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Opportunistic Pathogens Isolated from Peri-Implant and Periodontal Biofilm from Adjacent Teeth

Ana Maísa Malheiro de Sá

Dissertação conducente ao **Grau de Mestre** em **Reabilitação Oral**

Gandra**, julho de 2023**

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Nota

Esta tese teve por base o artigo "Opportunistic Pathogens Isolated from Peri-Implant and Periodontal Biofilm from Adjacent Teeth" submetido à revista "Applied Sciences" no dia 4 de julho de 2023, a aguardar aprovação neste momento.

Abstract

Even though most studies consider strict anaerobe Gram-negative bacteria as the main factor associated with peri-implantitis, other studies have identified other microorganisms present in implants and related to peri-implant disease that have the ability to reduce the effectiveness of treatment, such as *Candida* spp., *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Therefore, microbiologic diagnosis is important for the success of implant treatment. The main goal of this study was to detect *Candida* spp., *E. faecalis* and *P. aeruginosa* in periodontal and peri-implant biofilms in the presence or absence of disease and relate the presence of these microorganisms with demographic data, systemic diseases, hygiene habits, the type of implant connection and endodontic treatment. The study population consisted of 20 patients that filled out a questionnaire regarding gender, age, systemic diseases, and oral hygiene. Peri-implant and periodontal biofilms from an adjacent tooth, both with and without disease, were analysed for the presence of these three opportunistic pathogens. Microbiological analysis revealed a higher prevalence of *E. faecalis* in patients with and without periodontal and peri-implant disease. *Candida* spp. was identified in a higher degree in cases with disease, and *P. aeruginosa* was mostly detected in peri-implantitis. The detection of these three pathogens suggested a possible means of transmission of infection from adjacent teeth to implants, with implant design associated with rehabilitation as a primary cause of pathogen growth. Although this study did not relate pathogen growth directly to periodontal disease, the high values UFC/mL values of *E. faecalis* may reveal an etiologic role of this bacterium in peri-implantitis.

Keywords: periodontitis; peri-implantitis; opportunistic pathogens; biofilm; *Enterococcus faecalis*; *Pseudomonas aeruginosa*; *Candida* spp.

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1. Introduction

The success of oral rehabilitation in patients undergoing implant treatment largely depends on the health of the tissues. As the oral cavity is a dynamic system, continuously colonized by interacting and proliferating microorganisms, it is extremely important to understand the microbiota and control causal factors before, during, and after implant placement to prevent the development of peri-implant disease [1,2].

Oral microbiota differ in everyone and, when in balance, these microorganisms do not cause any harm to the oral structure, a phenomenon known as eubiosis, characterized by a mutual beneficial relationship and a defence mechanism against other species [1]. However, alterations in the host's immune system, pH changes, decreased salivary flow, altered activity of salivary proteins, diet (high carbohydrate consumption), poor oral hygiene, tobacco use, diabetes, prolonged use of oral antibiotics/antimicrobials/antiseptics, and genetic factors can lead to microbial imbalances. Under these circumstances, virulent and opportunistic microorganisms in dysbiosis can cause periodontal and peri-implant diseases [1–3].

In peri-implant disease, the biofilm has been reported to contain significant amounts of Gram-negative bacteria such as *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Aggregatibacter actinomycetemcomitans*, but *Candida* spp., *Enterococcus faecalis*, and *Pseudomonas aeruginosa* have also been found in implants with peri-implantitis [4–7].

Candida species in the oral cavity are commensal and it has been suggested that the subgingival environment can serve as a refuge, where, under favourable conditions, they can transform into opportunistic pathogens and induce oral diseases [8–10].

P. aeruginosa is one of the most common microorganisms in healthcare-associated infections, with high mortality rates, especially in severely ill and immunocompromised patients. It is an opportunistic human pathogen characterized by intrinsic resistance to multiple antimicrobial agents. A recent study found significantly higher levels of *P. aeruginosa* in oral epithelial cells of individuals with periodontitis compared to individuals with a healthy periodontium [11].

Enterococcus faecalis are facultative anaerobic Gram-positive cocci. They are rarely found in the oral cavity under healthy conditions, but have a high occurrence in failed endodontic treatments, persistent periapical lesions, chronic periodontitis, and have also been detected in cases of peri-implantitis [4].

If peri-implantitis is not diagnosed and treated early, extensive bone loss will occur and compromise implant stability; therefore, microbiological diagnosis is important for a more appropriate and effective treatment.

