PREVALENCE OF STAPHYLOCOCCUS SPP AND CANDIDA SPP IN THE ORAL CAVITY AND PERIODONTAL POCKETS OF PERIODONTAL DISEASE PATIENTS

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ABSTRACT
The oral cavity can act as a reservoir of certain pathogens that can cause systemic infections. The periodontal pocket is an ecological niche appropriate for hosting microorganisms that could act as opportunistic pathogens. The ability of Staphylococcus spp and Candida spp to form a biofilm and live within certain niches allows them to develop mechanisms that increase persistence, such as the evasion of host defenses and antibiotic efficacy. These microorganisms can easily become resistant to antibiotics and lead to superinfection. The aims of this study were to assess the presence of Staphylococcus aureus and Staphylococcus spp in biofilm in subgingival plaque and oral cavity of individuals with gingival-periodontal disease, to identify isolates and their relationship with Candida spp. The study included eighty-two patients, aged 18-70 years with periodontal disease and at least two sites with probing depth ≥3 mm.

Participants’ data were evaluated individually. Subgingival biofilm samples were obtained using Gracey curettes 7/8, after supragingival biofilm removal, and a sample from the oral cavity (buccal mucosa, tongue and cheek mucosa) by sterile swab. Of all the patients studied, 42.7% exhibited Staphylococcus spp in the periodontal pocket and 69.5% in the oral cavity while 25.6% exhibited Candida spp in the periodontal pocket and 42.7% in the oral cavity. However, 13.4% had both microorganisms in the periodontal pocket and 36.6% in the oral cavity. The prevalence of Staphylococcus aureus was 13.4% in the periodontal pocket and 15.8% in the oral cavity. Candida albicans was the most prevalent yeast in the periodontal pocket (76.2%) and in the oral cavity (63.0%).

Key words: Staphylococcus aureus, Candida, periodontal pockets, periodontal disease

INTRODUCTION
Several epidemiological studies suggest that periodontal disease may be a risk factor for developing systemic infectious diseases1,2. Infections of odontogenic origin in the oral cavity such as periodontal diseases and the chronic periapical process may spread some microorganisms through different routes such as intracranial, retropharyngeal or pleuropulmonary extensions as well as hematogenous dissemination from heart valves, prosthetic devices and other metastatic focuses. All these events clearly highlight the serious nature of potential infections that may put patients’ lives at risk3,4.
Gingival-periodontal diseases (GPD) involve a series of pathological processes of infectious nature which have an impact on the supporting tissues of the teeth. These clinical manifestations include gingivitis and chronic periodontitis. The periodontal pocket is an ecological niche appropriate for hosting some opportunistic pathogens. Periodontitis is diagnosed by means of clinical studies and radiography, and exhibits increased gingival and dental plaque rates, insertion loss, probing depth and bleeding on probing. Severe clinical cases, called chronic and aggressive periodontitis, might increase the possibility of toxin translocation, due to pathogenic and/or opportunistic microorganisms which act as a reservoir and as a potential septic focus. The periodontal pocket has a thin, permeable epithelium. When an ulcer appears in the inner epithelium, the microorganisms present in the biofilm pass through the connective tissue and blood capillaries. It is known worldwide that some opportunistic microorganisms, such as Staphylococcus spp and Candida spp, must be taken into consideration as probable pathogens, especially in individuals with different systemic impairments, e.g. diabetes mellitus, neutropenia, agranulocytosis, and AIDS. S. aureus and Candida spp may be part of the microbiota of the oral cavity and cause infections in that ecosystem. It is well documented that orthodontic/orthopedic treatment increases the levels of yeasts and Staphylococcus spp in the mouth. The use of dental devices is a risk factor for gingival-periodontal diseases and dental caries, since they facilitate the accumulation of microorganisms, in terms of quantity and type/diversity, altering the oral microbiota. Orthodontic devices can act as traps, since yeasts and other microorganisms adhere to their surface - whether acrylic, glass, composite, sealants, or membranes, and retain microorganisms, thus becoming a niche for colonization by normal or opportunistic microorganisms and facilitating infection. According to the literature on this subject, several epidemiological studies conducted in certain countries have demonstrated different prevalences of yeasts in individuals with periodontitis. Different Candida species have been isolated from biofilm of the subgingival plaque (7.1-19.6%) of patients with GPD. Staphylococcus aureus has become one of the community and nosocomial pathogens of epidemiological concern, since it is the agent that causes infective endocarditis with high morbidity and mortality rates, and also for its multi-resistance to antibiotics. The horizontal transfer of antibiotic resistance genes and the genetic capacity to form a biofilm enhance microorganism persistence, especially in Staphylococcus aureus and Candida spp. The biofilm significantly increases the pathogens’ ability to evade host defenses and antibiotic efficacy. Biofilm formation is also involved in pathogenesis and clinical manifestations of several infections. Many findings have suggested that yeast cells can modulate sensitivity to antibiotics, and bacteria can affect antifungal activity of fungi in mixed biofilms. Biofilm deactivation may increase the efficacy of current antibiotics against infections.

