ABSTRACT

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

Materials and Methods: A total of 102 Candida species were isolated from various clinical specimens (100) including stool, sputum, nasal, oral, ear swabs, diabetic foot, skin and nail scrapings. Speciation of Candida was done using CHROM agar and conventional method simultaneously.

Observations: Non albicans candida (NAC) predominated (54.1%) over Candida albicans (45.9%). Non albicans candida spp isolated were C.tropicalis (35.29%), C.krusei (10.78%), C.parapsilosis (7.84%), and C. dubliniensis (0.9%). Antifungal susceptibility testing was done using antymycotic sensitivity testing by disc diffusion method. Isolates were 100% sensitive to ketoconazole, clotrimazole, nystatin and amphotericin B. 87.5% of C.krusei, 36% C.tropicalis, 6.25% .C. albicans were resistant to itraconazole. 25% C.krusei and 8% C.tropicalis were resistant of fluconazole. C.dubliniensis was resistant to itraconazole only.

Conclusion: The advantage of using CHROM agar is that it facilitates the isolation and identification of Candida to species level. The performance of CHROM agar exactly 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.

CHROM 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 patients morbidity and mortality.

Key Words: Candida, CHROM agar, Antifungal susceptibility

INTRODUCTION

Candida albicans historically has been predominant cause of Candidiasis. In 1980s C.albicans accounted for greater than 80% of all candidal isolates recovered from nosocomial yeast infections [1]. More recently non albicans candida (NAC)species has been recovered with increasing frequency.

Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentation. These enzymes are species specific, allowing organisms to be identified to the species level by their colour and colony characteristics [1]. 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 CHROM agar over conventional method for speciation of Candida isolates and their susceptibility to antifungal agents by disc diffusion method.

MATERIALS AND METHODS

A total of 102 Candida spp isolated from randomly selected 100 clinical specimens which included stool, sputum samples, nasal, oral and ear swabs, diabetic foot, skin and nail scrapings formed the study group. The study was conducted from Jan 2008 to Dec 2008, in the department of Microbiology, Bowring and L.C. Hospital, Bangalore.

CHROM agar was prepared as per the instruction manual (Himedia India). Candida spp isolated were inoculated simultaneously to CHROM agar plates and SDA tubes. These were incubated at 37°C for 48 hours. Species were identified on CHROM agar by morphology and colour of the colony. Growth on SDA were speciated by standard methods using germ tube, corn meal agar, slide culture, sugar fermentation and assimilation test. Ten faecal samples and two oral swabs from AIDS patients were directly inoculated onto SDA and CHROM agar.

A variety of species specific colony were seen.

Appearances of Candida spp on CHROM agar were as follows:

- C.albicans – blue green
- C.tropicalis – dark blue grey centre with pink halo
- C. krusei – pink large rough spreading colonies with pale edge.
- C.parapsilosis – pale cream coloured colonies.
- C.dubliniensis – dark green colonies.

Antifungal susceptibility was performed by disc diffusion method using antymycotic sensitivity test agar. Discs used were amphotericin B (100 units), fluconazole (10mcg), clotrimazole (10mcg), nystatin (100mcg), itraconazole (10mcg), ketoconanole (10 mcg) was measured as for the instruction manual (HiMedia). ATCC strain of C.albicans was used as control.

RESULTS

[Table/Fig-1] shows Candida spp isolated in various clinical samples. All isolates of candida grew on CHROMagar after 48 hr of incubation at 37°C.

C.dubliniensis isolated from faecal sample of AIDS patient grew only on CHROMagar. C.dubliniensis was further identified by germ tube, chlamydospore formation and no growth at 45°C.
A mixed growth of *C.tropicalis* + *C.parapsilosis*, *C.albicans* + *C.tropicalis* were isolated in two sputum samples. Isolates were 100% sensitive to amphotericin B, clotrimazole, nystatin and ketaconozole. 87.5% of *C.krusei*, 36% *C.tropicalis*, 6.5% *C.albicans* were resistant to itraconozole. 25% of *C.krusei*, 36% *C.tropicalis* were resistant to fluconozole. *C.dubliiniensis* was resistant to itraconozole only.

