BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 5

DOI: 10.58240/1829006X-2025.21.5-292

ORIGINAL ARTICALE

COMPARATIVE ANALYSIS OF INDIVIDUAL REPRESENTATIVES OF THE MICROBIOTA OF THE PERIIMPLANT ZONE IN NORMAL AND INFLAMMATORY CONDITIONS

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Received: *May 15. 2025;* ***Accepted:*** *Jun 15, 2025;* ***Published:*** *Jun.30,2025*

ABSTRACT

Background: The incidence of periimplantitis has increased significantly in recent years, which has led to an increase in the number of implant rejection cases and a deterioration in patients' lives.

The purpose of the study. To conduct a comparative analysis of individual representatives of the microbiota of the peri-implant sulcus in normal and inflammatory conditions.

Materials and methods: Samples of the contents of the peri-implant sulcus from 20 patients with healthy peri-implant tissues and 20 patients with peri-implantation. The quantitative and qualitative composition of the microbiota in the samples was determined by the classical bacteriological method.

Results: It was found that *Streptococcus spp., Staphylococcus spp., Candida spp. and Escherichia coli* predominate in the samples obtained from patients of the main group. Moreover, the number of *Candida spp*. In the main group, the number of *Candida spp* is 20.8 times higher. in the comparison group. In addition, representatives of *Sarcina spp*., which had not previously been associated with the development of peri-implantitis, were found.

Conclusion: The microbiota plays a crucial role in the development of peri-implantitis. Further research aimed at understanding the role of various microorganisms and their mechanisms of action will make it possible to develop effective strategies for the prevention and treatment of this complex disease. Maintaining good oral hygiene and regular routine checkups remain key factors in preventing the development of peri-implantitis.

***Keywords*:** dental implants, peri-implantitis, microbiota, microbial biofilms.

1.INTRODUCTION

The use of dental implants is a highly effective method of restoring the integrity of the dentition in patients with missing teeth. Despite the high degree of success, implantological treatment is associated with the risk of complications, in particular, peri-implantitis. Numerous experimental and clinical studies have shown that peri-implantitis is associated with infection of peri-implanted tissues by microorganisms of the oral cavity due to unsatisfactory individual oral hygiene. Microbial biofilm forms on all surfaces of the implant structures, provoking inflammation followed by destruction of bone tissue [1-5]. The results of clinical studies have shown that the risk of peri-implantitis in patients with periodontitis, both in the active phase and in remission, is five times higher than in patients with healthy periodontitis [6-9].

Despite the opinion about the identity of the microorganisms that cause periodontitis and peri-implantitis, and the detection of similar pathogenic microorganisms in the periodontal pockets and in the area around the implants, a number of authors deny the possibility of infection of the peri-implant zone from periodontal foci of infection. In this regard, the study of the microbiota in the combined course of these diseases is of considerable scientific interest [10].

The pathogenesis of peri-implantitis is less studied, and its features are primarily related to the structural features of the peri-implant zone[1,4]. Canullo L, Penarrocha-Oltra D et al. suggested that the absence of periodontitis in the implant area causes the mobility of the soft tissues of the peri-implant zone. This, in turn, leads to greater traumatization, increasing the adhesive capabilities of microorganisms and contributing to contamination of the peri-implant sulcus [11]. Peri-implantitis, like periodontitis, is an opportunistic disease; therefore, the bacteria that cause it are part of the normal microbiota of the oral cavity [12]. Bacteria from the oral fluid and dental plaque colonize the surface of the implant, colonizing the peri-implantant groove. Different types of microorganisms form associations (biofilms) for joint survival in the oral cavity. Microbes interact closely in biofilms, which leads to an increase in their pathogenicity, mutual provision of nutrients, exchange of genetic information, and formation of intracolonial signaling links, which leads to further progression of inflammation [4,26]. The microbiome of a healthy peri-implant sulcus is characterized by a low ratio of anaerobic and aerobic species and a small number of periodontal pathogens [13]. However, with certain microecological shifts, bacteria associated with inflammation become dominant and, acting in concert, acquire pathogenic properties [12]. At the same time, the anaerobic microbiota creates an acidic environment around the implant, which poses a physico-chemical threat to the stability of the oxide layer of the implant, disrupting the connection of the implant to the gum and bone and leading to the loss of surrounding and supporting tissues of the implant [2].