The aims of this study were to determine the prevalence of Staphylococcus aureus and Staphylococcus spp in biofilm of subgingival plaque and oral cavity of individuals with gingival-periodontal disease, and to identify isolated microorganisms and their relationship with Candida spp. We also assessed the correlation between the use of dental devices and the kind of gingival-periodontal disease in the study population together with the increasing presence of these microorganisms.

MATERIALS AND METHODS

Study population

Adult immunocompetent individuals with gingival-periodontal disease were studied prior to basic periodontal therapy. Exclusion criteria included: individuals with systemic diseases altering the gingival-periodontal status, and those who were under antibiotic or antifungal therapy or anti-inflammatory drugs six months prior to samples collection, and patients who had received periodontal treatment six months prior to the study. The study population consisted of 82 immunocompetent adult individuals with gingival-periodontal disease who attended the Outpatients Unit at the Faculty of Dentistry, University of Buenos Aires. Ages ranged from 18-70 years and the mean age was 43.3 ± 15.4 (54.9% women and 45.1% men). The patients were divided into groups according to their gingival-periodontal disease: gingivitis (n=26) and periodontitis (n=56). This population was classified according to the inclusion criteria guidelines provided by the American Academy of Periodontology (AAP). The periodontal evaluation of the study population included clinical examination, radiographs and clinical indicator measurement using a periodontal catheter with controlled pressure: insertion loss (IL), probing pocket depth (PPD), plaque index (Silness and Loe). Measurements were performed on all the teeth, excluding the third molar, at 4 sites per tooth (mesial, vestibular, distal and palatine/lingual). Fifty -seven percent...
of the subjects used dental devices, 17 orthodontic appliances (9 fixed and 8 removable) and 30 prosthetic devices (14 fixed and 16 removable). The study protocol was reviewed and approved by the local ethics committee. Permission to collect and analyze samples from both groups was obtained. Participants were recruited on a volunteer basis and written informed consent was provided by all of them.

Sample collection
Subgingival biofilm samples were obtained with Gracey curettes 7/8, after supragingival biofilm removal and with relative isolation of the area with sterile cotton pad and a highly potent suction device. Patients had previously rinsed their oral cavities with sterile distilled water. Sampling was conducted through the oral cavity (buccal mucosa, tongue and cheek mucosa) using a sterile swab. The material collected from the subgingival biofilm and oral cavity was soaked in sterile PBS (phosphate buffer solution, pH 7.2), and stored at 4ºC until subsequent processing. Samples were immediately submitted to the microbiology laboratory for testing.

Microbiological method used
Direct microscopic studies were conducted on samples by Gram staining.

