

Indicators of the Oral Microbiome in Patients with Dental Implantation

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ABSTRACT

Relevance: The method of dental implantation is used almost totally. The main problem when carrying out this intervention is the insufficiency of the volume of bone tissue of the upper and lower jaw, necessary for the installation of dental implants. The incidence of this pathology reaches 70% with concomitant adentia. Various methods of bone grafting are used to compensate for bone tissue deficiency and create the necessary volume. It should be noted that each of these methods involves disrupting the integrity of bone and soft tissues, which can lead to microcirculatory disorders and an increased risk of inflammation. The oral cavity is normally inhabited by a diverse range of normal flora, which plays a crucial role in preventing the growth and prevalence of pathogenic microorganisms. However, under certain conditions, such as microcirculatory disorders, dysbiosis can occur. The risk of contamination with pathogenic microflora increases and an inflammatory complication develops.

The aim of the work: was to study the microbiome of the oral cavity and the gingival sulcus in patients with planned bone grafting and further dental implantation.

Materials and methods: 42 patients admitted for bone grafting operations in the oral cavity were examined. Before the operation, all patients underwent a microbiological examination. Clinical material samples were taken from the oral mucosa and from the gingival sulcus. The microbiological study evaluated the aerobic and conditionally anaerobic spectrum of bacteria and Candida fungi. The clinical material was cultured using a quantitative method to determine the concentration of the isolated microorganism.

Results: The conducted microbiological study of the material obtained from the oral mucosa and the gingival sulcus revealed that the frequency of isolation of microorganisms that determine the normocenosis is 76% and 48%, respectively. According to the list of species of the normoflora in both cavities, but with different frequencies of occurrence, mainly streptococci (*S.mutans*, *S.mitis*, *S.sangiis*, *S.viridans*) were identified at a concentration of 10⁵ CFU/ml. Microorganisms that cause dysbiosis were isolated in 24% of cases from the oral mucosa and in 52% of cases from the gingival sulcus, i.e., three times more frequently. The leading microorganisms from the gingival sulcus were *S. aureus*, isolated in 25% with a concentration of 10⁴-10⁵ CFU/ml, *Candida albicans* in 21% with a concentration of 10⁵ CFU/ml, and *E.faecalis* with a concentration of 10⁴-10⁵ CFU/ml. The incidence of *S. aureus* and *Candida albicans* on the oral mucosa was 15% at a concentration of 10³ – 10⁵ CFU/ml, and 9% at a concentration of 10³ CFU/ml, respectively. Based on these findings, 15 patients were treated with phage therapy in the pre- and postoperative periods using a specially selected phage preparation based on its lytic activity against *S. aureus*.

Conclusion: Comparison of the microbial spectrum and the concentration of microorganisms showed that the most informative are the indicators of contamination of clinical material obtained from the dentogingival furrow. It was noted that every 4 patients, there is a pronounced dysbiosis formed by *S. aureus* and *Candida albicans* with a concentration of 10⁴ -10⁵ CFU/ml, which may be a risk factor for the development of postoperative complications of an inflammatory nature. The use of bacteriophage according to the lytic activity of the phage preparation against *S. aureus* contributed to a decrease in inflammatory complications and improved healing of the postoperative area.

Keywords: Dental Implantation, Bone Grafting, Microbiology, Phage Therapy, Normal Flora, Dysbiosis, Microbiome.

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Introduction

Technological progress in the 21st century is rapidly being implemented in the processes of maintaining public health and improving the quality of medical care. However, oral and dental pathologies remain quite common, and new ways to achieve dental and oral health are being sought [1,2]. Today, dental implantation is an effective method of rehabilitation for patients with missing teeth, but a number of associated conditions often lead to complications and difficulties in performing this surgical procedure, making it impossible to achieve full rehabilitation, and sometimes even making it impossible to use dental implants as supports for orthopedic structures [1,2].

