Identifying the Different Kinds of Oral Candida Species in Denture Wearing Patients

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Abstract
Oral candidiasis is a common opportunistic infection in denture wearing patients. Presence of dentures in the oral cavity of many patients can promote the Candida colonization and results in higher incidence of oral and systemic candidiasis. This study was undertaken to assess the various species of fungus Candida associated with patients of denture stomatitis (both diabetic and non-diabetic) and also in healthy denture wearing patients.

Keywords: Candida albicans; Denture Stomatitis; Dental Plaque; Antifungal; Oral Yeast

Introduction
The oral cavity has a very complex environment and has a rich variety of Candida species [1]. C. albicans represents the most common causative agent of oral candidiasis; however, other species of Candida, like C. glabrata, C. tropicalis, C. krusei and C. parapsilosis have been isolated with relative frequency from patients with denture stomatitis [2].

Oral candidiasis in the form of Candida-associated denture stomatitis, is a common disease in a large percentage of denture wearers, which means the Candida species has an association with dentures and other oral prosthesis [3]. Yeast cells adhere and colonize oral surfaces including mucosa and acrylic dentures and have the ability to co-aggregate with oral bacteria. The prevalence of Candida species also significantly increases with the degree of inflammation and it plays an important role in progression of denture stomatitis [4,5].

Aims and Objectives
1) To find the most common kind of Candida species associated with denture stomatitis.
2) To assess the Candida species associated with non-diabetic denture stomatitis.
3) To assess the Candida species associated with diabetic denture stomatitis.
4) And also, to assess the Candida species found in healthy denture wearers without denture stomatitis.

Materials and Methods
A total of 60 denture wearing patients between the age of 50 - 69 years were taken. Out of which 20 (10 males and 10 females) patients were known diabetics and had denture stomatitis. Another group of 20 (10 males and 10 females) patients had denture stomatitis without debilitating systemic illnesses and the last group of 20 (13 males and 7 females) denture wearing individuals were having healthy oral mucosa under their dentures. All the 3 groups were selected from the department of Oral medicine and Radiology, V.S. Dental college and...
hospital, Bangalore. None of the above patients were receiving any treatment for stomatitis for last 3 months including steroids, antibiotics, antifungals or antiseptic mouthwashes. Patients with co-morbidities and on immunosuppressive drugs were also excluded. Patients wearing the dentures for a duration of more than 30 years were also excluded from the study. The comparative study was completed in a total of 18 months.

After obtaining the written study consent by the patient a thorough personal, medical and dental history was taken and recorded. Blood sugar levels of each patient at baseline (including random and fasting blood sugar) were recorded.

Each patient is asked to remove their denture before intraoral examination. Careful intraoral examination was done, if denture stomatitis encountered, it was been diagnosed and classified according to Newton.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Type I</td>
<td>A localized simple inflammation or pin point hyperemia</td>
</tr>
<tr>
<td>Type II</td>
<td>An erythematous or generalized simple type is seen as more diffuse erythema involving a part or the entire denture covered mucosa</td>
</tr>
<tr>
<td>Type III</td>
<td>A granular type (inflammatory papillary hyperplasia) commonly involving the central part of the hard palate and the alveolar ridge.</td>
</tr>
</tbody>
</table>

A sample is collected from the mucosal surface of denture using cotton oral swab. Cotton swab is placed in sterile swab tube, 5ml of sterile normal saline is added to the tube and vortexed for 1 minute. Container is sent to laboratory for microbiologic analysis. Immediately after collection of the samples it was transferred to the Department of Microbiology, Kempegowda Institute Of Medical Science Hospital, Bangalore for further evaluation.

Firstly, in a freshly collected sample, a gram stain was done and also inoculated onto Sabouraud’s Dextrose Agar media at 37°C. Cream color smooth, pasty growth was appeared in 48 - 72 hrs. Sometimes growth may be observed within an overnight incubation.

Sabouraud’s agar tube was prepared by dissolving 20 mg glucose, 10 mg peptone, 15 mg agar; and 1 litre of water with steam. After adjusting pH to 5.4 the agar was autoclaved at 115°C for 15 minutes, 20 ml amounts was dispensed into each test tube. Growth was identified by the colony characteristics and the growth of Candida was confirmed by Gram’s staining as gram positive budding yeast like cells and germ tube was put up.