Despite the recognized priority of biofilms in the etiology of peri-implant diseases, there is still no consensus on the differences in the composition of the submucosal biofilm around implants in normal and inflammatory conditions. A clear understanding of the microbial profiles of the peri-implant sulcus is necessary to understand the consequences of microecological changes and to develop effective strategies for the prevention, diagnosis and treatment of peri-implantitis.

The aims of the study: To conduct a comparative analysis of individual representatives of the microbiota of the peri-implant sulcus in normal and inflammatory conditions.

MATERIALS AND METHODS

The study was conducted on the basis of two clinics in Tver (Russia): Professor Strelnikov's Clinic and Morozovskaya Dentistry, as well as at the Department of Microbiology and Virology with a course in immunology at the Tver State Medical University of the Ministry of Health of the Russian Federation.

The study included 40 patients, including 26 women and 16 men aged 24 to 60 years, who received dental implants of the Dentium system (South Korea). The study was conducted in compliance with all the principles of the Helsinki Declaration and approved by the local Ethics committee of the Tver State Medical University of the Russian Ministry of Health. Before being included in the study, a "Voluntary informed consent for examination" was signed with each volunteer, and an individual dental patient's medical record was created for each patient to collect information about the presence of oral diseases, as well as concomitant acute and/or chronic diseases of internal organs and systems, and an allergic history.

The patients were divided into two groups: group 1 (main) – patients with peri–implantation (n=20), group 2 (comparison) - patients with implants without signs of inflammation of surrounding tissues (n=20). All patients included in the studies did not have a diagnosis of "chronic periodontitis" established earlier and/or at the time of the examination (k05.3).

The examination of patients was carried out according to a generally accepted scheme, including a survey, clinical and X-ray dental examination, including periodontal probing.

Inclusion criteria: (1) age 18 years and older, (2) the presence of at least 1 dental implant, the functioning of which with a load of suprastructure for at least 6 months.

Design of the selection of dental implants included in the study: One dental implant was evaluated for each patient. In the presence of several healthy implants, an implant with the best hygienic and periodontal parameters was selected. In the presence of several affected implants, an implant with the worst hygienic and periodontal parameters was selected to assess the most severe condition. If one patient had both implants with healthy surrounding tissues and peri-implantation, then this

patient was included in group 1 (the main one), and the affected implant was analyzed.

Exclusion criteria: (1) unsigned voluntary informed consent to participate in the study, (2) pregnancy, (3) diabetes mellitus, (4) HIV infection, (5) taking antibacterial drugs for 3 months prior to sampling, (6) patients receiving intravenous and/or oral bisphosphonate therapy, (7) patients who underwent any type of anti-inflammatory therapy for 3 months prior to sampling, (8) the presence of a history of chronic periodontitis according to medical documentation or identified after examination, (9) the depth of probing in the implant area is more than 6 mm.

Sample collection, microbiological monitoring:

The samples for the study were taken from the periimplant sulcus (Fig. 1).

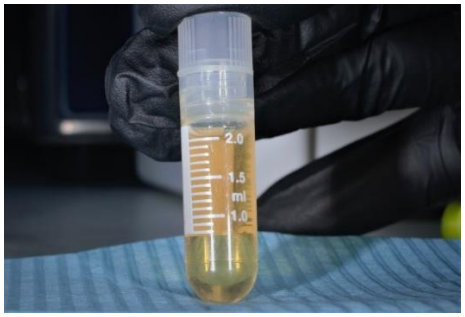

Figure 1. Tubes with Ames transport medium

The material was taken with sterile paper pins, which were placed in the sulcus for 30 seconds, and then into tubes with a special transport medium, Ames medium without coal (HiMedia, India) (Fig. 2).

