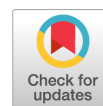


# THE IMPACT OF COVID-19 ON RESPIRATORY TRACT MICROBIOTA PATTERN IN PATIENTS WITH SEVERE PNEUMONIA



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**Abstract.** COVID-19 weakens the body's immune system and predisposes to bacterial infections. It has been increasingly evident about changes in the etiological pattern of pneumonia pathogens due to altered lung microbiota after viral pneumonia, the effect of SARS-CoV-2 on the immune system, and antibiotics taking as part of preventing secondary bacterial infection. Our study was aimed to analyze the dynamic change in the pattern of the respiratory tract microbiota in patients with severe pneumonia in the years from 2019 to 2023. There were enrolled 304 patients with pneumonia diagnosed after X-ray examination assessed from January 2019 to December 2023 inclusive. During the pandemic, all the investigated patients had a positive SARS-CoV-2 PCR result, and in the period after the pandemic all the examined patients were negative. Sputum samples delivered to the laboratory, where we performed microbiological culture with identification by MALDI-ToF mass spectrometry. In the pre-COVID-19 pandemic, 62 sputum samples were analyzed, among which *Klebsiella pneumoniae* and *Stenotrophomonas maltophilia* were the most common found in 21% and 17.7%, respectively. Both *Acinetobacter baumannii* and *Staphylococcus aureus* were isolated in 14.5% cases. *Streptococcus pneumoniae* was found in 8.1% cases. During the COVID-19 pandemic, 122 samples were evaluated allowing to observe that *K. pneumoniae* accounted for half of all isolated microorganisms. The second most common was *A. baumannii* (23.8%). In the post-pandemic period, 120 samples were analyzed, from which *K. pneumoniae* was mainly identified (31.7%). The next most frequent among pathogens were *S. aureus* and *A. baumannii* — 23.3% and 18.3%, respectively. In this way, there was a statistically significant change in the frequency of detection of Gram-positive and Gram-negative microorganisms in ICU patients during the study periods. Before the COVID-19 pandemic, the proportion of Gram-negative microorganisms in the pattern of pathogens was 67.7%, during the pandemic — 91.0%, in the post-pandemic period — approached the values of 2019–2020 and amounted to 70.0% ( $p < 0.001$ ). *K. pneumoniae* and *A. baumannii* were the most frequently found, however, the statistical significance of the changes was observed only for *K. pneumoniae* ( $p < 0.005$ ). An insignificant decline in the detection rate of pneumococcus was established as well. The frequency of staphylococcal discharge after coronavirus infection exceeded the pre-pandemic magnitude ( $p < 0.001$ ).

**Key words:** COVID-19, pneumonia, bacterial infection, *Klebsiella pneumoniae*, microbiota, respiratory tract.

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## ВЛИЯНИЕ COVID-19 НА СТРУКТУРУ МИКРОБИОТЫ РЕСПИРАТОРНОГО ТРАКТА У ПАЦИЕНТОВ С ТЯЖЕЛОЙ ПНЕВМОНИЕЙ