These conditions include insufficient bone volume in the alveolar process/jaw, which occurs in at least 70% of cases of adentia. For this purpose, various bone-plastic and barrier materials are widely used in the practice of dental implantation. At the same time, many clinicians find that the results of complex bone reconstruction methods are often unpredictable, and there are reports of numerous complications that occur after their implementation, despite all modern approaches to their prevention [3-6]. The need for bone-plastic surgeries in patients with indications for further dental rehabilitation using the dental implantation method ranges from 26% to 55%. The success rate of bone grafting is 71–90%, with implant survival rates of 85–97%. However, the incidence of complications is 45–59% [1,2,6]. Therefore, a thorough analysis of the relationship between surgical outcomes and individual risk factors, as well as the development of prognostic criteria for assessing clinical risks, is of great scientific and practical importance. Thus, the development, theoretical substantiation, and implementation of a set of clinically proven personalized prognostic criteria should increase the effectiveness of dental care for patients of various ages with tooth loss and significant bone tissue deficiency. All of the above served as the basis for conducting this study.

Materials and Methods

This work is based on the study of data obtained after processing the results (observation of complications and outcomes) of the treatment of 42 patients (men, women) with significant atrophy and/or defect of the alveolar process/part in need of bone-plastic surgery for subsequent dental rehabilitation using the method of dental implantation. The object of the study is the microbiological indicators obtained in the pre- and postoperative periods (N=42) in patients with missing teeth aged from 18 to 75 years. The subject of the study was the influence of the initial microbiological indicators on the results of bone grafting, including the indicators of the presence/structure of postoperative complications and the outcomes of the performed operations. The assessment of the results for the significance of prognostic criteria was carried out after comparing the clinical, radiological, and laboratory results of the study. All patients underwent oral sanitation before the study and the surgical stage of treatment, according to the approved protocols for oral sanitation before dental implantation [7]. Microbiological samples of clinical material from the oral mucosa and the gingival sulcus were taken in the preoperative and early postoperative periods. The sample was taken directly from the oral mucosa using a swab, and from the gingival sulcus using a dental probe. The obtained material

was placed in a sterile disposable tube with Ames transport medium. The use of transport medium was necessary because it was not always possible to deliver the material to the laboratory within 2 hours of taking the sample.

The studies were conducted in the laboratory of microbiological research methods at Moscow Regional Research Clinical Institute named after M.F. Vladimirov (MONIKI), Moscow, Russia. During the microbiological study, the aerobic and conditionally anaerobic spectrum of bacteria and fungi of the genus *Candida* was evaluated. The clinical material was cultured using a quantitative method to determine the concentration of the isolated microorganism. The results were expressed in colony-forming units per swab (CFU/swab). The primary seeding was carried out on dense nutrient media: 5% blood agar; Chistovich's egg-salt agar; chocolate agar; Saburo agar.

The identification of the isolated cultures was carried out using classical methods, as well as using test systems:

1. "Strepto test 16 kit" for biochemical identification of streptococci;
2. "ENTEROtest 24 kit" for biochemical identification of enterobacteria;
3. "Chromogenic agar for *Candida*", "CAN" for species identification of fungi of the genus *Candida*.

Results

The analysis of the research results showed the following results.

Table 1: Microflora of the oral mucosa in patients before surgical treatment (N=42)

Indicators	Frequency of occurrence (%)	Concentration (CFU/ml)
Frequency of occurrence of microorganisms that determine the normocenosis		
<i>S.mutans</i>	21	10 ⁵ - 10 ⁶
<i>S.mitis</i>	8	10 ⁵
<i>S.sangius</i>	20	10 ⁵
<i>S.epidermalis</i>	11	10 ⁵
<i>Neisseria</i>	4	10 ⁴
<i>S.viridans</i>	12	10 ⁴ – 10 ⁹
Total	76	
Frequency of occurrence of microorganisms that determine dysbiosis		
<i>S. aureus</i>	15	10 ⁴
<i>Candida albicans</i>	9	10 ³
Total	24	

A microbiological study of the material obtained from the oral mucosa revealed that the frequency of isolation of microorganisms that determine the normocenosis is 76%.

