017; MUK 4.2.1890–04, Assessment of Microorganisms' Susceptibility to Antibacterial Agents. Moscow, 2004.

[4] Federal Clinical (Methodological) Guidelines, Rational Use of Bacteriophages in Treatment and Anti-Epidemic Practice. Moscow, 2014.

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ry tract); therefore, the statistically processed results included the diagnostically significant titer of the microorganism count. The bacteria structure was dominated by gram-positive cocci mainly represented by the genus *Streptococcus* and fungi of the genus *Candida*. Most of the cultures of microorganisms from the genus *Streptococcus spp.* belonged to saprophytic microflora of the upper respiratory tract mucosa; *S. pneumoniae* was isolated from potentially pathogenic streptococci, only from 5 patients with laboratory-confirmed COVID-19. Based on the results of identification of clinical strains of fungi belonging to the genus *Candida*, a special place was taken by *C. albicans* (80%); besides, *C. kefyr, C. glabrata, C. dubliniensis, C. tropicalis, C. krusei* were isolated.

The comparative profile of the sputum microbiocenosis, bronchial washings and alveolar lavage fluids from patients with CAP, depending on laboratory confirmation of SARC-CoV-2, is presented in **Table 1**.

The assessment of the frequency of occurrence of different groups of microorganisms showed that the likelihood of detection of gram-positive microorganisms was higher when the samples had SARC-CoV-2 RNA ($p < 0.001$). The likelihood of detection of non-fermenting gram-negative bacteria and bacteria of the family *Enterobacteriaceae* in this category of patients was lower than in patients with negative PCR test results for coronavirus infection ($p = 0.040$ and $p = 0.012$) respectively. No statistically significant differences were found when the detection rates were compared for other bacteria, depending on the SARC-CoV-2 presence.

Table 2 shows the data on the main structure of potential CAP pathogens isolated from biological material collected from patients. The leading place is taken by *K. pneumoniae* bacteria of the family *Enterobacteriaceae*. Most of the non-fermenting gram-negative bacteria are represented by *Acinetobacter spp* strains.

The highest levels of resistance are detected in *Acinetobacter spp., Enterococcus spp*. and coagulase-negative *Staphylococcus spp.* strains (96.9, 75.8 and 75.4%, respectively) isolated from patients with SARS-CoV-2. In the meantime, among the patients without laboratory confirmed SARS-CoV-2, the highest resistance of bacteria was observed in *Acinetobacter spp.,* coagulase-negative *Staphylococcus spp.* and *K. pneumoniae* (92.3, 74.2 and 70%, respectively; **Table 3**).

The comparison of microflora resistance variables depending on the presence of SARS-CoV-2 in patients incorporated the estimation of occurrence likelihood and demonstrated the statistically confirmed difference in antibiotic resistance of strains in the group of non-fermenting gram-negative bacteria and bacteria of the family *Enterobacteriaceae* as well as among representatives of the genus *Streptococcus spp.* and *S. aureus* isolates.

The statistical analysis showed that in the group of patients with CAP associated with SARS-CoV-2, the likelihood of occurrence of resistant *Streptococcus spp*. strains was 1.5 times as high; considering the 95% confidence interval, the likelihood of occurrence was 1.1–2.1 times as high.

Among the patients with the negative PCR test for COVID-19, the likelihood of detecting antibiotic-resistant strains was higher in the group of non-fermenting gram-negative bacteria due to *Pseudomonas spp*.; among the strains of the family *Enterobacteriaceae*, this likelihood was high due to *K. pneumoniae*. In addition, a higher level of resistant *S. aureus* strains was observed in this category of patients.

The data on resistance of gram-positive and gram-negative bacteria, depending on the type of the antimicrobial agent, are presented in **Table 4** and **Table 5**.

Table 1. Results of comparison of the frequency of detection of microorganisms in biological samples of patients depending on the detection of SARC-CoV-2

Bacteria	SARS-CoV-2				*p*	% (95% CI)
	detected (*n* = 430)		not detected (*n* = 297)			
	abs	%	abs	%		
Gram-positive bacteria *(Staphylococcus* spp.*, Streptococcus* spp.*, Enterococcus* spp.)	156	36,3	69	23,2	<0,001*	1,88 (1,34–2,62)
Bacteria of the family *Enterobacteriaceae*	47	10,9	48	16,2	0,040*	1,56 (1,01–2,42)
Non-fermenting gram-negative bacteria	38	8,8	44	14,8	0,012*	1,79 (1,12–1,66)
Candida fungi	160	37,2	112	37,7	0,891	0,97 (0,72–1,32)
Others	29	6,7	24	8,1	0,496	0,82 (0,46–1,44)

Note. *Statistically significant differences.