Secondly the sample is plated on CHROMagar and incubated at 37°C for 48 hrs. Colonies will be presumptively identified by colony color. This media contains chromogenic substrates that react with specific enzymes secreted by yeast producing colonies with various pigmentation. These enzymes are species specific, allowing detection of yeast to species level. The chromogenic substance is a- glucosaminidase substrate, which is metabolized to give the colored colonies of different Candida species.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Colour and colonies on CHROM agar medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Light green colour (Apple green colonies)</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>Dark green</td>
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<tr>
<td>C. tropicalis</td>
<td>Blue with purple pigment diffusion</td>
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<tr>
<td>C. glabrata</td>
<td>Dark pink-purple</td>
</tr>
<tr>
<td>C. krusei</td>
<td>Large, fuzzy, rose coloured colonies with white edge</td>
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<tr>
<td>C. parapsilosis</td>
<td>Off white to pale - pink colonies</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>Small pink - purple colonies</td>
</tr>
<tr>
<td>Others</td>
<td>Small, creamy colonies that are white, pale pink, light lavender</td>
</tr>
</tbody>
</table>

Thirdly Candida species were also identified by carbohydrate assimilation test using Candida identification kit. KB006 is a standardized test system that can be used for identification of Candida species. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit. The tests are based on the principle of pH change and substrate utilization on incubation; organisms undergo metabolic changes which are indicated

by a spontaneous color change in the media. KB006 cannot be used directly on clinical specimens. The organisms to be identified were first isolated and purified on a common medium like Sabourauds dextrose agar. The inoculums was prepared by picking 2-4 well isolated colonies and homogenous suspension was made in 2-3 ml sterile saline. The density of the suspension was adjusted to 0.50D at 620 nm. The kit was opened aseptically by peeling off the sealing foil. Then each well was inoculated with 50 μl of above inoculums using surface inoculation method above inoculums was incubated at a temperature of 22.5°C ± 2.5°C for duration of 24 to 48 hours. Results were interpreted as per the standards given in the identification index.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Urase</th>
<th>Melobose</th>
<th>Lactose</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>Galactose</th>
<th>Cellobiose</th>
<th>Inositol</th>
<th>Xylose</th>
<th>Dulcitol</th>
<th>Raffinose</th>
<th>Trehalose</th>
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<td>C. albicans</td>
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<td>C. catenulata</td>
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<td>C. dubliniensis</td>
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<td>C. famata</td>
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<td>C. glabrata</td>
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<td>C. kefir</td>
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<td>C. krusei</td>
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<td>C. lambica</td>
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<td>C. lipolytica</td>
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<td>C. lusitaniae</td>
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<td>C. parapsilosis</td>
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<td>C. pintolopesi</td>
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<td>C. rugosa</td>
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<td>C. tropicalis</td>
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<td>C. zeylanoides</td>
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<td>C. pseudotropicalis</td>
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<td>C. stellatoidea</td>
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**Interpretation of results**

Results were interpreted as per the standards given in the identification index.

The data was then analyzed by turkey post hoc test, kruskal wallis test followed by duns multiple comparisons test, fisher exact test and x analysis.

**Results**

Among a total of 60 patients comprising study groups of 20 patients in group I (diabetic), 20 patients in group II (non-diabetic) and patients in group III (healthy denture wearers), a sum of 20 patients were negative for *Candida* organism. Out of 40 positive cases, 30 (50%) cases had *C. albicans*, 4 (6.6%) cases had *C. dubliniensis*, 2 (3.3%) cases had *C. tropicalis* and 3 (5%) cases had *C. glabrata* species and 1 (1.6%) cases had *C. parapsilosis*.

*Candida* species distribution in 20 group I diabetic patients: 4 types of *Candida* species were identified. *C. albicans* was seen in 12 (60%) patients, *C. dubliniensis* was seen in 1 (5%) patient, *C. tropicalis* was seen in 1 (5%) patient and *C. parapsilosis* was seen in 1 (5%) patient.

