Evaluation of the frequency of *Candida* spp. in hospitalized and non-hospitalized subjects


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Abstract

The aim of this study was to evaluate the frequency of *Candida* species between a non-hospitalized and a hospitalized population. For this purpose, samples of saliva were sampled through sterile swabs, moistened in peptone water and rubbed in the oral cavity of 140 individuals, from which, 70 were hospitalized patients from the Medical Clinic of a Teaching Hospital and the other 70 were non-hospitalized subjects. All saliva samples were plated in Sabouraud Dextrose agar added with Chloramphenicol and incubated at 36 °C for 48 hours. The morphology identification was performed through macroscopic and microscopic characterization, the CHROMagar *Candida* medium and the VITEK® system Yeast Biochemical Card (bio Mérieux SA, France). The results showed a colonization of *Candida* spp. in 85.7% the hospitalized individuals, where the species found were *C. albicans* (60%), *C. tropicalis* (23.4%), *C. krusei* (3.3%) and *Candida* spp. (13.3%). In the non-hospitalized individuals the colonization by *Candida* spp was 47.1%, and the species found were: *C. albicans* (45.5%), *C. krusei* (9.1%), *C. guilliermondii* (9.1% %), *C. tropicalis* (3.0%), *C. famata* (3.0%) and *Candida* spp. (30.3%). In spite of their presence in oral cavity in both groups, *Candida* spp. was more frequently isolated in hospitalized individuals, who were 6.73 times more likely to have this fungus in the oral cavity and were 3.88 times more likely to have *Candida albicans*.

Keywords: Oral *Candida* spp., colonization, hospitalized patients, nosocomial infections.
1. Introduction

The yeasts of Candida species are microorganisms of the oral microbiota of humans, in which colonization occurs immediately after birth. This microbiological condition usually offers a harmonious balance between the parasite and the host, maintaining the immune system and keeping the integrity of tissue barriers. This balance, however, may be disrupted due to a low immunity or by a mechanical, physical and iatrogenic change in the oral cavity, compromising the immune system and facilitating endogenous opportunistic infections caused by Candida spp. (Ribeiro et al., 2006; Moyes and Naglik, 2011; Simões et al., 2013). Thus, Candida spp. virulence may present pathogenic action when the host’s resistance is overcome (Santana et al., 2013).

Opportunist nosocomial infections are more frequent and severe in patients with poor oral health care, as well those who have been exposed to long intensive drug therapies. Some authors have stated that hospitalized patients tend to develop a higher oral candidiasis prevalence, due to both local and systemic alterations, changing the microbiota and contributing to this kind of infections (Grimoud et al., 2003; Meurman and Hamalainen, 2006; Moris et al., 2008). In addition, many intrinsic and extrinsic factors influence the composition, metabolic activity and pathogenicity of a variety of microorganisms in the oral cavity (Bodineau et al., 2009).

Hospital-acquired infections caused by yeasts may have an exogenous source, and are generally transmitted by the hands of health professionals, surfaces of materials, lab coats of health care practitioners (Savastano et al., 2016) and by people getting in contact with the patient, or by an endogenous source, such as the pre-existing microorganisms in the host, turning pathogenic for some reason. In view of the above, the aim of the present paper was to verify the occurrence and frequency of the yeasts of Candida spp., in the oral cavity of both non-hospitalized subjects and hospitalized patients from a Medical clinic of a Teaching Hospital situated in the city of Pelotas, Rio Grande do Sul.

2. Material and Methods

2.1. Individuals and sampling

A total of 140 subjects took part in this study; they were divided in two groups. One group of 70 patients were under hospitalization in the Federal University Hospital of Pelotas, and the second group of 70 non-hospitalized subjects, were mainly students and professionals from the same University; none of the 140 subjects presented oral lesions.

The evaluation was based on a questionnaire where the non-hospitalized subjects also declared that they did not carried any infectious diseases at the time of sampling nor in the preceding month; they also declared that they did not have any underlying diseases, they had not been submitted to antibiotic and corticoid therapy in the six months previous to evaluation, and had no contact with hospitalized patients who participated in this study. These individuals age ranged between 18-75 years.

From 70 hospitalized patients, 50 had some type of bacterial infection and underwent antibiotic therapy at the most one month ago, and 20 were submitted to cancer treatment; their age ranged of 19-86 years. The hospitalized patients with cancer were submitted to the same type of chemotherapy, in the General Medical Clinic of the hospital, between one week and one month of hospitalization. The collections were always performed after the chemotherapy.

This research was approved by the Research Ethics Committee of the Federal University of Pelotas (UFPeL), under registration CEP 48/10, and the participants signed an Informed Consent Agreement.

