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Review

Candida spp. in periodontal disease: a brief review

Janaina C. O. Sardi[1)], Cristiane Duque[2)], Flávia S. Mariano[1)], Iza T. A. Peixoto[1)], José F. Höfling[1)] and Reginaldo B. Gonçalves[1,3)]

[1)]Department of Oral Diagnosis, Microbiology and Immunology, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil
[2)]Department of Dentistry, Pediatric Dentistry, Federal Fluminense University, Nova Friburgo, Rio de Janeiro, Brazil
[3)]Periodontology, Faculty of Dentistry, University Laval, Québec, Canada

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Abstract: Although the main reservoir of *Candida* spp. is believed to be the buccal mucosa, these microorganisms can coaggregate with bacteria in subgingival biofilm and adhere to epithelial cells. Such interactions are associated with the capacity of *Candida* spp. to invade gingival conjunctive tissue, and may be important in the microbial colonization that contributes to progression of oral alterations caused by diabetes mellitus, some medications, and immunosuppressive diseases such as AIDS. In addition, immune deficiency can result in proliferation of *Candida* spp. and germination of forms that are more virulent and have a higher capacity to adhere to and penetrate cells in host tissues. The virulence factors of *Candida* spp. increase host susceptibility to proliferation of these microorganisms and are likely to be important in the study of periodontal disease. Herein, we briefly review the literature pertaining to the role of *Candida* spp. in periodontal disease, and consider the main virulence factors, the host immune response to these microorganisms, and the effect of concomitant immunosuppressive conditions. (J Oral Sci 52, 177-185, 2010)

Keywords: *Candida* spp.; periodontal disease; immune system; virulence; immunosuppression.

Correspondence to Dr. Reginaldo Bruno Gonçalves, Department of Oral Diagnosis, Microbiology and Immunology, Piracicaba Dental School, University of Campinas (UNICAMP), Av. Limeira, 901, Piracicaba, São Paulo, CEP: 13414-903, Brazil
Tel: +55-19-2106-5321
Fax: +55-19-2106-5322
E-mail: reginaldo.goncalves@fmd.ulaval.ca

Introduction

The *Candida* spp. are opportunistic pathogens that can cause disease in hosts who are compromised by underlying local or systemic pathological processes (1,2). *Candida albicans* is the species most often associated with oral lesions, but other *Candida* spp., including *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. dubliniensis* have also been isolated (2) in the saliva of subjects with and without oral candidiasis. The isolation of *Candida* from the oral cavity does not imply the presence of disease (3). Fungal organisms commonly colonize the tongue, palate, and buccal mucosa. Such colonization may also occur in subgingival plaque of adults with periodontitis (4).

The *Candida* spp. have virulence factors that facilitate colonization and proliferation in the oral mucosa and, possibly, in periodontal pockets. These fungal organisms can coaggregate with bacteria in dental biofilm and adhere to epithelial cells. These interactions, which are associated with their capacity to invade gingival conjunctive tissue, may be important in microbial colonization that contributes to progression of oral diseases (5,6). In addition to these properties, *Candida* spp. also produce enzymes, such as the collagenases and proteinases that degrade extracellular matrix proteins, and immunoglobulins (5). Barros et al. (7) investigated the genetic diversity and production of exoenzymes in *C. albicans* and *C. dubliniensis* isolated from the oral cavity of systemically healthy patients with periodontitis. They verified that genetically homogeneous strains of *C. albicans* were present in the oral cavity of these patients and that these strains were capable of producing high levels of exoenzymes.

The attachment of *Candida* spp. to oral epithelium is the first step in the colonization process, after which local and systemic host defense mechanisms are activated to combat fungal proliferation and infection. This local defense comprises lactoferrin, β-defensins, histatins, lysozyme, transferrin, lactoperoxidase, mucins, and secretory immunoglobulin A (sIgA) (8). The innate immunity system also recognizes specific cell-wall surface proteins of *Candida* spp. and participates in the response against *Candida* infection (9).

