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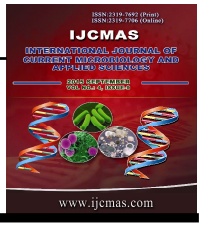

Original Research Article

Evaluation of Chrom Agar in Speciation of *Candida* Species from Various Clinical Samples in a Tertiary Care Hospital

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Keywords

Candida albicans, non-albicans *Candida*, immune-deficiencies, speciation, chromogenic agar

A B S T R A C T

Antibiotics, immunodeficencies, advances in medical practices have all contributed to increased incidence in fungal infections. New fungal pathogens have emerged dramatically, while the common ones have decreased in number. A decade ago*Candida albicans* was considered the most important etiological agent, non albicans species were considered as contaminants. These have emerged as important pathogens, expressing inherent or acquired resistance to azoles. Non albicans*Candida* are increasingly being isolated from clinical specimens. The conventional methods of identification are time consuming and difficult to perform. This study was done to evaluate the performance of conventional identification methods and commercially available chromogenic media for the speciation of*Candida* species in a routine clinical microbiology laboratory. This prospective study was conducted in the Department of Microbiology at VIMS & RC. A total of 100*Candida* isolates from various clinical specimens were taken up for the study over a period of 1 year from out patients and in patients 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, immunodeficiencies, HIV status, diabetes mellitus, pregnancy, malnutrition, any ongoing treatment, burns, cancer and any other co morbid conditions. The*Candida* isolates were speciated by using standard conventional methods and chromogenic agar. A total of 100 clinical isolates of *Candida* from various clinical specimens were processed during the study period. The rate of isolation of*Candida* was highest (31) in high vaginal swab followed by urine (24). Six species of *Candida* were characterized: *Candida albicans* was the predominant species (46%). The following non-albicans species were isolated – *Candida glabrata* (26%), *Candida tropicalis* (22%), *Candida krusei* (3%), *Candida kefyr* (2%) and *Candida parapsilosis* (1%). The overall isolation of non- albicans species was 54%. *Candida albicans* was more frequently isolated from high vaginal swabs (15/46, 33%) followed by urine (10/46, 22%) and sputum (9/46, 19.5%), *Candida glabrata* from urine samples (12/26, 46%). *Candida tropicalis* was found in high vaginal swab (11/22, 50%). Candidiasis was common between16 and 30 years followed by 31–45 years. Females were more affected when compared to males. Diabetes mellitus was the commonest underlying predisposing factor followed by previous use of antibiotics/steroids. There was an agreement in identification by chromogenic agar method in 98% strains. The species level identification of *Candida* is important due to variation in sensitivities of various species to different antifungals and also due to limited therapeutic options because of emergence of resistance to antifungals. Speciation can greatly influence the treatment options and have an impact on the patient care. This study highly recommends the use of chromogenic media which aids in early and easy species level identification of *Candida*.

Introduction

Yeast like fungi are ubiquitous in nature. Yeast like fungi particularly *Candida* species are the commonest cause of fungal infections. Candidiasis is a primary or secondary infection involving a member of the genus *Candida*, however, the disease is an infection caused by *Candida albicans*.

Man lives in a delicate balance with *Candida albicans* which is an integral part of human microbial flora. *Candida* is frequently isolated from the mouth, gut, vagina and skin of the normal population. Since *Candida albicans* is an endogenous species the disease represents an opportunistic infection. The genus *Candida* includes several species implicated in human pathology.

The pathological processes evoked are also diverse and vary from irritation and inflammation to chronic and acute suppuration or granulomatous response. Pregnancy, increasing incidence of HIV infections, widespread and indiscriminate use of broad spectrum antibiotics, use of therapeutic modalities for advanced life support, organ transplantation, implantation of prosthetic devices and the emergence of resistance to antifungal agents have continued to be important in the expanding incidence of Candidial infections.

Nosocomial infections with *Candida* species have been on a significant rise over the past decade.

The clinical manifestations of the disease are extremely varied ranging from acute, subacute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis (Rippon, 1974; Jagdish Chander, 2002; Topley and Wilson, 1998).