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**Резюме.** *Введение.* COVID-19, ослабляя иммунную систему организма, предрасполагает к присоединению бактериальных инфекций. Увеличивается количество сообщений об изменении этиологической структуры возбудителей пневмонии вследствие изменения микробиоты легких после перенесенной вирусной пневмонии, влияния SARS-CoV-2 на иммунную систему, приема антибиотиков в рамках профилактики вторичной бактериальной инфекции. Целью нашего исследования было проанализировать динамическое изменение структуры микробиоты дыхательных путей у пациентов с тяжелой пневмонией в период с 2019 по 2023 г. *Материалы и методы.* В исследовании участвовало 304 пациента с диагнозом «пневмония», установленным после рентгенологической диагностики, с января 2019 г. по декабрь 2023 г. включительно. В период пандемии у всех исследованных пациентов был зарегистрирован положительный результат ПЦР-теста на SARS-CoV-2, а в период после пандемии — отрицательный. Образцы мокроты доставляли в лабораторию, где проводили микробиологический посев с идентификацией методом MALDI-ToF масс-спектрометрии. *Результаты.* В период до пандемии COVID-19 было проанализировано 62 образца мокроты. Наиболее часто встречались *Klebsiella pneumoniae* и *Stenotrophomonas maltophilia* — 21 и 17,7%. В равной степени были выделены *Acinetobacter baumannii* и *Staphylococcus aureus* — 14,5%. *Streptococcus pneumoniae* высевался в 8,1% случаев. В пандемию COVID-19 была проведена оценка 122 анализов, по результатам которых половину от всех выделенных микроорганизмов составил *K. pneumoniae* (50,8%). Вторым по встречаемости являлся *A. baumannii* — 23,8%. В период после пандемии было проанализировано 120 образцов, из которых преимущественно высевался вид *K. pneumoniae* (31,7%). Следующими по частоте выявлений были *S. aureus* и *A. baumannii*. — 23,3 и 18,3% соответственно. Таким образом, было выявлено статистически значимое изменение частоты обнаружения грамположительных и грамотрицательных микроорганизмов у пациентов ОРИТ в исследуемые периоды. До пандемии COVID-19 доля грамотрицательных микроорганизмов в структуре возбудителей составляла 67,7%, в период пандемии — 91,0%, в постпандемийный — приблизилась к значениям 2019–2020 гг. и составила 70,0% ( $p < 0,001$ ). Наиболее часто высевались *K. pneumoniae* и *A. baumannii*, однако статистическая значимость изменений была установлена только для *K. pneumoniae* ( $p \leq 0,005$ ). Отмечалось снижение частоты обнаружения пневмококка, однако статистическая значимость не была установлена. Частота выделения стафилококков в период после коронавирусной инфекции превысила допандемийные значения ( $p < 0,001$ ).

**Ключевые слова:** COVID-19, пневмония, бактериальная коинфекция, *Klebsiella pneumoniae*, микробиота, респираторный тракт.

## Introduction

In December 2019, the coronavirus infection was first reported in Wuhan, China. On March 11 2020, the World Health Organization (WHO) declared a COVID-19 pandemic.

The causative agent of the infection is the severe acute respiratory syndrome virus SARS-CoV-2. SARS-CoV-2 has a tropism for lung tissue, where it causes diffuse alveolar damage, leading to extensive lung lesions. The pathogen binds to angiotensin-converting enzyme 2 receptors, penetrating the cell, triggers pattern recognition receptors responsible for the innate antiviral response. Start producing Interferons (IFN) types I, IFN III and proinflammatory cytokines [6]. The virus produced factor inhibition of IFN, which leads to dysregulation of the immune system and excessive release of proinflammatory cytokines, chemokines, and factors migration of immune cells (macrophages, neutrophils, monocytes and lymphocytes). Development of hyperactivation of the immune response (cytokine storm) leads to depletion of the immune system and an increase in the pathological process. SARS-CoV-2 also suppresses the cellular immune response impaired

lymphopoiesis and induction of apoptosis of CD4<sup>+</sup> T cells, causing lymphocyte depletion [2].

Bacterial complications develop in 6.9% patients with COVID-19 [3]. Bacterial co-infections are developing within 4–14 days from infection and leads to severe pneumonia. Complications occur significantly more often in patients undergoing treatment in intensive care unit (ICU). They reach 14–40%, and fatal outcomes in ICU patients occur in 50% or more cases [4, 10].

The development of bacterial pneumonia in COVID-19 patients is associated with a disruption of the mucous barrier and mucociliary clearance, as well as a weakening of specific defense factors. As a result, the respiratory tract favorable conditions are created for bacterial colonization [8]. The aggressive synergism of bacteria and viruses increases the hyperactivation of the immune response and leads to a “cytokine storm”, which negatively affects the outcome of the disease [11].

Before COVID-19 pandemic the most common pathogens of bacterial pneumonia were *Streptococcus pneumoniae* and *Haemophilus influenzae*. *S. pneumoniae* is isolated in 30–50% of cases, and *H. influenzae* — up to 25%. The role of gram-negative bacteria

is also increasing, including in patients undergoing inpatient treatment [7, 13].