The aimed of this study was to detect *Candida* spp., *E. faecalis*, and *P. aeruginosa* in the periodontal and peri-implant biofilm with or without disease presence and to seek a correlation between the presence of these microorganisms and demographic data, hygiene habits, endodontic treatment, and implant connection type.

2. Materials and Methods

2.1. Study Characteristics

This was an observational, analytical, and cross-sectional study conducted by a single calibrated dentist on patients of the University Clinic at IUCS/CESPU in Gandra who were undergoing oral rehabilitation treatment with implants.

2.1.1. Study Population

The patients who participated in this study were recruited between May and September 2022. Twenty individuals were selected from patients of the University Clinic at IUCS/CESPU in Gandra, Portugal, who were undergoing oral rehabilitation treatment with implants. A questionnaire was used to collect information about demographic data, oral hygiene habits, smoking habits, medical history, and dental history, obtained through an examination of the oral cavity. Additionally, the periodontal and peri-implant diagnosis was performed on patients who met the inclusion criteria, according to the **"**Classification of Periodontal and Peri-implant Diseases 2018**"** [12]. All participants were informed about the purpose of the research and signed informed consent, and all procedures were carried out with data protection in mind. This study protocol was previously approved by the Ethics Committee of the University Institute of Health Sciences (IUCS-CESPU), following the Helsinki guidelines.

2.1.2. Inclusion Criteria

The study included healthy patients or those with controlled chronic diseases (e.g., hypertension and diabetes) who were over 18 years of age and had implants placed less than 10 years ago and more than 6 months ago.

2.1.3. Exclusion Criteria

Patients with immunological disorders, those undergoing therapy with high-dose steroids, therapeutic levels of fluoride in bone, bisphosphonates, cyclosporine, phenytoin, and nifedipine, patients who had received antibiotic treatment in the last 30 days and/or mouthwash with antiseptics in the last 15 days, as well as patients with acute abscesses near the collection areas, were excluded. Patients who had undergone

periodontal or peri-implant treatment in the last six months and patients with incomplete or missing clinical information were also excluded.

2.2. Microbiological Examination

2.2.1. Sample Method

The collection was performed as follows: 3 sterile #30 paper cones were inserted into the peri-implant sulcus (for 20 s); 3 sterile #30 paper cones were inserted into the periodontal sulcus of the tooth adjacent to the studied implant (for 20 s). The 3 paper cones related to the implant and the tooth were separated into two vials with VMGA III transport medium.

2.2.2. Sample Processing

In the laboratory, the transport medium was vortexed for 60 **s** at maximum speed to homogenize the sample. Then, 100 µL of the transport medium was taken for CHROMID® CPS Elite (BioMérieux Marcy-l'Étoile-France), a chromogenic agar medium for the isolation and identification of *E. faecalis*, another 100 µL for CHROMID® Candida (BioMérieux Marcy-l'Étoile, France) a chromogenic agar medium for the isolation and identification of *Candida albicans*, and another 100 µL for Cetrimide Agar medium (BioMérieux Marcy-l'Étoile, France), which allows for the isolation and identification of *P. aeruginosa*. The inoculum deposited in each medium was then spread in a clockwise direction using a Drigalsky loop, and all media were incubated in an incubator at 35–37 °C for 24–48 h.

2.2.3. Statistical Analysis

Data analysis was performed using IBM® SPSS® (Statistical Program for Social Sciences), version 29.0 for Windows.

Descriptive statistics were used to estimate frequencies and percentages, mean, median, 1st quartile, 3rd quartile, standard deviation, minimum, and maximum. The Shapiro–Wilk test was used to assess the normality of the variables under study, including gender, age, smoking habits, hygiene habits, one or more teeth with endodontic treatment, implant connection type, and the use of removable prostheses.

Since normality was not observed, non-parametric analyses were chosen. Therefore, to compare the number of colony-forming units per millilitre (CFU/mL) of *Enterococcus faecalis*, *Candida* spp., and *Pseudomonas aeruginosa* according to the collection site (tooth or implant), the non-parametric Mann–Whitney test was used. In relation to the implant, to compare the CFU/mL of *Enterococcus faecalis* according to the presence or absence of endodontic treatment and the CFU/mL of *Candida* spp. according to the use or non-use of an irrigator, the Mann–Whitney test was employed. The non-parametric Kruskal–Wallis test, followed by Dunn's test with Bonferroni correction for multiple comparisons, was used to compare the CFU/mL of *Enterococcus faecalis*, *Candida* spp., and *Pseudomonas aeruginosa* according to the disease state (mucositis or peri-implantitis) and health, as well as to compare the CFU/mL of *Enterococcus faecalis*, *Candida* spp., and *Pseudomonas aeruginosa* according to the type of implant (Cone Morse, internal hexagon, and external hexagon). Spearman's correlation coefficient was used to assess the relationship between age and the different microorganisms (*Enterococcus faecalis*, *Candida* spp., and *Pseudomonas aeruginosa*). The significance level was set at 0.05.

