Detection of *Candida* Species by Hichrom Agar and Their Antimycotic Sensitivity in Hadoti Region

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**Abstract**

**Introduction:** Hichrom agar is a differential culture medium which facilitates the isolation and identification of some clinically important species of *Candida*.

**Materials and Methods:** A total of 100 *Candida* species were isolated from various mucocutaneous clinical specimens including oral thrush, vaginitis, balanitis, and angular cheilitis. Speciation of *Candida* was done using Hichrom agar and conventional methods simultaneously. Antifungal susceptibility testing was done by the disc diffusion method to amphotericin B, fluconazole, nystatin, itraconazole, ketoconazole, and clotrimazole.

**Results:** *Candida albicans* (54%) was the predominant species isolated. Non-albicans *Candida* spp. isolated was *Candida tropicalis* (22%), *Candida glabrata* (12%), *Candida krusei* (06 %), *Candida parapsilosis* (04%), and *Candida kefyr* (02%). Antifungal susceptibility testing was done using antimiyoic sensitivity testing by disc diffusion method. Overall antifungal drug resistance for *Candida* in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B.

**Conclusion:** The advantage of using Hichrom agar is that it helps in the isolation and identification of *Candida* to species level. The performance of Hichrom agar paralleled that of conventional methods. Use of this medium is rapid, technically simple, and cost-effective compared to time-consuming technically demanding expensive conventional method. Hichrom agar serves as a primary isolation and differentiation medium for clinical specimens that could allow mycology laboratories to rapidly identify *Candida* spp., enabling clinicians to choose appropriate antifungal agents, thus decreasing patient’s morbidity and mortality.

**Key words:** Antifungal susceptibility testing, *Candida*, Hichrom agar, Fluconazole

**INTRODUCTION**

*Candida* is the most common fungal infection found in the humans affecting mucosa, skin, nails, and internal organs. *Candida* species colonize the mucosal surfaces of all humans soon after birth, and the risk of endogenous infection is ever-present.¹ *Candida* species are a component of the normal flora of human beings and commonly found on the skin, gastrointestinal tract, and female genital tract, particularly higher in the vagina during pregnancy.¹ Carriage rate of *Candida* species tends to increase with age. *Candida* species are the fifth most common cause of blood stream infections and fourth common cause of nosocomial infections.¹⁻² *Candida* species produces various cutaneous, mucocutaneous, and systemic manifestations depending on the immune status of the host and underlying predisposing factors. In developed countries, *Candida albicans* accounts for 40-60% of yeasts isolates, whereas Indian reports show an increased predominance of non-*C. albicans* (NAC) isolates.³⁻⁴ Increase in the prevalence of non-albicans species such as *Candida glabrata* and *Candida krusei* has been noted during the past decade because of the extensive use of antimiyoic drugs particularly azoles for prolonged periods. *C. glabrata* is associated with severe complications than other species.³
Several brands of chromogenic media have been developed to produce rapid yeast identification. C. albicans produces an enzyme b-N-acetyl-galactosaminidase and incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of C. albicans isolates directly on primary isolation. Hichrom Candida differential agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of Candida species namely C. albicans, C. krusei, Candida Tropicalis, and C. glabrata on the basis of coloration and colony morphology.

It is necessary to identify Candida to species level as many NAC have decreased susceptibility to antifungal agents. The present study was undertaken to evaluate the advantages of Hichrom agar over conventional method for speciation of Candida isolates and their susceptibility to antifungal agents by disc diffusion method.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Government Medical College and M.B.S Hospital, Kota, from June 2012 to September 2013. A total of 100 strains of Candida were isolated from various mucocutaneous clinical samples of patients admitted in MBS Hospital and associated Group of Hospitals - JK Lone Hospital and NMC Hospital, Kota, Rajasthan.

Hichrom agar was prepared as per the instruction manual (Himedia India) (Table 3). Candida spp. isolated was inoculated simultaneously to Hichrom agar plates and Sabouraud dextrose agar (SDA) tubes. These were incubated at 37°C for 48 h. Species were identified on Hichrom agar by morphology and color of the colony. Growth on SDA was specified by standard methods using germ tube, corn meal agar, sugar fermentation, and assimilation test. Appearances of Candida spp. on Hichrom agar were as follows:8

- C. albicans - blue green
- C. tropicalis - dark blue-gray center with pink halo
- C. krusei - pink large rough spreading colonies with pale edge
- C. parapsilisis - pale cream colored colonies
- C. glabrata - cream to white smooth colonies

Antifungal susceptibility was performed by disc diffusion method using antymycotic sensitivity test agar. Discs used were amphotericin B (100 units), fluconazole (10 mcg), clotrimazole (10 mcg), nystatin (100 mcg), itraconazole (10 mcg), ketoconazole (10 mcg), and sensitivity zones were measured as for the instruction manual (Himedia).8

RESULTS

Table 1 shows Candida spp. isolated in various clinical samples. All isolates of candida grew on Hichrom agar after 48 h of incubation at 37°C.

Overall antifungal drug resistance for Candida in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B. The results of this study have been presented in Tables 1 and 2.

DISCUSSION

C. albicans (54%) was the most prevalent species of Candida reported in the present study (Table 1). This finding was consistent with the findings of other workers who reported that the incidence of C. albicans was 61.3% (Biradar et al.), 49.3% (Feglo and Narkwa),11 and 47% (Dominic and Dharwad). However, Kashid et al. found that C. tropicalis was the most prevalent species accounted for 46.2% followed by C. albicans (29.2%). C. tropicalis (22%) was the second most common species reported in the present study. This finding was comparable with other workers, Bobade et al. (22.9%), Babin et al. (26.4%), and Khan and Baqai et al. (21%). However, C. glabrata was reported as second most common species by Feglo and Narkwa et al. (17.9%) and Saldhei et al. (11.9%).