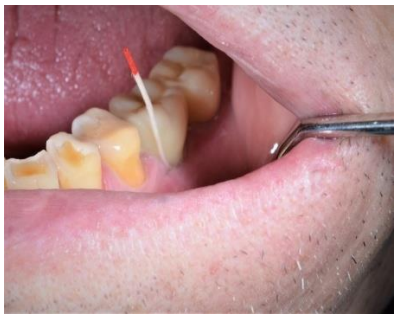

Figure 2. Sampling of the contents of the peri-implantation sulcus

The test tubes were tightly closed with lids and delivered to the laboratory within 2 hours to isolate pure cultures of microorganisms using the classical bacteriological method. 0.1 ml of the test material was taken for seeding (from the transport medium) and seeded on nutrient media: mannitol salt agar (M118) (HiMedia, India), blood agar (HiMedia, India), Saburo agar with glucose and chloramphenicol (HiMedia, India), streptococcal agar (HiMedia M304) (HiMedia, India), followed by incubation. Smears were prepared from isolated colonies grown on appropriate nutrient media and stained using the Gram method. Morphological and tinctorial properties of microorganisms were studied using the Diamorphocito® software and hardware complex (Russia) (1:1000 magnification using a Biolam binocular microscope). The number of bacteria was determined by counting colony-forming units per 1 ml of the test material (lg CFU/ml). The identification of microorganisms was carried out by biochemical activity using API® test systems (bioMérieux Vitek, Inc.) and API® WEB PC software.

Statistical processing of results:

Statistical characteristics based on samples, hypothesis testing based on statistical criteria was performed using Microsoft Excel [Microsoft Excel 2013, Microsoft Corp., USA]. Statistical differences between the groups were determined using the Student's criterion. Statistical significance was determined at the level of $p < 0.05$ to compare the quantitative composition of the microbiota and at the level of $p < 0.001$ to compare the indicators of dental status.

RESULTS

The results of the assessment of the dental status of patients in the two groups are presented in Table 1. The values of all the studied indicators significantly differed in patients of the first and second groups. The depth of probing in the implant area in patients of group 1 did not exceed 6 mm, in patients of group 2 – 3 mm against the background of general periodontal health. These indicators correlate with the value of the oral hygiene index and correspond to an average "satisfactory" level of oral hygiene for patients in group 1, with individual patients having "unsatisfactory" oral hygiene. In the comparison group, the average hygiene index values corresponded to "good" oral hygiene, and only some patients had a "satisfactory" level of oral hygiene.

In our study, when comparing the results obtained in patients with peri-implantation (the first group, n = 20) and patients in the comparison group (the second group, n = 20), significant differences in the microbial diversity of the contents of the peri-implant sulcus were revealed.

Table 1. The results of the assessment of the dental status of patients in two groups

Indicator	Group 1 (main) n=20			Group 2 (comparisons) n=20			p-value
	M_1	Std_1	Max_1	M_2	Std_2	Max_2	
Depth of periodontal probing (PPD), mm	3,55	1,145	6	2,05	0,686	3	≤0.001
Depth of sensing in the area of implants, mm	4,95	1,234	6	2	0,561	3	≤0.001
The level of radiological bone loss in the area of implants, mm	4,65	1,268	6	0,775	0,734	2	≤0.001
Silness-Loe Oral Hygiene Index, points	1,54	0,949	3	0,52	0,242	1,2	≤0.001
Mühlemann bleeding index, points	1,97	0,989	3,5	0,51	0,35	1,4	≤0.001

The values of all the studied indicators significantly differed in patients of the first and second groups. The depth of probing in the implant area in patients of group 1 did not exceed 6 mm, in patients of group 2 – 3 mm against the background of general periodontal health. These indicators correlate with the value of the oral hygiene index and correspond to an average "satisfactory" level of oral hygiene for patients in group 1, with individual patients having "unsatisfactory" oral hygiene. In the comparison group, the average hygiene index values corresponded to "good" oral hygiene, and only some patients had a "satisfactory" level of oral hygiene. In our study, when comparing the results obtained in patients with peri-implantation (the first group, n = 20) and patients in the comparison group (the second group, n = 20), significant differences in the microbial diversity of the contents of the peri-implant sulcus were revealed (Fig. 3). It was found that the dominant representatives of the microbiota in the studied samples were Peri-implantitis are *Streptococcus spp., Staphylococcus spp., Candida spp. and Escherichia coli* ($p < 0.05$).