3. Results

A total of 20 individuals, 70% of whom were female (n = 14) and 30% of whom were male (n = 6), with ages ranging from 26 to 86 years (mean = 52.25; SD = 15.1), agreed to participate in this study. The demographic characteristics, clinical parameters of the study population, and collection sites are presented in Tables 1 and 2, respectively. The analysed data regarding oral hygiene habits showed that 65% (n = 13) brushed their teeth twice a day, with the majority, 85% (n = 17), not using dental floss and 70% (n = 14) not using an irrigator. Regarding smoking habits, 90% (n = 18) were non-smokers, while the remaining 10% (n = 2) had this habit. Regarding systemic pathologies, 85% (n = 17) reported not having any pathology, while one patient reported cardiovascular disease and type two diabetes mellitus, another reported hypertension, and another had been hospitalized 2 months prior due to pneumonia. A total of 90% of the patients (n = 18) did not have removable prostheses, while 10% (n = 2) did. As for the presence of endodontic treatment, 80% (n = 16) had one or more treated teeth. The study population included 25% (n = 5) of patients with edentulous maxillae rehabilitated with prostheses supported by internal connection implants, 25% (n = 5) with single-unit Cone Morse connection implants, 20% (n = 4) with single-unit internal connection implants, 15% (n = 3) with single-unit external connection implants, 10% (n = 2) rehabilitated with pontics up to three elements with internal connection, while 5% (n = 1) were Cone Morse implants.

Table 1. Comparison of CFU/mL of different microorganisms according to the sampling site.

	Sampling	**n**	**Median (IQR)**	***p-Value***
E. faecalis	Tooth	14	10.E+7 [10. E+6; 32,500,000.0]	0.796
	Implant	20	10.E+7 [10. E+6; 77,500,000.0]	
Candida spp.	Tooth	6	500,040.0 [18.75; 25,750,000.0].	0.093
	Implant	6	10.E+7 [7,750,000.0; 10. E+7]	
P. aeruginosa	Tooth	2	55,000,000.0[10. E+7]	0.571
	Implant	5	10.E+7 [64.5; 10. E+8]	

Summary data as median and interquartile range (IQR); *p*-value derived from Mann-Whitney test.

Table 2. Comparison of *E. faecalis* UFC/mL according to the presence or absence of endodontic treatment.

	Endodontic Treatment	**n**	**Median (IQR)**	***p-Value***
E. faecalis	Yes	15	10.E+7 [10. E+6; 10. E+8]	0.214
	No	5	10.E+6 [10. E+6; 10. E+7]	

Summary data include median and interquartile range (IQR); *p*-value derived from the Mann–Whitney test.

Subsequently, the microbiological results were analysed after 24–48 h of sample processing in culture media. Using a colony counter, colony-forming units per millilitre (CFU/mL) of the isolated microorganisms were calculated. Figure 1 shows the microbiological result of the three opportunistic pathogens isolated from the same patient, from the periodontal and peri-implant biofilms, respectively. In the upper part of the image corresponding to the implant, there were positive results for Pseudomonas aeruginosa and Enterococcus faecalis, and negative results for *Candida* spp. In the lower part of the image, corresponding to the adjacent tooth, there was a negative result for Pseudomonas aeruginosa and positive result for Enterococcus faecalis and *Candida* spp.

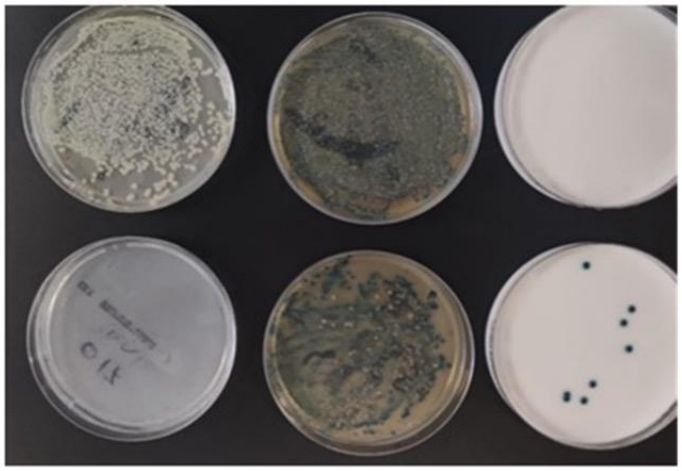

Figure 1. Three culture media (from left to right, respectively Cetrimide Agar, CPSE, and Chrom Candida), in the upper part corresponding to the implant and the lower part corresponding to the adjacent tooth of sample number 17.