During the pandemic, there was a change in the structure of microorganisms isolated from the sputum of COVID-19 patients. In the first months of the pandemic, there was a decrease in the number of pneumonias caused by *S. pneumoniae*. In the spring of 2021, their number reached pre-pandemic levels. This was due to the circulation of seasonal viral infections, such as influenza [12].

During pandemic, there was also an increase in bacterial concomitant complications caused by *Klebsiella pneumoniae* and *Acinetobacter baumannii* [1, 5]. *K. pneumoniae* was mainly isolated from patients with severe pneumonia and comorbidities. It has been established that coinfection of pneumonia caused by *K. pneumoniae* and COVID-19 is accompanied by a high 28-day mortality rate [9]. The highest mortality was observed in the group of patients with hospital-acquired pneumonia — 39.6%. Mortality from community-acquired pneumonia was 27.9%. The addition of bacterial pathogens worsens the condition of patients and increases the risk of death [14].

After pandemic changes in the etiological structure of pneumonia pathogens are possible. This could be due to the uncontrolled use of antibiotics during the pandemic, as well as the effect of SARS-CoV-2 on the immune system and lung tissue.

The aim of this study was to analyze the dynamic change in the structure of the respiratory tract microbiota in patients with severe pneumonia from 2019 to 2023 years.

## Materials and methods

We analyzed the results of microbiological examination of sputum from 304 patients treated in the ICU with a diagnosis of pneumonia from January 2019 to December 2023 inclusive. The study stages were divided into three periods: before (January 2019–March 2020), during (April 2020–June 2022) and after (July 2022–December 2023) the COVID-19 pan-

demic. The diagnosis of COVID-19 was confirmed by polymerase chain reaction. In the post-COVID period, all patients had negative test results of SARS-CoV-2. Pneumonia was confirmed by X-ray diagnostic methods.

Sputum samples were collected in sterile containers and delivered to the laboratory within two hours. The material was plated on nutrient media including 5% blood agar with defibrinated sheep blood (HiMedia, India), chromogenic agar for urinary tract infections (CondaLab, Spain) and Mueller-Hinton agar (CondaLab, Spain). The grown cultures were identified by MALDI-ToF mass spectrometry on a Microflex LT mass spectrometer (Bruker, Germany). During the study, 304 strains of microorganisms were identified.

Statistical analysis was performed using StatTech v. 4.7.2 (developer: StatTech LLC, Russia). Comparisons of percentages in multifield contingency tables were performed using the Pearson chi-square test. Post hoc comparisons were performed using the Pearson chi-square test with Holm's correction. Differences were considered statistically significant at  $p < 0.05$ .

## Results

The dynamics of changes in microorganisms isolated from clinical material are presented in Fig. In the pre-COVID-19 period, 62 samples were examined. The most frequently detected bacteria were *K. pneumoniae* and *Stenotrophomonas maltophilia*, isolated in 21.0% and 17.7% of cases, respectively. *A. baumannii* and *Staphylococcus aureus* were detected with the same frequency of 14.5%. *Streptococcus pneumoniae* was isolated in 8.1% of cases. Order *Enterobacterales*, excluding the genus *Klebsiella*, was isolated in 9.7%.

During the coronavirus pandemic 122 sputum samples were evaluated. *K. pneumoniae* was detected in half of all samples. The second most common pathogen was *A. baumannii* — 23.8%. Order

