

**ОПЫТ ДИНАМИЧЕСКОГО НАБЛЮДЕНИЯ КОЛОНИЗАЦИИ
ВЕРХНИХ ДЫХАТЕЛЬНЫХ ПУТЕЙ ПАЦИЕНТА С
МУКОВИСЦИДОЗОМ ШТАММОМ ROSEOMONAS
AEROFRIGIDENS НА ФОНЕ ПРИЕМА ПРЕПАРАТОВ ТАРГЕТНОЙ
ТЕРАПИИ**

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**DYNAMIC OBSERVATION OF UPPER AIRWAYS COLONIZATION IN A
CYSTIC FIBROSIS PATIENT INFECTED WITH ROSEOMONAS
AEROFRIGIDENS UNDER TARGETED THERAPY**

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Резюме

Муковисцидоз является заболеванием, для которого характерно широкое микробиологическое разнообразие. В него входят такие бактерии как *Pseudomonas aeruginosa*, *Burkholderia cepacia complex*, *Achromobacter xylosoxidans/ruhlandii*. Кроме них также присутствуют микроорганизмы с неустановленным при муковисцидозе клиническим значением, к которым относят *Roseomonas aerofrigidens*. При этом, в свободных источниках практически отсутствуют описания выделения представителей рода *Roseomonas* от пациентов с муковисцидозом. Целью данного исследования является оценка распространённости указанных микроорганизмов среди пациентов с муковисцидозом, проходящих регулярное обследование на базе микробиологической лаборатории Клиник ФГБОУ ВО СамГМУ Минздрава России. Был проведен анализ результатов 12094 посевов клинического материала, идентификация проводилась с помощью масс-спектрометра MALDI-ToF, рассчитан составной индекс корреляции. Также, для оценки характера симбионтных или антагонистических взаимодействий использовался коэффициент сходства Жаккара. В ходе исследования было выделено 20 штаммов бактерий *Roseomonas spp.* В четырех из двадцати случаев штамм был выделен со слизистой оболочки задней стенки глотки, в остальных случаях - из жидкости назального лаважа. Пациент с генетикой F508del/F508del с октября 2022 года получал двухкомпонентную таргетную терапию. До начала терапии не наблюдалось клинически значимых грамотрицательных видов организмов, также как и штаммов *Roseomonas aerofrigidens* в анамнезе. Однако, на третьем, пятом, шестом, восьмом, девятом, одиннадцатом и восемнадцатом месяцах наблюдения отмечены высевы *Roseomonas aerofrigidens* из жидкости назального лаважа, при отсутствии роста указанного штамма в мокроте. При изучении особенностей белковых профилей изолятов установлено, что они имеют высокую степень родства с индексом корреляции более 0,8, что подтверждает гипотезу о

продолжительной (в течении нескольких месяцев) колонизации параназальных синусов одним и тем же штаммом *Roseomonas aerofrigidens*. При оценке критерия постоянства установлено, что среди 30 видов микроорганизмов, выделенных за 18 месяцев наблюдения из жидкости назального лаважа, два микроорганизма были отнесены к представителям постоянной микробиоты, четыре (среди которых был *Roseomonas aerofrigidens*) отнесены к добавочной микробиоте. Остальные виды относятся к случайной микробиоте. Исходя из индекса Жаккара, установлено, что для четырех пар микроорганизмов характерен синергидный характер взаимоотношений. Данные наблюдения демонстрируют случай многомесячной колонизации уникальным штаммом *Roseomonas aerofrigidens* пациента с муковисцидозом, получающего таргетную терапию.

Ключевые слова: муковисцидоз, дыхательные пути, микробиота, *Roseomonas aerofrigidens*, MALDI-ToF, таргетная терапия.

Abstract

Cystic fibrosis (CF) is a disease characterized by a varying microbiological diversity, including bacteria such as *Pseudomonas aeruginosa*, *Burkholderia cepacia complex*, *Achromobacter xylosoxidans/ruhlandii*. Additionally, there are microorganisms with unknown clinical significance, including *Roseomonas aerofrigidens*. The main goal of the study is to evaluate the prevalence of such microorganisms among CF patients undergoing regular examinations at the microbiological laboratory of the Samara State Medical University, Ministry of Health of the Russian Federation. An analysis of 12,094 clinical material cultures was carried out, with identification done by using MALDI-ToF mass spectrometry. A composite correlation index was calculated. The Jaccard similarity coefficient was used to assess the nature of symbiotic or antagonistic interactions.