In Figure 2, we can observe the distribution of the studied microorganisms. However, it is important to mention that numbers 4, 6, 16, 19, and 20 correspond to implant-supported complete dentures, which means the absence of teeth (which were not analysed).

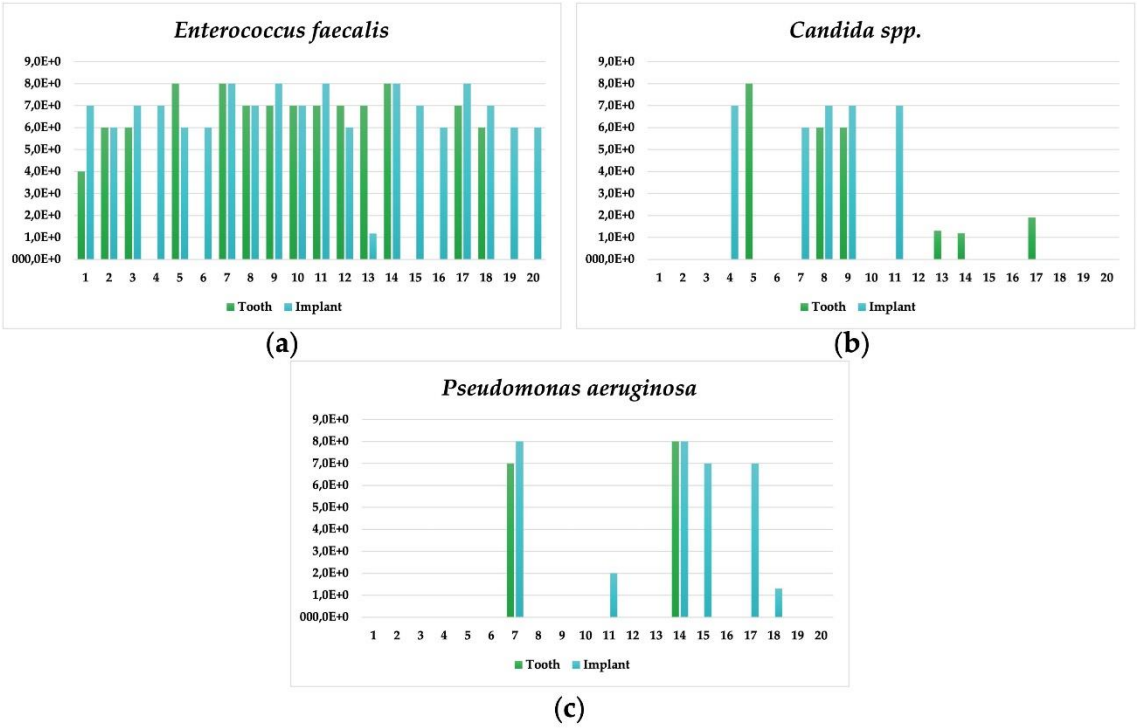

Figure 2. Individual results for *E. faecalis* (**a**), *Candida* spp. (**b**), and *P. aeruginosa* (**c**) for the implant and adjacent tooth.

For all positive cases of *E. faecalis*, *Candida* spp., and *P. aeruginosa*, their relationship with periodontal and peri-implant diagnosis was analysed through the following graphs, which showed a higher presence of these three opportunistic pathogens in both periodontal and peri-implant disease (Figure 3).

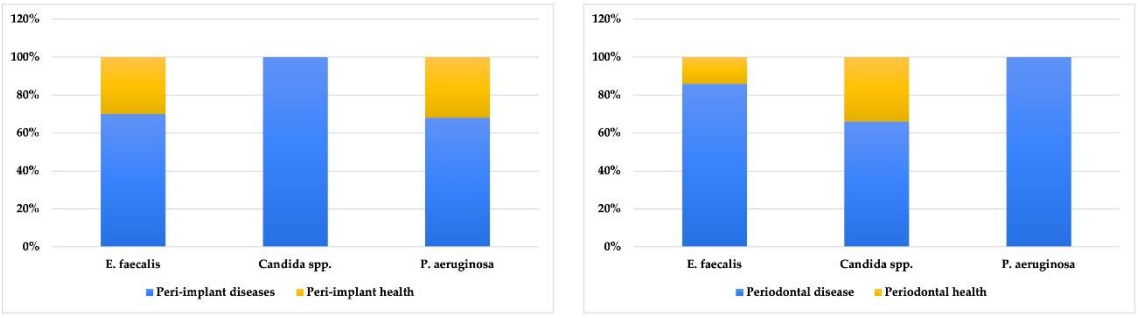

Figure 3. Percentage distribution of microorganisms for peri-implant disease/health (**a**) and periodontal health/disease (**b**) in positive individuals.