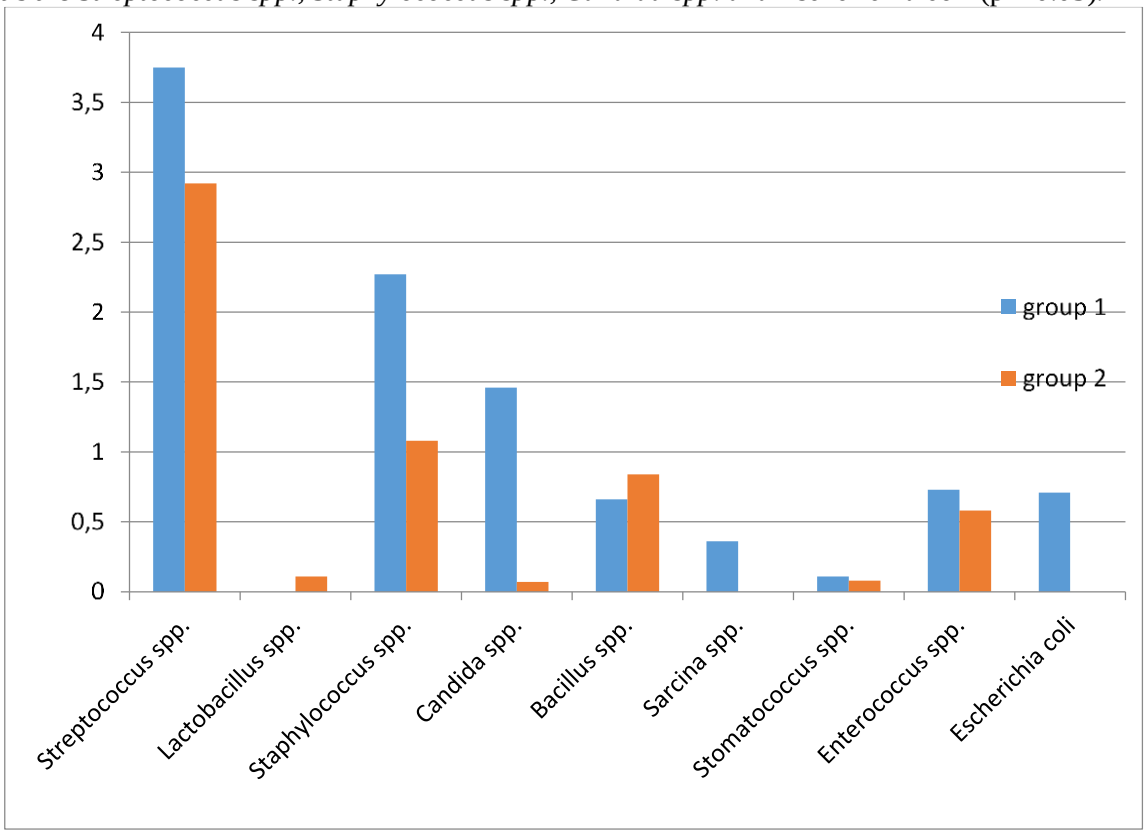

Figure 3. Comparison of the quantitative composition of isolated microorganisms of the main group and the comparison group (lg CFU/ml).

Also, representatives of *Sarcina sp*. were found in the area of the peri-implant sulcus in patients with peri-implantation, with their complete absence in the case of a healthy peri-implant zone. The specific and quantitative composition of the microbiota isolated from the peri-implant sulcus of the patients of the two groups is presented in Table 2.