A total of 20 strains of *Roseomonas* spp. bacteria was isolated. Four strains were obtained from the mucous membrane of the posterior pharyngeal wall, while the remaining strains – from nasal lavage fluid. The patient with F508del/F508del genetics had been receiving dual targeted therapy since October 2022. Before treatment, no clinically significant Gram-negative species or *Roseomonas aerofrigidens* strains were noted in the patient's anamnesis. However, during observation, *Roseomonas aerofrigidens* cultures were obtained from nasal lavage fluid at the third, fifth, sixth, eighth, ninth, eleventh, and eighteenth months of observation, with no concurrent bacterial strain growth in sputum samples.

The study of the protein profiles demonstrated a high degree of kinship among the isolates, with a correlation index exceeding 0.8, confirming the hypothesis on long-term colonization of the paranasal sinuses by same *Roseomonas aerofrigidens* strain. While assessing the consistency criterion, it was found that among 30 microbial species isolated from nasal lavage fluid over 18-month observation, two microorganisms were classified as representatives of the constant microbiota, four (including *Roseomonas aerofrigidens*) as additional

microbiota. The remaining species belong to random microbiota. Based on the Jaccard coefficient, it was found that four pairs of microorganisms are characterized by a synergistic nature of the relationship. These observations demonstrate a case of multi-month colonization with a unique strain of *Roseomonas aerofrigidens* in a patient with cystic fibrosis receiving targeted therapy.

Keywords: cystic fibrosis, respiratory tract, microbiota, *Roseomonas aerofrigidens*, MALDI-ToF, targeted therapy.

1 Introduction

Cystic fibrosis remains one of the most significant genetic diseases, characterized by a unique microbiological landscape and significant species breadth. In addition to the "classic" species for this disease, such as *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Achromobacter xylosoxidans/ruhlantii*, whose clinical significance in the development of bacterial complications of the respiratory tract is beyond doubt. In recent years, the question of the role of environmental microorganisms with unspecified clinical significance in cystic fibrosis has become relevant. These include bacterium *Roseomonas aerofrigidens*. These microorganisms are gram-negative coccobacilli belonging to class *Alphaproteobacteria*. They have a low growth rate, giving visible growth no earlier than 2-3 days of incubation, depending on the type of growth media used. According to the literature, the strains can grow on simple nutrient media, on MacConkey medium, but the best growth noted when culturing on Sabouraud medium. Growth is given in the form of slimy colonies, moist with a shiny surface from pale pink to coral-salmon shade. When examined microscopically, they look like gram-negative coccobacilli or thick rods, located in pairs or in the form of separate chains. Many of them can be mobile due to one or two polar flagella. Their biochemical activity is well studied. Meanwhile, representatives of the genus *Roseomonas* are quite rarely isolated from biological samples from humans. There are descriptions of the isolation of strains from blood, wound discharge, exudate, fragments of ureters and fluid for peritoneal dialysis. [1-6] However, in the available sources there are practically no descriptions of the isolation of these strains from respiratory samples from patients with cystic fibrosis, especially cases of chronic colonization.

2 Materials and methods

To assess the prevalence of these microorganisms among patients with cystic fibrosis in the Russian Federation undergoing regular microbiological examination

29 at the microbiological laboratory of the Clinics of the Federal State Budgetary
30 Educational Institution of Higher Education Samara State Medical University of the
31 Ministry of Health of the Russian Federation, an analysis of the results of 12,094
32 clinical material cultures was performed for the period from January 2019 to July
33 2024. The isolated cultures were identified using MALDI-ToF mass spectrometry
34 (Bruker, Germany). Visualization of the results of statistical proteomic comparison
35 of the mass spectra of the isolated strains was carried out using MALDI Biotyper
36 3.0 Offline Classification software (Bruker Daltonik GmbH, Germany). For the
37 studied strains, the Composite Correlation Index was calculated using the BioTyper
38 Composite Correlation Standard Method. To assess the biological diversity of the
39 microbiota isolated from nasal lavage fluid over time, the consistency criterion C
40 was used. When assessing the indicator with a prevalence of microorganisms less
41 than 25% in the overall structure of isolated representatives, the species was
42 regarded as a transient microbiota participant. With an isolation frequency from 25
43 to 50%, the species was regarded as a representative of additional microbiota, and
44 in the case of presence in more than 50% of samples from the locus, as a permanent
45 participant in the microbiome. In addition, to assess the nature of symbiotic or
46 antagonistic interactions within the considered community of the biotope, the
47 Jaccard similarity coefficient (q) was calculated. With a value of $q < 30\%$, the
48 species were considered as antagonists, $q = 30-70\%$ - as synergists, $q > 70\%$ as
49 mutualists.