All positive cases for the three microorganisms mostly had a diagnosis of periodontal and peri-implant disease. When comparing the presence of microorganisms according to the sampling site (periodontal biofilm or peri-implant biofilm—Table 1), it was observed that Enterococcus faecalis had equal median values of CFU/mL in the adjacent tooth and the implant, although the values in the implant showed greater variability. *Candida* spp. had higher median values in the implant (10.E+7 [10.E+6; 77,500,000.0]), compared to the adjacent tooth (500,040.0 [18.75; 25,750,000.0]), but these differences were not statistically significant. *P. aeruginosa* also showed a higher CFU/mL in the peri-implant biofilm compared to the periodontal biofilm of the adjacent tooth, but these differences did not reach statistical significance.

When comparing the values of *E. faecalis* in individuals who underwent endodontic treatment with those who did not undergo endodontic treatment, it was found that those who underwent treatment had higher median values of *E. faecalis* UFC/mL (10. E+7 [10. E+6; 10. E+8]) compared to those who did not undergo treatment (10. E+6 [10. E+6; 10. E+7]). However, these differences are not statistically significant (Table 2).

Through Table 3, we can observe that *E. faecalis* had higher median values of UFC/mL in individuals with mucositis (10.E+7 [10.E+7; 10.E+8]), compared to individuals with peri-implant health (10.E+6 [750,003.75; 32,500,000.0]) and peri-implantitis (5,500,000.0 [10.E+6; 32,500,000.0]); however, these differences are not statistically significant.

Table 3. Comparison of UFC/mL of Enterococcus faecalis and *Candida* spp. in individuals with mucositis, peri-implantitis, and peri-implant health.

	Diagnostic	**n**	**Median (IQR)**	***p-Value***
E. Faecalis	Mucositis	8	10.E+7 [10. E+7; 10. E+8]	0.148
	Peri-implantitis	6	5,500,000.0 [10. E+6; 32,500,000.0]	
	Peri-implant health	6	10.E+6 [750,003.75; 32,500,000.0]	
Candida spp.	Mucositis	3	10.E+7 [10. E+6]	0.607
	Peri-implantitis	2	10.E+7 [10. E+7; 10. E+7]	
	Peri-implant health	1	10.E+7 [10. E+7; 10. E+7]	

Summary data as median and interquartile range (IQR); *p*-value derived from the Kruskal–Wallis test.

When analysing the presence or absence of differences in the number of UFC/mL of *Candida* spp. between individuals who use or do not use an irrigator, it was found that out of the six individuals who were positive for *Candida* spp., the five individuals who did not use an irrigator had higher median values (10.E+7 [10.E+7; 10.E+7]) compared to the individual who used an irrigator (10.E+6 [10.E+6; 10.E+6]), and these differences were statistically significant ($p = 0.025$) (Table 4).

Table 4. Comparison of UFC/mL of *Candida* spp. according to the use or non-use of an irrigator.

	Use an Irrigator	**n**	**Mean Rank**	***p-Value***
Candida spp.	Yes	1	10.E+6 [10. E+6; 10. E+6]	0.025
	No	5	10.E+7 [10. E+7; 10. E+7]	

Summary data as median and interquartile range (IQR); *p*-value derived from the Mann–Whitney test.

The comparison of the number of UFC/mL of the microorganisms among the different types of implant connection was performed using the Kruskal–Wallis test (Table 5), which revealed that individuals with Cone morse implants had significantly higher UFC/mL values of *E. faecalis* (10.E+8 [10.E+7; 10.E+8]) compared to individuals with external hexagon (10.E+7 [10.E+7; 10.E+7]) and internal hexagon (10.E+6 [10.E+6; 10.E+7]) implants (H = 10.3; p = 0.007). These differences were observed specifically between cone morse and internal hexagon implants (p = 0.005). Regarding the number of UFC/mL of Candida spp., all three types of implants showed equal median values. As for *P. aeruginosa*, individuals with Cone morse implants had higher UFC/mL values compared to internal hexagon and external hexagon implants, respectively, but these differences did not reach statistical significance.

Table 5. Comparison of UFC/mL of the three microorganisms according to the type of implant connection (cone morse, internal hexagon, and external hexagon).

