Original research article

Isolation and Speciation of Candida -using Chrome Agar in a tertiary care hospital

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Abstract:

Introduction Candida species are the most common cause of fungal infections world wide. It is the fourth leading cause of health care associated infections. Candida species have emerged as a major cause of human disease, especially among the immunocompromised and those hospitalized with serious co –morbid conditions.

Aim: To isolate and identify the various candida species from clinical samples by using chromogenic media.

Materials and Methods: Various samples received in laboratory from patients of all age group and both sexes with suspected Candida infection were included in this studyand the positive isolates were identified by using chromogenic media.

Results: 89 candida isolates were studied. 21-40 years was the most common age group affected and female predominance was seen C.tropicalis was most commonly found followed by C.albicans.

Conclusion: It can be concluded from our results that the species level identification of the Candida isolates using chrome agar medium would enable the laboratories to rapidly identify and speciate the clinically important candida species. This can greatly influence the treatment options for the clinician and may have an impact on the patient care that can potentially reduce the patient's morbidity and mortality.

Keywords: Candida species, chromogenic media, clinical samples.

Introduction:

Candida species are the most common cause of fungal infections worldwide. It is the fourth leading cause of health care associated infections and the third most common cause of central line-associated bloodstream infections.[1]Candida species are the members of the normal flora of skin, mucous membrane, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. As result general risk factors for Candida infections are associated with compromised immune system like diabetes mellitus, febrile neutropenia, patients on chemotherapy, patients on broad spectrum antibiotics, patients on steroid, post transplantation, malignancy, HIV infected people, pregnancyand extremes of age (infancy, old age). The genus is composed of a heterogeneous group of organisms and more than 17 different Candida species are known to bethe etiological agents of human infection. In the past decademajority of the infections were caused by Candida albicans. However, recently there has been an upsurge in infections caused by non- albicans species ofCandida.

Candida albicans and non- albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility. All Candida species cause disease ranging

from superficial infections such as oral thrush to invasive disease, yet they show differences in disease severity and susceptibility to different antifungal agents.

Candida species identification is therefore important for successful management. Speciation helps to understand the epidemiology of candida, particularly the source and mode of transmission. This in turn facilitates the development of effective measures to prevent and control transmission of resistant pathogens.[2]

Identification of yeast pathogens by traditional methods requires several days and specific mycological media. These methods are thus labor intensive and time consuming. Several brands of chromogenic media are available for rapid identification of Candida Species.

Chrome Agar is a new differential culture medium that allows the isolation and presumptive identification of yeast species of clinical importance rapidly. Hence the aim of this study wasto rapidly isolate and identify the distribution of candida species from various clinical samples at a tertiary care hospital[3]. In this study, we evaluated the performance of a commercially available chromogenic Candida speciation media for the identification of medically important yeasts and yeast like organisms in a routine clinical microbiology laboratory.

Material and method:

This prospective study was conducted in the Department of Microbiology at Coimbatore medical college Hospital ,Coimbatore. A total of 89 Candida isolates from various clinical specimens (urine, highvaginalswabs, pus, wound swabs, sputum, blood, BAL and skin scraping) were taken up for the study over a period of 6 months from out patients and inpatients admitted into various wards and intensive care units. A detailed clinical history was taken with regards to the age of the patient, sex, underlying disease /conditions, immune deficiencies, HIV status, diabetes mellitus, pregnancy, malnutrition, anyon-going treatment, burns, cancer and any other co- morbid conditions. The various clinical specimens we recollected and processed as per the standard microbiological procedures. They were further speculated by the germ tube test and Hi –chrome agar .

1) Direct examination by KOH Mount
2) Gram stain
3) Culture on SDA(at 25°Cand37°C)
4) Growth on blood agar
5) Germ tube test
6) Growth on chrome agar: Isolated species were inoculated on Hi Chrome Candida differential agar to improve species identification based on coloured colony morphology. These agar plates were incubated at37°C for 48hours. The species were identified by characteristic colony colour as per Hi Media technical data MI1297A.

Variety of species specific colonies was seen.

Appearance of Candida species on chrome agar were as follows

- C. albicans- Light green coloured smooth colonies
- C. tropicalis- Blue to metallic blue coloured raised colonies
- C. glabrata – Cream to white smooth colonies
- C. krusei – Purple fuzzy colonies

  We used ATCC strains of Candidaalbicans,ATC10231,Candida glabrataATCC15126,Candida kruseiATCC14243andCandida tropicalisATC50 as control.