Table 2: Microflora of the gingival crevice mucosa in patients before surgical treatment (N=42)

Indicators	Frequency of occurrence (%)	Concentration (CFU/ml)
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Frequency of occurrence of microorganisms that determine the normocenosis		
<i>S.mutans</i>	10	10 ⁵ - 10 ⁶
<i>S.mitis</i>	2	10 ⁵
<i>S.sangius</i>	12	10 ⁵
<i>S.epidermalis</i>	7	10 ⁵
<i>Neisseria</i>	19	10 ⁵
<i>S.viridans</i>	48	10 ⁵ – 10 ⁹
Total	76	
Frequency of occurrence of microorganisms that determine dysbiosis		
<i>S. aureus</i>	25	10 ⁴ – 10 ⁵
<i>Candida albicans</i>	21	10 ⁵
<i>E.faecalis</i>	6	10 ⁴ – 10 ⁵
Total	52	

The microbiological study of the material obtained from the gingival sulcus revealed a frequency of 48% of microorganisms that determine the normal microflora. According to the list of

species of the normal microflora in both cavities, but with different frequencies of occurrence, streptococci were predominantly identified. Among the streptococci, *S.mutans*, *S.sangius*, *S.viridans*, and *S.mitis* were predominant, with a concentration of 10⁵ CFU/ml. The microorganisms that determine dysbiosis were 3 times less common than normoflora, and accounted for 24% on the oral mucosa and in equal proportions with the mucous membrane of the dental sulcus (48 and 52%, respectively). The leading microorganisms from both cavities were *S. aigeis*. On the oral mucosa, the incidence of *S. aureus* was 15% at a concentration of 10³ – 10⁵ CFU/ml, and *Candida albicans* was 9% at a concentration of 10³ CFU/ml. From the gingival sulcus, *S. aureus* was identified in 25% with a concentration of 10⁴-10⁵ CFU/ml, *Candida albicans* in 21% with a concentration of 10⁵ CFU/ml, and *E. faecalis* with a concentration of 10⁴-10⁵ CFU/ml. The differences in frequency of occurrence were identified when the data were analyzed according to their concentration. For this purpose, all the results were divided into two groups. The first group included patients with a concentration of microorganisms below the diagnostic titer ($\geq 10^3$ CFU/ml), and the second group included patients with a concentration above the diagnostic titer ($\leq 10^4 - 10^5$ CFU/ml).

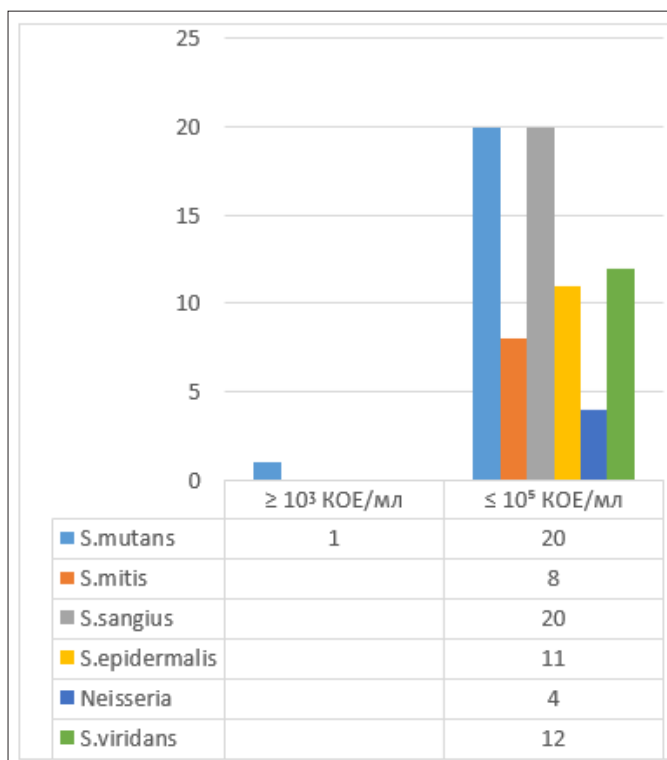


Figure 1.1 Frequency of occurrence of microorganisms that determine the normocenosis (%)

