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 be or 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 the 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

PREVALENCIA DE *STAPHYLOCOCCUS SPP* Y *CANDIDA SPP* EN LA CAVIDAD ORAL Y BOLSAS PERIODONTALES DE PACIENTES CON ENFERMEDAD PERIODONTAL

RESUMEN

La cavidad bucal puede actuar como reservorio de ciertos patógenos que pueden producir infecciones sistémicas. La bolsa periodontal es un nicho ecológico propicio para albergar microorganismos que podrían actuar como patógenos oportunistas. La posibilidad que Staphylococcus spp y Candida spp puedan formar un biofilm o biopelicula y vivir dentro de ciertos nichos les permite a estos microorganismos desarrollar ciertos mecanismos que aumentan su persistencia como ser la capacidad de eludir las defensas del huésped y la terapia antimicrobiana. Estos microorganismos pueden ser o fácilmente convertirse en resistentes a los antibióticos y dar origen a una superinfección. El propósito de este estudio fue evaluar la presencia de Staphylococcus aureus y Staphylococcus spp en biofilm placa subgingival y en cavidad oral en sujetos con enfermedad gingivoperiodontal, identificar los microorganismos aislados y su relación con la portación de Candida spp. El estudio incluyó ochenta y dos pacientes, de edades entre 18 a 70 años de edad, con enfermedad periodontal, y al menos dos sitios con la profundidad de sondaje ≥ 3 mm. Se evaluaron los datos individuales. Las muestras de biofilm subgingival fueron obtenidas con cureta tipo Gracey 7/8, previa remoción del biofilm supragingival y una muestra de cavidad oral (mucosa, lengua y carrillo) mediante hisopo estéril. Del total de los pacientes estudiados, el 42,7% mostraron Staphylococcus spp en la bolsa y el 69,5% en la cavidad oral, mientras que 25,6% mostraron Candida spp en la bolsa y 42,7% en la cavidad oral. Sin embargo, el 13,4% tenían ambos microorganismos en la bolsa y el 36,6% en la cavidad oral. La prevalencia de Staphylococcus aureus en la bolsa periodontal fue de 13,4% y 15,8% en la cavidad oral. Candida albicans fue la levadura más frecuente en la bolsa periodontal (76,2%) y en la cavidad oral (63,0%).

Palabras claves: Staphylococcus aureus, Candida, bolsa periodontal, enfermedad periodontal.

INTRODUCTION

Several epidemiological studies suggest that periodontal disease may be a risk factor for developing systemic infectious diseases[1,2].

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 risk[3,4].

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[5,6]. 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[7,8].

S. aureus and *Candida spp* may be part of the microbiota of the oral cavity and cause infections in that ecosystem[9]. It is well documented that orthodontic/orthopedic treatment increases the levels of yeasts and *Staphylococcus spp* in the mouth[10,11]. 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[12,13]. *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[14,15].

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[16,17]. 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)[18]. 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)[19]. 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 7.5.
CHROMagar™ MRSA and CHROMagar™ Staph aureus are commercial chromogenic selective and differential culture media aimed at the qualitative detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* respectively. The hyper-salted broth is a liquid selective culture medium for *Staphylococcus spp* which was used to increase the sensitivity of the method. Samples were grown and incubated at 35-37 ºC for 24-48 hours. Suspicious colonies were identified by Gram staining, catalase test, coagulase test (bioMérieux, Marcy-L'Etoile, France) and later, antibiotic susceptibility testing was performed[20].
For the isolation of yeast species, a portion of the sample was grown on differential chromogenic solid medium (CHROMagar Company, Paris, France). They were incubated at 37ºC for one week, screening the presence of their development daily.
Isolated yeasts were identified according to the chromogenic medium color, screening for the presence of one or more species, micromorphology in agar milk 1%-Tween 80[21], and carbohydrate assimilation profile using a commercially available kit, API ID 32D (bioMérieux, Marcy-L'Etoile, France). When green color yeast was observed in CHROMagar *Candida*, additional studies were conducted in order to complete the predictable identification of *C. dubliniensis*, such as xylose assimilation, growth at 45ºC, and chlamydoconidia formation in the black birdseed medium after incubating the medium at 28ºC for 72 hours[22] and PCR using specific primers for *C. dubliniensis*[23,24].
With the aim of assessing *Staphylococcus spp* antimicrobial susceptibility, diffusion tests were carried out following the CLSI guidelines. The diffusion method in agar Mueller-Hinton (Merck, Darmstadt, Germany) was performed using 1 ug oxacillin discs (Laboratorios Britania, Buenos Aires, Argentina). A direct colony suspension method with 24 hours incubation was used to prepare the inoculum. Oxacillin susceptibility was determined. For *Staphylococcus aureus*, an oxacillin-inhibition area >13 mm was susceptible, and ≤10 mm resistant or MRSA.
Antifungal sensitivity testing study: The studies to determine antifungal sensitivity were performed on yeast isolates by means of a disk diffusion method in the plaque and by MIC. Procedures were followed according to the CLSI guidelines ("Clinical and Laboratory Standards Institute")[25].

