Speciation of Candida by Hicrome agar and Sugar assimilation test in both HIV infected and non infected patients.

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ABSTRACT

Background: Candida spp. are the fourth most common pathogens isolated from blood cultures and the numbers of non-albicans Candida (NAC) with decreased susceptibility to antifungal agents are also increasing. Rapid identification of yeast isolates to the spp. level is essential in order to optimize the antifungal treatment. Aim: To speciate the isolates obtained from both HIVinfected and non-infected patients using chrom agar and to carry out the antifungal susceptibility by disk diffusion method and also to detect the common candidal species in HIVinfected patients in our hospital. Materials and Methods: A total of 150 samples were collected from both HIV infected and non infected patients and were subjected to culture on Chromagar, germ tube test, sugar assimilation test and their antifungal susceptibility for fluconazole and voriconazole. Results: A total of 136 isolates were obtained from 150 samples. Of which 57 were from HIV infected and 79 from patients with suspected candidiasis. C.albicans was the most common isolates in both the study groups. Among non-albicans candida in HIV infected patients, C.glabrata was the next common isolate seen whereas in the non inflected patients it was C.tropicalis. They all showed increased resistance to fluconazole but were sensitive to voriconazole except for one glabrata isolate. C.albicans in both (HIVinfected and non-infected) the groups showed 98% and 96% sensitivity to voriconazole respectively but its resistance pattern to fluconazol was noted more in case of HIV Infected patients.

1. Introduction

Infections due to Candida spp are increasing in the recent few decades. The most probable reason being the increasing incidence of HIV infection worldwide and other immunocompromised conditions like use of steroids and broad spectrum antibiotics, drug abuse and organ transplantation. Although the isolation of non-albicans candida is increasing in the recent years, Candida albicans is the most common cause of candidiasis accounting for about 60%-80% of infections[1]. However, recent data shows a shift in the distribution of yeast species. More than 30% of nosocomial infections are due to non-albicans species such as C.glabrata, C.tropicalis and C.parapsilosis [2].There has also been an increase in the incidence of candidaemia over the last two decades[3]. Growing population of immunocompromised patients and advances in medical and surgical managements have contributed to the increased incidence of candidaemia which is also associated with high mortality [4,5]. The mortality attributed to candidaemia ranged from 5% to 71% in various reviewed studies[6]. Isolation and prompt identification of the infecting organism to the species level is essential to optimize the early antifungal therapy.

2. Materials and Methods

A total of 150 clinical samples were collected from patients attending the counseling center for HIV /AIDS patients and non-HIV/AIDS patients attending dermatology department of Bapuji hospital, Davangere from Nov-2011 to Feb-2012. Samples were collected with all aseptic precautions using sterile swabs (in house made). The swabs were dispensed in a test tube containing 5 ml of sterile saline. The samples were inoculated on HiCrome Candida differential agar obtained from Hi-Media, Mumbai. From the same swab a smear was made and gram stained. Germ tube test was done using the same broth to identify the albicans group. Those tested positive were inoculated on Corn-Meal agar for chlamydospore production. The remaining broth was kept at room

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2.1. Sugar Assimilation Test

The saline containing the clinical sample was incubated at room temperature for about 24 hrs to exhaust the carbohydrate reserves so that the sugar supplemented will be utilized properly and this rules out false negative results. Yeast extract agar (Hi-Media, Mumbai) was prepared following the manufacturer’s instructions. A lawn culture of the preincubated broth was made on the Yeast Extract agar plate and the sugar disks- Glucose, Maltose, Sucrose, Lactose, Melibiose, Xylose, Trehalose, Raffinose, Cellobiose, were put and incubated for 24-72 hrs. Most of the isolates showed increased growth around the sugars they used except for few which required incubation for up to a week. The results were noted and tabulated. For sugar assimilation test, the sugar disks were obtained from Hi-Media, Mumbai.

