Identification and Antifungal Sensitivity Pattern of Candida Species Isolated from Various Oro-dental Conditions.

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Received: June 2016
Accepted: June 2016

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ABSTRACT

Background: Oral candidiasis is the most common oral opportunistic infection seen in immunocompromised patients. Apart from C. albicans the non albicans Candida species, which are less susceptible to the commonly used antifungal drugs are major etiological agent for candidiasis. Thus, in recent years there has been an increased interest in spectrum of infections caused by Candida species. However with the recognition, that Candida spp. differ in the production of virulence factor and sensitivity to antifungal agents, greater emphasis has been placed on identification of isolates up to species level. In the past identification of various species of Candida other than C. albicans has not been attempted in oral lesions. Methods: A total of 158 swabs were collected from oral cavity of patients having lesions suggestive of candidiasis. One swab was subjected for direct microscopy using Gram staining. The second swab was inoculated on two tubes of Sabouraud Dextrose agar (SDA) with antibiotics (Hi-Media). Results: Candida albicans though was the commonest species isolated. NAC is also emerging as important opportunistic pathogens in oro-dental infections. Conclusion: In view of the changing pattern, it is strongly recommended that species identification and sensitivity test can help in much better treatment strategies, and thus, gain a good control over the disease. Keywords: Candida albicans, Non albicans Candida, oro-dental infections.

INTRODUCTION

Candidiasis is the commonest fungal disease found in humans affecting mucosa, skin, nails and internal organs. Oral candidiasis is the most common form of disease, produced by colonization of Candida species also known as oral thrush. First known description of candida infection as oral thrush has been found in Hippocrates “epidemics” from 4th century B.C.¹ Candida infection may be acute or chronic, superficial or deep and its clinical presentation is wide.²,³ Candida species are opportunistic pathogens that can cause disease in host who are compromised by underlying local or systemic pathological process.⁴ It is reported that it affects very young, very old and very sick.⁵

MATERIALS AND METHODS

The present study was conducted in department of Microbiology Subharti Medical college in collaboration with department of Oral Medicine and Radiology Subharti Dental college over a period of 12 months. A total of 158 swabs were collected from oral cavity of patients attending the outpatient clinics and having lesions suggestive of
oral candidiasis. The patient presented with different oro-dental conditions such as denture wearers, smokers, immunocompromised patient, patients of gingivitis and periodontitis. Two swabs were collected from the oral lesions of each patient, transported and processed for isolation and identification of Candida species. One swab was subjected for direct microscopy using Gram staining. The second swab was inoculated on two tubes of Sabouraud Dextrose agar (SDA) with antibiotics (Hi-Media). The inoculated tubes were incubated at 25°C and 37°C. Any growth appearing on the tubes were identified using standard mycological technique such as colony morphology, Gram staining, germ tube test (GTT) to differentiate C. albicans from NAC species, morphological identification on corn meal agar, colour differentiation on CHROM agar and sugar fermentation tests. Antifungal sensitivity testing was done by disc diffusion method against amphotericin B, clotrimazole, fluconazole, itraconazole, voriconazole and ketoconazole (Hi-Media). The inoculated tubes were subjected for direct microscopy using Gram staining. The second swab was inoculated on two tubes of Sabouraud Dextrose agar (SDA) with antibiotics (Hi-Media). Culture was considered as negative and tubes were discarded when there was no growth for maximum of 3 weeks.

RESULTS
A total of 92/158 (58.2%) swabs yielded Candida species, out of which 66/92 (71.73%) isolates were identified as Candida albicans and 26/92 (28.26%) were identified as NAC spp. [Table 1] Among the 26 NAC species, Candida tropicalis (10) was the predominant Candida spp. isolated followed by Candida parapsilosis (8), Candida glabrata (6) and Candida dublinensis (2) [Table 2].

