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STAPHYLOCOCCUS SPP. IN THE ORAL CAVITY AND PERIODONTAL POCKETS OF CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Staphylococcus spp are not usually isolated from the oral cavity, and when this occurs, they are considered to belong to the transitory microbiota. Individuals with periodontal disease represent possible reservoirs of these opportunistic bacteria in the oral cavity. The use of antibiotics for treatment of periodontal disease or other infections may predispose to the increase of the number of *Staphylococcus* spp. in the oral cavity. These microorganisms easily become resistant to antibiotics, and may result in superinfection. The purpose of this study was to evaluate the presence of *Staphylococcus* spp. in the oral cavity and in periodontal pockets of patients with chronic periodontitis, identify the isolates and verify the relationship between the presence of *Staphylococcus* spp. in the oral cavity and presence of periodontal pockets. The study included eighty-eight patients, 25-60 years of age, with chronic periodontitis, and at least two sites with probing depth ≥ 5mm. Individual data examination was assessed. Then, samples were colleted from the periodontal pocket with the aid of paper tips and from the oral cavity through mouth rinses. Out of the total of patients, 37.50% presented *Staphylococcus* spp. in the periodontal pocket and 61.36% in the oral cavity, 27.27% presented the bacteria in both sites. *S. epidermidis* was the most prevalent specie in the periodontal pocket (15.9%) and oral cavity (27.27%). The occurrence of higher proportions of nonresident's microorganisms in subgingival samples and oral sites may represent significant problem in causing and maintaining periodontal infections.

Key words: *Staphylococcus*, opportunistic bacteria, periodontitis

INTRODUCTION

Staphylococcus spp. display important virulence properties and cause a wide range of human infectious diseases including pneumonia, septicemia, and endocarditis (1,11,12,13,17,18, 21,22,23). They are not usually isolated from the oral cavity and when it occurs they are considered a part of the transient microbiota. However, a change in the oral microbiota may develop for several reasons. In immunocompromised individuals these microorganisms may occur in higher number. Patients with periodontal disease represent possible reservoirs of these opportunistic bacteria in the oral cavity. They can also be an infection source to other individuals (3,7,20).

The purpose of this study was to evaluate the presence of *Staphylococcus* spp. in the oral cavity and in periodontal pockets of patients with chronic periodontitis, identify the isolates and verify the relationship between the presence of *Staphylococcus* spp. in the oral cavity and periodontal pocket.

MATERIALS AND METHODS

The study included 88 individuals with chronic periodontitis from the Clinic of Periodontology of the University of Taubaté (UNITAU). They were from 25 to 60 years of age and had not received periodontal nor antibiotic treatment. Following the examination, all patients were given instruction regarding to a

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proper self-performance plaque control. Prior to participation, the purpose and procedures were fully explained to all the patients. They volunteered themselves to participate in the trial and gave their informed consent (16). This study was carried out with the approval of the Ethics Committee of São José dos Campos Dentistry School (UNESP). Probing pocket depth and clinical attachment level were measured using a Williams' probe. Just one examiner, previously calibrated, examined all the patients. After individual data collection and clinical examination, samples of subgingival dental biofilm were obtained in those individuals that had at the time of referred at least two sites with probing depth ≥ 5mm. At the selected sites, supragengival plaque was removed with the aid of sterilized gauze compress and isolated with cotton rolls. Subgingival dental biofilm samples were collected by inserting 3 sterile paper points (number 30 Áureo) consecutively into the periodontal pocket, for 30 seconds.