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The antifungal susceptibility testing was done on Mueller Hinton Aar supplemented with 2% glucose and 0.5 μg/ml methylene blue (as per CLSI guidelines M44-A document) using the colonies directly from the Chromagar plates. Drug disks tested were- Voriconazole() and Fluconazole(), obtained from Hi-Media, Mumbai.

To identify the albicans group, germ tube test and cornmeal agar were carried out apart from their colony appearance on Chrom agar and sugar assimilation test. Whereas to identify the non-albicans group, their colony appearance on Chrom agar and Sugar assimilation test was considered.

3. Results:

Of the 150 non replicate clinical samples collected, 136 yielded growth. Among 136, 57 were from HIV patients attending the counseling center and 79 were from non-HIV patients attending to Dermatology department for various diseases. The details are shown in table-1.

Table-1 showing the source of samples.

<table>
<thead>
<tr>
<th>HIV</th>
<th>After Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-HIV-Intertrigo</td>
<td>57</td>
</tr>
<tr>
<td>- Onychomycosis</td>
<td>62</td>
</tr>
<tr>
<td>- Vulvovaginitis</td>
<td>8</td>
</tr>
<tr>
<td>- Angular Cheilitis</td>
<td>5</td>
</tr>
<tr>
<td>- Balanoposthitis</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
</tr>
</tbody>
</table>

Table-2 Of the 71 strains of C.albicans 68 produced bluish green colonies and 3 produced light green colonies. All the C.tropicalis showed metallic blue colored colonies whereas C.glabrata produced pink to lilac colour and C.parapsilosis produced colourless to pinkish white colonies. All four C.krusei also produced pink colonies but they were pale, flat and spreading with the characteristic fringe. C.gullermondi and C.kefyr produced purplish colonies. All four of C.dubliniensis showed dark green colonies. The interpretation was found to be difficult in case of differentiating between C.glabrata - C.parapsilosis and C.Gullermodi - C.kefyr. But the final identification was done with the help of assimilation test. Refer table-3 for the details of assimilation test.

Photos: 1-4

C.krusei (pink) and C.dubliniensis(dark green)

C.glabrata(lilac) and C.gullermondi/kefyr
Voriconazole-S (≥17MM) , Fluconazole-R (≤13mm)

C.albicans(light green) and C.tropicalis(metallic blue)

Table-2 showing the different species from both HIV and non HIV patients

<table>
<thead>
<tr>
<th>Species isolated</th>
<th>Total</th>
<th>HIV</th>
<th>Non-HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.albicans</td>
<td>71(52%)</td>
<td>32(45%)</td>
<td>39(55%)</td>
</tr>
<tr>
<td>C.tropicalis</td>
<td>26(19%)</td>
<td>7(27%)</td>
<td>19(73%)</td>
</tr>
<tr>
<td>C.glabrata</td>
<td>17(13%)</td>
<td>14(82%)</td>
<td>3(18%)</td>
</tr>
<tr>
<td>C.parapsilosis</td>
<td>11(8%)</td>
<td>4(100%)</td>
<td>0</td>
</tr>
<tr>
<td>C.dubliniensis</td>
<td>4(3%)</td>
<td>0</td>
<td>11(100%)</td>
</tr>
<tr>
<td>C.krusei</td>
<td>4(3%)</td>
<td>0</td>
<td>4(100%)</td>
</tr>
<tr>
<td>C.kefyr</td>
<td>2(1.5%)</td>
<td>0</td>
<td>2(100%)</td>
</tr>
<tr>
<td>C.guillermondi</td>
<td>1(0.7%)</td>
<td>0</td>
<td>1(100%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>136</td>
<td>57</td>
<td>79</td>
</tr>
</tbody>
</table>

Table-3 showing the results of sugar assimilation test

<table>
<thead>
<tr>
<th>Species</th>
<th>Glu</th>
<th>Malt</th>
<th>Sucr</th>
<th>Lac</th>
<th>Melib</th>
<th>Raffi</th>
<th>Cellob</th>
<th>Tre</th>
<th>Xy</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.albicans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.tropicalis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.glabrata</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C.parapsilosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.dubliniensis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.krusei</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C.kefyr</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.guillermondi</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Glu-Glucose, Malt-Maltose, Sucr-Sucrose, Lac-Lactose, Melib-Melibiose, Raffi-Raffinose, Cellob-Cellobiose, Tre-Trehalose, Xy-Xylose.
4.Discussion