50 3 Results and discussion

51 During the period of research, 20 strains of *Roseomonas spp.* bacteria were
52 isolated. In four cases, the strain was isolated from the mucous membrane of the
53 posterior pharyngeal wall, in all other cases isolated from nasal lavage fluid. Single
54 episodes of isolation were noted among 13 patients, which could be explained by
55 transient carriage, and one patient, at the time of describing this case, had a history
56 of seven-fold cultures from nasal lavage fluid.

57 Patient K, born in 2013, has genetics F508del/F508del. Since October 2022,
58 he has been receiving two-component targeted therapy. Before the start of therapy,
59 patient had no cultures of clinically significant gram-negative types of
60 microorganisms from respiratory samples. He also had no cultures of strains of
61 *Roseomonas aerofrigidens* in his medical history. Since the start of targeted
62 therapy, the patient has undergone regular microbiological monitoring. At the time
63 of the case description, the patient had 18 months of microbiological monitoring of
64 sputum and nasal lavage fluid. At the third, fifth, sixth, eighth, ninth, eleventh and
65 eighteenth months of observation, *Roseomonas aerofrigidens* cultures were noted
66 from nasal lavage fluid, with no growth of the strain in sputum. Growth was
67 obtained on OFPBL medium (HiMedia, India) in the form of pink mucous colonies,
68 shown in figure (**figure 1**). No hemolytic activity was detected when reseeded on
69 Columbia agar with 5% sheep blood, and no phospholipase activity was detected
70 when culturing on yolk-salt agar. When culturing on chromogenic agar (Conda,
71 Spain), mucous pink colonies identical to those obtained on OFPBL medium were
72 obtained.

73 When studying the characteristics of the protein profiles of the isolates, it was
74 found that they have a high degree of kinship (correlation index over 0.8), which
75 confirms our hypothesis that the patients paranasal sinuses had been colonized the
76 with the same strain of *Roseomonas aerofrigidens* for several months (CCI matrix
77 is shown in the figure) (**figure 2**). When assessing the consistency criterion C, it
78 was found that 2 out of 30 microorganism isolated from nasal lavage fluid during
79 18 months of observation, were classified as representatives of the constant
80 microbiota (*Staphylococcus aureus* - consistency criterion of 75.0%, *Sphingomonas*
81 *paucimobilis* - consistency criterion of 50.0%). Four representatives were classified
82 as additional microbiota. Among them are *Roseomonas aerofrigidens*,
83 *Staphylococcus epidermidis*, *Chryseobacterium taihuense* and *Acinetobacter junii*
84 with criterion values of 44.8%, 37.5%, 31.3% and 25.0%, respectively. The
85 remaining species: *Kocuria rhizophila*, *Brevibacterium rhamnosus*, *Acinetobacter*

86 *pitti*, *Staphylococcus pasteurii*, *Brevundimonas aurantiaca*, *Neisseria flavescens*,
87 *Microbacterium paraoxydans*, *Microbacterium aurum*, *Commamonas aquatica*,
88 *Acinetobacter johnsonii*, *Sphingobacterium miltivorans*, *Rhizobium radiobacter*,
89 *Brevundimonas albigilva*, *Pseudomonas stutzeri*, *Staphylococcus intermedius*,
90 *Chryseobacterium hamamense*, *Kocuria marinae*, *Acidovorax temperans*,
91 *Streptococcus oralis*, *Acinetobacter ursungii*, *Microbacterium estacenum*,
92 *Moraxella catarrhalis*, *Sphingomonas panni* were classified as representatives of
93 random microbiota, having C criterion less than 25%. To determine the conjugacy
94 of taxa for pairs of species belonging to the constant and additional microflora, the
95 Jaccard coefficient was calculated, the results of which were presented in the figure
96 (**table 1**). It was found that a synergistic nature of the relationship was determined
97 for four pairs of microorganisms highlighted in orange. Presented observations
98 demonstrate an example of a very rare and previously undescribed case of multi-
99 month colonization by a unique strain of *Roseomonas aerofrigidens* of a patient
100 with cystic fibrosis receiving targeted therapy. It is noted that before the start of
101 pathogenetic treatment, the patient had no episodes of excretion of the presented
102 microorganism. At the same time, from a clinical point of view, despite
103 colonization, the patient has not yet shown any deterioration in condition, both in
104 terms of subjective signs (absence of complaints) and from the standpoint of the
105 conclusion of computed tomography of the sinuses. It should be noted that, in all
106 likelihood, we are talking about isolated sinonasal colonization, since during the
107 entire observation period, we did not obtain any growth of the strain from sputum
108 or oropharyngeal samples.