Statistical analysis
Statistical analysis was performed using statadisticx 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

ANOVA test ($p<0.05$). Out of the 82 patients with gingival-periodontal disease, *Staphylococcus spp* isolates were recovered from subgingival samples of 35 patients (42.7%); from the oral cavity of 57 patients (69.5%), while 36.6% (n=30) exhibited *Staphylococcus spp* at both sites and 14.6 % (n=12) had *Candida spp* at both sites. *Candida spp* isolates were found in 25.6% (n=21) and 42.7% (n=35) of oral cavities. *Staphylococcus spp* and *Candida spp* were present in 13.4% of periodontal pockets (n=11) and 36.6% of oral cavities (n=30). *Staphylococcus aureus* isolates associated to *Candida spp* were found in 8.5% (n=7) of subgingival samples and 2.4% (n=2) of oral cavities.

The prevalence of *Staphylococcus aureus* found in periodontal pocket was 13.4% (n=11) and in oral cavity 15.8% (n=13), methicillin-resistant *Staphylococcus aureus* (MRSA) was found in periodontal pocket of 7.3% (n=6) and in oral cavity of 7.3% (n=6). The most prevalent yeast was *Candida albicans,* found in periodontal pocket of 76.2% (n=16) and in oral cavities of 58.3% (n=22) (Table 2).

The presence of *Staphylococcus spp* and *Candida spp* in periodontal pocket samples among the groups of individuals with gingivitis and chronic periodontitis did not differ significantly. The data were statistically analyzed using Chi-square test. Differences were considered statistically significant when $p \leq 0.05$ (Table 3).

Fig. 1 shows the number of isolates of *Staphylococcus aureus* and/or yeast species from the oral cavity of patients with periodontal disease according use of dental devices. An increase in yeast and *S. aureus* prevalence was observed in samples corresponding to patients who used dental appliances (n=47); these increases attained statistical significance in samples from the oral cavity of subjects with gingivitis and chronic periodontitis using dental appliances (p(Fisher)= 0.000002; CHI2 (Yates) = 19.9392; GL= 1 ; 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 1: Periodontal clinical parameters (Mean ± SD and IC95%) of subjects at the time of sampling according to periodontal disease status (n=82).

Clinical Parameters	Gingivitis**	Chronic Periodontitis***
Pocket depth (mm)	3.8 ± 0.6 (3.16 - 4.36)	7.0 ± 1.5 (5.45 - 8.47)
Clinical attachment level (mm)	0	6.7 ± 2,5 (4.21 - 9.25)

* A significant difference was observed between the 2 groups (gingivitis and chronic periodontitis) in pocket depth according to the ANOVA test ($p<0.05$).
** Gingival index >0 and BOP positive.
*** Gingival index>0, BOP positive, furcation and dental movement Class II or III.

Table 2: Distribution of absolute (n) and relative (%) frequencies of *Staphylococci spp* and/or *Candida spp*isolates from the oral cavity and periodontal pocket of patients with periodontal disease (n=82)

Staphylococci / Yeast Species	Isolates			
	Oral cavity		Periodontal pocket	
	n	%	n	%
MSSA	2	2.44	4	4.88
MSSA/ Ca	2	2.44	1	1.22
MSSA/ Ck	1	1.22	0	0.00
MSSA/ Ct/ Cguil	1	1.22	0	0.00
MSSA/ Cd/ Cguil	1	1.22	0	0.00
MRSA	3	3.66	5	6.10
MRSA/ Ca	2	2.44	1	1.22
MRSA/ SCN	1	1.22	0	0.00
SCN	21	25.61	15	18.29
SCN / Ca	14	17.07	5	6.10
SCN / Cd	2	2.44	2	2.44
SCN / Ct	2	2.44	0	0.00
SCN / Cg	1	1.22	1	1.22
SCN / Ck	1	1.22	0	0.00
SCN / Cg/ Cd	1	1.22	0	0.00
SCN / Ca/ Cg	0	0.00	1	1.22
SCN / Ca/ Cguil	1	1.22	0	0.00
SCN / Cg/ Cguil	1	1.22	0	0.00
Ca	1	1.22	5	6.10
Cd	0	0.00	1	1.22
Cg	1	1.22	0	0.00
Cp	1	1.22	0	0.00
Ct/ Ca	0	0.00	1	1.22
Cd/ Cg	0	0.00	1	1.22
Rd/ Ca	1	1.22	0	0.00
Cp/ Ca	1	1.22	2	2.44
Total of positive patients	62	75.61	45	54.88