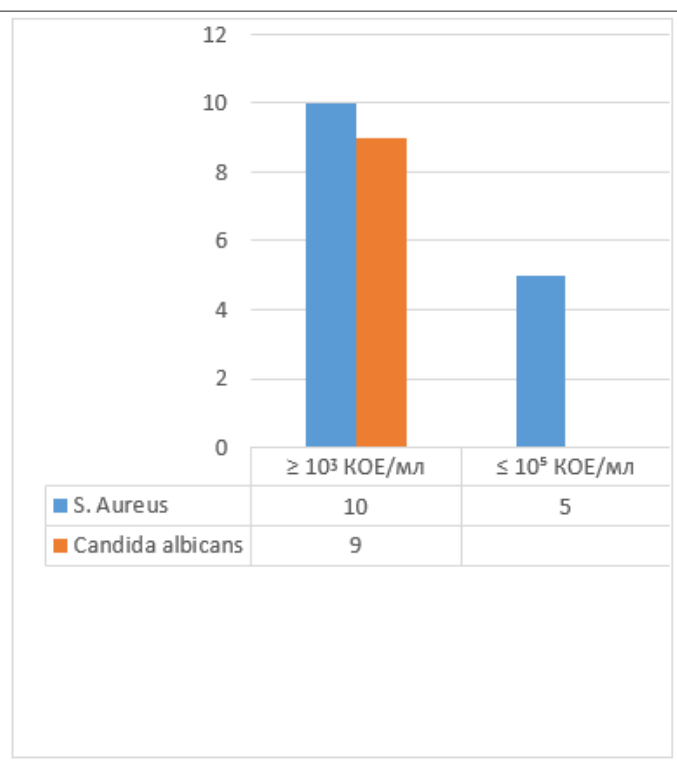


Figure 1.2 Frequency of occurrence of microorganisms that cause dysbiosis (%)

Figure 1: The oral mucosa microflora in patients, depending on the concentration of isolated microorganisms

As can be seen from the materials of Figures 1 and 2, the main conditional pathogens (*S. aureus*, *Candida albicans*) were identified in the oral mucosa at a concentration of 10³ CFU/ml in 19% of cases, and at a concentration of 10⁵ CFU/ml in 5% of cases. From the dental-gingival canal, the isolation of *S. aureus* and *Candida albicans* at a diagnostic titer was 21%, and *E. faecalis* was isolated at an equal frequency (3%) in each group. Based on the data obtained, all patients were prescribed antibiotic prophylaxis, including antifungal medications, to prevent complications in the preoperative period. In the postoperative period, appropriate postoperative antibiotic therapy was prescribed based on the results of the microbiological examination.

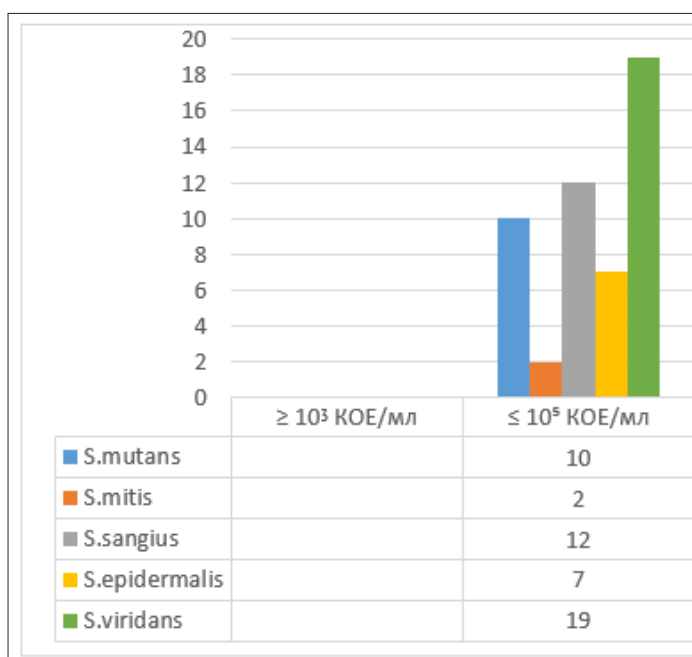


Figure 2.1 Frequency of occurrence of microorganisms that determine the normocenosis (%)

