Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(8F):3460-3468 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i08.085

Original Research Article

Isolation, Identification and Speciation of Candida Species from Various Clinical Specimens in a Tertiary Care Hospital in Chennai

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Abstract: The aim and objective of the study was to isolate Candida species , find its frequency from various clinical samples (sputum, urine, high vaginal swab, ET tube swab, nail clippings, pus, etc).,their speciation and to bring out the various risk factors associated with candidiasis A total of 250 Candida species isolated from various clinical specimens in our institute from June 2016 to June 2017 were included in the study. Standard yeast identification protocol and Hichrom agar were used for speciation. Out of the total 2350 clinical samples, 250 candida species were isolated , thus the prevalence of 9.4%. Among the 250 culture positive cases, 118 (47.2%) C. albicans, 64 (25.6%) C. tropicalis, 75 (30%), C. glabrata, 38 (15.2%), C. krusei 14 (5.6%) and C. parapsilosis 5 (2%) were obtained. Non albicans Candida predominated (52.8%) over Candida albicans(47.2%). Males were affected more than the females. In both the sexes maximum patients belonged to the age group of >60 years. In the present study prevalence of candidiasis is 9.4%. The risk factors for candidiasis noted in this study were diabetes mellitus, prolonged antibiotic therapy, steroids and pregnancy. Along with Candida albicans , non-albicans Candida(NAC) species like C. glabrata, C. krusei, C. tropicalis and C. parapsilosis are increasingly being isolated from clinical samples. Characterization upto species level is important for early treatment decisions and effective management as some NAC species are intrinsically resistant to antifungal agents. Hichrom Candida agar is useful for the primary isolation and differentiation of medically important Candida species.
Keywords: Candida, Speciation, Hichrom agar, non-albicans Candida

INTRODUCTION:

Candida is one of the most commonly encountered opportunistic fungi that cause superficial mucosal infections usually, but can invade tissue and produce life threatening pathology caused by alteration of immune defenses. There are various conditions in which the normal equilibrium between Candida and the host is altered and it leads to pathologic state : extremes of age, pregnancy, diabetes, prolonged administration of antibiotics secondary to bacterial infections, steroid therapy and AIDS [1]. Species level identification of Candida is important as Non albicans Candida (NAC) species have replaced Candida albicans as the predominant pathogen and are more resistant to antifungal drugs. Speciation of candida can be done using Hichrom agar, which is a differential culture medium and facilitates the isolation and identification of some clinically important Candida species.

Candidiasis refers to infection caused by any of the species of the genus Candida. Incidence of Candidiasis is increasing worldwide. Two major medical events have revived the interest in fungal diseases in general and Candida infections in particular .The first was the introduction of antibacterial drugs in the second half of the twentieth century. These drugs may act as a predisposing factor for mycotic infections causing an imbalance of host's natural microbial flora in favour of fungi, upon which they have inhibitory activity .The second event was the increase in the prevalence of immunosuppressed patients during the last few decades, as a result of chemotherapy or disease [AIDS], which led to a parallel increase in the incidence of Candida infections in general and non- albicans Candida species in particular[2]. The shift of Candida species from

commensal to potent pathogen is facilitated by a number of virulence factors such as adherence to host tissues and medical devices , biofilm formation and secretion of extracellular hydrolytic enzymes[3].

Candida albicans accounts for 40-60% yeasts isolated in developed countries, whereas Indian reports show an increased predominance of non albicans Candida spp. The emergence of non-albicans Candida spp. may represent selection of less susceptible species like C. glabrata and C. Kruse. C.glabrata is less susceptible and Candida krusei is intrinsically resistant to Fluconazole. C. tropicalis has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and is 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 [4-6]. Identification of Candida to species level is definitely warranted as there is increase in the incidence of non-Candida albicans infections [7].

Since molecular techniques are very expensive, usage of CHROM agar for species identification would be of benefit for easy and rapid speciation [8]. These rapid speciation methods can also provide information on the susceptibility to Fluconazole and hence aid in early treatment decisions.

MATERIALS AND METHODS:

A prospective study was undertaken at our institute for a period of 1 year from JUNE 2016 to june 2017. Specimens were collected from OPD/IPD of Sree Balaji medical college and hospital, Chrompet ,Chennai.Out of the total 2350 samples received in the central laborartory , 250 Candida species isolated from various clinical specimens like urine,sputum,high vaginal swab, pus etc.were included in the study.Gram stain was performed from direct samples and inoculated on sabourads dextrose agar at 37^0C for 24 hours. The plates were read at 24 and 48 hrs after incubation.