**DISCUSSION**

In the present study NAC (55.8%) was isolated at a higher rate than *C.albicans* as reported by other workers. *Candida spp* isolated by various workers is shown in [Table/Fig-2] [2-9]. In our 1997 study *C.albicans* predominated [10]. Among the stool samples received 90% were from antibiotic associated diarrhoea. *C.dubliiniensis* isolated on CHROM agar did not grow on SDA. Isolation of this species has been reported by Raut [11]. Using CHROM agar species like *C.dubliiniensis* can be isolated as in the present study. Mixed growth of *Candida spp* found in faecal specimens also has been reported by Frank [6].

<table>
<thead>
<tr>
<th>Specimen(100)</th>
<th>C.albicans</th>
<th>C.tropicalis</th>
<th>C.krusei</th>
<th>C.parapsilosis</th>
<th>C.dubliiniensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool-47</td>
<td>12</td>
<td>24</td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Sputum-23</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CSOM-10</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nasal swab-6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Oral swab-5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic foot-5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin&amp;Nail -4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>O</td>
<td>0</td>
</tr>
<tr>
<td>Total 100%</td>
<td>46</td>
<td>36</td>
<td>11</td>
<td>8</td>
<td>1</td>
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</tbody>
</table>

[Table/Fig-1]: Candida spp isolated in various clinical samples

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>C.albicans</td>
<td>45.9</td>
<td>3.4</td>
<td>25.8</td>
<td>46</td>
<td>43.15</td>
<td>24</td>
<td>39.25</td>
<td>47.6</td>
<td>76.92</td>
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<tr>
<td>C.tropicalis</td>
<td>35.29</td>
<td>35.6</td>
<td>38.7</td>
<td>30</td>
<td>9.47</td>
<td>24</td>
<td>7.43</td>
<td>35.4</td>
<td>17.94</td>
</tr>
<tr>
<td>C.krusei</td>
<td>10.78</td>
<td>–</td>
<td>6.45</td>
<td>10</td>
<td>1.05</td>
<td>10</td>
<td>5.92</td>
<td>4.9</td>
<td>–</td>
</tr>
<tr>
<td>C.parapsilosis</td>
<td>7.84</td>
<td>28.8</td>
<td>25.8</td>
<td>–</td>
<td>21.05</td>
<td>6</td>
<td>10.19</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>C.dubliiniensis</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.12</td>
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</tr>
</tbody>
</table>

[Table/Fig-2]: Candida spp isolated by various workers in percentage

<table>
<thead>
<tr>
<th></th>
<th>C.albicans</th>
<th>C.tropicalis</th>
<th>C.krusei</th>
<th>C.parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconozole</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Itraconozole</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Fluconozole</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Itraconozole</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>100</td>
<td>93.75</td>
<td>100</td>
<td>92</td>
</tr>
</tbody>
</table>

[Table/Fig-3]: Antifungal susceptibility as reported by various workers (%)

The advantages of CHROMagar are easy to prepare by boiling and dispensing in Petri plates, facilitates the rapid isolation and identification of yeast species. CHROM agar facilitates identification between yeast spp from specimens containing mixture of yeast spp as in the present study and do not affect the viability on subsequent subcultures [6]. CHROM 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.

Though the results on CHROM agar exactly paralleled that of conventional method, it is superior to SDA in terms of suppressing the bacterial growth. Use of CHROM agar medium would allow mycology laboratories to identify rapidly, clinically important *Candida Spp* while potentially decreasing laboratory cost. More...
importantly this capability will also enable clinicians to choose appropriate anti fungal agents, thus decreasing patient’s morbidity and mortality [Table/Fig-4, 5, 6].

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REFERENCES

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DECLARATION ON COMPETING INTERESTS:
No competing Interests.