	Rehabilitation Type	n	Mean Rank	*H*	*p-Value*
E. faecalis	Cone morse	6	10.E+8 [10. E+7; 10. E+8] *	10.03	0.007
	Internal hexagon	11	10.E+6 [10. E+6; 10. E+7] *		
	External hexagon	3	10.E+7 [10. E+7; 10. E+7]		
Candida spp.	Cone morse	4	10.E+7 [3,250,000.0; 10. E+7]	0.50	0.779
	Internal hexagon	1	10.E+7 [10. E+7; 10. E+7]		
	External hexagon	1	10.E+7 [10. E+7; 10. E+7]		
P. aeruginosa	Cone morse	3	10.E+8 [107.0]	2.25	0.325
	Internal hexagon	1	10.E+7 [10. E+7; 10. E+7]		
	External hexagon	1	22.0 [22.0; 22.0]		

Summary data as median and interquartile range (IQR); p-value derived from the Kruskal–Wallis test, followed by the Dunn's test with Bonferroni correction. * Statistically significant differences were found between the internal hexagon and Morse taper implants (p = 0.005).

When we examined the relationship between age and the presence of these three microorganisms (Table 6), a weak to moderate negative correlation was found with *E. faecalis*, *Candida* spp., and *P. aeruginosa*, but this did not reach statistical significance. The older the age, the higher the presence of these microorganisms in both the periodontal biofilm of adjacent teeth and the peri-implant biofilm.

Table 6. Spearman correlation between age and the presence of the three analysed microorganisms in the total samples.

	N	Age
E. faecalis	20	−0.401
Candida spp.	6	−0.131
P. aeruginosa	5	−0.616

In Figure 4, the relationship between the type of rehabilitation and the type of implant connection with the peri-implant diagnosis is presented, where it can be observed that there is a higher number of peri-implantitis cases in implants with internal connection.

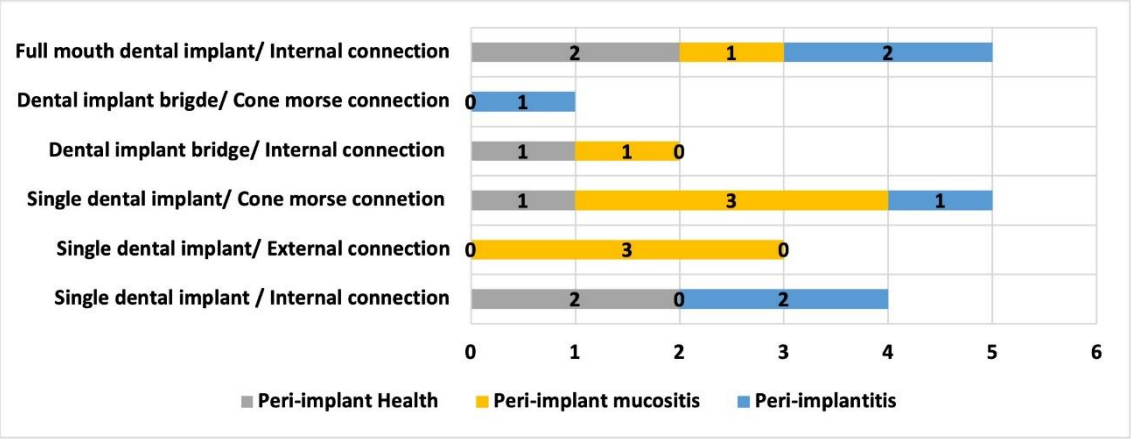

Figure 4. Distribution of results regarding the type of oral rehabilitation/connection type with peri-implant diagnosis.

4. Discussion

Approximately 10% of titanium implants present with premature failure, mainly due to bacterial infection within the first year of placement [13].

Leonhardt et al., in 2003, evaluated the microflora in peri-implant lesions and demonstrated that facultative anaerobic periodontal pathogens and opportunistic species such as *Staphylococcus* spp., *Enterococcus* spp., *Candida* spp., and *P. aeruginosa* were also found around compromised implants [14]. A recent study also found significantly higher levels of *P. aeruginosa* in oral epithelial cells of individuals with periodontitis compared to those with healthy periodontium [11]. In our study, when we correlated the number of CFU/**mL** of *E. faecalis* with peri-implant health, peri-implant mucositis, and peri-implantitis, the median values were higher in peri-implant mucositis than in peri-implantitis, although these differences were not statistically significant. However, it should be noted that undiagnosed and untreated peri-implant mucositis can progress to peri-implantitis.Based on the Consensus Report of the Sixth European Workshop on Periodontology, Lindhe and Meyle reported an incidence of peri-implant mucositis of up to 80% and incidence of peri-implantitis between 28% and 56% [12]. Several studies have quantified the incidence of peri-implantitis development in patients with a history of periodontitis, indicating that it is about six times more prevalent in patients with periodontitis than in patients without a history of periodontal disease. Other research indicates that teeth can be a source of bacteria in partially edentulous patients who have been rehabilitated with dental implants [15,16]. Regarding our study, we found that the majority of individuals diagnosed with periodontal disease (gingivitis and periodontitis) also had a diagnosis of peri-implant disease in the selected implant (peri-implant mucositis and peri-implantitis).