Species of *Candida*, especially *C. albicans*, have been recovered from periodontal pockets in 7.1% to 19.6% of patients with chronic periodontitis (4,10). Urzúa et al. (11) observed that *C. albicans* and *C. dubliniensis* were capable of colonizing periodontal pockets in patients with chronic periodontitis, while only *C. albicans* was identified in the subgingival microflora of healthy individuals and patients with aggressive periodontitis. Cancer, diabetes mellitus, and immunosuppressive conditions such as acquired immunodeficiency syndrome (AIDS) increase host susceptibility to these infections. Feller et al. (12) observed higher prevalences of *Candida* spp. in the oral cavity, and specifically in the subgingival biofilm, of human immunodeficiency virus (HIV)-seropositive patients. In the present report, we briefly review the literature on the role of *Candida* spp. in periodontal disease, consider the main virulence factors and host immune responses to these microorganisms, and describe the effects of concomitant immunosuppressive conditions.

Virulence factors of *Candida* spp.

Candida albicans is frequently found in humans, and often resides on skin, mucosa, and normal gingival sulcus of otherwise healthy individuals. In compromised hosts, however, *Candida albicans* can cause serious disease, ranging from deep-seated mucosal infection to systemic infections (1,13). Several factors have been proposed as virulence factors of *Candida* spp., including adhesion; phenotypic diversity; hyphal formation; production of phospholipases, proteinases, or other metabolites; synergistic coaggregation or competition with bacteria; and mechanisms for adaptation in the host environment (13). The capacity of *Candida* spp. to adhere to different cells is important in its dissemination, infection, and persistence in oral and other tissues. Nikawa et al. (14) quantitatively evaluated the adhesion of oral isolates of *C. albicans*, *C. tropicalis*, and *C. glabrata* to human gingival epithelial cells, gingival fibroblasts, and pulmonary fibroblasts. They observed that most *Candida* strains had significantly higher adherence to gingival epithelial cells than to either type of fibroblast. However, environmental factors such as diet, composition of body fluids, and presence of antifungal agents may also cause changes in the cell surface and thereby modulate *Candida* adhesion.

Candida spp. have developed several virulence traits that facilitate invasion of host tissues and evasion of host defense mechanisms. One such group of virulence factors is the hydrolytic enzymes, which are secreted extracellularly by these microorganisms. Important hydrolytic enzymes include the phospholipases and secreted aspartyl proteinases (SAPs): 7 phospholipase genes (PLA, PLB1, PLB2, PLC1, PLC2, PLC3, and PLD1) and 10 SAP genes (SAP1 to SAP10) have been identified in *C. albicans* (15,16). Although their roles in pathogenesis have not been fully elucidated, it is known that phospholipases facilitate adherence to tissue, in addition to degrading phospholipids present in the cell membrane, which ultimately leads to cell lysis. Similarly, SAP9 and SAP10 collaborate in adherence, tissue damage, and evasion of host immune response (17). Adherence to epithelial cells is the first step in *C. albicans* colonization, and is followed by the establishment of mucocutaneous infection. It has been proposed that SAPs produced by *C. albicans* digest the surface of epithelial cells, thereby providing an entrance into the cell (18). In an investigation of the interaction between this microorganism and epithelial cells, it was found that hyphae are the invasive form of the organism, and that blastospores are generally found either on the surface of or between epithelial cells (18). Invasion of epithelial cells by *C. albicans* can also occur by means of endocytosis, in which pseudopods surround the organism and pull it into the cell (19). Although both yeast and hyphal-phase organisms are capable of inducing endocytosis, hyphae are more efficient at stimulating this process, suggesting that, in this form, *C. albicans* expresses specific invasive molecules on its surface, and that these bind to 1 or more epithelial cell receptors and induce endocytosis (20).

In addition to these virulence factors, coaggregation has been observed between some species of *Candida* and other oral microorganisms, but the extent of this coaggregation depends on growth conditions such as temperature (21). These interspecies interactions may be important in the microbial colonization that contributes to the progression of oral diseases. It has been suggested that the initial interspecies association is followed by a tight adhesion-receptor interaction, mediated by a mannoprotein in *C. albicans* (22). Hydrophobic proteins in the polysaccharide matrix of the *C. albicans* cell wall contribute to the strength of this adhesion receptor, and increase the pathogenicity of the yeast (22). Indeed, hydrophobicity has been found to be correlated with increased virulence in

Candida spp., because hydrophobic cells are more adherent to host cells and substrates, including mucin and extracellular matrix proteins (23).