White to cream-colored, pasty and smooth colonies appeared in within 24 hours to 48 hrs.[Fig 2,3] These colonies morphologically resembling the members of genus candida were subjected to gram staining. On microscopic examination they appeared as gram-positive budding yeast cells with or without pseudohyphae (Fig 4). Germ tube test was done and the positives were identified as either C.albicans or C.dubliniensis.C.albicans was further identified by growth at 45^0C and chlamydospore formation on corn meal agar. All the isolates were subjected to sugar fermentation and sugar assimilation tests for final confirmation of the species.Simultaneously the Candida spp. were inoculated on Hichrom Candida agar and incubated at 37^0C for 24 hrs and the species were identified by type and colour of the colonies on Hichrom Candida agar as per manufacturer's instructions[TABLE 1,Fig 5, 6,7,]. Hichrom Candida agar is a novel, differential culture medium that is claimed to facilitate the isolation by colorimetric presumptive identification [9,10].

Table-1: appearance of candida species on hichrom candida agar

S.No	Candida species	Colony colour of Candida species
1	C. albicans	Light green
2	C. krusei	Pink
3	C. parapsilosis	White
4	C. tropicalis	Dark blue
5	C. glabrata	Cream coloured

RESULTS:

250 clinical isolates of candida species confirmed by standard yeast identification protocols were included in the study and the prevalence of Candidiasis was 9.4% in various clinical specimens. Non-albicans candida (52.8%) isolates was more than Candida albicans (47.2%)[table 2]. Among non-albicans species, C. tropicalis was 30% followed by C. glabrata 15.2% , C. krusei 5.6% and C. parapsilosis 2% were the major isolates.[table 3] Males (51.2%) predominated over females (48.8%) patients.[table 4] Males were affected more than females. Maximum number of Candida isolates were from the age group >60 years[table 5]. Maximum number of Candida isolates was from urine, followed by sputum, high vaginal swab and pus .Split up of the Candida isolates is shown in [Fig.1]. Candida albicans was the major isolate from sputum. Candida tropicalis, Candida glabrata & Candida krusei was the major isolates from urine. Maximum Candida isolates were from medicine followed by nephrology, urology, surgery, ICU,ENT ,paediatrics ,chest and TB, gynaecology & dermatology[table 6]. Diabetes mellitus was the most commonly associated risk factor,

followed by the use of broad-spectrum antibiotics and indewelling Foleys cathter. Pregnancy and the use of steroids were the other risk factors recorded by our study [table 7]

Table-2: Distribution of candida albicans and non albicans candida isolates

CANDIDA SPECIES	NUMBER OF ISOLATES	PERCENTAGE(%)
CANDIDA ALBICANS	118	47.2%
NON ALBICANS CANDIDA	132	52.8%
TOTAL	**250**	**100%**

Table-3: Distribution of candida species in clinical specimens

CANDIDA SPECIES	TOTAL	PERCENT(%)
Candida albicans	118	47.2%
Candida tropicalis	75	30%
Candida glabrata	38	15.2%
Candida krusei	14	5.6%
Candida parapsilosis	5	2%
TOTAL	**250**	**100%**

Table-4: Gender distribution of the study population

GENDER	NUMBER	PERCENT(%)
MALE	128	51.2
FEMALE	122	48.8
TOTAL	**250**	**100**

Table-5: Age distribution of the study population

AGE(years)	NUMBER OF CASES	PERCENT(%)
0-10	13	5.2
11-20	4	5.6
21-30	5	2
31-40	31	12.4
41-50	33	13.2
51-60	71	28.4
>60	93	37.2
TOTAL	**250**	**100**

Table-6: Wardwise distribution of the study population

WARD	NUMBER OF PATIENTS
MEDICINE	50
NEPHROLOGY	40
UROLOGY	30
GYNAECOLOGY	28
ICU	26
SURGERY	22
DERMATOLOGY	20
CHEST AND TB	18
PAEDIATRICS	16
TOTAL	**250**

Table-7: Associated risk factors for candidiasis

Risk factors	Number= n/250	Percentage
Diabetes mellitus	94	37.6%
Broad spectrum antibiotics	42	16.8%
Urinary catheterization	36	14.4%
Pregnancy	32	12.8%
Steroids	46	18.4%

