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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

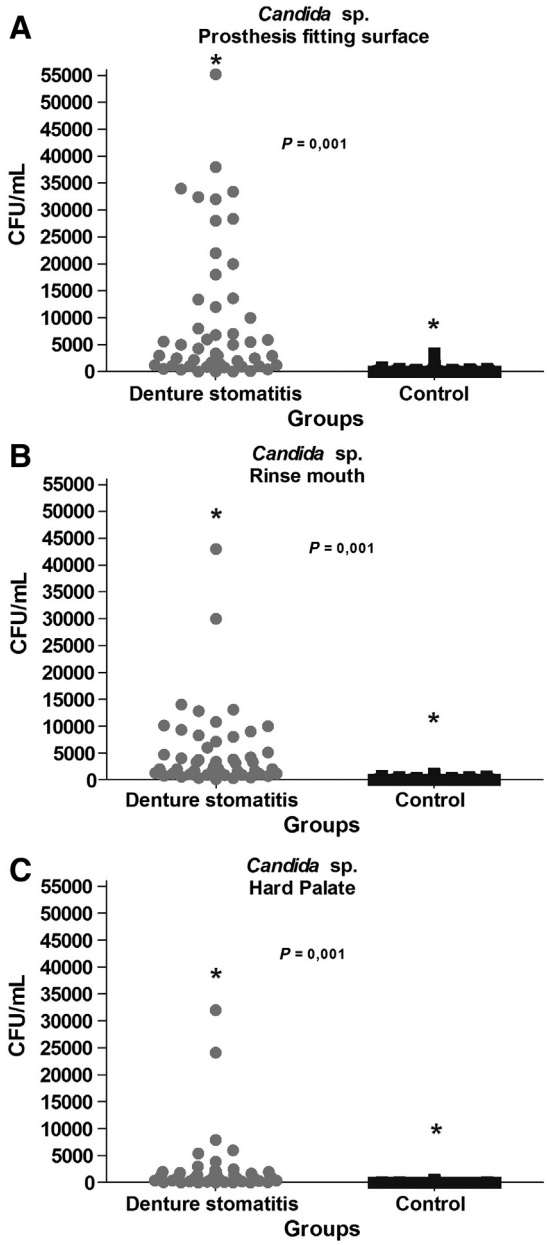

Fig. 1. Oral counts of *Candida* spp. (colony-forming units per milliliter; CFU/mL) obtained for the control and denture stomatitis groups, in the different source of isolation. (A) Oral counts of *Candida* spp. in prosthesis fitting surface. (B) Oral counts of *Candida* spp. in rinse mouth. (C) Oral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

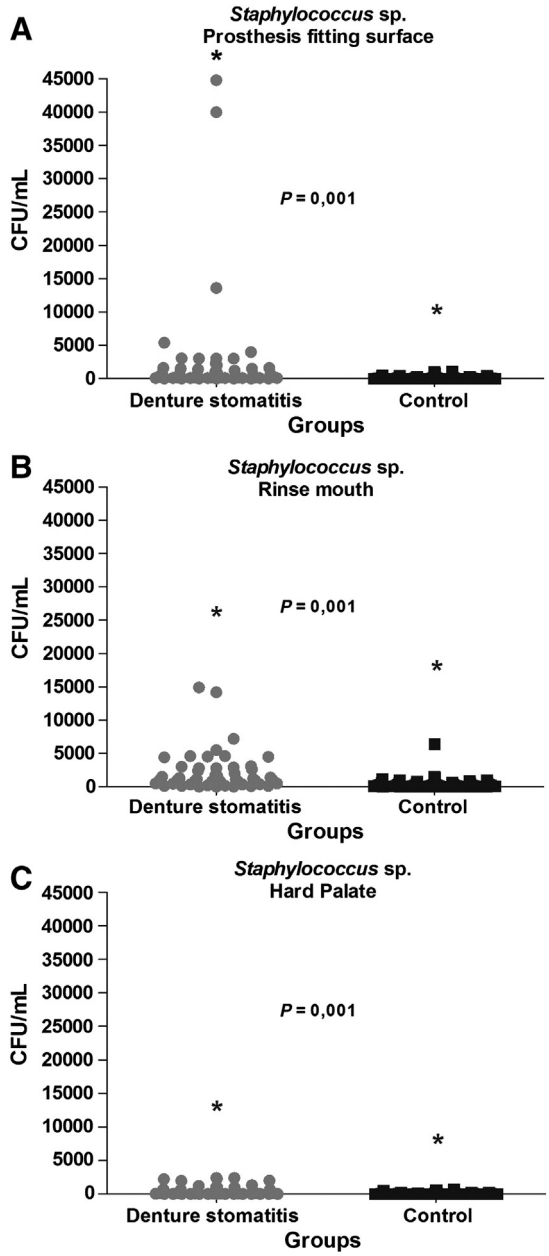

Fig. 2. Oral counts of *Staphylococcus* spp. (colony-forming units per milliliter; CFU/ml) obtained for the control and denture stomatitis groups, in the different source of isolation. (A) Oral counts of *Staphylococcus* spp. in prosthesis fitting surface. (B) Oral counts of *Staphylococcus* spp. in 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.).