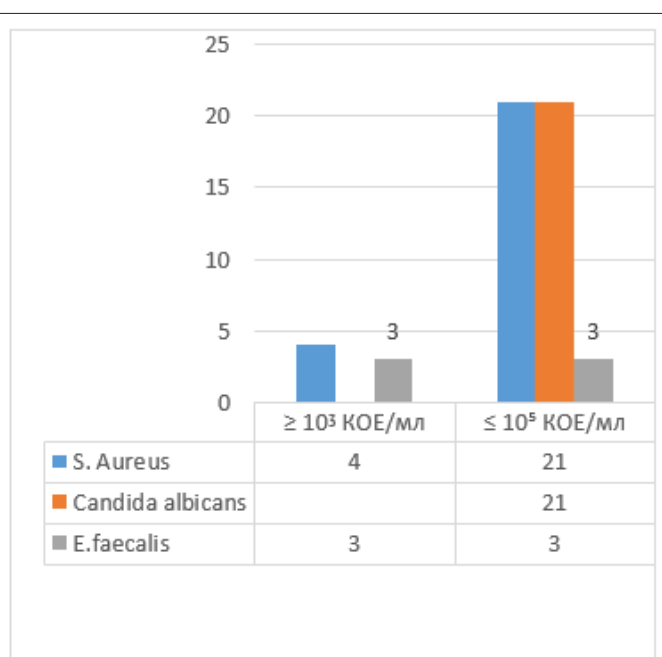


Figure 2.2 Frequency of occurrence of microorganisms that cause dysbiosis (%)

Figure 2: Microflora of the gingival sulcus in patients, depending on the concentration of isolated microorganisms

Despite the preoperative sanitation of all patients, according to the accepted clinical guidelines, pathogenic microflora continued to be isolated from the oral mucosa and, to a greater extent, from the gingival sulcus at a diagnostic titer [7].

This fact contributed to the prescription of phagotherapy in the pre- and postoperative period for 15 patients. The drug was specifically selected based on the lytic activity of the phagopreparation against *S. aureus* [8].

The clinical assessment was conducted according to the following criteria: 1. Pain: intensity and duration. 2. Mucous membrane edema: intensity and duration. 3. Primary or secondary healing

Table 3: Comparative assessment of patients' conditions in the postoperative period

Criteria	Patients with phagotherapy in the pre- and postoperative period (N=15)	Patients without phagotherapy in the pre- and postoperative period (N=27)
Pain	up to 5 days, moderate	up to 7 days or more, intense
Mucous membrane edema	moderate, maximum on days 1-3, up to 5 days	maximum on days 1-3, up to 10 days
Healing	by primary tension	by primary tension (N=22); by secondary tension (N=5)

The results of the study showed that the use of a specially selected phage preparation for preventive purposes before and after bone-plastic surgeries, based on its lytic activity against *S. aureus*, helps to normalize the mucosal microbiome and increases the effectiveness of surgical interventions, as well as improves the quality of life for this patient population [9-11].

Conclusion

Thus, a comparison of the microbial spectrum and the concentration of microorganisms showed that the most informative indicators are the levels of contamination in the clinical material obtained from the gingival sulcus.

When assessing the microflora, it was noted that every 4 patients had a pronounced dysbiosis caused by *S. aureus* and *Candida albicans*, with a concentration of 10⁴ - 10⁵ CFU/ml, which may be a risk factor for the development of postoperative inflammatory complications.

The administration of bacteriophage, based on the lytic activity of the phage preparation against *S. aureus*, helped to normalize the microbiome of the mucous membrane and increased the effectiveness of the performed oral surgery.

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