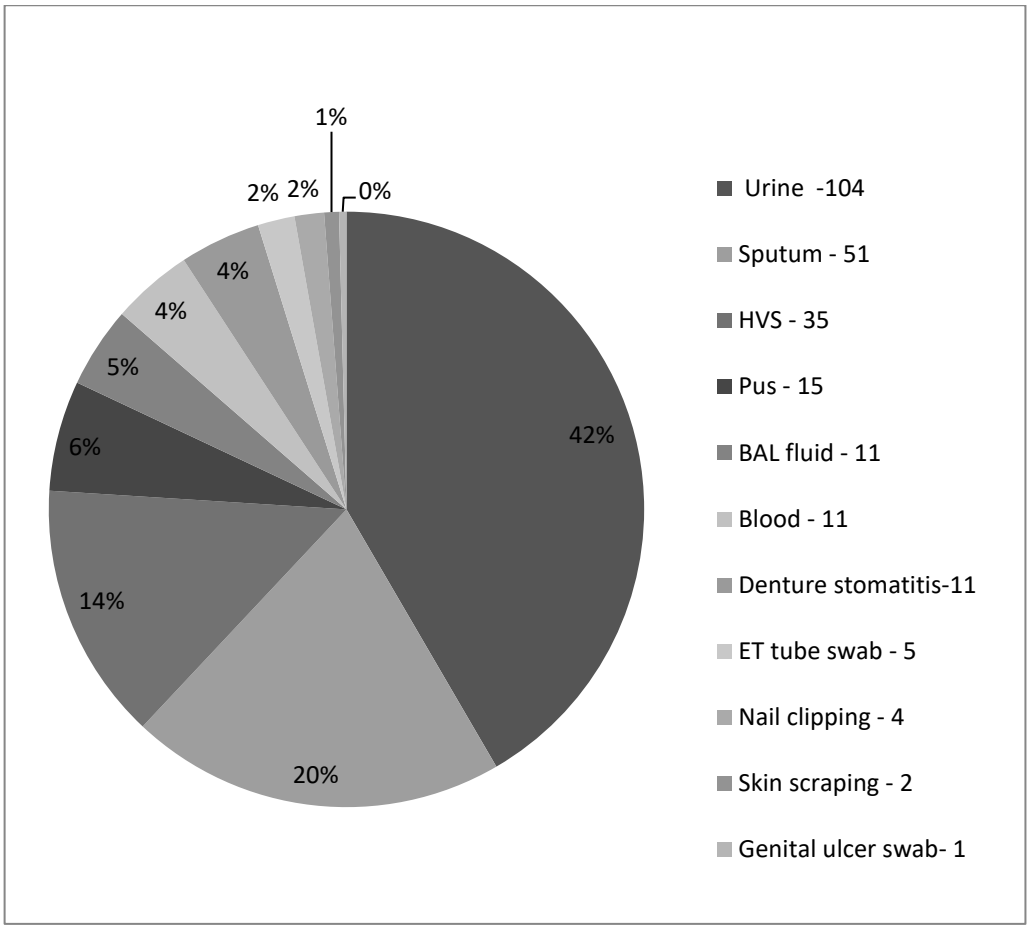

Fig-1: Distribution of Candida Species among Various Samples

Fig-2: growth in SDA

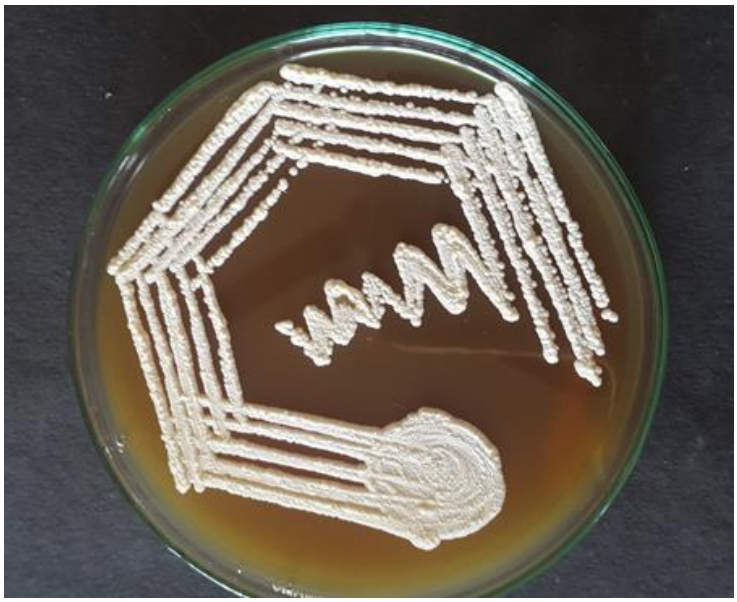

Fig-3: Dry wrinkled colonies of non-albicans candida in SDA

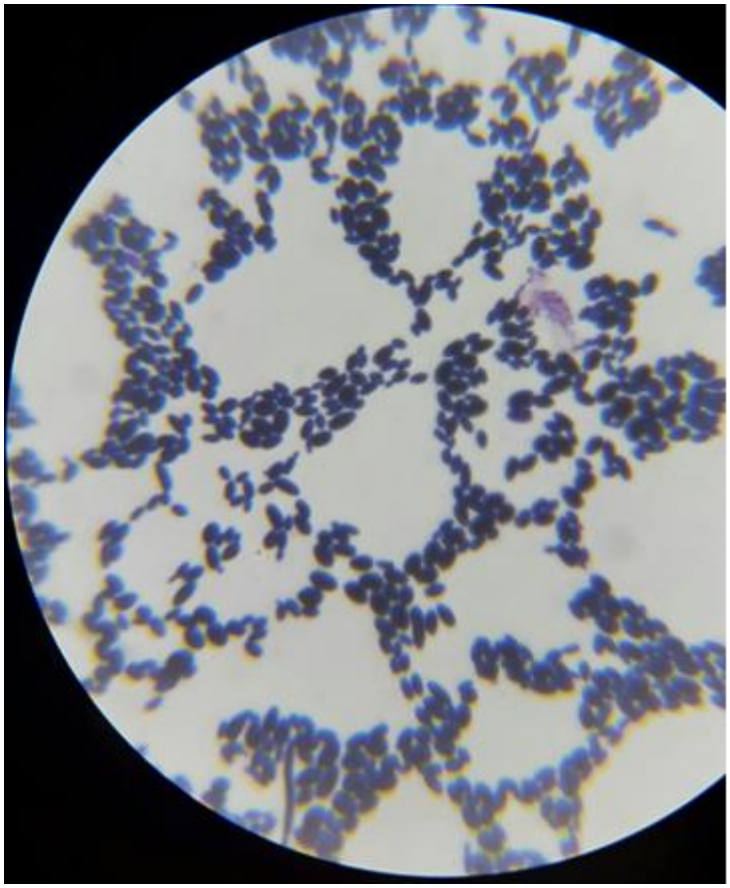