The collected material was placed in Eppendorf tubes containing 1mL of sterile solution (PBS 0.1M/pH 7.2). Material from oral cavity was collected through oral rinses, during 1min, in 10mL of sterile solution (PBS 0.1M/pH 7.2) previously distributed in sterile universal containers. The samples were maintained in ice until the process in the laboratory of Microbiology. The maximum period of 3 hours between collection and processing was respected. Microbiological analysis of the samples were performed in the laboratory of Microbiology, Department of Biology (UNITAU) or in the laboratory of Biosciences and Oral Diagnosis (São José dos Campos School of Dentistry, UNESP)

The material obtained from periodontal pocket was mechanically dispersed with the aid of a Vortex mixer for 30 seconds. After this procedure, the paper points were removed. Each sample (oral rinse and periodontal pocket sample) was centrifuged by 10 min (8000 Xg) and the supernatant was discharged. The pellet was ressuspended in 2.5 mL and 0.6mL PBS respectively, obtaining the final suspension.

From each sample 0.1 mL aliquots were plated in duplicate copy onto Baird-Parker agar (Difco) supplemented with egg yolk (12.5 egg yolks in 25ml saline solution o.85%) and potassium tellurite (0.1g of potassium tellurite in 10 mL of distilled water) and were incubated by 24 to 72 hours at 37ºC. After the incubation period, the identification of *Staphylococcus* isolates was based on colonial morphology and Gram stain characteristics. The strains were then on plated in Tryptic Soy Agar (TSA - Difco) and incubated by 24 hours at 37ºC. Pure cultures were identified according Koneman *et al.* (6). Coagulase negative *Staphylococcus* isolates were identified using the system API Staph (Bio-Merièux, France). The bacterial suspension preparation and the inoculation of the trips were carried out as recommended by the manufacturer. The strips were incubated by 24 hours at 37ºC. Based on the biochemical reactions, a seven-digit profile number was assigned to each isolate and the Code book were used for speciation. Chi-square homogeneity test and Chi-square tendency test were used with critical level at $p< 0.05$.

RESULTS

Out of the 88 patients with chronic periodontitis, staphylococci were isolated from the subgingival sample of 33 patients (37.50%); from the oral cavity of 54 patients (61.36%), and 27.27% (n=24) presented staphylococci in both sites. S. *epidermidis* (n=14, 15.90%) were isolated from the subgingival samples and 27.27% (n=24) from the oral cavity. Five patients (5.68%) present *S. epidermidis* in both sites. *S. aureus* were isolated from 4.55% of the subgingival samples and 25% (n=22) of the oral cavity samples (Table 1). From the 88 studied patients, 39.77% were males (n=35) and 60.23% (n=53) females. The prevalence of *Staphylococcus* spp in the oral cavity of males was significantly higher in relation to the females. The prevalence of subgingival *Staphylococcus* according to the gender was not statistically significant (Tables 2 and 3). The individuals ranged from 25 to 60 years of age (mean age 40.95) The presence of these microorganisms among the age groups was not statistically different (Tables 4 and 5). In the present

Table 1. Distribution of absolute (n) and relative (%) frequencies of species staphylococci isolated from the oral cavity and periodontal pocket of patients with chronic periodontitis (n=88).

Staphylococci Species	Isolates			
	Oral Cavity		Periodontal Pocket	
	n	%	n	%
S. epidermidis	19	21.59	14	15.91
S. epidermidis / S. aureus	4	4.55	0	0.0
S. epidermidis / S. capitis	1	1.14	0	0.0
S. aureus	20	22.73	4	4.55
S. aureus / S. hyicus	1	1.14	0	0.0
S. aureus / S. schleiferi	1	1.14	0	0.0
S. intermedius	2	2.27	6	6.82
S. capitis	2	2.27	4	4.55
S. schleiferi	1	1.14	0	0.0
S. caprae	1	1.14	0	0.0
S. haemolyticus	1	1.14	0	0.0
S. xylosus	1	1.14	1	1.14
S. hyicus	0	0.0	1	1.14
S. cohini	0	0.0	1	1.14
S. warneri	0	0.0	2	2.27
Total of patient positive	54	61.36	33	37.50

Table 2. Number of individuals with *Staphylococcus* spp. in the oral cavity according to the gender.

Gender	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
Males*	26	(74.28)	9	(25.71)	35	(100)
Females	28	(52.83)	25	(47.16)	53	(100)
Total	54	(61.36)	34	(38.63)	88	(100)

(Significantly statistically (p <0.05). Chi-square homogeneity test (2 = 4.09; gl=1; p=0.0431).

Table 3. Number of individuals with subgingival *Staphylococcus* spp. according to the gender.

Gender	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
Males	13	(37.14)	22	(62.85)	35	(100)
Females	20	(37.73)	33	(62.26)	53	(100)
Total	33	(37.50)	55	(62.50)	88	(100)

Chi-square homogeneity test (2 = 0.00; gl=1; p=0.955).

Table 4. Number of individuals that presented *Staphylococcus* spp. in the oral cavity in according to the age group.

Age group (years)	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
25-30	6	(60)	4	(40)	10	(100)
31-40	20	(62.50)	12	(37.50)	32	(100)
41-50	22	(59.45)	15	(40.54)	37	(100)
51-60	6	(66.66)	3	(33.33)	9	(100)
Total	54	(61.36)	34	(38.63)	88	(100)

Chi-square tendency test (2 = 0.01; gl=1; p=0.918).

study, no significant correlation was detected between age and increase of the microorganisms (Table 6). The higher number of positive samples for *Staphylococcus* spp was observed among the individuals between 31 and 40 years old (56,25%). For the probing depths between 5 to 6mm, 7 to 8mm and 9 to 10mm, the prevalence of *S. epidermidis* was of 15.15%, 17.07% and 14.29% respectively, for *S. aureus* was respectively of 6.06%, 0% and 14.29% .

Table 5. Number of individuals that presented *Staphylococcus* spp. in the pocket periodontal according to the age group.

Age group (years)	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
25-30	6	(60)	4	(40)	10	(100)
25-30	1	(10)	9	(90)	10	(100)
31-40	18	(56.25)	14	(43.75)	32	(100)
41-50	12	(32.43)	25	(67.56)	37	(100)
51-60	2	(22.22)	7	(77.77)	9	(100)
Total	33	(37.50)	55	(62.50)	88	(100)

Chi-square tendency test (2 = 0.25; gl=1; p=0.617).

Table 6. Number of individuals that presented *Staphylococcus* spp. in the periodontal pocket in according to probing depth.

Probing depth (mm)	Positive cases		Negative cases		Total	
	n	(%)	n	(%)	n	(%)
5-6	12	(36.36)	21	(63.63)	33	(100)
7-8	15	(36.58)	26	(63.41)	41	(100)
9-10	6	(42.85)	8	(57.14)	14	(100)
Total	33	(37.50)	55	(62.50)	88	(100)

Chi-square tendency test (2 = 0.13; gl=1; p=0.722).

DISCUSSION

Although staphylococci, mainly *S. aureus* and *S. epidermidis,* are frequently reported as pathogen causing nosocomial infections and acute problems, they are not frequently studied in the oral cavity. Subgingival staphylococci may not necessarily represent superinfection, but rather a colonization of indigenous oral bacteria. In the view of the virulence properties of these microorganisms, the present study examined the occurrence of these in oral cavity of patients with periodontal diseases. Slots *et al.* (19) found *Staphylococcus* spp. in 28.3% of individuals that were between 25 and sixty years of age. *S. aureus* was the second most common subgingival staphylococcal species, after *S. epidermidis.* In the present study, the microorganisms were present in 37.5% of individuals with similar age group and using similar methodology for the collection of the samples. *S. epidermidis* was present in 15.91% of the individuals, and was also the most commonly isolated subgingival staphylococcal specie. Our results are in accordance with others studies. Sánchez-Cordero *et al.* (15) isolated *Staphylococcus* from subgingival plaque from three groups: control group (10%),