109 4 Conclusion

110 In recent years, due to the optimization and improvement of quality of
111 microbiological diagnostics, the number of isolation of rare species of
112 microorganisms with unknown clinical significance has increased. To understand
113 their potential role in the development of infectious complications, it is necessary

114 to accumulate experience in isolation and knowledge of their biological properties,
115 pathogenetic potential not only in isolation within microbiological laboratories, but
116 also by assessing the relationship between the indicated cases of strain isolation and
117 the clinical dynamics of the patient, the results of his instrumental and physical
118 examination methods.

ТАБЛИЦЫ

Table 1. Jaccard coefficient for species isolated from patient K. during 18 months of observation.

Species	a	b	c	q
<i>S.paucimobilis</i> + <i>R.aerofrigidens</i>	8	7	5	50.0%*
<i>S.paucimobilis</i> + <i>S.aureus</i>	8	12	4	25.0%
<i>S.paucimobilis</i> + <i>S.epidermidis</i>	8	6	2	16.7%
<i>S.paucimobilis</i> + <i>C.taihuense</i>	8	5	1	8.3%
<i>S.paucimobilis</i> + <i>A.junii</i>	8	4	1	9.1%
<i>R.aerofrigidens</i> + <i>S.aureus</i>	7	12	5	28.6%
<i>R.aerofrigidens</i> + <i>S.epidermidis</i>	7	6	4	44.4%*
<i>R.aerofrigidens</i> + <i>C.taihuense</i>	7	5	3	33.3%*
<i>R.aerofrigidens</i> + <i>A.junii</i>	7	4	1	9.1%
<i>S.aureus</i> + <i>S.epidermidis</i>	12	6	4	28.6%
<i>S.aureus</i> + <i>C.taihuense</i>	12	5	3	21.4%
<i>S.aureus</i> + <i>A.junii</i>	12	4	2	14.2%
<i>S.epidermidis</i> + <i>C.taihuense</i>	6	5	2	22.2%
<i>S.epidermidis</i> + <i>A.junii</i>	6	4	0	0%
<i>C.taihuense</i> + <i>A.junii</i>	4	4	2	33.3%*

