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Research Article

IDENTIFICATION AND SPECIATION OF CANDIDA ISOLATED FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

Ngairangbam Gopeshwor, Tarana Sarwat, Dalip K Kakru and Saheli Bhadra

Department of Microbiology, SMSR, Sharda University

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A B S T R A C T

Infections due to Candida species have increased substantially over a few decades due to increased use of broad-spectrum antibiotics, immune-suppressants and indwelling devices which attribute to high rates of invasive candidiasis. *Candida albicans* accounts for the majority of superficial and systemic infections. Both,*Candida albicans* and non-albicans is associated with high morbidity and mortality because of the increasing antifungal resistance. Hence, it is important to correctly identify and speciate them. The present study was therefore conducted to identify various species ofCandidaisolated from various clinical specimens.

The study was conducted over a period of one year from November 2020 to November 2021. The various species of Candida isolated, were identified by using conventional tests (germ tube and chlamydospore test, sugar fermentation and assimilation tests and commercially available CHROM agar)and automated methods (Vitek 2 Compact system).

A total of 79 strains of Candida were isolated during the study period. *Candida tropicalis*was the most frequently isolated species, 41(51.8%), followed by *Candida albicans,*16(20.2%), *Candida krusei,*11 (13.9%),*Candida glabrata,*8(10.12%) and*Candida dubliniensis,*3(3.7%). Early identification and reporting of infections due to Candida can help the clinicians in better patient management. Now a days, a wide range of molecular techniques have been used including non-DNA-based methods and DNA-based methods because they generate unambiguous and highly reproducible typing data. CHROM agar is a conventional and rapid method of identification of *Candida* species even in poor resource settings, hence its use may help in making an early diagnosis.

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INTRODUCTION

Candida is one of the most commonly encountered opportunistic fungi thatusuallycauses superficial mucosal infections, but can invade tissues and produce life threatening diseases caused by alteration of immune defences. There are various conditions in which the normal equilibrium between *Candida* and the host is altered and it leads to pathological state e.g. extremes of age, pregnancy, diabetes, prolonged administration of antibiotics, steroid therapy and AIDS[1-2].

Species level identification of *Candida* isimportant as non-albicans Candida (NAC) has found to bemore resistant to antifungal drugs. Speciation of Candida can be done using Hichrom agar, VITEK system and various other biochemical reactions. Use of CHROM agar for species differentiation would be of benefit for easy and rapid speciation since it contains chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentation [3].

Candida albicans accounts for 40-60% yeasts isolated in developed countries, whereas Indian reports show an increased predominance of non albicans *Candida* species.

C. tropicalis shows highest adherence rate to inanimate materials such as urinary and vascular catheters, and often involved in biofilm formation, that is more resistant to antifungal agents. Resistance to azoles in *C. tropicalis* and *C. albicans* has also been increasingly reported. So, it is necessary to identify and speciate Candida which shows drug resistance [5].

The present study was therefore conducted to identify and speciate various strains of Candida isolated from clinical specimens so that it may aid the clinicians in starting appropriate antifungal treatment on time.

MATERIALS AND METHODS

The study was conducted from November 2020 to November 2021 in Department of Microbiology, Sharda Hospital, Greater Noida, UP. All clinical samples received in the Mycology laboratory were including in the study. These specimens were processed for the isolation of *Candida species*using standard mycological methods. The specimens were inoculated on blood agar and Sabouraud's dextrose agar and were incubate at

Corresponding author:* **Ngairangbam Gopeshwor
Department of Microbiology, SMSR, Sharda University

37°C for 24-48hrs. Gram staining was performedto identify the yeast-like microorganisms (fig 1).