Because microorganisms exist in polymicrobial communities, the capacity of yeasts to coexist with commensal or pathogenic bacteria is an important virulence factor. The quantitative and qualitative characteristics of coexisting microorganisms may therefore influence *Candida* biofilm formation. Thein et al. (24) evaluated the effects of oral bacteria, including the periodontopathogens *Prevotella nigrescens* and *Porphyromonas gingivalis*, on the development of *Candida albicans* biofilm *in vitro*. They observed a reduction in yeast counts when these microorganisms were cocultured with *Candida* biofilm, possibly because metabolites produced by anaerobes interfere with biofilm physiology or because the physical presence of bacteria inhibits biofilm growth. In assessing the effectiveness of antifungals, studies using mixed biofilms are of greater value than those using isolated species.

Hemolysin is another virulence factor that contributes to the pathogenesis of *Candida*. The secretion of hemolysin, followed by iron acquisition, facilitates hyphal invasion in disseminated candidiasis (25). An elevated blood glucose concentration may contribute, directly or indirectly, to increased hemolysin activity among *C. albicans* isolates in diabetic patients (26). In addition to physiological factors, the study of genotypic diversity, as assessed by molecular typing techniques, has become fundamental in elucidating the epidemiology of *Candida* isolates. Pizzo et al. (27) suggested that heterogeneity within subgingival *C. albicans* isolates results not only from the spreading of *Candida* microorganisms from saliva or biofilm, but also from new strains adapting to subgingival pockets and developing different virulence properties.

Studies have demonstrated that *Candida* spp. can adapt to an adverse host environment by altering pH, oxygen concentration, and nutrient availability. Environmental pH has profound effects on cells. Firstly, proteins have pH optima for activity, and become nonfunctional when pH is changed (28). Alterations in pH also stress fungi by disturbing the acquisition of nutrients, including iron (28). Iron is stored intracellularly in ferritin complexes. It is bound by transferrin in tissues and by lactoferrin on mucosal surfaces, and is an important facet of innate immunity (28). The effects of pH and innate immunity limit iron acquisition by pathogenic fungi. However, signaling pathways allow fungi to sense alterations in environmental pH and change the expressions of the genes – that regulate modifications in the morphology of *Candida* spp. (i.e., PHR1 and PHR2), resulting in either acidic pH-promoting yeast cell growth or neutral-alkaline pH-promoting filamentous growth (28). Moreover, *Candida* spp. can grow in both aerobic and anaerobic conditions, and have developed adaptive mechanisms to survive in both conditions. Oxygen can generate reactive products during infection and induce an oxidative stress response. Treatment of *C. albicans* with low concentrations of either hydrogen peroxide or menadione (a superoxide-generating agent) induces a redox potential with the activation of antioxidant enzymes, which protects cells from the lethal effects of a subsequent challenge with higher concentrations of these oxidants (29). The presence of anaerobic environments, such as those in root canal systems and periodontal pockets, can lead to polymicrobial infections. Rosa et al. (30) found that SAP secretion consistently increased in cultures of *C. albicans* strains, when strains recovered from periodontal pockets and intraoral sites not associated with the periodontium were grown under anaerobic conditions. This suggests that oxygen concentration in the atmosphere surrounding cells influences the virulence attributes of *C. albicans*.

The presence of a large amount of carbohydrate in the oral cavity influences several virulence factors of *Candida* spp. Incubation in sucrose, glucose, fructose, or maltose promotes adhesion of *C. albicans*, *C. tropicalis*, and *C. krusei* to epithelial cells (31), increases acid production, and lowers pH, with consequent activation of acid proteinases and extracellular phospholipases – factors involved in yeast adhesion (32). Polysaccharides such as as [DK1]rhamnose, mannose, and N-acetyl-glucosamine are present in the skeletal cell wall (33) and biofilm matrix (34), which make them targets for the development of therapies capable of disrupting cells and biofilms. In the tridimensional structure of the biofilm, there are a variety of covalently linked cell wall proteins (CWPs) that play a direct role in the response to several stress conditions (1). Genomic transcript analysis and, to a lesser extent, cell wall proteome analysis have shown that the response of *C. albicans* to certain forms of stress often includes dramatic changes in the protein levels of CWPs, which confirms that these proteins play a crucial role in virulence and that their expression is tightly controlled (1).