In our study, when we analysed the number of CFU/mL of *Candida* spp. among individuals who used an irrigator and those who did not, we found that those who did not use an irrigator had higher median values compared to the individual who used an irrigator, and these differences were statistically significant. These data, although requiring further investigation regarding the usefulness of the irrigator in reducing the colonization of peri-implant tissues, particularly by *Candida* species, may indicate an effect of this hygiene method, like the cleansing action of saliva, preventing the presence of yeast in peri-implant biofilm formation.

Alrabiah et al. reported that the subgingival environment can serve as a refuge for various *Candida* species [8]. In addition to adhering to teeth and oral mucosal surfaces, yeast can also adhere to non-biological surfaces such as titanium implants. While the presence of oral *Candida* species in the subgingival region plays a role in the etiopathogenesis of periodontal diseases (such as chronic periodontitis and aggressive periodontitis), the contribution of oral yeast to the occurrence and progression of peri-implant diseases remains uncertain [17]. As in the study by Alrabiah et al., a higher presence of *Candida* was found in individuals with peri-implantitis compared to those

without peri-implantitis. In our study, all cases of peri-implantitis revealed the presence of *Candida* spp.

Some risk factors associated with an increased oral presence of *Candida* include smoking and compromised oral hygiene status. These are the same risk factors that have been shown to increase the risk of peri-implant diseases. The results of the present study are in line with the study by Darwazeh et al., which showed a significantly higher presence of *Candida* in patients with poor oral hygiene [18].

According to Flanagan et al., *Enterococcus faecalis* is present in the majority of endodontic infections and is difficult to eliminate through endodontic treatment, so it can persist in the root canals and the surrounding alveolar bone. This bacterium often remains in the alveolar bone after the extraction of these teeth and can colonize the implant after its placement, which can lead to marginal bone loss and, consequently, implant loss. Although there are few studies linking *E. faecalis* to peri-implant disease, it appears to play a key role in bone loss around the implant or in peri-implantitis. This author even suggests that *E. faecalis* can cause infection both individually and in multi-species [19]. When comparing the median CFU/mL values of *E. faecalis* in individuals with endodontically treated teeth and those without endodontic treatment, it was found that those who underwent endodontic treatment had higher median CFU/mL values of *E. faecalis* than those who did not. Although these differences were not statistically significant, the role of teeth adjacent to endodontically treated implants should be further analysed to evaluate their role in the colonization of peri-implant crevicular fluid and the development of peri-implantitis.

Various modifications to implant design have been made in recent years to reduce the space between the implant and the prosthetic component to reduce bacterial proliferation, but with limited success. Generally, implants have a polished cervical collar that prevents the adhesion of microorganisms, as the connector region is in contact with soft tissues and not intraosseous [20]. High roughness and hydrophilicity are suggestive of an important role in bacterial adhesion and colonization on implant surfaces, but they also have benefits for the process of osseointegration [21]. In addition, although studies suggest that there is no significant difference regarding the shape or macrostructure of the implant (external or internal connection), the external connection shows a greater response of the soft tissues due to infiltration [22,23].

In our study, the highest bacterial colonization by *E. faecalis* and *P. aeruginosa* was found in implants with Morse taper connections, while the lowest was associated with implants with internal hexagon connections, contradicting the results of the study by Romanos et al. in 2016, which found that prosthetic components with Cone morse connections had lower bacterial counts, since this type of connection has a frictional locking system that allows for intimate adaptation in the deeper internal portions of the system, reducing micro-movements during loading. However, in the study by Romanos et al., a higher quantity of *Prevotella*, *Selenomonas, Eubacterium*, and *Fusobacterium* was detected in the internal connection, and only Ochrobactrum was detected in the Cone morse taper connection, which were not analysed in our study. Khorshidi et al., in

2016, also concluded that, overall, the Cone morse connection seems to have an obvious advantage in terms of microbial sealing capability, although their study mainly focused on the presence of *Streptococcus mutans* [24].