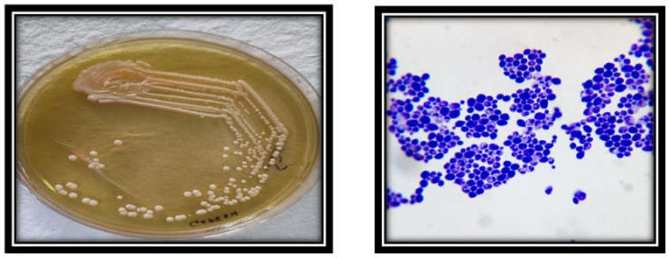

Figure 1 Creamy white colony of Candida on SDA plate and b) Gram stain showing Gram-positive budding yeast cells.

A germ tube test was done and the test positive organisms were identified as either *C. albicans or C. dubliniensis*(fig 2). *C. albicans* was further confirmed by growth at 45°C and chlamydospore formation on corn meal agar (fig 4). The isolates were subjected to sugar fermentation and assimilationtests (fig. 5, 6) as per the standard methods [6]. Simultaneously the strains were inoculated on CHROM agar at 37°C for 24 hrs and the species were identified by the type of colour of the colonies on CHROM agar media as per manufacturer's instructions (fig. 3). All the Candida isolates were confirmed by VITEK2 Compact system (fig. 7).

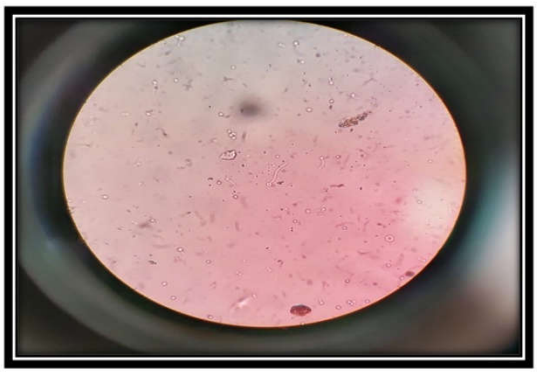

Figure 2 Germ tube test

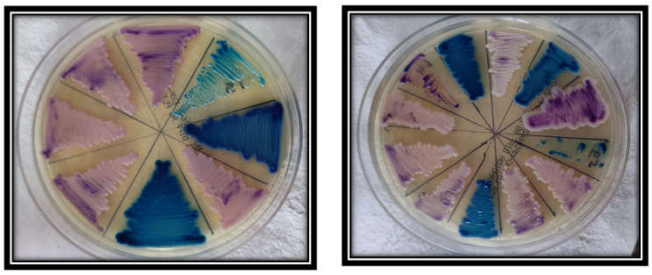

Figure 3 Candida CHROM agar

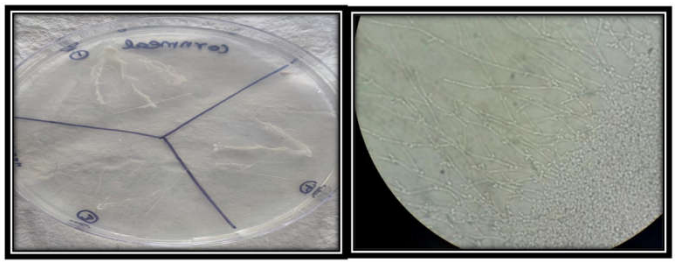

Figure 4 Corn meal agar showing Chlamydospore

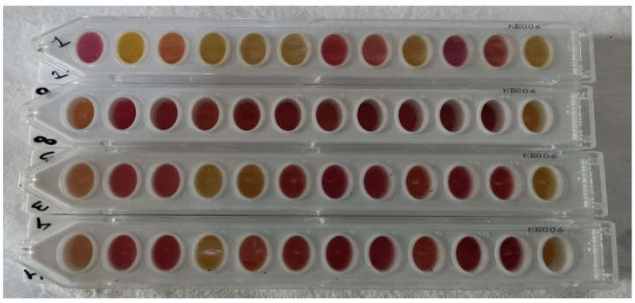

Figure 5 Carbohydrates fermentation test showing positive and negative Reaction.