Recognition of *Candida* spp. by the immune system

The oral epithelium is an effective primary barrier against invasion by a number of oral commensals, including *Candida* spp. Once this barrier is breached, other innate immune mechanisms, such as the interleukins (ILs) and colony-stimulating factors of epithelial cells, come into play (35). Other local defense mechanisms against mucosal infection include the salivary proteins (e.g., lactoferrin, β-defensins, histatins, lysozyme, transferrin, lactoperoxidase,

and mucins) and secretory immunoglobulin A (sIgA), which are activated in the immune response against *Candida* infection (8). These salivary proteins can impair adhesion and growth of *Candida* in the oropharyngeal cavity (8). The adherence of *C. albicans* to oral epithelia is the first step in the infection process and enables the yeast to overcome the normal flushing mechanism of body secretions (36). Host defense mechanisms against mucosal candidiasis are not well understood, but include both innate and adaptive responses.

Phagocytic cells recognize pathogens by means of a variety of pattern recognition receptors (PRRs), including toll-like receptors (TLRs). The TLR family is a class of 13 receptors that are abundantly expressed on innate immune cells – such as macrophages, dendritic cells (DCs) (37), monocytes, neutrophils – and in the mucosal epithelium of the mouth, middle ear, and nasopharynx (38). Neutrophils strongly express phagocytic receptors such as complement receptor 3 (CR3) and Fcγ-receptors (FcγRs), which facilitate uptake into the fungus. Complement binding and activation is mediated by the alternative pathway. Complement activation is mainly important for chemotaxis and opsonization in *C. albicans*, but not in *C. albicans* lyses, in which it is prevented by the thick and complex cell wall (8,39). Several membrane-bound receptors localized in macrophages, monocytes, neutrophils, and dendritic cells contribute to the phagocytosis of *C. albicans*. These include dectin 1, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and mannose receptor (MR). Dectin 1 is a myeloid-expressed transmembrane receptor that, in response to fungi, induces a respiratory burst resulting in production of toxic oxidants (40). DC-SIGN is a receptor that is specifically expressed on the cell membrane of dendritic cells (DC), which have been shown to directly mediate uptake of fungal particles in transfected cell systems (40). MR is found in macrophages and dendritic cells, but its ability to mediate phagocytosis has recently been questioned (41). Recognition of *C. albicans* by the immune system triggers the production of the Th1 and Th2 cytokines, mainly by $CD4^+$ T cells. The TLRs (TLR2, TLR4, TLR6, and TLR9) are also involved in triggering these cytokine responses – which are linked to mechanisms of innate and acquired immunity – together with the participation of cells such as macrophages, neutrophils, and dendritic cells (9).

In general, upon recognition of microbial structures, TLRs activate either the NFκB (nuclear factor κB) or MAPK (mitogen-activated protein kinase) pathway, which leads to the production of different cytokines (42). Furthermore, some studies have found that TLR2 and TLR4 play roles in modulating Th1 and Th2 immune responses against *Candida*. There is also evidence indicating that TLR2 can recognize blastoconidia and hyphae of *C. albicans*, while TLR4 recognizes only blastoconidia. Van der Graaf et al. (43) showed that the recognition of hyphae and blastoconidia by TLR2 induces a Th2 immune response, culminating in the synthesis of IL-10 and IL-4 cytokines, which are capable of inhibiting the Th1 pattern response. As a result, elimination of *C. albicans* is slowed and the microorganism can disseminate in the host. Blastoconidia are recognized by TLR4, which markedly stimulates proinflammatory cytokines such as IFN-γ (interferon-γ), IL-6, TNF-α (tumor necrosis factor-α), and IL-12. Th1 or Th2 responses seem to be an important determinant of the host's ability to contain infection. Th1 responses are correlated with protection of the host, and the progression of infection is associated with the predominance of Th2 responses (44). A balance between Th1 and Th2 cytokines may thus be important in ensuring optimal antifungal protection while minimizing immune-mediated damage. Studies have shown that *C. albicans* induces immunosuppression through TLR2-mediated IL-10 release, and that this can lead to the generation of $CD4^+CD25^+$ regulatory T cells with immunosuppressive potential (9). In addition, *in vivo* models indicate that regulatory T cells attenuate Th1 antifungal responses, induce tolerance to the fungus, and participate in the development of long-lasting protective immunity after yeast priming (8).