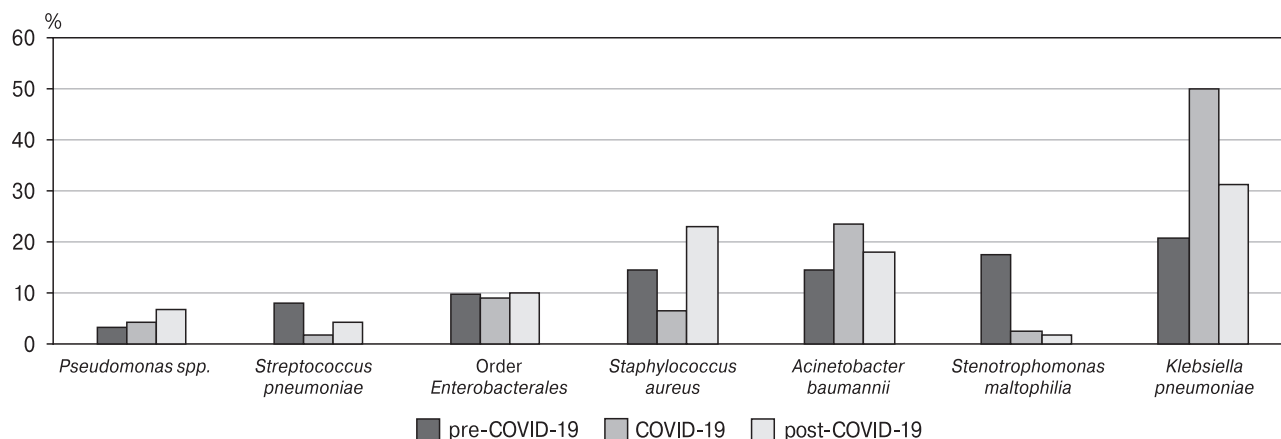


Figure. Structure of isolated microorganisms

**Table 1. Changes in the frequency of isolation of gram-positive and gram-negative microorganisms during the study periods**

Microorganisms	Presence in clinical material	Periods			p
		Before COVID-19	COVID-19	After COVID-19	
Gram-positive	Not found	45 (72.6%)	112 (91.8%)	84 (70.0%)	< 0.001
	Found	17 (27.4%)	10 (8.2%)	36 (30.0%)	
Gram-negative	Not found	20 (32.3%)	11 (9.0%)	36 (30.0%)	< 0.001
	Found	42 (67.7%)	111 (91.0%)	84 (70.0%)	

**Table 2. Changes in the frequency of pathogen isolation**

Microorganisms	Presence in clinical material	Periods			p-test value
		Before COVID-19	COVID-19	After COVID-19	
<i>Staphylococcus aureus</i>	Not discovered	53 (85.5%)	114 (93.4%)	92 (76.7%)	< 0.001
	Found	9 (14.5%)	8 (6.6%)	28 (23.3%)	
<i>Streptococcus pneumoniae</i>	Not discovered	57 (91.9%)	120 (98.4%)	115 (95.8%)	0.105
	Found	5 (8.1%)	2 (1.6%)	5 (4.2%)	
<i>Stenotrophomonas maltophilia</i>	Not discovered	52 (83.9%)	119 (97.5%)	118 (98.3%)	≤ 0.001
	Found	10 (16.1%)	3 (2.5%)	2 (1.7%)	
<i>Klebsiella pneumoniae</i>	Not discovered	48 (77.4%)	60 (49.2%)	82 (68.3%)	≤ 0.005
	Found	14 (22.6%)	62 (50.8%)	38 (31.7%)	
<i>Acinetobacter baumannii</i>	Not discovered	53 (85.5%)	93 (76.2%)	98 (81.7%)	0.291
	Found	9 (14.5%)	29 (23.8%)	22 (18.3%)	
<i>Escherichia coli</i>	Not discovered	57 (91.9%)	117 (95.9%)	113 (94.2%)	0.536
	Found	5 (8.1%)	5 (4.1%)	7 (5.8%)	
<i>Pseudomonas aeruginosa</i>	Not discovered	60 (96.8%)	117 (95.9%)	112 (93.3%)	0.513
	Found	2 (3.2%)	5 (4.1%)	8 (6.7%)	

*Enterobacteriales* (except *K. pneumoniae*) were identified in 9.0%. Pneumococcus was isolated in 1.6% of cases, *Stenotrophomonas maltophilia* — 2.5%.

In post-COVID period, 120 patients were examined. *K. pneumoniae* was isolated in 31.7% of cases. Also dominated *S. aureus* (23.3%) and *A. baumannii* (18.3%). Order *Enterobacteriales*, except for *K. pneumoniae*, were isolated in 10.0% of cases, *S. pneumoniae* — 4.2%. The share of *Stenotrophomonas maltophilia* was 1.7%.