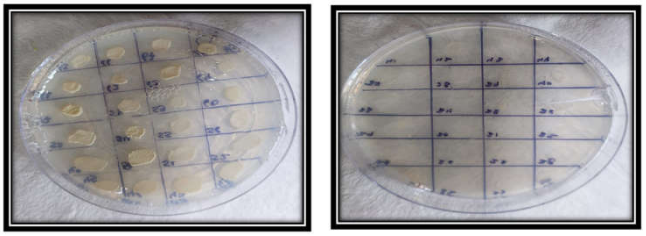

Figure 6 Carbohydrates assimilation test

Figure 7 Rapid automated Identification system

RESULTS

Out of 7051 samples received in laboratory for culture, 5041(71.5%) showed growth of pathogens. Out of these 5041 isolates, 4962 (98.43%)were bacterial pathogens and 79(1.57%) were identified as Candida species.

Most strains of Candida were isolated in the age group of 40-49(22.78%), followed by 50-59(18.98%), 60-69(16.45%), 20-29(16.45 %), 70-79 (5.06%), 0-9 (5.06%) and 80 above (2.53%) as shown in table 1.

Table 1 Age wise distribution of Candida isolates

Age in years	No. of Isolates (N)	Percentage (%)
0-9	4	5.06
10-19	2	2.5
20-29	13	16.5
30-39	7	8.9
40-49	18	22.8
50-59	15	18.9
60-69	13	16.5
70-79	4	5.06
Above 80	2	2.5

The maximum number of strains were isolated from urine samples 37(43.03 %) followed by sputum 9 (11.39%), blood 7 (8.86%), pus 7(8.86%) and ETT 7(8.86%), catheter tip 7(8.86%), nasal swab 3(3.79%), vaginal swab 1(1.26%) and BAL 1(1.26%) as shown as Table 2.

Table 2 Sample wise distribution of Candida isolates

Sample	No. of Isolates (N)	Percentage (%)
Urine	37	46.08
Sputum	9	11.4
Catheter Tip	7	8.9
Blood	7	8.9
ETT	7	8.9
Pus	6	7.6
Nasal Swab	3	3.8
BAL	1	1.3
Vaginal Swab	1	1.3
Maxillary tissue	1	1.3

Maximum no. of isolates, 43 (54.43%) were obtained from patients admitted in ICU followed by General Surgery and Medicine wards, 20 (9.09%), OPD patients, 10 (12.6%) and least were isolated from Paediatrics 2 (2.53%) and Orthopaedics wards 1(1.26%)as shown in Table 3.

Table 3 Ward/OPD wise distribution of Candida isolates

Ward	No. of isolates (N)	Percentages (%)
Emergency Zone	16	20.3
ICU	27	12.7
OPD	10	12.7
Surgery ward	8	5.06
Medicine Ward	12	7.6
Orthopedics	1	1.3
ENT	5	6.3

Candida tropicalis was the most commonly isolated species (41, 51.8%) in Urine 37(20, 54.5%), Catheter tip 7(5, 71.4%), Blood and ETT 7(4, 57.1%),sputum 9 (3, 33.3%), pus 6 (2, 33.3%), nasal swab 3 (1,33.3%) and BAL and vaginal Swab 1(1, 100%)followed by *Candida albicans* (16, 20.2%) in urine 37 (7,18.9%), followed by Sputum 9 (2, 22.2%), Pus 6(2, 22.2%) and blood 7(2, 22.2%), ETT and Catheter Tip 7(1, 14.3%). *Candida krusei*(11, 13.9%) in Urine 37 (5, 13.7%), Sputum 9 (2, 22.2%) followed by Blood, ETT and Catheter Tip 7 (1,14.3%), *Candida glabrata* (8, 10.12%) in Urine 37 (2, 5.4%), Sputum 9 (2, 5.4%), Pus 6 (2, 5.4%), ETT 7 (1 ,14.3%) and Nasal swab 3 (1, 33.3%)and *Candida dubliniensis* was the least isolated from urine 37 (3, 3.7%) as shown in Table 4.