Fig-4: Gram positive budding yeast cells in gram stain

Fig-5: Pink colour colonies of candida krusei in hichrom agar

Fig-6: Blue colour colonies of c. tropicalis in hichrom agar

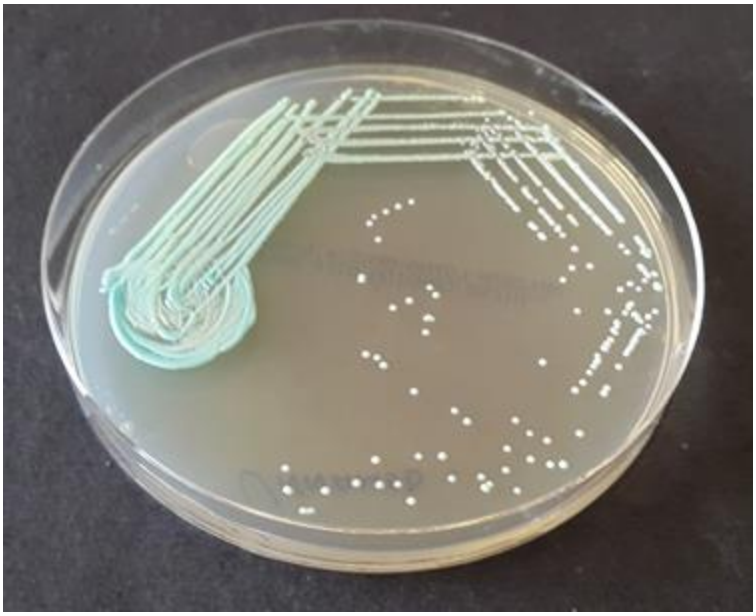

Fig-7: light green colour colonies of c. albicans in hichrom agar

DISCUSSION:

The incidence of yeast infection has increased in the last two decades. Incidence and prevalence of all forms of Candidal infections has risen abruptly. In the present study, prevalence of candidiasis was 9.4% in various clinical specimens. NAC (52.8%) was isolated at higher rates than Candida albicans (47.2%) which correlates with reports by other workers .Vijaya D *et al* [9] showed 46% C. albicans isolates, Grace *et al* [11] showed 43.15% C. albicans, Prasad et

al. showed 47.6% [12] C. albicans. and Sachin C. Deorukhkar *et al* [13] showed 39.2% C. albicans.

In our study most common species isolated was C. albicans 47.2% . The predominant NCA isolated in our centre was C.tropicalis 30%, which was similar in studies done by Ragini et al,[14] Vijaya et al,[9]and Saroj et al. Other non-albicans candida species isolated were C. glabrata 15.2%, C. krusei 5.6% and C.parapsilosis 2%. Similar observation was documented by Dasari Sarada et al[15]. , Sachin C. Deorukhkar et al.[13] and C A Kauffman *et al.*[16] In the study by Sachin C. Deorukhkar et al.[13] out of 523 Candida spp. isolated from various clinical specimens, 192 (36.7%) were C. albicans and 331 (63.3%) were NAC spp. Among the NAC spp., C. tropicalis (35.1%) followed by C. glabrata (28.1%) and C. krusei (16.3%) was the major isolates, which is comparable with our study.

In our study majority of candida spp. was isolated from urine 41.6%, followed by sputum 20.4%, high vaginal swab 14% and pus 6%. Of these >50 % of urinary candida isolates belongs to NAC spp. Our observation is similar to that of F Alvarez-Lerma et al. [17]and C A Kauffmann et al.[16] , where >50% of urinary Candida isolates belonged to NAC spp.

In the present study, most of the candida isolates was found to be higher in male patients 128(51.2%) as compared to female patients 122 (48.8%) with male to female ratio of 1:0.95. This [12] correlates well with from R A Kashid *et al* [14] who reported the isolation of Candida species was higher in males 81 (55.10%) as compared to females 66 (44.8%) with male to female ratio of 1:0.81. Our study differed from Amar C S *et al*. who isolated Candida species more from female 62 (60.2%) than male 41 (39.8%) patients in ratio of (M: F) 0.66:1[18].

We observed that the frequent isolation of Candida species was in the age group above 60 years (37.2%) which was similar with the study of R A Kashid *et al* [14]. who reported highest incidence in the age group above 60 years (24.48%). In a study done by Aikaterini Flevari *et al* [19] it is said that Candida species remain the most important cause of opportunistic infections worldwide, affecting predominantly patients over 65 years of age.

The potential clinical importance of species-level identification of Candida species lies in the fact that they differ in the expression of virulence factors and antifungal susceptibility. For differentiation between different species of candida conventionally Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests are being used which are laborious and time consuming. Hichrom agar is a rapid method to differentiate between different candida species. It facilitates identification of candida species in 24-48 hours. As per our study, Hichrome agar was found to have the advantage of being technically simple and rapid and cost effective as compared to the conventional methods In the present study, Hichrome agar Candida identified all Candida albicans, C. tropicalis, C. glabrata, C. krusei and Candida parapsilosis which correctly correlates with study by Willinger B [20] *et al* [22], Momani OM *et al* [21] and Gultekin *et al* [22].

CONCLUSION:

This study emphasizes the requirement of rapid and precise identification of Candida isolates upto species level. Prevalence of candidiasis is 9.4% in various clinical samples and non albicans candida predominated Candida albicans. There is an increase in the prevalence of non- albicans candida. Prevalence of candidiasis was found to be higher in patients associated with predisposing factors like indwelling vascular catheters, prolonged antibiotic therapy and diabetes mellitus. The advantages of Hichrom candida agar is that it provides for the rapid isolation and identification of medically important candida species in a resource-limited setting and it potentially decreases the laboratory cost which is required for effective management strategies. Effective treatment requires both early diagnosis and prompt initiation of therapy against fungal infection thus decreasing patients' morbidity and mortality.

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