*Candida* species distribution in 20 group II non-diabetic patients: *C. albicans* was seen in 11 (55%) patients, *C. tropicalis* was seen in 1 (5%) patient, and *C. glabrata* was seen in 2 (10%) patients. Prevalence of *C. glabrata* was higher in group II following *C. albicans*.
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*Candida* species distribution in 20 group III healthy denture wearers: *C. albicans* was seen in 7 (35%) patients, *C. dubliniensis* was seen in 3 (15%), and *C. glabrata* was seen in 1 (5%) patient. Prevalence of *C. dubliniensis* was higher in control group next to *C. albicans*.

*Graph 1:* Candida species identified in group I patients.

*Graph 2:* Candida species identified in group II patients.

*Graph 3:* Candida species identified in group III patients.

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Discussion

The presence of a denture in the oral cavity, associated with the local alterations of the oral mucosa and the systemic complications, may render the denture wearer patient with diabetes even more prone to candidal infection [2,5,7,8]. A significantly higher incidence of Candida infection and increased levels of Candida spp. were found in diabetic patients wearing removable dentures. The presence of a removable denture may decrease the salivary pH and saliva flow rate and impede the mechanical cleaning of the soft tissue surfaces by the tongue [3,5,7]. In addition, denture induced trauma may reduce tissue resistance against infection because of the increase in permeability of the epithelium to soluble candidal antigens and toxins [3]. Moreover, the tissue surface of the acrylic resin denture acts as a reservoir that harbors microorganisms, enhancing their infective potential and aggravating a previously existing condition. For this reason, both systemic and local predisposing factors might promote an increase in the number of microorganisms and therefore the risk of oral candidiasis in diabetics, especially in those patients wearing removable dentures [5,7].

The relationship between dentures and oral candidiasis has been extensively studied in the literature [1-6], particularly because diabetic patients are more susceptible to fungal infections than those without diabetes [1-3].

Yeast adhesion to epithelial cell surfaces is recognized as an essential first step in the process of candidal colonization and subsequent infection. Salivary glucose levels in diabetic patients favors yeast growth owing to increased numbers of available receptors for Candida. Consequently, buccal cells from diabetic patients have shown an increased adherence of C. albicans compared with buccal cells from non-diabetics [7].

The presence of a denture in the oral cavity, associated with the local alterations of the oral mucosa and the systemic complications, may render the denture wearer patient with diabetes even more prone to candidal infection [2,5,7,8]. A significantly higher incidence of Candida infection and increased levels of Candida spp. were found in diabetic patients wearing removable dentures. The presence of a removable denture may decrease the salivary pH and saliva flow rate and impede the mechanical cleaning of the soft tissue surfaces by the tongue [3,5,7]. In addition, denture induced trauma may reduce tissue resistance against infection because of the increase in permeability of the epithelium to soluble candidal antigens and toxins [3]. Moreover, the tissue surface of the acrylic resin denture acts as a reservoir that harbors microorganisms, enhancing their infective potential and aggravating a previously existing condition. For this reason, both systemic and local predisposing factors might promote an increase in the number of microorganisms and therefore the risk of oral candidiasis in diabetics, especially in those patients wearing removable dentures [5,7].

Different Candida species have been frequently isolated from the oral cavities of patients with diabetes. Candida albicans is the most commonly recovered species in diabetic patients, with a prevalence of up to 80%. This oral fungal pathogen is the most virulent of the Candida species and is able to grow as biofilm, which consists of a complex community of cells embedded in a matrix of extracellular polysaccharide [1-3,9,11].

The epidemiology of Candida infections has changed with emergence of non albicans species which have been increasingly described in both compromised and non-compromised hosts. Non-albicans species have been consistently observed in diabetes patients and in those with denture stomatitis [1,3,5,6,8,9].

Although C. albicans is still the most frequently isolated species from patients with Candida infections [1,2,5,7,13-17], the growing prevalence of non-albicans species is clearly a concern. In the present study, C. glabrata was isolated from 5% and 15% in healthy and denture stomatitis patients respectively. C. tropicalis was isolated from 5% in denture stomatitis patients.