Overall antifungal drug resistance for Candida in the present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole, and 10% for nystatin. No resistance was observed for amphotericin B. In present study, 22.2% of C. albicans was found to be fluconazole resistant which is in consonance with Kashid et al. and Babin et al. However, a higher resistance was observed by Saldhei et al. (81.1%). For NAC, the resistance varies from 0% in Candida kefyr to 100% in C. krusei. For AmB, no resistance was observed in the present study. This was in consonance with Kashid et al. Bobade et al. reported 7.5% resistance in C. albicans.

The conventional methods and the CHROM agar method were compared and were found to give similar results. This was similar to the findings of Nayak et al., who found that CHROM agar showed 100% specificity and 100% sensitivity when compared to SDA and conventional methods. The advantages of Hichrom agar are easy to prepare, i.e., boiling, facilitate the rapid isolation, and identification of yeast species. Hichrom agar facilitates identification between yeast spp. from specimens containing mixture of yeast spp. and do not affect the
Table 1: Candida species isolated from different clinical conditions

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>C. albicans (%)</th>
<th>C. tropicalis (%)</th>
<th>C. glabrata (%)</th>
<th>C. krusei (%)</th>
<th>C. parapsilosis (%)</th>
<th>C. kefyr (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral thrush</td>
<td>23 (42.5)</td>
<td>17 (31.4)</td>
<td>6 (11.1)</td>
<td>6 (11.1)</td>
<td>0</td>
<td>2 (3.7)</td>
<td>54 (54)</td>
</tr>
<tr>
<td>Vaginitis</td>
<td>26 (68.4)</td>
<td>3 (7.8)</td>
<td>6 (15.7)</td>
<td>0</td>
<td>3 (7.8)</td>
<td>0</td>
<td>38 (38)</td>
</tr>
<tr>
<td>Balanitis</td>
<td>4 (80)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (20)</td>
<td>0</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Angular cheilitis</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (3)</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>54 (54)</td>
<td>22 (22)</td>
<td>12 (12)</td>
<td>6 (6)</td>
<td>4 (4)</td>
<td>2 (2)</td>
<td>100</td>
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</tbody>
</table>


Table 2: Antifungal sensitivity profile of Candida isolates (in percentage)

<table>
<thead>
<tr>
<th>Antifungal drugs</th>
<th>Fluconazole (25 µg)</th>
<th>Itraconazole (10 µg)</th>
<th>Clotrimazole (10 µg)</th>
<th>Ketoconazole (10 µg)</th>
<th>Nystatin (100 U/disc)</th>
<th>AmB (100 U)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>C. albicans</td>
<td>48.1</td>
<td>29.6</td>
<td>22.2</td>
<td>29.6</td>
<td>44.4</td>
<td>25.9</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>54.5</td>
<td>31.8</td>
<td>13.6</td>
<td>54.5</td>
<td>31.8</td>
<td>13.6</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>50</td>
<td>16.6</td>
<td>33.4</td>
<td>50</td>
<td>16.6</td>
<td>33.4</td>
</tr>
<tr>
<td>C. krusei</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total (in percentage)</td>
<td>48</td>
<td>26</td>
<td>26</td>
<td>38</td>
<td>38</td>
<td>24</td>
</tr>
</tbody>
</table>


Table 3: Comparison of various studies showing Candida species recovered from clinical samples (in percentage)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>54</td>
<td>36.6</td>
<td>79.1</td>
<td>45.9</td>
<td>29.2</td>
<td>49.3</td>
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<td>47</td>
<td>30</td>
<td>61.3</td>
<td>62.5</td>
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<tr>
<td>C. tropicalis</td>
<td>22</td>
<td>22.9</td>
<td>5.9</td>
<td>35.29</td>
<td>46.2</td>
<td>11.9</td>
<td>22.9</td>
<td>30</td>
<td>21</td>
<td>18</td>
<td>15.6</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>12</td>
<td>13.7</td>
<td>11.9</td>
<td>-</td>
<td>6.12</td>
<td>17.9</td>
<td>20.6</td>
<td>9</td>
<td>8</td>
<td>10.6</td>
<td>9.3</td>
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<tr>
<td>C. krusei</td>
<td>6</td>
<td>0.87</td>
<td>2.9</td>
<td>10.78</td>
<td>-</td>
<td>4.5</td>
<td>15.7</td>
<td>14</td>
<td>03</td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4</td>
<td>0.36</td>
<td>-</td>
<td>7.84</td>
<td>10.2</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>2</td>
<td>1.46</td>
<td>-</td>
<td>1.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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REFERENCES

9. Glaismacher A, Molitor E, Meeger J, Marklein G. Antifungal prophylaxis viability on subsequent subcultures.19 Hichrom agar has the advantage of rapid identification of Candida species, technically simple, rapid, and cost-effective compared to technically demanding time consuming and expensive conventional method.

CONCLUSION

Although the results on Hichrom agar exactly paralleled that of the conventional method, it is superior to SDA in terms of suppressing the bacterial growth. Use of Hichrom agar medium would allow mycology laboratories to identify rapidly, clinically important Candida spp. while potentially decreasing laboratory cost. Furthermore, the species level identification of the Candida isolates along with their antifungal susceptibility patterns can greatly influence the treatment options for the clinician and may have an impact on the patient care.


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