MSSA: Methicillin Susceptible *Staphylococcus aureus*; MRSA: Methicillin Resistant *Staphylococcus aureus*; SCN: *Staphylococus Coagulase* negative; Ca: *Candida albicans*; Cd: *Candida dubliniensis*; Cg: *Candida glabrata*; Cguil: *Candida guillemondii*; Ct: *Candida tropicalis*; Ck: *Candida krusei* Cp: *Candida parapsilosis*; Rd: *Rhodotorula spp.*

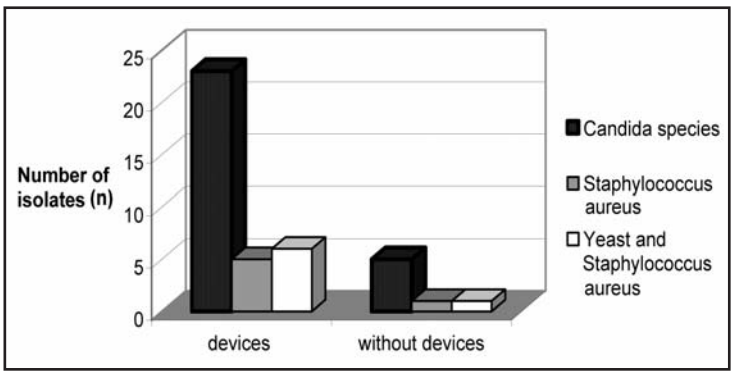

Fig. 1: Number of isolates of Staphylococcus aureus and/or yeast species from the oral cavity of patients with gingival-periodontal disease associated or not to the use of dental appliances. Candida species with devices (n= 23); S. aureus with devices (n=5), Yeast and S. aureus with devices (n=6), Candida species without devices (n= 5); S. aureus without devices (n=1), Yeast and S. aureus without devices (n=1).

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

Table 3: Number of individuals with subgingival *Staphylococcus spp* and/or *Candida spp* isolates according to periodontal disease (n=82).

Periodontal disease	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
Gingivitis	15	(57.69)	11	(42.31)	26	(100)
Chronic Periodontitis	30	(53.57)	26	(46.43)	56	(100)
Total	45	(54.88)	37	(45.12)	82	(100)

p(Fisher)= 0.457223; (CHI2 (Yates) = 0.0122; GL= 1 ; p = 0.912)

Table 4: Number of individuals with subgingival *Staphylococcus spp* and/or *Candida spp* isolates according to the presence or absence of dental appliances (n=82).

Dental appliances	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
Presence	24	(72.34)	23	(27.66)	26	(100)
Absence	6	(17.14)	29	(82.86)	56	(100)
Total	30	(36.59)	52	(63.41)	82	(100)

*A significant difference (p<0.05) was observed between the 2 groups (devices and without devices).
p(Fisher)= 0.001427; (CHI2 (Yates) = 8.5409; GL= 1 ; p = 0.0035).

Table 5: Number of individuals with subgingival *Staphylococcus spp* and/or *Candida spp* isolates from the oral cavity according to the presence or absence of dental appliances (n=82).

Dental appliances	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
Presence	34	(82.93)	13	(31.71)	47	(100)
Absence	7	(17.07)	28	(68.29)	35	(100)
Total	41	(50.00)	41	(50.00)	82	(100)

*A significant difference (p<0.05) was observed between the 2 groups (devices and without devices).
p(Fisher)= 0.000002; (CHI2 (Yates) = 19.9392; GL= 1 ; p = 0).

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[26].

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.[27]; 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.[28] 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[29]. 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[30,31].

Although *C. albicans* is the most prevalent yeast species, other species such as *C. dubliniensis*, *C. glabrata*, *C. guillemondii*, *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 monoculture biofilms in serum, it formed a substantial polymicrobial biofilm in the presence of *C. albicans*[32]. 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[17,18].

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[33].

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[34,35].

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[36]. This microbial association enhances the infectious capacity and antimicrobial resistance of individual cells, hindering the efficacy of treatments.

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