The sampling was performed from the mucosa of the cheek in the oral cavity, through sterile swabs, moistened in peptone water. Each sample was identified with the corresponding number of the clinical report within the information of the patient. The reports were referred, along with the sampled material, to the Laboratory of Mycology of the Biology Institute from the UFPeL.

2.2. Identification of yeasts

The samples were plated in Sabouraud Dextrose agar added with Chloramphenicol and incubated at 36 °C for 48 hours. The macro morphology was analysed by Gram-stain smears observed by light microscopy (1000X), followed by the germ tube test and micro culture in cornmeal media. After, the samples were replicated in CHROMagar Candida medium for an initial differentiation of the species (Araújo et al., 2005; Mimica et al., 2009). Unidentified isolates were evaluated through the assimilation of carbohydrates by the Vitek® system Yeast Biochemical Card (bio Mérieux SA, France) (Spolidorio et al., 2009; Gonçalves et al., 2015).

2.3. Statistical analysis

A descriptive comparison of both study groups (hospitalized patients and non-hospitalized subjects) of different variables was developed, expressing frequency (value - n) and percentages (%) values. The statistical tests between the groups was performed using the chi-square test (p ≤ 0.05), and the evaluation of the odds ratio (OR) with 95% of confidence intervals (CI 95%).

This research was approved by the Research Ethics Committee of the Federal University of Pelotas (UFPeL), according to the Declaration of Helsing, and the participants signed an Informed Consent Agreement.

3. Results

Candida isolates were found in 33 (47.1%) of the 70 samples from the non-hospitalized individuals. The species isolated were: C. albicans in 15 (21.4%) individuals; C. krusei in three (4.3%); C. Guilliermondii in three (4.3%); C. tropicalis in one (1.4%); C. famatain one (1.4%), and 10 (14.3%) were identified as Candida spp.
Candida spp. was isolated in 60 (85.7%) from the 70 samples of the hospitalized patients. The identified species were: C. albicans in 36 (51.4%) samples; C. tropicalis in 14 (20%) samples; C. krusei in two (2.9%), and Candida spp. in eight (11.4%). From patients hospitalized due to cancer chemotherapy (20 individuals), 14 were positive for fungus (70%) and C. albicans was isolated in eleven of the samples from these patients and Candida spp. in three. Yeasts were isolated in 46 (92%) of the 50 samples from patients undergoing antibiotic therapy due to infection (p-value = 0.017, OR = 4.93, CI_{95%} = 1.22 - 19.99). Of these 46 samples, 25 (54.3%) were by Candida albicans.

Bacterial diseases and the location of tumors were not considered as a whole for a disease that can be statistically analyzed for characteristics.

In both groups, no mixed colonization by Candida species was detected, only individual mono colonization occurred in the analyze. A statistical significant difference (p-value<0.0001) was observed in the isolation of the yeast from oral cavity in both groups included in this study – non-hospitalized individuals and hospitalized patients from the Medical Clinic (Table 1). Hospitalized patients were 6.73 (p-value<0.0001, CI_{95%} = 2.97 – 15.24) times more likely to have Candida spp., and 3.88 (p-value<0.0001, CI_{95%} = 1.85 – 8.13) times more likely to have C. albicans compared to the non-hospitalized subjects.

### 4. Discussion

Candida species live as commensals and are part of the normal microbiota of healthy subjects. A great variety of microorganisms with the ability of moving out and reintroducing themselves rapidly into the human system are regularly found in the oral cavity. This relation of microbial coexistence with the human health is possible by the immunological mechanisms through a continuous process of adaptation (Bodineau et al., 2009; Moyes and Naglik, 2011; Naglik and Moyes, 2011). Fungal infections have increased in recent years, and are responsible for high morbidity and mortality rates. Candida still causes most of the fungal nosocomial infections; the presence of these yeasts in human microbiota favours the incidence of these infections, mainly due to the virulence factors of the fungus and its interaction with the hospitalized patients who presented a weak immune system (Hinrichsen et al., 2009; Heo et al., 2011; Silva et al., 2014).

In this study, Candida spp. was found in 47.1% of the non-hospitalized subjects, and this agrees with other studies showing a 10 to 70% variation of Candida spp., in healthy individuals (Motta et al., 2010). 47.3% of positivity of Candida spp. in the oral cavity was also found in a group of children of a school from different socioeconomic status without candidiasis lesions, in which C. albicans was the prevailing specie, corresponding to 95% of all the isolates (Moreira et al., 2001). Yeasts of Candida play an extremely important role in high frequent infections and colonization in humans (Moris et al., 2008; Lass-Fiörl, 2009; Savastano et al., 2016). These findings agrees with the reported by several authors related to the colonization prevalence of C. albicans over other species in the oral cavity, and consequently on the subsequent candidiasis development (Moreira et al., 2001; Grimoud et al., 2003; Naglik et al., 2003; Moris et al., 2008; Moyes and Naglik, 2011).