Table 2. Species composition of microorganisms found in the contents of the lower respiratory tract of patients diagnosed with community-acquired pneumonia

Types of bacteria	SARS-CoV-2 detection frequency			
	detected		not detected	
	abs	%	abs	%
Bacteria of the family *Enterobacteriaceae*				
Klebsiella pneumoniae	20	42,55	30	62,50
Escherichia coli	7	14,89	7	14,58
Enterobacter spp.	12	25,53	6	12,50
Proteus mirabilis	3	6,38	2	4,17
Other (single)	5	10,64	3	6,25
Non-fermenting gram-negative bacteria				
Pseudomonas spp.	10	26,32	11	25,00
Acinetobacter spp.	16	42,11	28	63,64
Stenotrophomonas maltophilia	5	13,16	4	9,09
Other (single cases)	7	18,42	1	2,27
Gram-positive bacteria				
Staphylococcus aureus	10	6,41	9	13,04
Staphylococcus spp., except *S. aureus*	20	12,82	7	10,14
Streptococcus pneumoniae	5	3,21	2	2,90
Streptococcus spp., except *S. pneumoniae*	107	68,59	43	62,32
Enterococcus spp.	14	8,97	8	11,59

Table 3. Antibiotic resistance of bacteria isolated from the biosamples of patients with community-acquired pneumonia

Bacteria	SARS-CoV-2				*p*	% (95% CI)
	detected (*n* = 299)		not detected (*n* = 242)			
	abs	%	abs	%		
Non-fermenting gram-negative bacteria	31	67,5	43	78,6	0,011*	1,8 (1,14–2,76)
Acinetobacter spp.	16	96,9	28	92,3	0,131	2,6 (1,39–9,37)
Pseudomonas spp.	10	30,9	11	49,3	0,027*	2,2 (1,09–4,35)
Bacteria of the family *Enterobacteriaceae*	47	31	48	48,4	<0,001*	2,1 (1,52–2,87)
Escherichia coli	7	25	7	32,7	0,377	1,5 (1,59–3,37)
Klebsiella pneumoniae	20	41,9	30	69,9	<0,001*	3,2 (2,04–5,1)
Enterobacter spp.	12	15,8	6	8,1	0,259	2,1 (1,78–8,05)
Gram-positive cocci:						
Enterococcus spp.	14	75,8	8	66,7	0,174	1,6 (1,22–3,01)
Staphylococcus aureus	10	16,7	9	29,0	0,036*	2,0 (1,04–4,02)
Staphylococcus spp., except *S. aureus*	20	75,4	7	74,2	0,854	1,1 (1,78–2,01)
Streptococcus spp.	112	51,2	45	41,0	0,019*	1,5 (1,07–2,13)

Note. *Statistically significant differences.

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Table 4. Antibiotic resistance of gram-positive bacteria isolates from patients diagnosed with community-acquired pneumonia, %

Antibiotics	*Streptococcus* spp.		*Staphylococcus* spp.		*Enterococcus* spp.	
	COVID-19 (+)	COVID-19 (–)	COVID-19 (+)	COVID-19 (–)	COVID-19 (+)	COVID-19 (–)
Ampicillin	79,6	70,7	–	–	86,7	75
Ampicillin/sulbactam	–	–	75	63,6	–	–
Amoxicillin/clavulanic acid	79,6	70,7	75	63,6	85,7	71,4
Amoxicillin/sulbactam	–	–	–	–	100	71,4
Ofloxacin	–	–	54,2	41,7		
Ciprofloxacin	–	–	57,7	41,7	85,7	75
Levofloxacin	17,4	14,5	54,2	41,7	83,3	75
Imipenem	–	–	55,6	46,2	100	100
Meropenem	–	–	46,7	46,2	100	100
Cefoxitin	–	–	62,0	60,0	–	–
Cefotaxime	46,9	28,2	–	–	–	–
Clindamycin	16,1	13,3	46,7	46,7	–	–
Azithromycin	15,3	9,6	70	62,5	–	–
Amikacin	–	–	6,7	18,8	–	–
Gentamicin	–	–	–	–	60	75
Vancomycin	–	–	–	–	0	0

Table 5. Antibiotic resistance of gram-negative bacteria isolates from patients diagnosed with community-acquired pneumonia, %