Table 1: Distribution of Candida albicans and Non albicans Candida (NAC) species in clinical isolates. (n=46)

<table>
<thead>
<tr>
<th>Name</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>66</td>
<td>71.73%</td>
</tr>
<tr>
<td>Non albicans Candida (NAC)</td>
<td>26</td>
<td>28.26%</td>
</tr>
</tbody>
</table>

Table 2: Distribution of various Non albicans Candida species isolated (n=26)

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Number</th>
<th>Percentage among NAC’s (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tropicalis</td>
<td>10</td>
<td>38.46%</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>08</td>
<td>30.76%</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>06</td>
<td>23.07%</td>
</tr>
<tr>
<td>C. dublinensis</td>
<td>02</td>
<td>7.69%</td>
</tr>
</tbody>
</table>

A total of 80% cases of Candida species was isolated from immunocompromised individuals in which the predominant species was Candida glabrata [4] followed by Candida albicans and Candida dublinensis (2 each). Candida albicans, Candida tropicalis and Candida parapsilosis were the predominant species isolated from 69% cases of gingivitis and periodontitis. Candida albicans and Candida tropicalis were isolated from 60% cases of denture wearers. However, C. albicans was the common species seen in patients with habit of smoking (26%) [Table 3]. On antifungal sensitivity testing it was observed that Candida albicans isolated was 100% sensitive to amphotericin-B and voriconazole but showed high level of resistance to clotrimazole (30%) and ketoconazole (42%). However, the NAC spp. has shown high level of resistance to fluconazole (61%) and ketoconazole (69%). Candida glabrata and Candida dublinensis, isolated from case of immunocompromised patient showed resistance to all azoles and was sensitive only to amphotericin-B.

Table 3: Prevalence of Candida species in different oro-dental conditions.

<table>
<thead>
<tr>
<th>S. no</th>
<th>Condition</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunocompromised status(n=10)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>2</td>
<td>Patients with Gingivitis and Periodontitis(n=52)</td>
<td>36(69%)</td>
</tr>
<tr>
<td>3</td>
<td>Denture wearers(n=30)</td>
<td>18(60%)</td>
</tr>
<tr>
<td>4</td>
<td>Smokers(n=38)</td>
<td>10(26%)</td>
</tr>
</tbody>
</table>

DISCUSSION
Candida spp. lives commensally in the human oral cavity with high carriage rate (>70%); responsible for candidiasis, the most common opportunistic infection seen in the immunocompromised patients. Although, C. albicans is the predominant etiologic agent of candidiasis, numerous records indicated that epidemiology of candidiasis has changed with increased incidence of non-Candida albicans Candida species among immunocompromised patients.

In the present study, the prevalence of oro dental candidiasis was 58.2%. This is slightly higher to that reported by Hamester et al[10] where they reported yeast of genus Candida in 52% of their samples. This may be because our study included symptomatic patients with some predisposing factors responsible for oro- dental lesions.

In our study Candida albicans (72%) was the predominant species of candida isolated from patients with oro-dental lesions compared to NAC species (28%) . This is in accordance with the findings of Batool et al[8] who reported 75% prevalence of Candida albicans and 25% prevalence of NAC species.

Among NAC species Candida tropicalis was the predominant species with prevalence of 11%. Similar findings have been reported by Hamester et al[10] and Shaheen et al[11] who reported prevalence of 12% for Candida tropicalis. In the present study the second predominant NAC species isolated was Candida parapsilosis (8%) followed by Candida
The positivity rate of Candidiasis was 60% among complete denture wearers with predominance of Candida albicans as the predominant species isolated. Similar finding has been reported by Hamester et al. They reported 35% prevalence of Candida albicans among patients with periodontitis and gingivitis. Among immunocompromised patient incidence of Candidiasis was found in 80% patients in the present study which is similar to that reported by Badarkar et al, who reported 83.3% incidence rate among immunocompromised individuals. The antifungal sensitivity pattern of Candida species isolated from samples was studied. The susceptibility pattern of these isolates revealed all isolates of Candida albicans were sensitive to amphotericin-B. However, Candida albicans has shown high level of resistance to clotrimazole (30%) and ketoconazole (42%) while NAC species has shown high level of resistance to fluconazole (61%) and ketoconazole (69%). Resistance pattern obtained was similar to that reported by Berry et al. Peter G Pappas et al also reported high level of resistance to fluconazole in NAC species.

**CONCLUSION**

*Candida albicans* though was the commonest species isolated. NAC is also emerging as an important opportunistic pathogens in oro-dental infections. NAC species show increase in the resistance to various commonly used antifungal drugs posing a therapeutic problem which is a matter of concern. In view of this changing pattern, it is strongly recommended that species identification and sensitivity test can help in much better treatment strategies, and thus, gain a good control over the disease. Moreover in future similar studies on a larger group of patients and for a longer duration needs to be carried out to exactly know the prevalence of different Candida species in oro-dental conditions.

**REFERENCES**

5. Burkett’s textbook of oral medicine.


**Source of Support:** Nil, **Conflict of Interest:** None declared