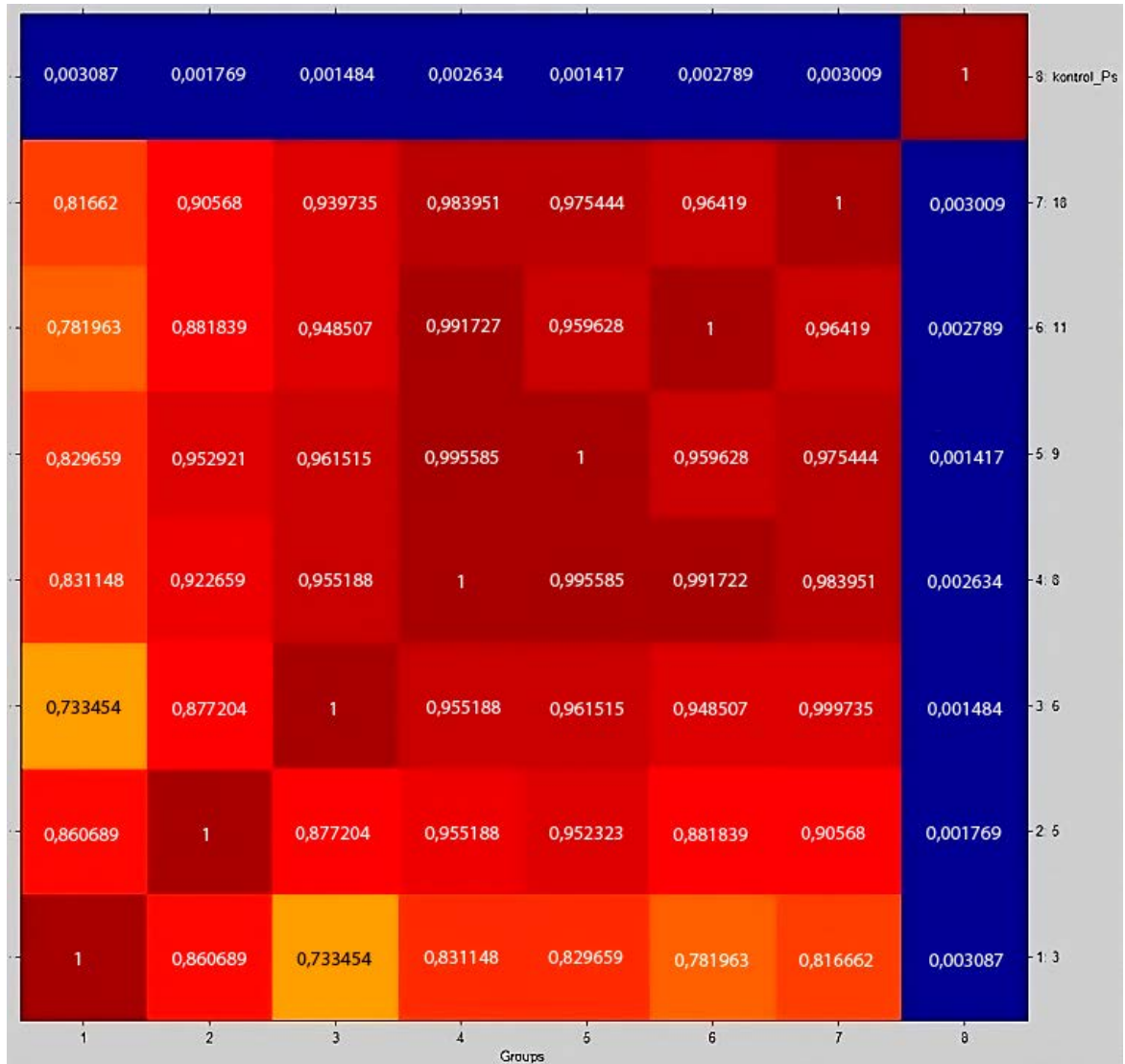
a - number of samples in which the first microorganism was isolated; b - number of samples in which the second microorganism was isolated; c - number of samples in which both microorganisms of the pair were isolated; q - Jaccard coefficient; * - species for which synergistic relationships have been determined.

РИСУНКИ

Figure 1. Growth of *Roseomonas aerofrigidens* on OFPBL agar.



Figure 2. CCI matrix for *Roseomonas aerofrigidens* strains isolated from patient K. at 3, 5, 6, 8, 9, 11 and 18 months of therapy.



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Блок 3. Метаданные статьи

ОПЫТ ДИНАМИЧЕСКОГО НАБЛЮДЕНИЯ КОЛОНИЗАЦИИ ВЕРХНИХ
ДЫХАТЕЛЬНЫХ ПУТЕЙ ПАЦИЕНТА С МУКОВИСЦИДОЗОМ
ШТАММОМ ROSEOMONAS AEROFRIGIDENS НА ФОНЕ ПРИЕМА
ПРЕПАРАТОВ ТАРГЕТНОЙ ТЕРАПИИ

DYNAMIC OBSERVATION OF UPPER AIRWAYS COLONIZATION IN A
CYSTIC FIBROSIS PATIENT INFECTED WITH ROSEOMONAS
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Сокращенное название статьи для верхнего колонтитула:

ROSEOMONAS ПРИ МУКОВИСЦИДОЗЕ
ROSEOMONAS IN CYSTIC FIBROSIS

Ключевые слова: муковисцидоз, дыхательные пути, микробиота,
Roseomonas aerofrigidens, MALDI-ToF, таргетная терапия.

Keywords: cystic fibrosis, respiratory tract, microbiota, Roseomonas aerofrigidens,
MALDI-ToF, targeted therapy.

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