Table 4 Isolation of Candida spp. from clinical samples wise distribution

Sample	No. of isolates	*C.tropicalis*	*C.albicans*	*C.krusei*	*C.glabrata*	*C.dubliniensis*
Urine	37	20 (54.5%)	7 (18.9%)	5 (13.7%)	2(5.4%)	3 (8.1%)
Sputum	9	3(33.3%)	2(22.2%)	2(22.2%)	2(22.2%)	0
Pus	6	2(33.3%)	2(33.3%)	0	2(33.3%)	0
Blood	7	4(57.1%)	2(28.6%)	1(14.3%)	0	0
ETT	7	4(57.1) %	1(14.3%)	1(14.3%)	1(14.3%)	0
Catheter tip	7	5(71.4%)	1(14.3%)	1(14.3%)	0	0
Nasal Swab	3	1(33.3%)	0	1(33.3%)	1(33.3%)	0
BAL	1	1(100%)	0	0	0	0
Maxillary Tissue	1	0	1(100%)	0	0	0
Vaginal Swab	1	1(100%)	0	0	0	0

DISCUSSION

The incidence of non -albicans candida has risen due to increasing immunocompromised states. Non albicans Candida shows more resistance to fluconazoles, therefore species level identification has impact on choice of empirical antifungal treatment. Also, there may be geographic variation in the species isolated which necessitates that we have data on the distribution of candida species in different geographic regions [6-7].

The present study was undertaken to speciate the Candida isolated from various clinical samples. The study also concentrated on the changes observed in species distribution, the shift towards non albicans Candida species in our hospital. *Candida species* differentiated conventionally by Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests which are being used is laborious and time consuming. CHROM AGAR is a rapid method to identify different *candida species*. So, it facilitates the detection and identification of candida from mixed cultures and provides result in 24-48 hours[8-10].

Urinary tract infection by candida known to be most frequent nosocomial fungal infection world wide. Most Candida isolated were from urine 37 (46.03%) isolates, out of which 15 (44.12%) isolates were of *Candida tropicalis*, 9 (11.4%) isolates were from sputum and 7(8.9%) isolates were blood in which 4 were *Candida tropicalis*(57.14%).Among non-albicans Candida; *Candida tropicalis* was the most common species isolated in our study which was consistent with the study by Graf B *et al*[9].Use of broad-spectrum antibiotics, prior ICU admission and use of central venous catheters are the most prevalent predisposing factors of candidemia[10]. Present study shows there was an increased number of non-*albicans Candida* in blood overtime with *Candida tropicalis (7, 57.1%) and Candida krusei (1, 14.2%).*

In our study *Candida tropicalis* was the commonest species isolated followed by *Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida dubliniensis*. Also, a greater number of Candida were isolated in 40-49 years group (22.78%), followed by 50-59 (19%), 60-69 (16.5%), 20-29 (16.5%) and below 20 (7.59%) as shown in table 1.

We did not face any difficulty in using conventional methods for speciation of Candida and the results were consistent with those of automated methods. CHROM agar gives the advantage of being technically simple, rapid and cost effective. CHROM agar has given a valuable method for identification of *Candida species* even in resource poor settings.

CONCLUSION

Along with *Candida albicans*, non albicans Candida species like *Candida tropicalis, Candida krusei, Candida glabrata* are increasingly being isolated from clinical specimens. CHROM agar is a simple, rapid and inexpensive methods with good sensitivity and specificity for identification of such species. Clinical microbiologists will be able to save time and costs for the diagnosis of fungi in clinical specimens especially blood cultures in which early identification may require change of antifungal agents as *Candida glabrata* and *Candida krusei* are inherently resistant to azoles.

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Conflict of Interest: Nil

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