RAPID IDENTIFICATION AND SPECIATION OF CANDIDA ISOLATED FROM VARIOUS CLINICAL SAMPLES USING HICROME CANDIDA DIFFERENTIAL AGAR AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

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Abstract: Fungi are world-wide distribution but only few of them are considered as pathogenic. The incidence of fungal infections has increased dramatically in the past 20 years partly because of increase in the number of immunocompromised persons due to ageing, AIDS, Diabetes mellitus, organ transplantation and cancer therapy. Candidiasis is an important opportunistic fungal infection most commonly caused by Candida albicans and occasionally caused by nonalbicans species of Candida. The species identification of Candida is usually carried out by conventional methods which are all more time taken procedures. So for rapid identification and speciation of candida, Hicrome Candida Differential Agar was used. From the period of July 2013-November 2013, Candida was isolated in 72 samples out of 110 samples (oral thrush, high vaginal swab, urine, pus, sputum and otomycosis). Antifungal Susceptibility Testing was done by Disc Diffusion Method using Glucose-Methylene Blue-Mueller Hinton Agar (GMB-MH agar-2 glucose and 0.5gm per ml in Mueller Hinton Agar)1,4. The predominant species isolated was Candida albicans followed by Candida tropicalis, Candida glabrata, Candida krusei and Candida dubliniensis. Among the 72 isolates of Candida, 18 (27.7) were resistant to Fluconazole with the Minimal Inhibitory Concentration (MIC) of more than 64mcg/ml.

Keyword: Candida species, Candida Crome agar, Antifungal Susceptibility Testing, GMB-MH Agar, MIC of Fluconazole [if gte mso 9] Normal 0 false false false MicrosoftInternet Explorer4

INTRODUCTION:
Candidiasis is an important opportunistic yeast like fungal infection most commonly caused by Candida albicans and occasionally caused by nonalbicans species of Candida both in immunocompetent and immunocompromised states (diabetes, HIV, on chronic use of steroids etc).2,3. The types of Candidiasis are cutaneous candidiasis, mucocutaneous candidiasis, candidiasis of urinary tract, candiditis, candida meningitis, pulmonary candidiasis, disseminated candidiasis, candidemia and nosocomial candidiasis etc. About 46% of systemic candidiasis is mainly caused by non-albicans species of Candida. The species identification of Candida is usually carried out by conventional methods like germ tube test, chlamydospore formation in Corn Meal Agar, sugar assimilation and sugar fermentation tests, which are all more time taken procedures. So for rapid identification and speciation, Hicrome Candida Differential Agar have been developed. Nowadays as the emergence of drug resistant albicans and nonalbicans species of Candida is coming up, Antifungal Susceptibility Testing is very essential. The accuracy of antifungal susceptibility tests are important for accurate surveillance and for the clinical management of seriously infected patients of Candida10.

AIM AND OBJECTIVES:
1. To evaluate the usefulness of Hicrome Candida Agar over conventional methods for detection and speciation of Candida isolated from various clinical samples.
2. To evaluate their Antifungal Susceptibility Testing by using GMB-MH Agar by Disc Diffusion Method.

MATERIALS AND METHODS:
From the period of July 2013-November 2013, totally 110 samples were received in Microbiology Lab at Hospital for fungal culture. The received samples were processed and analysed by Gram staining, 10% KOH wet mount and routine fungal culture. Out of 110 samples, Candida[72(67%)] was isolated from 19(26.3%) of oral thrush of HIV infected persons (CD4 Count less than 200/μl), 18(25%) from high vaginal swab, 17(23.7%) from urine, 10(14%) from sputum, 5(7%) from pus sample and 3(4%) from otomycosis. The isolated Candida were further processed and speciated by germ tube test, sugar assimilation tests, sugar fermentation tests, chlamydospore formation in Corn Meal Agar and also in cromogenic medium (HiMedia-Hicrome Candida Agar- M1297A). The color produced in cromogenic medium by the each isolates of Candida were noted and interpreted with the manufacturer’s guidelines. Antifungal Susceptibility Testing by Disc Diffusion Methodmodified Kirby-Bauer technique was done for the isolates of Candida species as per CLSI Guidelines (CLSI vol.31 No.1 Jan.2011) using Glucose-Methylene Blue-Mueller Hinton Agar 3 and HiMedia - Hexadiscs (Hexa Antimyco-01)-contain Ketoconazole (10mcg), Itraconazole (10mcg), Fluconazole (25mcg), Nystatin (100units), Amphotericin-B (100units) and...
Clotrimazole (10mcg). Hi-comb strip (MD072) was used for the MIC determination of Fluconazole in all the isolated Candida species except Candida krusei (as it is intrinsic resistant to Fluconazole) as per CLSI Guidelines. Candida albicans ATCC 9002811 was used as control strain. The results were recorded and analysed.