Antibiotics	Bacteria family *Enterobacteriaceae*		*Klebsiella pneumoniae*		Non-fermenting gram-negative bacteria		*Acinetobacter* spp.	
	COVID-19 (+)	COVID-19 (–)	COVID-19 (+)	COVID-19 (–)	COVID-19 (+)	COVID-19 (–)	COVID-19 (+)	COVID-19 (–)
Ampicillin	80	77,8	–	–	–	–	–	–
Amoxicillin/clavulanic acid	69,2	80	76,5	84,6	–	–	–	–
Amikacin	20	36,5	31,6	56,7	69,2	74,4	93,8	85,7
Ciprofloxacin	35,5	54,9	57,9	79,3	73,1	82,1	100	96,4
Imipenem	15,2	40,4	25	66,7	73,1	79,5	100	92,9
Meropenem	17,4	38,5	30	66,7	76,9	79,5	100	92,9
Cefepim	12,5	0	–	–	73,1	82,1	100	92,9
Cefotaxime	48,4	72,5	50	80	–	–	–	–
Ceftazidime	–	–	–	–	33,3	54,5	–	–
Cefoperazone/sulbactam	20	30	23,1	60	22,2	50,0	–	–
Co-trimoxazole	–	–	–	–	0	25	–	–
ESBL	41,2	42,9	33,3	37,5	–	–	–	–

The *Streptococcus spp.* group is primarily represented by the following species: *S. mitis, S. parasanguinis, S. vestibularis, S. salivarius,* and *S. oralis*, which demonstrated the highest levels of resistance to antimicrobial agents of the penicillin family. All the above bacteria were recognized as dominant flora of the upper respiratory tract mucosa, providing normal biocenosis in healthy people. *S. pneumoniae* strains isolated from patients with confirmed COVID-19 were distinct by their resistance to azithromycin. Among the bacte-

ria belonging to the genus *Staphylococcus*, there were *S. aureus, S. haemolyticus,* and *S. warneri*, which more frequently demonstrated resistance to the penicillin family and azithromycin. There were 2 *S. aureus* strains detected, which were susceptible only to amikacin. Strains of bacteria belonging to the genus *Enterococcus* were mainly represented by species *E. faecium* and were distinct by their multiple resistance, with vancomycin being an exception.

Bacteria of the family *Enterobacteriaceae* exhibit high-level tolerance to penicillins due to natural resistance of *K. pneumoniae* strains; they are also resistant to third-generation cephalosporins, though fourth-generation cephalosporins demonstrated the highest susceptibility rates. Pneumonia caused by non-fermenting gram-negative bacteria is effectively treated with ceftazidime and cefoperazone/sulbactam. Most of the *Serratia marcescens* strains were susceptible to co-trimoxazole, which is a specific feature of this subtype.

The susceptibility of 30 *K. pneumoniae* strains isolated from patients with CAP associated with SARS-CoV-2 was assessed for two commercially available bacteriophages (Klebsiella pneumoniae-specific, purified bacteriophage and sextaphage) using the Spot-test. One clinical strain of *K. pneumoniae* was detected as the strain exhibiting susceptibility to the above bacteriophages. The titers of phage particles were estimated by using Gratia's double-layer technique [24]. The bacteriophage titer was 108 PFU/ml. Thus, the solution of the problem addressing the expansion of the bank of bacteriophages for the most significant pathogens of bacterial infections needs further thorough research.

Conclusions

The LRT microbiota of patients with moderate and severe CAP associated with SARS-CoV-2 showed prevalence of gram-positive coccal flora mainly represented by bacteria of the genus *Streptococcus,* which significantly differed by high resistance to antibiotics.

The microbiome of the discharge from LRT of patients with moderate and severe CAP, without confirmed presence of SARS-CoV-2, was characterized by more frequent detection of gram-negative bacteria: the family *Enterobacteriaceae* (*K. pneumoniae*) and non-fermenting gram-negative bacteria (*Acinetobacter spp*).

High antibiotic resistance of gram-positive isolates, regardless of coronavirus presence, was primarily displayed to the penicillin family of antimicrobial agents.

Gram-negative isolates belonging to the family *Enterobacteriaceae* are characterized by resistance to the penicillin family of antimicrobial agents and third-generation cephalosporins; non-fermenting gram-negative bacteria showed multi-drug resistance.

The studied *K. pneumoniae* strains have demonstrated a high level of resistance to commercially available bacteriophages.

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