5. Conclusions

Despite the limitations of this study, it was possible to find some correlations between the presence of *Candida* spp., *E. faecalis*, and *P. aeruginosa* in the periodontal and peri-implant biofilm and the presence or absence of disease. Although we cannot conclude that these microorganisms can promote periodontal/peri-implant disease, their abundant presence cannot be overlooked, and their etiological role in peri-implantitis should be further investigated. The relationship of these microorganisms with demographic data, medical history, hygiene habits, implant connection type, and endodontic treatment has been established, although with low statistical relevance due to the small sample size.

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7. Annex

PARECER DA COMISSÃO DE ÉTICA

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

Comissão de Ética

Exma. Senhora Investigadora
Ana Maísa Malheiro de Sá

N/Ref.ª: CE/IUCS/CESPU-17/22 **Data:** 2022/março/27

Assunto: - **Parecer relativo ao Projeto de Investigação:** 10/CE-IUCS/2022
- **Título do Projeto:** *"Identificação de Candida, Enterococcus faecalis e Pseudomonas aeruginosa em implantes e sua relação com Peri-implantite"*
- **Investigadora responsável:** Ana Maísa Malheiro de Sá
- **Orientadora responsável:** Prof Doutora Cristina Maria Leal Moreira Coelho

Exma. Senhora,

Informo V. Exa. que o projeto supracitado foi analisado na reunião da Comissão de Ética do IUCS, da CESPU, Crl, no dia 21/04/2022.

A Comissão de Ética emitiu um parecer **favorável** à realização do projeto tal como apresentado.

Com os melhores cumprimentos.

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Prof. Doutor José Carlos Márcia Andrade
Presidente da Comissão de Ética do IUCS

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QUESTIONÁRIO

Número de identificação:

Género:

FEMININO ___ MASCULINO ___

Data de nascimento:

Patologia sistémica:

DIABETES ___ DOENÇAS CARDIOVASCULARES ___ DOENÇAS AUTOIMUNES ___ GRAVIDEZ ___

OUTRAS: _____

Medicação:

Antibiótico nos últimos 3 meses?

Fumador:

SIM ___ NÃO ___

Frequência de Escovagem:

3x/dia ___ 2x/dia ___ 1x/dia___

Uso de irrigador:

SIM ___ NÃO ___

Internamento recente:

SIM ___ NÃO___

Presença de dentes desvitalizados:

SIM ___ NÃO ___

Próteses removíveis:

SIM ___ NÃO ___

Data de colocação dos implantes:

Tipo de implante:

HEXAGONO INTERNO ___ EXTERNO ___

APARAFUSADO ___ CIMENTADO ____

Reabilitação:

UNITÁRIO ___ PONTE ___ TOTAL ____

Localização

Maxila ____ Mandibula ____

DIAGNOSTICO DE PERIDONTITE PRÉVIO

SIM ___ NÃO ___

Tratamento periodontal prévio:

SIM ___ NÃO ___

Diagnóstico perimplantar:

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CONSENTIMENTO INFORMADO

Eu,_____,

fui informado pela Dra Ana Maísa de Sá com cédula profissional nº 10724 sobre a realização do estudo "Identificação de *Candida, Enterococcus faecalis e Pseudomonas aeruginosa* em implantes e sua relação com a Peri-implantite" no âmbito da tese de Mestrado em Reabilitação Oral.

Os procedimentos são dirigidos aos implantes para diagnóstico de doença peri-implantar. Esta divide-se em: Mucosite que se caracteriza por um processo inflamatório reversível induzido por bactérias, que se manifesta com eritema, edema e hemorragia à sondagem periodontal. Esta se não for diagnosticada nem tratada pode evoluir para Peri-implantite, que é doença progressiva e irreversível dos tecidos duros e moles ao redor do implante e é acompanhada de reabsorção óssea, hemorragia e aumento da profundidade de sondagem, e possibilidade de purulência e mobilidade.

O estudo consiste na colheita de uma amostra microbiana da região peri-implantar que será enviada e posteriormente analisada em laboratório. Tem como objetivo identificar a presença de *Candida* spp, *Enterococcus faecalis* e *Pseudomonas aeruginosa* e verificar a sua relação com a doença peri-implantar.

Após diagnóstico serei informado e orientado para a melhor técnica de higiene oral e tratamento.

Compreendo que devo informar o médico dentista acerca da existência de patologias e medicação associada.

Esta participação no estudo é de carácter voluntário e será garantida a confidencialidade dos dados recolhidos e dos participantes.

Assinatura:

Data: