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# Opportunistic microorganisms in individuals with lesions of denture stomatitis

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ABSTRACT

The aim of this study was to isolate, quantify, identify, and compare opportunistic microorganisms (*Candida* and *Staphylococcus* genera and Enterobacteriaceae/Pseudomonadaceae families) from prosthesis-fitting surfaces, the hard palate, and mouth rinses of individuals wearing removable maxillary prosthesis with (50) and without (50) lesions of denture stomatitis (DS). The strains were collected and identified using phenotypic, biochemical and molecular tests. The counts of microorganisms were significantly higher in the group of individuals with DS ($P < 0.05$). *C. albicans* was the most frequently isolated yeast species in both groups, following by *C. tropicalis* and *C. glabrata*. Six isolates were identified as *C. dubliniensis*. *S. aureus* and *S. epidermidis* were the most frequent *Staphylococcus* species in both groups. *Klebsiella pneumoniae* was the predominant species in both groups. The association between *Candida* spp. and bacteria isolated in this study with DS suggests that these microorganisms may play important roles in the establishment and persistence of this disease.



## 1. Introduction

Wearing intra-oral prosthesis is commonly associated with denture stomatitis (DS). This disease is characterized by inflamed mucosa, particularly under the upper denture, and patients may complain of a burning sensation, discomfort, or bad taste, but in the majority of cases, they are unaware of the problem. DS is an inflammatory lesion of the palatal mucosa under complete or partial removable dentures and affects up to 65% of denture wearers. There are various factors that influence the onset and severity of DS: denture trauma, continuous denture wearing, salivary flow, denture cleanliness, denture base material, denture age, cellular immunity, smoking, dietary factors, pH of the denture plaque and oral microbiota (Coco et al., 2008; Gasparoto et al., 2009).

In the oral cavity, most colonizing and infecting microorganisms are not found as single living cells but rather as complex structured microbial communities that are often encapsulated within a matrix of exopolymeric material and attached to biotic or abiotic surfaces (Kolenbrander, 2000). These communities are referred to as biofilms. Biofilms are a well-described phenomenon that have gained notoriety due to their ability to resist antimicrobials and immune cell challenge (Ramage et al., 2004). Biofilms can be up to 1000 times more resistant to toxicants than planktonic cells (Mah et al., 2003).

Yeasts and bacteria coaggregate as biofilms on the fitting surface of the denture rather than on the mucosal surface and have the ability to cause damage to the oral mucosa, which is typified by inflammation and hyperplasia of the denture-bearing tissue (Pereira-Cenci et al., 2008).

Approximately 90% of cases of DS are thought to be caused by yeasts, typically *Candida albicans*, although other species, such as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii* and *C. dubliniensis*, may also contribute to the pathogenesis of the disease (Figueiral et al., 2007; Freitas et al., 2008; Ramage et al., 2004).

Many publications have focused on the presence of *Candida* spp. in lesions of DS, but the oral cavity is a complex environment that has a rich variety of species, and its population densities are most likely dynamic and change frequently. Therefore, it is necessary to isolate and identify other opportunistic microorganisms that can colonize the oral cavity and the prosthesis of individuals with DS lesions to identify a better treatment method.

The aim of this study was to isolate, quantify, identify, and compare opportunistic microorganisms (*Candida* and *Staphylococcus* genera and Enterobacteriaceae/Pseudomonadaceae families) from individuals wearing removable maxillary prosthesis with and without lesions of DS.

## 2. Materials and methods

This study was approved by the local ethics committee (protocol number n°012/2010- PH/CEP) and was undertaken with the written informed consent of each subject. One hundred individuals wearing removable maxillary prosthesis were included in the study. In the clinical examination, 50 individuals presented DS. In the control

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group, 50 healthy individuals who had similar characteristics as the individuals with DS in relation to age and gender were studied. Individuals with diabetes mellitus, HIV-positive individuals, pregnant or lactating women, individuals who were undergoing any type of chemotherapy or radiotherapy were not included. Individuals under treatment with antimicrobials/antifungals or any prior therapy during the 60 days that preceded the sampling were not included. The Individuals with DS present this clinical condition approximately six months.

A specific physical examination was carried out in an odontological chair with direct lightning. The Newton (1962) classification was used to describe the denture stomatitis lesions. This classification is subdivided into 3 clinical groups: class I, punctiform hyperemia; class II, diffuse hyperemia; and class III, granular hyperemia.

Samples were collected from each individual from the hard palate mucosa and the prosthesis-fitting surface by swabs and from mouth rinses in phosphate-buffered saline (PBS, 0.1 mol/L, pH 7.2) for 1 min (Back-Brito et al., 2011).

The samples were centrifuged for 10 min at 8000×*g*, and the supernatant was discarded. The pellets were resuspended in 2.5 ml of PBS. To count the number of colony-forming units per milliliter (CFU/ml) and isolate species, dilutions of $10^{-1}$ and $10^{-2}$ were made in PBS, and an aliquot (0.1 mL) of each dilution was plated in duplicate on selective culture agar: a) Sabouraud dextrose agar (SDA; Difco, Detroit, MI, USA) with 50 mg/l chloramphenicol (União Química, São Paulo, Brazil) to count the CFU/ml of *Candida* spp. and CHROMagar *Candida* (Difco) for isolation of this species; b) Mannitol salt agar (Difco) for *Staphylococcus* spp.; and c) MacConkey

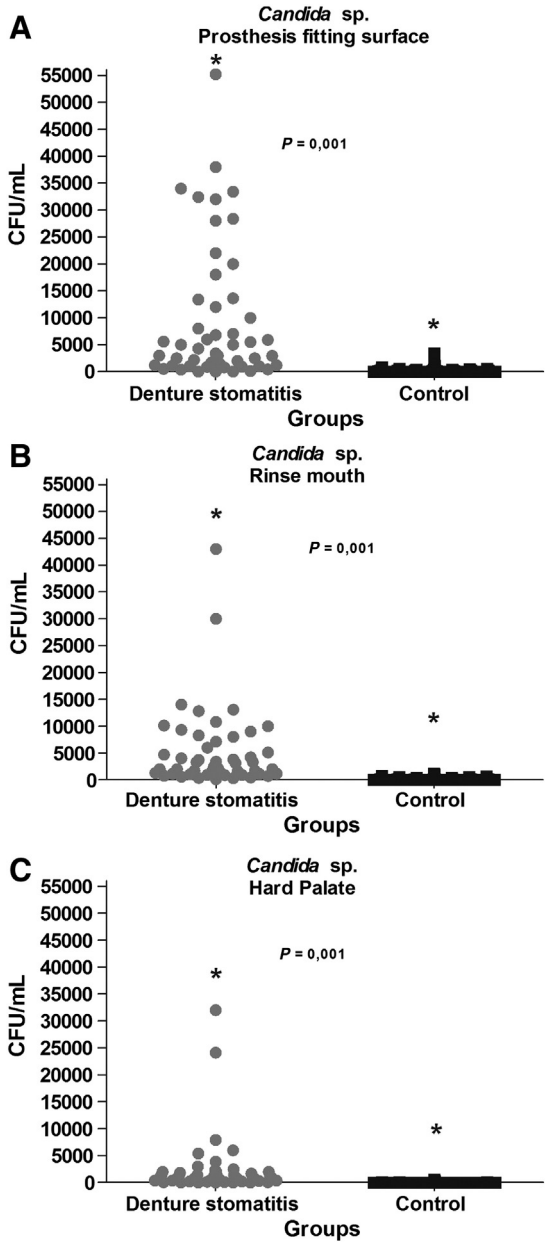


**Fig. 1.** Oral counts of *Candida* spp. (colony-forming units per milliliter; CFU/mL) obtained for the control and denture stomatitis groups, in the different source of isolation. (A) Oral counts of *Candida* spp. in prosthesis fitting surface. (B) Oral counts of *Candida* spp. in rinse mouth. (C) Oral counts of *Candida* spp. in hard palate. *Values of $P \leq 0.05$ were considered statistically significant (Mann–Whitney *U* test; Minitab, Inc.).

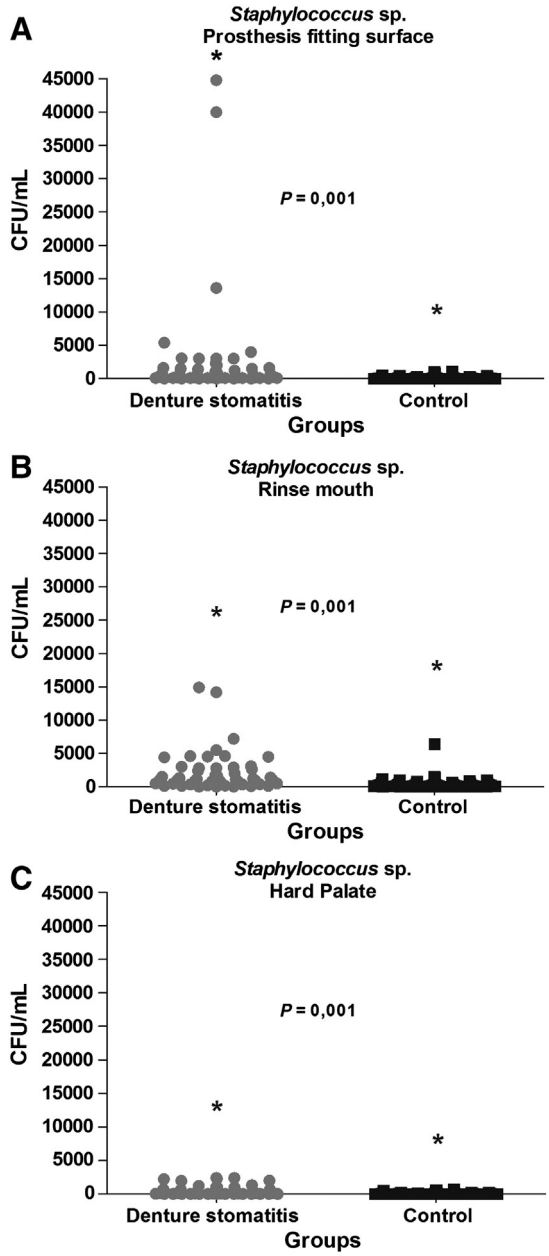


**Fig. 2.** Oral counts of *Staphylococcus* spp. (colony-forming units per milliliter; CFU/ml) obtained for the control and denture stomatitis groups, in the different source of isolation. (A) Oral counts of *Staphylococcus* spp. in prosthesis fitting surface. (B) Oral counts of *Staphylococcus* spp. in rinse mouth. (C) Oral counts of *Staphylococcus* spp. in hard palate. *Values of $P \leq 0.05$ were considered statistically significant (Mann–Whitney *U* test; Minitab, Inc.).

agar (Difco) for Enterobacteriaceae/Pseudomonadaceae families. The plates were incubated at 37°C for 48–72 h. Next, characteristic colonies were counted, and the CFU/ml was obtained. Colonies with different morphologies were subjected to microscopic confirmation and identification.

Isolates suggestive of *Staphylococcus* spp. were subjected to catalase and coagulase tests and subsequently identified using the API®Staph system (bioMérieux, Marcy-l'Etoile, France). Isolates suggestive of Enterobacteriaceae/Pseudomonadaceae families were identified using the API®20E system (bioMérieux, Marcy-l'Etoile, France).

*Candida* spp. were grown on CHROMagar, and differently colored colonies were identified using morphological tests, germ tube and chlamydospore production and biochemical tests, including carbohydrate assimilation, using the API® 20C AUX system (bioMérieux, Marcy-l'Etoile, France). Isolates that were phenotypically identified as *C. dubliniensis* were molecularly identified. These isolates were analyzed by multiplex polymerase chain reaction (PCR) according to the methodology proposed by Mähnß et al. (2005), with modifications. Briefly, the isolates were plated on SDA agar and incubated for 24 h at 37°C. Then, a single colony was transferred to 100 μL of lysing solution (0.9 M sorbitol, 0.1 M EDTA, 50 mmol/L dithiothreitol (Fermentas Inc, Hanover, MD, USA); Zymolyase™ 165 μg mL-1 (ICN, Costa Mesa, CA, USA)). After incubation at 37°C for 30 minutes, 10 μL 10% sodium dodecyl sulfate was added, and the tubes were kept at 65°C for 30 minutes. The D1/D2 region of 28S rRNA was amplified under standard conditions using a set of primers. Two pairs of primers were used: two universal primers, Uni-f: 5′-GCATATCAATAAGCGGAGGAAAA-3′ and Uni-r: 5′-GGTCCGTGTTTCAAGACG-3′, and two *C. dubliniensis*-specific primers, DUBF Act-f: 5′-GTATTTGTCGTTCCCCTTTC-3′ and DUBR Act-r: 5′-GTGTTGTGTGCACTAACGTC-3′. Amplification was carried out in a final volume of 10 μL containing 5 pmol of each primer, 5.0 μL of PCR Master Mix (Promega, São Paulo, SP, Brazil) 3.2 μL ultra-pure water, and 1 μL of DNA template. The cycling conditions consisted of 3 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 60 s at 72 °C, followed by 72°C for 10 min. *C. albicans* (ATCC 18804) and *C. dubliniensis* (NCFP 3108) were included as controls in all reactions. The amplification products were separated by electrophoresis through 2% (w/v) agarose gels (Promega, São Paulo, SP, Brazil) containing 25 μM ethidium bromide (Promega, São Paulo, SP, Brazil) and visualized on a UV transilluminator. A DNA ladder of 100 bp was

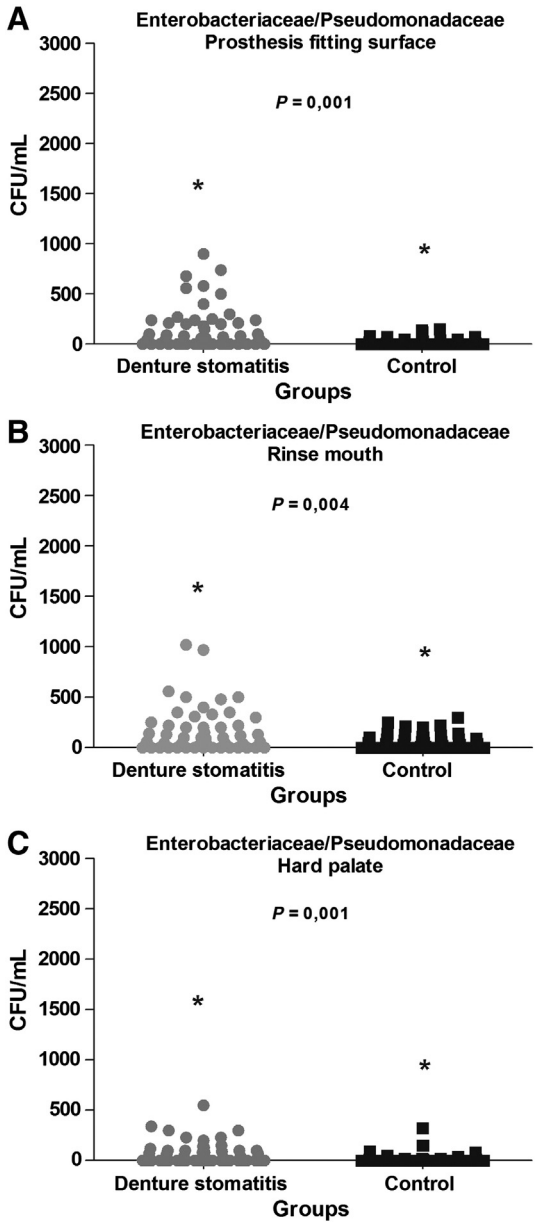


**Fig. 3.** Oral counts of Enterobacteriaceae/Pseudomonadaceae strains (colony-forming units per milliliter; CFU/ml) obtained for the control and denture stomatitis groups, in the different source of isolation. (A) Oral counts of Enterobacteriaceae/Pseudomonadaceae strains in prosthesis fitting surface. (B) Oral counts of Enterobacteriaceae/Pseudomonadaceae strains in rinse mouth. (C) Oral counts of Enterobacteriaceae/Pseudomonadaceae strains in hard palate. *Values of $P \leq 0.05$ were considered statistically significant (Mann–Whitney test; Minitab, Inc.).

**Table 1**
*Candida* species found in individuals with lesions of denture stomatitis and control group, according to the location of isolation.

| Microbial growth *Candida* spp. | Specimen (n) from | | |
|---|---|---|---|
| | Prostheses (with/without DS) | Mouth Rinse (with/without DS) | Hard Palate (with/without DS) |
| *C. albicans* | 21/14 | 26/10 | 29/03 |
| *C. glabrata* | 03/04 | 01/05 | 01/02 |
| *C. tropicalis* | 04/03 | 02/07 | 04/03 |
| *C. dubliniensis* | 01/00 | 00/01 | 00/00 |
| *C. guilliermondii* | 00/01 | 00/01 | 00/01 |
| *C. krusei* | 00/02 | 00/03 | – |
| *C. lusitaniae* | 00/03 | 02/00 | 03/00 |
| *C. albicans* + *C. glabrata* | 03/01 | 03/00 | – |
| *C. albicans* + *C. tropicalis* | 04/01 | 01/02 | 02/00 |
| *C. albicans* + *C. dubliniensis* | 00/01 | – | – |
| *C. albicans* + *C. guilliermondii* | 02/00 | 01/00 | – |
| *C. albicans* + *C. krusei* | – | 01/00 | – |
| *C. albicans* + *C. lusitaniae* | 04/00 | 01/00 | 01/00 |
| *C. albicans* + *C. parapsilosis* | – | 01/00 | – |
| *C. albicans* + *C. glabrata* + *C. tropicalis* | – | 01/00 | 01/00 |
| *C. albicans* + *C. glabrata* + *C. lusitaniae* | – | – | 01/00 |
| *C. albicans* + *C. glabrata* + *C. dubliniensis* | 01/00 | – | – |
| *C. albicans* + *C. lusitaniae* + *C. tropicalis* | – | 01/00 | – |
| *C. albicans* + *C. glabrata* + *C. lusitaniae* + *C. tropicalis* | – | 01/00 | – |
| *C. glabrata* + *C. tropicalis* | – | 01/00 | 01/00 |
| *C. glabrata* + *C. lusitaniae* | 01/00 | – | – |
| *C. glabrata* + *C. dubliniensis* + *C. tropicalis* | 01/00 | – | – |
| *C. tropicalis* + *C. guilliermondii* | – | 01/00 | – |
| *C. tropicalis* + *C. krusei* | 00/01 | – | – |
| *C. tropicalis* + *C. lusitaniae* | 01/00 | 02/00 | 01/00 |
| *C. dubliniensis* + *C. glabrata* + *C. tropicalis* | – | 01/00 | – |

**Table 2**
*Staphylococcus* species found in individuals with lesions of denture stomatitis and control group, according to the location of isolation.

| Microbial growth *Staphylococcus* spp. | Specimen (n) from | | |
|---|---|---|---|
| | Prostheses (with/without DS) | Mouth Rinse (with/without DS) | Hard Palate (with/without DS) |
| *S. aureus* | 13/13 | 10/20 | 11/11 |
| *S. epidermidis* | 12/04 | 12/05 | 13/06 |
| *S. xylosus* | 11/01 | 09/04 | 07/02 |
| *S. lentus* | 01/00 | – | – |
| *S. cohnii* spp. *cohnii* | 01/00 | – | 01/00 |
| *S. saprophyticus* | 02/00 | 01/00 | – |
| *S. caprae* | 01/00 | 01/00 | – |
| *S. capitis* | 01/00 | 01/01 | 01/00 |
| *S. hominis* | 00/02 | 02/00 | – |
| *S. chromogenes* | 00/04 | 00/04 | 00/05 |
| *S. warneri* | 00/02 | 02/02 | 02/03 |
| *S. lugdunensis* | – | 01/01 | – |
| *S. aureus* + *S. capitis* | – | 00/01 | – |
| *S. aureus* + *S. chromogenes* | – | 00/01 | 01/00 |
| *S. aureus* + *S. epidermidis* | 00/02 | 00/01 | – |
| *S. aureus* + *S. xylosus* | 00/01 | 00/01 | 00/01 |
| *S. aureus* + *S. warneri* | 01/01 | 01/00 | – |
| *S. epidermidis* + *S. xylosus* | 00/01 | 02/00 | 01/00 |
| *S. epidermidis* + *S. warneri* | – | 01/01 | – |
| *S. epidermidis* + *S. cohnii* spp. *urealyticus* | – | 01/00 | – |
| *S. xylosus* + *S. chromogenes* | 01/00 | – | 01/00 |
| *S. xylosus* + *S. haemolyticus* | – | 01/00 | – |
| *S. xylosus* + *S. hominis* | – | 01/00 | – |
| *S. xylosus* + *S. cohnii* spp. *cohnii* | – | 01/00 | – |
| *S. hominis* + *S. sciuri* | – | – | 01/00 |
| *S. hominis* + *S. warneri* | – | 00/01 | – |
| *S. hycus* +*S. cohnii* spp. *urealyticus* | 01/00 | – | – |

used as the molecular size standard. The gels were photographed for analysis and documentation.

Counts of the microorganisms obtained for DS and control groups were compared by a Mann–Whitney *U* test (Minitab, Inc., State College, PA, USA). Values of $P \leq 0.05$ were considered statistically significant.

## 3. Results

In the present study, the microbiota of 50 individuals wearing removable maxillary prosthesis and presenting with denture stomatitis were evaluated, and they were compared with 50 healthy individuals wearing removable maxillary prosthesis. The mean age of the individuals with DS was 63.48±9.34 years. There was a higher frequency of women with DS (n = 32), and the predominant lesions in this group were type II (n = 16). Among men (n = 18), the most frequent lesions were type I (n = 09).

The counts of microorganisms were significantly higher at different sites of isolation in the group of individuals with DS ($P < 0.05$). *Candida* (Fig. 1A, B and C) and *Staphylococcus* (Fig. 2A, B and C) were the most frequently isolated genera in the prosthesis-fitting surface in both groups. However, strains of the Enterobacteriaceae and Pseudomonadaceae families (Fig. 3A–C) were more frequent in mouth rinses.

The *Candida* species isolated from individuals with DS and the control group are shown in Table 1. *C. albicans* was the most frequently isolated species in both groups. *C. tropicalis* and *C. glabrata* were also frequently isolated. Six isolates, 4 from individuals with DS and two from control groups, were identified as *C. dubliniensis*.

Table 2 shows the *Staphylococcus* species isolated from individuals with and without DS. *S. aureus* and *S. epidermidis* were the most frequent species in both groups.

Strains of the families Enterobacteriaceae/Pseudomonadaceae isolated from individuals with DS and the control group are shown in Table 3. *Klebsiella pneumoniae* was the most predominant species in both groups. *P. aeruginosa* and *P. fluorescens* were the only species of the Pseudomonadaceae family isolated in both groups in this study.

## 4. Discussion

The present study assessed the microbiota of individuals wearing maxillary dentures with and without lesions of DS. In this study, 64% (n = 32) of the individuals with DS were women, with an average age of 63± 8.9 years. This finding confirms that DS mainly affects women and the elderly (Baena-Monroy et al., 2005; Gasparoto et al., 2009; Marcos-Arias et al., 2009; Zomorodian et al., 2011). It is likely that women are more prone to this illness due to hormonal changes that occur in association with menopause, which can be reflected in the oral microbiota. Older age is associated with the development of systemic illnesses and changes in oral nutritious and hygienic habits and salivary composition, which along with the use of dental prosthesis, facilitate changes in the oral microbiota and the appearance of oral lesions (Vitkov et al., 1999).

According to Newton (1962), DS may be classified as localized simple inflammation or punctiform hyperemia (type I), generalized simple inflammation or diffuse hyperemia (type II), or inflammatory papillary hyperplasia or granular hyperemia (type III). In this study, clinical evaluation showed that type II DS was detected most often in our individuals (48%, n = 24), followed by type I (42%, n = 21) and type III (10%, n = 5).

The present study identified several strains of the *Candida* and *Staphylococcus* genera, and species of the Enterobacteriaceae and

**Table 3**
Enterobacteriaceae/Pseudomonadaceae strains found in individuals with lesions of denture stomatitis and control group, according to the location of isolation.

| Microbial growthEnterobacteriaceae/ Pseudomonadaceae strains | Specimen (n) from | | |
|---|---|---|---|
| | Prostheses(with/without DS) | Mouth Rinse(with/without DS) | Hard Palate(with/without DS) |
| *K. pneumoniae* | 05/06 | 07/09 | 07/05 |
| *Shigella* spp | 07/01 | 06/03 | 06/02 |
| *Escherichia coli* | 05/03 | 04/05 | 05/01 |
| *Enterobacter cloacae* | 04/02 | 05/02 | 02/02 |
| *Enterobacter aerogenes* | 02/00 | 02/02 | – |
| *K. oxytoca* | 02/01 | 01/01 | 01/00 |
| *Enterobacter sakazakii* | 01/00 | 01/00 | 01/00 |
| *Pantoe* spp. | 01/00 | – | 01/00 |
| *Pseudomonas fluorescens* | 03/01 | 03/02 | 03/01 |
| *P. aeruginosa* | 01/00 | 02/02 | 02/01 |
| *Proteus penneri* | – | 01/00 | – |
| *K. pneumoniae* + *P. fluorescens* | – | 01/00 | – |
| *K. pneumoniae* + *Escherichia coli* | – | 02/00 | – |

Pseudomonadaceae families. Differently from previous studies, this is the first comprehensive report of the microbiota of individuals wearing maxillary dentures with and without lesions of DS. *Candida* and *Staphylococcus* were the most frequently isolated genera in the prosthesis-fitting surface in both groups. This finding reinforces the role of dentures (prosthesis) in promoting the development of DS. A primary role is the ability of bacteria and yeast to colonize denture materials, forming a biofilm. Biofilms adhere to denture surfaces, forming plaque deposits, which provide a source of continued exposure of the mucosa to the organisms contained within these biofilms (Gendreau and Loewy, 2011). Thus, dentures can serve as a reservoir for pathogens responsible for aspiration pneumonia, one of the major causes of reduced quality of life in the elderly.

In this study, we observed a high frequency of *Candida* yeasts in addition to a large number of *Staphylococcus* spp. bacteria. Strains of *C. albicans* and *S. aureus* were isolated in 94% and 42% of individuals with DS, and 34% and 62% of individuals of control group. The percentage of individuals with DS and the control group that presented strains of *C. albicans* in association with *S. aureus* were of 40% and 20%, respectively. *Candida* spp. and *Staphylococcus* spp. are common in the human oral microbiota. However, in some situations, as in individuals with periodontitis treated with the systemic administration of penicillin and erythromycin, they can act as opportunistic microorganisms and produce superinfection (Helovuo et al., 1993). Other studies have also found a high frequency of these microorganisms in individuals with DS, ranging from 60–100% and 48.3–84%, respectively (Baena-Monroy et al., 2005), which is similar to the results found in this study.

Several species of the *Staphylococcus* genus can be isolated from the human oral cavity and are part of the transient microbiota (Loberto et al., 2006). These microorganisms are found in the oral cavity of approximately 95% of healthy individuals and have become a matter of concern because of their capacity to develop resistance to antimicrobial agents (Casey et al., 2007). In addition to their involvement in oral diseases, such as angular cheilitis, parotiditis, staphylococcal mucositis and periodontitis, these bacteria are the causative agents of severe skin and soft tissue infections, which are associated with an increased risk of complications, such as bacteremia, pneumonia and endocarditis (Smith et al., 2003). With respect to the *Staphylococcus* spp. identified in this study, *S. epidermidis* and *S. aureus* were the most prevalent coagulase-negative and coagulase-positive species, respectively, in both groups. Previous studies reported the presence of this species in the oral cavity of individuals with DS (Baena-Monroy et al., 2005) and HIV-positive patients (Back-Brito et al., 2011). *S. aureus* causes a wide range of infections, including acute, chronic, and toxin-mediated disease (Chambers and Deleo, 2009). The ability of this pathogen to cause such a diverse array of problems is due to its arsenal of virulence factors, such as pore-forming toxins, superantigens, matrix-binding surface adhesins, and tissue-degrading enzymes (Lowy, 1998). *S. epidermidis* is a commensal microorganism of the human skin and mucous membranes. As a recognized opportunistic pathogen, it is responsible for nosocomial infections of indwelling medical devices (Otto, 2009), such as peripheral or central intravenous catheters, prosthetic joints, vascular grafts, and central nervous system shunts, and cardiac device infections, such as prosthetic valve endocarditis, as well as ventricular assisted device driveline-related infections. A large variety of other *Staphylococcus* species was also observed in both groups. Some of these species, although isolated infrequently, may cause infections in humans, such as urinary tract infections, bacteremia, endocarditis, osteomyelitis, cellulitis and cerebral empyema (Cone et al., 2005; Seifert et al., 2005).

Enterobacteriaceae and Pseudomonadaceae families were identified in 78% of individuals with DS and 54% of individuals in the control group. The presence of these bacteria in the oral cavity is not considered indicative of pathogenicity; however, they may serve as a reservoir and can severely compromise the lives of immunocompromised individuals (Senpuku et al, 2003). Some studies have reported an association between Enterobacteriaceae and oral ulcerations in HIV-positive patients, but this association may not necessarily be causal, as enterobacteria may be secondary invaders (Tsang and Samaranayake, 2000). Microorganisms of the Enterobacteriaceae and Pseudomonadaceae families may act as opportunistic pathogens and colonize the human oral cavity, especially in patients with debilitating diseases who are submitted to prolonged treatment with antibiotics or cytotoxic medications (Slots et al., 1990). Previous studies reported the presence of this group of microorganisms in the oral cavity. A clinical study conducted by Goldberg et al. (1997) evaluated the prevalence of Enterobacteriaceae in four different populations, and they detected this family in 48% of patients with complete dentures, 27.1% of patients with halitosis, 16.4% of controls and 13% of orthodontic patients. A higher prevalence of Enterobacteriaceae and Pseudomonadaceae families was observed on the tongue dorsum of adult individuals (Conti et al., 2009) and oral cavities of HIV-positive patients (Back-Brito et al., 2011). In this study, the most frequent species in both groups was *K. pneumoniae*. According to Zhu et al. (2008), there is an important correlation between the presence of *K. pneumoniae* in the oral cavity and the risk of pneumonia by aspiration of these bacteria in people suffering from a stroke. *P. aeruginosa* and *P. fluorescens* were the only species of the Pseudomonadaceae family isolated in both groups in this study. Due to its low virulence, *P. fluorescens* is an infrequent cause of human infection and has been mainly implicated in outbreaks of pseudobacteremia (Smith et al., 2002). *P. aeruginosa* is an opportunistic pathogen and one of the principal agents of nosocomial infection. The clinical importance of infection by *P. aeruginosa* is characterized by the expression of multiple antimicrobial resistance elements associated with difficult disease eradication, leading to high rates of morbidity and mortality (Livermore, 2002).

Among the opportunistic microorganisms isolated in this study, the genus *Candida* was isolated in large numbers. *Candida* species are often isolated from DS lesions, and their role in the disease has been suggested. In extremely rare cases, *Candida* yeast can be recovered from the denture plaque and aspirated to the lower respiratory tract, causing pneumonia (Sumi et al., 2003). We obtained 202 isolates of *Candida* spp. from individuals with lesions of DS and 75 from individuals in the control group. *C. albicans* was the most prevalent species in both groups, with a frequency of 53.5% ($n = 108$) in individuals with lesions of DS and 42.7% ($n = 32$) in control individuals. This finding is in agreement with the literature. Denture stomatitis is a disease of predominantly fungal origin, and *C. albicans* is the most frequently isolated yeast from the oral cavities of patients with DS (Baena-Monroy et al., 2005). *C. albicans* is also the predominant biofilm-forming yeast pathogen due to its ability to form hyphae, a key determinant of these complex consortia. Candidal biofilms are clinically problematic, as they are intrinsically resistant to antimicrobial agents (Ramage et al., 2005). In addition, other virulence characteristics of *C. albicans*, such as adhesion (adhesion to mucosal cells), dimorphism (ability to convert from a single-celled yeast form to a filamentous form), and the secretion of hydrolytic extracellular enzymes (which are utilized in the process of host tissue invasion and liberation of nutrients), make *C. albicans* a pathogen that causes a broad spectrum of infections in different host sites (Tsang et al., 2007). Following *C. albicans*, *C. glabrata* and *C. tropicalis* were the most frequently isolated species in both groups, which corroborates with similar studies carried out by other authors, in which these three yeasts were also the most common (Marcos-Arias et al., 2009; Zomorodian et al., 2011). *C. tropicalis* has been identified as the most prevalent pathogenic yeast species of the *Candida*-non-albicans group (Kothavade et al., 2010). The virulence of *C. tropicalis* may be due to its ability to adhere to epithelial cells in vitro and its ability to secrete moderate levels of proteinase (Moran et al., 2002). *C. glabrata* is often the second or third most common cause of candidiasis after

*C. albicans*. *C. glabrata* infections are difficult to treat and are often resistant to many azole antifungal agents, especially fluconazole (Hitchcock et al., 1993). Consequently, *C. glabrata* infections have a high mortality rate in compromised, at-risk hospitalized patients (Fidel et al., 1999). Another species isolated in this study and that has been widely studied recently is *C. dubliniensis*. *C. dubliniensis* is closely related to *C. albicans* in evolutionary terms, and both have characteristics of commensalism and opportunistic infection (Sullivan et al., 1995). *C. dubliniensis* is now well recognized as an opportunistic pathogen associated with recurrent oral candidiasis in AIDS patients. It has also been isolated from the oral cavity of diabetic patients (Manfredi et al., 2002) and the sputum of cystic fibrosis patients (Peltroche-Llacsahuanga et al., 2002). The fact that *C. dubliniensis* has been isolated from upper respiratory tract specimens (Polacheck et al., 2000) and blood (Cimolai et al., 2002) suggests that it can disseminate to other sites as well.

The association between the *Candida* spp. and bacteria isolated in this study with DS suggests that these microorganisms may play important roles in the establishment and persistence of this disease.

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