It must be emphasized that in candidiasis, the different mechanisms of the immune system, as described above, act synergistically, i.e., they cooperate with and modulate each other in the process of combating fungal infection (45). The immune response to *Candida* spp. is related mainly to the different cytokines and chemokines produced by Th1 or Th2 cells; however, concomitant humoral immune responses to oral candidiasis and *C. albicans*-specific IgA and IgG antibodies have been observed (46,47). The exact mechanisms by which these antibodies protect against *Candida* infection are unknown, but are likely to include inhibition or germ tube formation, opsonization, neutralization of virulence-related enzymes, and direct yeast activity (45).

Inhibition of *C. albicans* adhesion to host surfaces is mediated by antibodies, and the extent of this inhibition has been analyzed in saliva samples. Fungi can also activate the complement system by the classical and alternative pathways, with deposition of C3 on the cell fungal surface. Complement activation facilitates the recruitment of phagocytes to infected tissues and enhances their anticandidal activity (8). It has been observed that the

protective potential of antibodies with enhanced phagocytosis and the killing of fungus depends upon epitope specificity, serum titer, and the ability of the complement system to bind in the fungal surface (8).

Few studies have been designed to investigate the immune response against *Candida* spp. in periodontal disease. Phagocytosis and killing of *C. albicans* by polymorphonuclear (PMN) cells were compared in patients who received organ transplants and those with periodontal disease. PMN cells were isolated and, after 20 min incubation, the phagocytosis of *C. albicans* and the intracellular killing rate were determined. The authors found no significant decrease in phagocytosis between transplant patients and those with periodontal disease. However, the killing activity of PMN cells was lower in these 2 patient groups than in healthy controls, an effect that was unrelated to the severity of periodontal disease (48). These results suggest that a reduction in killing activity, whether spontaneous or drug-induced, contributes to the development of periodontal disease (42). Using an enzyme-linked immunosorbent assay and whole unstimulated and stimulated saliva, Hägewald et al. (49) analyzed total IgA, IgA subclass 1, IgA subclass 2, and IgA reactivity to *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, and *C. albicans*. Significantly low concentrations and secretion rates of total salivary IgA, IgA1, and IgA2 were found in aggressive periodontitis. For all 3 microorganisms tested, the proportion of bacteria-reactive IgA in total IgA was significantly higher in the aggressive periodontitis group. In saliva, the pattern of humoral IgA response to *C. albicans* was similar to that of the *A. actinomycetemcomitans* and *T. denticola* antibodies. In addition, during activation of the bacteria-reactive humoral immune system in saliva, the authors observed inhibition of total secretory IgA, in particular IgA subclass 1, in aggressive periodontitis. Further studies are needed to elucidate the mechanisms of the immune system that control and eliminate *Candida* spp. from the host.

Immunosuppressive conditions and proliferation of *Candida* spp. in periodontal patients

Periodontal alterations are believed to be the result of an exacerbated immune response against host tissues. Changes in cellular and humoral immune responses (50) may allow different species, such as *Candida*, to colonize the subgingival environment (51). It has been reported that the proportion of yeasts in periodontal pockets is similar to that of some bacterial periodontopathogens, which suggests a role for *Candida* spp. in the pathogenesis of the disease (52,53). However, it is not yet possible to determine the role of *Candida* in the development or progression of periodontal disease, because only few studies have investigated the presence of yeasts in periodontitis patients, and they do not clear indicate whether their patients suffered the chronic or aggressive form of the disease (4,3,52). It is unclear if yeasts contribute to the development of periodontal disease, or if they show specificity for the chronic or aggressive forms of the disease (6,10,54-56). However, individuals with cancer, diabetes mellitus, and immunocompromising conditions such as HIV/AIDS are more susceptible to a wide spectrum of infections, including fungal infections (12,57,58).

Periodontal conditions were studied in 2 cross-sectional studies of adult, insulin-dependent, diabetics and age-and sex-matched controls (57). In one study, 154 diabetics and 77 controls participated; the other comprised 83 diabetics and 99 controls. There was a higher percentage of individuals with severe periodontal disease in the diabetic group than in the control group (57). However, there was no association between diabetes mellitus, periodontal disease, and the presence of *Candida* spp. In addition, a moderate increase in the glucose content of saliva did not result in higher mean numbers of *C. albicans*. Similar results were obtained by Yuan et al. (59), who found no significant differences between diabetic and nondiabetic individuals in the prevalence of a number of microorganisms, including *C. albicans*.