To isolate Staphylococcus spp, samples were grown on selective and differential mannitol salt agar culture media (Biokar Diagnostics, Beauvais, France), CHROMagar™ MRSA (CHROMagar Company, Paris, France), CHROMagar™ Staph aureus (CHROMagar Company, Paris, France) and in hyper-salted broth containing 5-peptone (Merck, Darmstadt, Germany), 10 g meat extract (Merck, Darmstadt, Germany), 65 g NaCl (Merck, Darmstadt, Germany) and distilled water (to 1 liter), pH 7.2, and stored at 4°C until subsequent processing. Samples were immediately submitted to the microbiology laboratory for testing.

Statistical analysis
Statistical analysis was performed using stastadisticx 7.0 and SPSS 11.0 versions. Confidence intervals (CI) were calculated at 95% employing the Epi-Info 6.04 program (Atlanta University, GA). The sample size was estimated with a confidence level of 95% for a Type I error: a of 0.05% and type II: between 0.10 and 0.20.

RESULTS
Table 1 shows periodontal indexes obtained from the study population: 82 individuals of whom 31.7% had gingivitis (n=26) and 68.3% chronic periodontitis (n=56). The two groups exhibited a significant difference (gingivitis and chronic periodontitis) in the parameter probing (or pocket) depth according to
Chi-square test (p<0.05) regarding the
Significant differences were found using
\( n=35 \) (Table 5).

\( \text{CHI2 (Yates)} = 19.9392; \text{GL}= 1; \text{p = } 0 \) compared to the remaining patients
(\( n=47 \)) and \( 42.7\% \) (\( n=35 \)) of oral cavities. \( S.\text{aureus} \) and Candida spp isolates
were present in 13.4\% (\( n=11 \)) and in oral cavity
15.8\% (\( n=13 \)), methicillin-resistant \( S.\text{aureus} \) (MRSA) was
found in periodontal pocket of 7.3\% (\( n=6 \)) and in oral cavity of 7.3\% (\( n=6 \)).

The prevalence of \( S.\text{aureus} \) found in periodontal pocket was 13.4\% (\( n=11 \)) and in oral cavity
15.8\% (\( n=13 \)), methicillin-resistant \( S.\text{aureus} \) (MRSA) was
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** Differences were considered statistically signifi-
cantly when p≤0.05 (Table 3).

Fig. 1 shows the number of isolates of
\( S.\text{aureus} \) and/or yeast species from the oral cavity of patients
with periodontal disease according use
do dental devices. An increase in yeast
and \( S.\text{aureus} \) prevalence was observed in samples corresponding to patients
who used dental appliances (\( n=47 \));
these increases attained statistical sig-
nificance in samples from the oral
cavity of subjects with gingivitis and chronic periodontitis using dental
appliances (p(Fisher)= 0.000002;
\( \text{CHI2 (Yates)} = 19.9392; \text{GL}= 1; \text{p = } 0 \) compared to the remaining patients
(\( n=35 \)) (Table 5).

** Significant differences were found using
Chi-square test (p<0.05) regarding the

<table>
<thead>
<tr>
<th>Yeast Species</th>
<th>Isolates</th>
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<tr>
<td>Oral cavity</td>
<td>Periodontal pocket</td>
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<tr>
<td>( n )</td>
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<tr>
<td>MSSA</td>
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<td>MSSA/ Ca</td>
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** Total of positive patients: 62 **

** MSSA: Methicillin Susceptible \( S.\text{aureus} \); MRSA: Methicillin
Resistant \( S.\text{aureus} \); SCN: \( S.\text{aureus} \) Coagulase negative;
Ca: Candida albicans; Cd: Candida dubliniensis; Cg: Candida glabrata;
Cguit: Candida guillenmondii; Ct: Candida tropicalis; Ck: Candida kruessel
 Cp: Candida parapsilosis; Rd: Rhodotorula spp.**
presence of Staphylococcus aureus and/or Candida spp in periodontal pocket sample among patients with gingivitis and chronic periodontitis with and without dental appliances. (p(Fisher) = 0.001427; (CHI2 (Yates) = 8.5409; GL= 1 ; p = 0.0035) (Table 4).