Table 2. Species and quantitative composition of microorganisms isolated from the periimplant sulcus (lg CFU/ml)

Microorganisms	Group 1 (main) n = 20		Group 2 (comparisons) n =20		p-value
	M_1	Std_1	M_2	Std_2	
Streptococcus spp.	3.75	0.74	2,92	0.85	**0.000441***
Lactobacillus spp.	0	0	0.11	0.50	0.165282
Staphylococcus spp.	2.27	1.14	1.08	0.96	**0,002051***
Candida spp (Candida albicans)	1.46	1.32	0.07	0.31	**0,00009***
Bacillus spp.	0.66	1.24	0.84	1,36	0,340765
Sarcina spp.	0.36	0.89	0	0	**0,042056***
Stomatococcus spp.	0.11	0.49	0.08	0.39	0,164938
Enterococcus spp.	0.73	1.57	0.58	1.26	0,352221
Escherichia coli	0,71	1,42	0	0	**0,018657***

* the differences are statistically significant times greater ($p<0.05$).

DISCUSSION

The results of our research differ from the results obtained in the study by Kensara A et al. [14]. There were no differences in microbial diversity between the healthy peri-implant zone and the area of implants with peri-implant ($p = 0.82$). A controlled cross-sectional clinical trial conducted by Kensara A et al. [15] involved 23 patients: 11 with healthy implants and 12 with affected peri-implantitis. The aim of the study was to characterize the composition of the microbiome in the field of dental implants with peri-implant and healthy implants. It was noted that higher levels of Gram-positive bacteria, especially *Enterococcus spp*, were observed around peri-implanted implants compared to healthy implants. In contrast to this study, our study did not find significant differences in the number of *Enterococcus spp*. between healthy and affected implants.

In addition, Sousa V. et al. [16] proved that in conditions simulating implant health, *Staphylococcus spp*. and *Candida spp*. they are present in smaller quantities. This situation is in sharp contrast to biofilms in peri-implantitis, which are denser, more diverse and contain more pathogenic species, which is consistent with the results of our study, which contains the presence of *Staphylococcus spp*. and *Candida spp*. It was higher in peri-implantitis than in the area of healthy implants.

Candida albicans is a fungal pathogen often involved in various infectious processes of the oral cavity, including peri-implantitis. This opportunistic yeast fungus is able to attach and colonize both mucosal surfaces and inert materials, as well as form biofilms, which is a critical factor in its pathogenicity. Indeed, biofilms protect *Candida albicans* from environmental stresses, including antifungal drugs and the host immune response [4,26,28,29]. Biofilms containing *Candida albicans* provide a protective environment not only for the yeast itself, but also for other representatives of the microbiota, including bacterial pathogens, which complicates treatment and leads to chronic infection [25]. It is important to draw a parallel with the course of periodontitis and endo-periodontal lesions: *Candida albicans* is found in 20% of cases with periodontitis and in 33-55% of cases with reinfected root canals [26]. In the context of peri-implantitis, *Candida albicans* can attach to the implant surface and form biofilms containing a complex community of microorganisms, which contributes to the maintenance and aggravation of the infectious process [27, 28].

However, the research results turned out to be contradictory: in some studies, *Candida* species were detected with the same frequency in both affected and

healthy areas, while in others, with a higher frequency in peri-implantitis areas ($p < 0.05$) [18, 19], which is consistent with the results of our study: the number of *Candida spp*. In the main group, the number of *Candida spp*. is 20.8 times higher. in the comparison group. It is interesting to note that *Candida spp*. It is capable of colonizing even areas of clinically healthy peri-implantant tissues.

The stability and stability of the microbial community in healthy areas around the implant are crucial for maintaining the health of peri-implant tissues [17]. The presence of opportunistic and saprophytic bacterial species associated with conditional oral health, such as *Streptococcus spp.*, probably plays a protective role by preventing colonization and overgrowth of pathogenic species. These bacteria contribute to maintaining a balanced microbial ecosystem that supports healthy tissues and prevents inflammation [20].