The yeast was isolated from the hospitalized patients in 85.7%, being twice the frequency observed in the non-hospitalized individuals. The isolated species were: C. albicans, C. tropicalis, C. krusei and Candida spp. The most frequent isolated specie was again C. albicans, found in 51.4% of the patients. C. albicans is without a doubt the most frequently isolated specie in both superficial and invasive infections from different anatomic sites, and the most common cause of candidiasis and candidemia worldwide (Moris et al., 2008; Hinrichsen et al., 2009; Savastano et al., 2016).

A study performed by Domaneschi et al. (2011) revealed the prevalence of Candida spp., in the oral cavity of immunocompromised patients (aids paediatric patients), and found it in 62% of the analysed samples and the most frequent isolated specie in this study was C. albicans. Another study by St-Germain et al. (2008), in which a total of 453 episodes of candidemia from 54 laboratories from hospitals in Quebec, Canada, founded Candida albicans in 62% of the isolates, and the authors reported that in North America and in most countries worldwide, C. albicans continues to be the single most common specie causing candidemia.

The presence of yeasts of Candida spp. on human skin and mucosa, facilitates the occurrence of oral or systemic infections, mainly due to the virulence factors of the fungus and its interaction with the hospitalized patients who presented a weak immune system (Hinrichsen et al., 2009; Heo et al., 2011; Silva et al., 2014).

### Table 1. Frequency of Candida spp. and Candida albicans in hospitalized (n = 70) and non-hospitalized individuals (n = 70) from the city of Pelotas, Rio Grande do Sul state, Brazil.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-hospitalized individuals</th>
<th>Hospitalized</th>
<th>p-value</th>
<th>OR (CI_{95%})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Candida spp.</td>
<td>33 (47.1)</td>
<td>60 (85.7)</td>
<td>&lt;0.0001</td>
<td>6.73</td>
</tr>
<tr>
<td>Presence of Candida albicans</td>
<td>37 (52.9)</td>
<td>10 (14.3)</td>
<td>(2.97 - 15.24)</td>
<td></td>
</tr>
</tbody>
</table>

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candidiasis (Moris et al., 2008). The virulence factors of the fungus and the immune status of the individual are two parameters which controls the onset of Candida spp. (Hube, 2004; Pfäffler and Diekema, 2007). The occurrence of nosocomial infections is a worrying factor, once it is directly connected to the onset of resistant infections in most of the cases (Soll et al., 2003; Cantón et al., 2006). The fact that the hospitalized patients undergoing antibiotic therapy had a higher significant prevalence of Candida spp. (p value = 0.017), deserves attention. This fact was verified by other authors; however, this prevalence can depend of the quantity of administrated antibiotics per day, type of drug used (mainly those with broad spectrum), the length of treatment, the Candida specie (C. albicans or non-albicans Candida species), among others (Kremmyer Junior et al., 1998; Singh, 2001; Al Thaqafi et al., 2014).

Commensal microorganisms become pathogenic due to alterations in the host defence mechanisms (immunosuppression), or in the secondary anatomical barriers – such as burns or invasive medical procedures. Alterations in the host defence mechanisms may also result from physiological changes in childhood (prematurity), aging, often associated with degenerative, congenital or acquired immune deficiencies, as well as hospitalizations or immune depression induced by medical action (Bodineau et al., 2009; Lund et al., 2010; Moyes and Naglik, 2011; Savastano et al., 2016). Therefore, the importance of knowing the microorganisms that are part of the microbiota of patients with a differentiated immune condition.

From the patients diagnosed with cancer, 70% (fourteen cases) had Candida spp. This result is higher than those obtained by Schelenz et al. (2011), who found a prevalence of oral yeast colonization in 56.8% (227/400) of cancerpatients. C. albicans was also the predominant (74%) specie in this research, followed by C. glabrata (11.5%), C. tropicalis (2.6%), C. krusei (2.6%) and C. parapsilosis (1.9%). Finally, this and others authors reported a significant increased risk of clinical oral fungal infection during cancer therapy (Lalla et al., 2010; Schelenz et al., 2011).

In conclusion, the present study showed that hospitalized patients have 6.73 more chances to have Candida spp. compared with the non-hospitalized individuals and 3.88 more chances to have Candida albicans. Besides that, the prevalence of Candida spp., is higher in those hospitalized patients under antibiotic therapy (p-value<0.0001). This finding deserves an important attention in the clinical and medical area due to the association of the frequency of the gender Candida spp. in hospitalized patients, since the infection caused by this fungus is associated with a higher severity of clinical response and by the immunosuppression that these individuals have.

References