Chromagar Candida is a differential medium being widely used to differentiate candida species. Non-albicans candida are being on the rise due to increasing immunocompromised conditions. Currently two chromogenic media are widely used in the clinical laboratories for the presumptive detection and identification of candida species. These are –Candida ID agar (bioMerieux) [7,8] and Chromagar [7,9,10,11]. These chromogenic media yield colonies of different colours secondary to chromogenic substances that react with enzymes secreted by the organisms [12,13].

Ideally, laboratories should be able to simultaneously detect and identify C. albicans and the major Canadia species other than C. albicans in clinical specimens. A recently developed differential agar medium, Chromagar Candida, appears to meet these criteria. It facilitates the detection and identification of yeasts from mixed cultures and can provide results 24 to 48 h sooner than standard isolation and identification procedures [9,14,15]. Although chromagar appears to be quite accurate in identifying the most common Candida species, it is not proposed as a substitute for standard identification protocols [9]. A major advantage of chromagar is the ability to detect mixed cultures of yeasts in clinical specimens. In our present study, out of 150 samples isolated, 14 showed no growth and 16(11%) of the samples showed either two types or three types of mixed isolates. Similar finding was noticed by Among these Pfaller et al [10] where in their study the mixed population was about 18%. Yamne and Saith [16] as well as Samaranayake [17] also found in their study the association of different yeast species to be ranging from 8-15.3%. Mixed population of isolates was more frequent in samples from HIV and from intertrigo. Calbacins was the most common isolate from both HIV and non-HIV infected patients. In HIV infected patients this was followed by Cglabrata and tropicalis. Although C. glabrata and C. parapsilosis could have been misidentified, it was observed that C. glabrata colonies were shiny and had a darker pink to lilac whereas C. parapsilosis produced pinkish white colonies. This observation was also reported by Odds & Bernarets [18]. Thus with the Chromagar, the speciation of candida was done with ease and within 48 hrs.

In treating the infections due to Candida species the clinicians are usually most interested in quickly knowing the species and the susceptibility pattern of the isolates. We tested for fluconazole (25μg) and voriconazole (1μg) as per the CLSI M-44 document guidelines [19].

Azoles are safe and effective agents for the treatment of oropharyngeal candidiasis and have gradually replaced amphotericin B. However, resistance to azoles is now becoming common. Several reports suggest that susceptibility rates of Candida spp. to triazole antifungal amongst cancer patients have remained high with fluconazole resistance restricted to Ckrusei [20, 21] and Cglabrata [20-22]. Other investigators have notified that Calbicins isolates from HIV positive and cancer patients are resistant to fluconazole and itraconazole [23, 24]. In our study we tested only for two drugs available as disks in Hi-Media, Mumbai. Our results indicate that both Calbicins and non-albicains candida showed increasing resistance to fluconazole(≤13mm) among HIV patients compared to non-HIV patients as noted by Germania et al and Kennedy et al in their study. Susceptibility of Calbicans to voriconazole(≥17mm) was 98% in HIV infected whereas in non-infected is was 96%. Non-albicains candida all showed 100% sensitivity to voriconazole in both the study groups.

5.Conclusion

Chrom agar when used to speciate can give excellent results within short time. Presumptive identification becomes easier especially in case of non-albicains candida. Hence Chromagar can be routinely used instead of Sabourad’s Dextrose agar. If this is corroborated with additional tests like germ tube and sugar assimilation test, identification upto species level can be made appropriately. Thus their optimum identification and isolation will help the clinicians to know the pathogen and their susceptibility pattern will help them to institute proper drugs thereby avoiding any treatment failures.

Acknowledgement

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6. Reference