RESULTS:
Out of 110 samples, Candida was isolated in 72 (67%) samples. Among the 72 isolates, males were 40 (55.5%) and females were 32 (44.5%). Among the 72 isolates, 65 isolates were in the age group of 25-45 years (90%). Among the 72 isolates, 19 (26.3%) were isolated from oral thrush of HIV infected persons (CD4 Count less than 200/μl) and 15 (21%) persons were uncontrolled Type I Diabetes mellitus. The predominant species isolated was Candida albicans 45 (62%) followed by Candida tropicalis 15 (21%), Candida krusei 7 (10%), Candida glabrata 3 (4%) and Candida dubliniensis 2 (3%). Among the 72 isolates of Candida, 69 isolates (96%) were sensitive to Amphotericin B, 59 (82%) isolates were sensitive to Ketconazole, 58 (80.5%) isolates were sensitive to Clotrimazole. 57 isolates (79%) were sensitive to Nystatin, 55 isolates (76%) were sensitive to Itraconazole and 47 isolates (72.3%) were sensitive to Fluconazole. The MIC of Fluconazole for all the isolated species of Candida except Candida krusei (as it is intrinsic resistant to Fluconazole) was detected by using Fluconazole-Hicomb Strip. The MIC of the Candida isolates which were sensitive to Fluconazole was in the range of 0.25-32mcg/ml. The MIC of the Candida isolates which were intermediate sensitive to Fluconazole was 16-32mcg/ml and the MIC of the Candida isolates which were resistant to Fluconazole was more than 64mcg/ml.

1. TOTAL NUMBER OF CANDIDA ISOLATED - 72 OUT OF 110 SAMPLES:

2. TOTALLY ISOLATED CANDIDA SPECIES

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Total (%)</th>
</tr>
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<tbody>
<tr>
<td>Candida albicans</td>
<td>45 (62%)</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>15 (21%)</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Total</td>
<td>72 (100%)</td>
</tr>
</tbody>
</table>

DISCUSSION:
In this present study, Candidiasis is more prevalent with male predominance [40 (55.5%)] and more prevalent in the adult age group of 25 to 40 years [65 (90%)]. In this present study, oral Candidiasis 7, 12 [19 (26.3%)] was found to be more common followed by vaginal candidiasis [18 (25%)]. In this present study, Candidiasis is predominantly caused by Candida albicans 45 (62%) and also by nonalbicans species of Candida 27 (33.5%) [2, 3]. When compared the cromogenic medium with routine conventional methods for the speciation of Candida, cromogenic medium yielded earlier results (within 48 hours) than conventional methods (4 to 5 days) [3]. The results of cromogenic medium (Hicrome Candida Agar) was exactly correlated with the results of routine conventional methods for identification and speciation of Candida [3]. Bacterial contamination was not noticed in the cromogenic medium of Candida [3]. In this present study, among the 72 isolates of Candida, 69 (96%) isolates were sensitive to Amphotericin B and 47 (72.3%) isolates were sensitive to Fluconazole. In this present study, more Fluconazole resistance [18 (27.7%)] [8, 13, 14] were noted on compared with the other antifungal drugs. The MIC of the Candida isolates resistant to Fluconazole was more than 64mcg/ml [8, 13, 14]. Resistance to azole antifungals continues to be a significant problem in the common fungal pathogen like Candida albicans due to alteration in the gene encoding the target enzyme ERG11 or over expression of efflux pump genes of CDR1, CDR2 and MDR115.
CONCLUSION:
The commonest species isolated in this present study was Candida albicans and more prevalent in the adult age group with male predominance. The Hicrome Candida Agar is very useful, easy, reliable, consumes less time and less cost for the speciation of Candida than the routine conventional methods and hence it may replace the routine conventional methods. The Antifungal Susceptibility Testing showed more Fluconazole resistance [18(27.7%)] were noted(with the MIC of more than 64 mcg/ml) on compared with the other antifungal drugs. In this present study, about 96% of isolates were sensitive to Amphotericin B. The identification of Candida to its species level and the detection of their Antifungal Susceptibility Testing is important because of proper management of Candidiasis at the earliest and thereby prevent the emergence of multi drug resistant form of Candida species.

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