## Discussion

Our study revealed a statistically significant change in the frequency of detection of microorganisms in ICU patients (Table 1). Before the COVID-19 pandemic, the proportion of gram-negative microorganisms was 67.7%. During the pandemic, it reached 91.0%, and in the post-pandemic period it approached the pre-COVID-19 values and amounted to 70.0% ( $p < 0.001$ ). Despite the decrease in the frequency of isolation of gram-positive microorganisms during the pandemic, in the post-pandemic period their proportion increased and began to correspond to pre-COVID-19 values.

The following results were obtained when analyzing the frequency of isolation of various microorganisms (Table 2). Before the COVID-19 pandemic, a high frequency of detection of *Stenotrophomonas maltophilia* was observed. However, the frequency

of isolation of the pathogen decreased from 17.7% in the pre-COVID-19 period to 1.7% in the post-pandemic period ( $p < 0.001$ ).

*K. pneumoniae* was detected with high frequency in the pre-COVID-19 period. During the COVID-19 period, the proportion increased from 21.0% to 50.8%, but in post-COVID-19 period, it decreased to 31.7%. The changes in the frequency of *K. pneumoniae* isolation were statistically significant ( $p \leq 0.005$ ).

There is a slight change in the frequency of occurrence of other representatives of the order *Enterobacteriales*, except *K. pneumoniae*. During the COVID-19 period, their number decreased from 9.7% to 9.0%, and in the post-pandemic period, it exceeded pre-pandemic values — 10% ( $p = 0.966$ ).

The dynamics of *A. baumannii* detection varied from 14.5% in the pre-COVID-19 period and reached a maximum during the pandemic — 23.8%. At the end of the pandemic, a decrease in the frequency of pathogen isolation to 18.3% was revealed ( $p = 0.291$ ).

*Pseudomonas* spp. was isolated from 3.2% in pre-COVID-19 to 4.1% during the pandemic and amounted to 6.7% in the post-COVID-19 period. These changes were not statistically significant ( $p = 0.513$ ).

During the COVID-19 period, a decrease in the proportion of isolated *S. aureus* was noted from 14.5% to 6.6% compared to pre-COVID-19 values. In the post-COVID-19 period, the frequency of microorganism isolation exceeded pre-pandemic values and reached 23.3% ( $p < 0.001$ ).

There was a downward trend in the pneumococcal detection rate ( $p = 0.105$ ). It decreased from 8.1% in the pre-COVID-19 period, reached a minimum during the pandemic (1.6%), and was 4.2% in the post-COVID-19 period. The findings are consistent with literature data, which also noted a decrease in *S. pneumoniae* during COVID-19 and an increase in the subsequent period [12].

Thus, in all the studied periods, there was a high frequency of detection of pathogens of nosocomial pneumonia in ICU patients. Before the COVID-19 pandemic, gram-negative pathogens (*K. pneumoniae*, *A. baumannii*, *S. maltophilia*) were most often isolated. Among gram-positive microorganisms, *S. aureus* and *S. pneumoniae* were dominated.

During the COVID-19 period, a decrease in the diversity of bacterial pathogens of pneumonia and an increase in the isolation of nosocomial pathogens, especially *K. pneumoniae* and *A. baumannii*, were noted. In our opinion, this is due to the infection of patients with COVID-19 with bacterial microflora

in hospitals, where gram-negative pathogens dominate. The transmission of *S. aureus* from staff to patients was difficult, since personal protective equipment was used.

In the post-COVID-19 period, changes in the ratio of pathogen detection rates with preservation of their generic structure have been identified. However, further monitoring of pathogens causing severe pneumonia is required.

## Conclusion

Thus, one of the global consequences of COVID-19 may be the spread of gram-negative flora often having antibiotic resistance. To prevent the further spread of these strains, it is necessary to conduct mandatory microbiological testing of clinical material in patients with pneumonia, use modern diagnostic methods, determine the sensitivity of bacteria to antibiotics and the minimum inhibitory concentration for justified targeted therapy.

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