including young adults clinically healthy, without evidence of loss of attachment, periodontitis nondiabetics group (17.5%), presented a pocket depth ≥ 5mm, and finally, periodontitis diabetics group (60%), showed pocket depth ≥ 5mm and medically supervised diabetic. There was no significant difference between the control and the periodontitis nondiabetics groups in the incidence of *S. epidermidis.* The diabetic group showed a significantly higher incidence when compared to the pooled control and periodontitis nondiabetics groups and when compared to the control group alone. According to the authors, in the diabetic group, insulin-injecting diabetics made up 62.5% of the whole group. Thus, it would be reasonable to assume that injections readily penetrate the skin barrier and may easily introduce staphylococci in the subject, but the authors observed that the distribution of the *S. epidermidis* was independent of the duration of the diabetic state and of oral or parenteral diabetic therapy. Dahlém and Wikström (3) observed positive samples of *S. epidermidis* in 54.4% of patients with chronic periodontitis although a lot of them demonstrated recurrent of periodontal attachment and use of antibiotic prior to the sutdy. *S. aureus* was observed in 8.2% of the patients. Listgarten *et al.* (8), Edwardsson *et al.* (4) observed low prevalence of *S. aureus* (< 5%) in individuals with different probing depth. We found *S. aureus* in only 4.5% (n=4) of individuals and we could not correlate with the probing depth. Many species isolated in this study are not considered part of oral microbiota such as *S. warneri* (6% of positive sample) and *S. capitis* (12.12% of positive samples). Other studies also found other staphylococci species in the periodontal pocket (2,5,8,10,14). *S. hycus* (17%) and *S. scheleiferi* subspecies *coagulans* (6.1%) were also found in the oral cavity in the study of Martins *et al.* (9) in health individuals using methodology similar to collect the oral rinses.

Rams *et al.* (14) reported approximately 50% of periodontal lesions harbored subgingival staphylococci. In the present study, 33% of the individuals with probing depth ≥ 5mm presented staphylococci. There was not found a correlation between the presence of the microorganisms, age and probing depth. The presence of staphylococci in higher proportions in subgingival dental biofilm cannot be considered super-infection. However, as they can act as an opportunistic microorganism, their presence must be considered during antibiotic therapy. The antibiotic administration can cause selection of resistant strains. The occurrence of higher proportions of nonresident's microorganisms in subgingival samples and oral sites may represent a significant problem in causing and maintaining periodontal infections.

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RESUMO

Staphylococcus spp. na cavidade bucal e na bolsa periodontal de indivíduos com periodontite crônica

Staphylococcus spp. não são usualmente isolados a partir da cavidade bucal. Quando presentes, são considerados pertencentes à microbiota transitória. Indivíduos que apresentam doença periodontal representam possíveis reservatórios dessas bactérias oportunistas na cavidade bucal. O uso de antibióticos para o tratamento da doença periodontal ou outras infecções pode predispor o aumento do número de *Staphylococcus* spp. na boca, pois estes adquirem facilmente resistência aos antibióticos, podendo resultar em superinfecção. O objetivo deste estudo foi verificar a presença de *Staphylococcus* spp. na cavidade bucal e nas bolsas periodontais de pacientes com periodontite crônica; identificar as cepas isoladas; verificar a relação entre a presença de *Staphylococcus* spp. na cavidade bucal e presença de bolsa periodontal. Participaram deste estudo 88 pacientes, entre 25 e 60 anos de idade e apresentando periodontite crônica, com pelo menos dois sítios com profundidade de sondagem maior ou igual a 5mm. Após anamnese e exame clínico periodontal foram feitas coletas de material da bolsa periodontal com cones de papel e da cavidade bucal por meio de bochechos. Do total de pacientes 37,50% apresentaram *Staphylococcus* spp. na bolsa periodontal e 61,36% na cavidade bucal, sendo que 27,27% apresentaram a bactéria nos 2 sítios. *S. epidermidis* foi a espécie mais prevalente para bolsa periodontal (15,9%) e cavidade bucal (27,27%). Não houve diferença estatística significante quanto à presença desses microrganismos entre as faixas etárias e aumento da profundidade de sondagem. A presença de bactérias oportunistas na cavidade bucal pode representar dificuldades para a manutenção do tratamento periodontal.

Palavras-chave: *Staphylococcus*, bactérias oportunistas, periodontite

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