**DISCUSSION**

Although Staphylococcus spp and Candida spp, mainly S. aureus, S. epidermidis, Candida albicans are frequently reported as nosocomial pathogens, and MRSA as a community pathogen, causing infections and serious problems, they are not frequently studied in the oral cavity. Staphylococcus spp and Candida spp at subgingival levels do not necessarily represent superinfection.
risk at a local level, but rather they colonize and are part of the indigenous microbial flora. However, in severe and aggressive chronic periodontitis cases, translocation towards the bloodstream of pathogenic and/or opportunistic microorganisms in the periodontal pocket are more likely to occur. The oral cavity may act as a reservoir and as a potential septic focus. Since these microorganisms colonize and persist by the formation of a biofilm, and taking into account their virulence properties, the present study examined the prevalence of Staphylococcus spp and Candida spp in the oral cavity of patients with gingival-periodontal disease.

The results shown in this study match the studies conducted by Loberto J. et al. for the prevalence of Staphylococcus spp isolates in subgingival and oral cavity samples, and at both sites, as well as the predominance of the CNS on Staphylococcus aureus. Rams et al. reported that about 50% of periodontal lesions harbored subgingival Staphylococci. In the present study, 39.3% (n=22/56) of the individuals with probing depth ≥5 mm exhibited Staphylococcus spp. In our study, neither gingival-periodontal disease nor the presence of dental orthodontics has been reported. The high number of Staphylococcus spp and Candida spp isolates found in this population suggests that these microorganisms are regular colonizers of the oral cavity, especially periodontal pockets. This fact leads us to hypothesise that this colonization can act as a potentially dangerous reservoir for the population as a source of dissemination towards other body locations and a source of transmission to other individuals, food and objects. The association among these microorganisms might promote and boost persistence. Other authors observed that C. albicans and S. aureus are microorganisms with an elevated adhesion capacity to the oral mucous.

Although C. albicans is the most prevalent yeast species, other species such as C. dubliniensis, C. glabrata, C. guillermontii, C. tropicales, C. krusei, C. parapsilosis and the genus Rhodotorula were isolated from the samples. Harriott M. et al. observed that Candida albicans readily forms biofilms on the surface on indwelling medical devices such as dental devices, and these biofilms serve as a source of local and systemic infections. These authors tested whether S. aureus and C. albicans are able to form a polymicrobial biofilm. Although S. aureus formed poor monocolonial biofilms in serum, it formed a substantial polymicrobial biofilm in the presence of C. albicans. In the present study the prevalence of Candida spp and S. aureus is higher in oral cavity and subgingival biofilm of patients with periodontitis who use dental devices. Our results indicate that dental devices serve as an artificial niche, increasing Candida spp and S. aureus carriage in subgingival plaque and oral mucosa. This result is in agreement with a number of reports.

The prevalence of MRSA in periodontal pockets of immunocompetent individuals with gingival-periodontal disease from Argentina has not been assessed yet, and no data regarding its pathogenesis is available. Moreover, it is important to highlight that the administration of antibiotics may cause the selection of resistant strains.

In this study, the rate of methicillin-resistant Staphylococcus aureus (MRSA) with regard to Staphylococcus aureus isolates was 54.5% (n=6/11) in periodontal pocket and 46.1% (n=6/13) in oral cavity. These strains exhibit a high rate of multiresistance compatible with those described in this geographic region. Epidemiologic surveillance is very important to determine the prevalence of Staphylococcus spp and yeasts in periodontal pockets since they constitute a reservoir for opportunistic microorganisms which, in particular clinical situations, play an important role in specific gingival-periodontal diseases and systemic diseases. The presence of MRSA represents a risk factor and an additional health problem of serious concern.

The association of Staphylococcus spp and Candida spp may be the origin of a stable bacterial community through the formation of a mixed biofilm. Periodontal disease is a common example of a disease where the formation of a biofilm plays an important role. Due to its resistance in the oral environment, the periodontal biofilm is responsible for the creation and evolution of systemic infections in individuals with immune disorders. This microbial association enhances the infectious capacity and antimicrobial resistance of individual cells, hindering the efficacy of treatments.

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