Representatives of the genus *Streptococcus spp*. act as "pioneers" in the formation of biofilms, initially attaching to the surface of teeth through the production of extracellular polysaccharides using glycosyltransferases, which, in turn, ensures the attachment of other microorganisms. By producing organic acids such as lactic acid, *Streptococcus spp*. helps regulate the local pH environment [21]. In addition, representatives of the genus Streptococcus produce antimicrobial substances such as hydrogen peroxide, which inhibit pathogenic bacteria, including cariogenic and periodontal microbiota [22, 23]. In addition to these functions, *Streptococcus spp.* It also interacts with the host's immune system to strengthen the integrity of the mucosal barrier and prevent excessive activation of inflammatory reactions [24]. The results obtained in our study show that *Streptococcus spp*. They are present both in the field of healthy implants and in the field of implants with periimplantitis. At the same time, the number of *Streptococcus spp*. in the main group, the number of *Streptococcus spp*. exceeds 1.28 times. in the comparison group, which suggests their role in the transition from health to disease.

A systematic review by Lafaurie GI et al. [29] showed that peri-implant tissues in the area of affected implants are also colonized by gram-negative microorganisms such as *Enterobacteriaceae spp.*, especially Escherichia coli, which are infrequently found in the area of teeth with periodontitis or healthy implants. Similarly, a study by Ardila CM et al. [30] showed that the presence of gram-negative E. coli in the implant area was associated with a deterioration in clinical parameters in patients with peri-implantation ($p <0.001$). In a cross-sectional study, Ramón-Morales CA et al. [31] the presence of *Escherichia* coli has been associated with peri-implantitis. Our results in this study also confirm that in patients with peri-implantitis, the microbial landscape is characterized by the appearance of gram-negative rods, including *Escherichia coli*.

Despite the limitations of this study, it was possible to find some correlations between the presence of *Sarcina* in the peri-implant area and the presence or absence of the disease. Although we cannot conclude that these microorganisms contribute to the development of peri-implantitis, their abundant presence should not be ignored, and their etiological role in peri-implantitis requires further investigation.

The results of our study are consistent with the conclusions of numerous domestic and foreign studies conducted in recent years, which compared the microbiome of healthy implants and implants affected by peri-implantitis. These studies indicate that the biofilm in peri-implantitis is characterized by a greater microbial diversity, a greater microbial load, and a more complex structure compared to healthy areas. [13, 15, 32 - 35].

1. The biofilm of the peri-implant zone during inflammation is characterized by a large microbial diversity and a greater microbial load.
2. The presence of *Candida albicans* in the peri-implant zone during inflammation may be associated with the severity and duration of the course of peri-implantitis.
3. The role of *Sarcina spp*. The development of the infectious process in peri-implantitis requires additional study.
4. The depth of tissue probing in the implant area and the severity of peri-implantitis correlate with the level of oral hygiene.
5. In order to develop effective and scientifically based protocols for the prevention and treatment of tissue pathology surrounding implants, a detailed understanding of the etiology, pathogenesis and diagnosis of these diseases is necessary.

CONCLUSION

The microbiota plays a central role in the pathogenesis of peri-implantitis, and its composition differs significantly from that of the microbiota in the healthy tissues surrounding the implants. Disruption of the balance of microorganisms, biofilm formation, activation of the immune response and metabolic processes caused by bacteria lead to inflammation and tissue destruction in the implant area. A comprehensive analysis of microbiological data is an important tool for understanding the etiology and pathogenesis of peri-implantitis. This will make it possible to develop more effective strategies for the treatment and prevention of peri-implantitis and, as a result, ensure the durability of dental implants.

Limitations of the study. This study is limited to a small sample size and focuses exclusively on the microbiome of healthy and peri-implanted sites. In addition, the main group included patients with a depth of probing in the implant area not exceeding 6 mm. Future research should eliminate these limitations by increasing the sample size and conducting a comparative analysis of microbiome profiles from peri-implant sites (both healthy and

diseased), depending on the method of fixation of orthopedic structures on dental implants.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Competing interest

The authors declare that there are no competing interests.

Acknowledgments

The article was prepared based on the results of M. Ibrahim's dissertation research.

Funding

None

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