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Results:

Table. 1 Age and Sex wise distribution of patients with Candidiasis

SI.no	Ageofpatients	Males	Females
1	0-20years	4	3
2	21-40 years	17	28
3	41-60 years	7	15
4	60 years	8	7
	Total	36	53

In ourstudy candidacies was found to be more common among 21-40years (50%) followed by 41-60 years (28%). The rate of isolation of the candida species was more in females (60%) than in males (40%) (Table 1)

Table: 2 Distribution of candida species in various clinical isolates

S.No	Samples	C.albicans	C.tropicalis	C.glabrata	C.Krusei	Total
1	Urine	7	23	3	0	33
2	High vaginal swab	14	4	2	0	20
3	Pus	7	3	2	0	12
4	Wound swabs	4	3	2	1	10
5	Sputum	4	3	1	0	8
6	Blood	3	0	0	0	3
7	BAL	0	0	0	2	2
8	Skin scraping	1	0	0	0	1
	Total	40	36	10	3	**89**

The distribution of Candida species in various clinical samples were as follows urine 37% , high vaginal swab 23% Pus 14%, wound swab 11% sputum9%,Blood 3%,BAL2%,andskinscraping 1% [Table 2]

Table.3 Distribution of predisposing factors in patients with Candidacies

SI .No	Predisposing factors	No. of patients
1	Diabetesmellitus	28
2	Historyofantibiotics/steroidsintake	20
3	In dwellingcatheters	12
4	Sepsis	9
5	Prolongedhospitalization	8
6	Pregnancy/ IUCD/ Oral contraceptive pills	6
8	HIV positive	6

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Analysis of the risk/predisposing factors in patients from whom the Candida species wereisolated showed, 31% with underlying diabetes mellitus, 22% on multiple antibiotics and13% with history of catheter usage.(Table 3) Among candida species isolated 49 (55%) were found to be candida nonalbicansand40 (45%) were candida albicans (Table 4).

Table 4: Distribution of Candida albicans and Non albicans Candida isolates

Isolates		**No. of isolates**	**Percentage**
Non albicans Candida (49)	**c. tropicalis**	**36**	**41%**
	c. glabrata	**10**	**11%**
	c. krusei	**3**	**3%**
Candida albicans (40)		**40**	**45%**
Total		**89**	**100%**

The most common species among the candida isolates wereC. tropicalis (41%) followed byC.glabrata (11%) and C.krusei (3%).

Table 5: Colony colour of Candida isolates on Hi chrome Candida differential agar

S.no	Candida species	Colony colour on Hi chrome Candida differential agar	No. of Candida isolates(n=89)
1	Candida albicans	Light green	40
2	Candida tropicalis	Blue	36
3	Candida glabrata	White to cream	10
4	Candida krusei	Purple fuzzy	3

Discussion:

In our study prevalence of candida was predominant in females than in males. We found female (60%)preponderance in our study which was concordant with the study conducted by Sajjan AC et al [1](65%)But Patel et al recorded the high prevalence of candida in malein theirstudy[2].

In our study the distribution of candida species in different samples showed that the highest number of isolates fromurine 37% followed by high vaginal swab 23%, Pus 14%, wound swab 11%, sputum9%, Blood 3%, BAL2%,and skin scraping 1% .

This is similar to study done by Pfalleret al[3]whohas highest Candida isolates from urine(25 %)But Jaggi Tetal[4] isolated Candida mostly from blood (33.6%) and respiratory samples (20%) and least from urine 8%[8].SimilarlySaldhanaetal[5]isolated highest number of Candida from high vaginal swabs (38%) followed by blood (16%) and urine (12%).

Considering the predisposing factors in association with Candida infection 31% of them had underlying diabetes mellitus, 22%wereonmultiple antibiotic therapy and 13% had a history of catheter usage in our study. In a study conducted bySajjanACetal[1] diabetes was the most common risk factor followed by pregnancy and drug in take

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.A studydonebyAroraDetal[6]found intra venous catheter as most common risk factor followed by prolonged antibiotic usage and immunosuppressant.

In our study , Candida non – barbicans species (55%) were more than Candida albicans species (45%) Similarly studies by saldhanaetal[5] and mokadas et al [7] showed higher rate of Candida non albicans species53% and 60.5% respectively. 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 .It is superior to SDA in terms of suppressing the bacterial growth.

Use of CHROM agar medium would allow mycology laboratories to identify clinically important Candida Species rapidly and potentially decreasing laboratory cost.

Conclusion:

Percentage of non – albicans species (55%)has increased in prevalence as compared to C. albicans(45%) from clinical samples. The use of chrome agar medium would enable the laboratories to rapidly identify and speciate the clinically important Candida species. Therefore isolation and prompt identification of the infecting organism to the species level is essential to optimize early anti fungal therapy.

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