In our previous study (60), using a polymerase chain reaction (PCR) assay, we found that quantities of some *Candida* spp. were higher in chronic periodontal disease patients with diabetes than in those without diabetes. Among diabetic patients, *C. albicans* was found in 57%, *C. dubliniensis* in 75%, *C. tropicalis* in 16%, and *C. glabrata* in 5% of periodontal pockets. Among nondiabetic patients, *C. albicans* and *C. dubliniensis* were present in 20% and 14% of periodontal sites, respectively; there was no evidence of *C. tropicalis* or *C. glabrata* colonization. Urzúa et al. (11) used phenotypic and genotypic methods to analyze the composition of yeast microbiota in the mucosa and subgingival sites of healthy individuals and in patients with aggressive and chronic periodontitis. Although the profiles of the species present in the mucosa of the 3 groups varied, they noted that only *C. albicans* and *C. dubliniensis* were capable of colonizing periodontal pockets in patients with chronic periodontitis, and that only *C. albicans* was identified in the subgingival pockets of healthy individuals and patients with aggressive periodontitis. It has been reported that the proportion of yeasts in periodontal pockets is similar to that of some bacterial periodontal pathogens, which suggests a role for *Candida* spp. in the pathogenesis of this disease (11).

Certain *Candida* spp. are believed to be commensal organisms within the oral cavity. Indeed, the prevalence of oral yeast in the general population is about 34%. However, certain patient subgroups have higher levels of oral colonization. Peterson et al. (61) noted that the prevalence of oral yeast in hospitalized patients was 55%. Oral yeast carriage is particularly common in patients with advanced cancer, among whom reported levels of oral colonization range between 47% and 87%.

In 24 patients with acute periodontal infection and chemotherapy-induced myelosuppression, high concentrations of microorganisms were detected in subgingival pockets. *Staphylococcus epidermidis*, *C. albicans*, *S. aureus*, and *Pseudomonas aeruginosa* were predominant, and combinations of these were detected in some patients (62). Drugs such as corticosteroids, azathioprine, and cyclosporine are used to prevent the rejection of transplanted organs; however, these agents can alter the immune system and modify the characteristics of dental biofilm, thereby altering its effects on periodontal tissues. Renal transplant patients who received immunosuppressive drugs had more periodontal inflammation than did immunocompetent subjects (62). Infections can also occur in immunosuppressed patients, and these frequently involve microorganisms that have little or no pathologic significance in immunocompetent hosts. The prevalence of microorganisms in the periodontal sites of patients receiving immunosuppressive therapy may increase in local disease or disseminated infections, and some cultivable species, including *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Campylobacter rectus*, *Fusobacterium* spp., *Streptococcus* spp., *Pseudomonas* spp., and *Candida* spp., have been detected (62).

The *Candida* spp. are one of the most common AIDS-defining fungal opportunistic infections in HIV-positive individuals. One study found that the prevalence of *Candida* spp. in subgingival sites was 42.3% in HIV-positive children and 7.1% in control individuals (63). In another study, the authors observed a higher prevalence of *Candida* species in the oral cavity of HIV-seropositive patients, specifically in the subgingival biofilm, although the prevalence of periodontal disease in HIV-seropositive and HIV-seronegative subjects was very similar (12). Using conventional mycological methods and a specific PCR assay, Jewtuchowicz et al. (64) studied immunocompromised patients, such as those with advanced HIV infection, to identify the different species of yeast present at periodontal disease sites. Among the 76 fungal organisms isolated, 10.5% were *C. dubliniensis*, which was present in 4.4% of patients studied; *C. albicans* was the most frequently isolated yeast species.

The *Candida* spp. are ubiquitous fungal organisms that often colonize the oral mucosa of normal individuals, without causing disease. These opportunistic microorganisms might influence the inflammatory process, as they possess several virulence factors by which they invade tissues and evade host defense mechanisms, thereby facilitating proliferation and release of exoenzymes that promote tissue degradation. Moreover, in immunosuppressed patients, the higher prevalence of *Candida* spp. (mainly *C. albicans*) in the oral cavity, and specifically the subgingival biofilm of periodontal pockets, could indicate their coparticipation in the progression of periodontal disease in these patients.

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