Vanden Abbeele., et al. also observed that C. albicans was the commonest yeast found on patients’ dentures, followed by C. glabrata and C. tropicalis. In another study, C. albicans, C. glabrata and C. tropicalis represented 80% of isolates from clinical infections. In terms of frequency distribution, some studies have shown that C. tropicalis was the second most prevalent species identified [2,5,14,15]. However, contrasting results have been found in other studies, in which C. glabrata was the most common yeast after C. albicans [7,13,14,17].

Suárez B., et al. observed in his study that C. albicans were the largest isolated species followed by C. parapsilosis in uncontrolled diabetic patients [18].

In the present study, C. albicans (50%) was the most prevalent species identified and second most prevalent was C. dubliniensis species (6.6%), followed by C. glabrata (5%), C. tropicalis (3.3%) and C. parapsilosis (1.6%).

The differences in findings among studies are likely to be related to a combination of factors, such as sample techniques and culture media used. Conventional sampling techniques used in various studies include oral rinses [3,8,13,17]. Although this sampling technique provides adequate qualitative information, it can be argued whether adherent biofilm cells or loosely adherent cells residing at the peripheries of the biofilm are removed during this procedure. To overcome this limitation, swabbing of the denture fitting surfaces was used for sampling in the present investigation [5,13,15,16].

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Because Chromagar *Candida* is generally more sensitive than other media in detecting mixed populations of yeasts, this improved culture medium was used in the present study [1,5,8,13,15,16] and provided differential staining and highlighted colonial morphologic variations between *Candida* species, thus increasing the sensitivity of detection of yeasts.

A review of the literature published to date, on the relationship between DM and oral candidal infection reveals a continuing debate. Studies on candidal colonization are often contradictory, which may be a result of the variety of sampling techniques employed [1,4,10,18]. The present study comprehensively investigated microbiologically oral yeast colonization and evaluated the effect of diabetes and denture stomatitis, which could potentially influence the candidal carriage rate and species variation.

Conclusions

Based on the observation of present study to compare the Candida species in diabetics with denture stomatitis, non-diabetics with denture stomatitis and healthy denture wearing individuals the following conclusions can be drawn:

1) *Candida* species were most prevalent in denture stomatitis patients than in patients with healthy oral mucosa below dentures, but among the two stomatitis groups the prevalence was equal.

2) Most commonly identified *Candida* species was *C. albicans* among all the groups.

3) In group I, i.e. diabetics with denture stomatitis, most common species identified was *C. albicans*, followed by *C. dubliniensis*, *C. tropicalis* and *C. parapsilosis*.

4) In group II, i.e. nondiabetics with denture stomatitis, most common species was *C. albicans*, followed by *C. glabrata*, *C. tropicalis*.

5) In group III, i.e. denture wearing individuals without diabetes and healthy mucosa, most common species was *C. albicans* followed by *C. dubliniensis* and *C. glabrata*.

6) The prevalence of *C. glabrata* is higher in denture stomatitis i.e. group II, indicates higher prevalence with degree of inflammation.

7) The isolation of four different types of species in group I suggests that the greater diversity of *Candida* species was present in diabetic patients.

Thus, it can be inferred that both albican and non-albican species of *Candida* can be identified in denture stomatitis with or without diabetes. But more diversity of *Candida* species were observed in patients with diabetes and denture stomatitis which indicates there is influence of diabetes on *Candida* species variation. Even in healthy denture wearing individuals also showed presence of non albican *Candida*. Most common species was *C. albicans* as supported by various studies. Prevalence of *C. glabrata* was higher in the presence of inflammation. Presence of *C. parapsilosis* was seen in patients with diabetes mellitus.

Species variation was not that significant but still different species were identified among groups, which indicates the influence of diabetes and denture stomatitis on species of *Candida* occurrence. The knowledge of prevalence species distribution, rapid species identification, antifungal susceptibility testing and the development of new antifungal drugs are mandatory to achieve a decrease in *Candida* infections and an increase in quality of life of denture-wearing individuals with and without diabetes mellitus. Our study included small group of 60 patients, so further studies are required to identify more species associated with diabetes and denture stomatitis so that better treatment plan can be provided.

Bibliography


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