Majority of the infections are caused by *Candida albicans*, however, past decade has seen a transition wherein there has been an upsurge in infections caused due to non-albicans species of *Candida*. *Candida* species have, become resistant to the anti-fungal agents, in particular to the azole compounds, by the expression of the efflux pumps that reduce drug accumulation, the alteration of the structure or concentration of the anti-fungal target proteins and by the alteration of the membrane sterol composition. The clinical consequences of the anti-fungal resistance can be seen as the treatment failure in the patients (Srujana Mohanty *et al.,* 2007).

The longer turnaround time taken by conventional methods of identification makes them less popular among the clinicians as early diagnosis is essential for initiating appropriate therapy. In order to facilitate rapid identification, several chromogenic substrate containing culture media are used (Sagar *et al.,* 2013).

Hence this study was undertaken to identify and speciate *Candida* in various clinical isolates based on their biochemical properties and to evaluate the importance of chromogenic media in rapid speciation of *Candida*, such a study is needed to evaluate the incidence of Candidial infections and to establish its properties which might be helpful in elucidating the role of this organism in the causation of nosocomial infections and its effect on the patient and society in large, especially in this era of antibiotics, medical developments and immunedeficiencies, in turn facilitating the development of effective measures to prevent and control transmission of resistant pathogens.

Material and Methods

This prospective study was conducted in the Department of Microbiology at VIMS & RC. A total of 100 *Candida* isolates from various clinical specimens (urine, high vaginal swabs, sputum, wound swabs, in dwelling catheters, aspirated fluids, blood, BAL, skin scraping and nail clippings) were taken up for the study over a period of 1 year from out patients and in patients 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, immunodeficiencies, HIV status, diabetes mellitus, pregnancy, malnutrition, any ongoing treatment, burns, cancer and any other co morbid conditions.

Processing of samples

The various clinical specimens were collected and processed as per the standard microbiological procedures. The *Candida* isolates which were obtained were further speculated by the germ tube test (Figure 1), chlamydospore formation on corn meal agar (Figure 2& 3), sugar assimilation (Figure 4) and sugar fermentation test.

Growth on chromogenic agar: Isolated species were inoculated on Hi Chrom *Candida* differential agar to improve species identification based on coloured colony morphology which is based on the reactions between the specific enzymes of the different species and the chromogenic substances. These agar plates were incubated at 37°C for 48 hours. The species were identified by characteristic colony colour as per HiMedia technical data (Sagar *et al.,* 2013; Shyamala K. Shettar *et al.,* 2012).

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

Results and Discussion

A total of 100 clinical isolates of *Candida* from various clinical specimens were processed during the study period. Total of 31 isolates were obtained from high vaginal swab followed by urine (24), sputum (17), wound swabs (10), in dwelling catheters (05), aspirated fluids (04), blood (03), broncho alveolar lavage (BAL) (03), skin scraping and nail clippings (02) and (01) respectively (Table 1).

In the present study six species of *Candida* were characterized using standard conventional methods. *Candida albicans* was the predominant species (46%). The following non-albicans species were isolated -*Candida glabrata* (26%), *Candida* tropicalis (22%), *Candida krusei* (3%), *Candida kefyr* (2%) and *Candida parapsilosis* (1%). The overall isolation of non- albicans species was 54% (Table 2).

Higher incidence of *Candida albicans* was found in the high vaginal swabs (15/46, 33%) followed by urine (10/46, 22%) and sputum (9/46, 19.5%) (Table 1).

Candida glabrata was more frequently isolated from urine samples (12/26, 46%) (Table 1).

Candida tropicalis was found in high vaginal swab (11/22, 50%) (Table 1).

Candida krusei was isolated from blood (1/3, 33%), in dwelling catheters (1/3, 33%) and aspirated fluids (1/3, 33%) (Table 1).

Age wise distribution showed 35 %

prevalence of Candidiasis in the age group of 16-30 years and 29 % and 17% in the age group of 31-45 years and 46-60 years respectively. The rate of isolation of the *Candida* species was more in females (64%) than in males (36%) (Table 3).

Diabetes mellitus was the commonest underlying predisposing factor accounting up to 28% followed by previous use of antibiotics/steroids (26%), in dwelling catheters (13%), usage of oral contraceptive pills (OCPs) and pregnancy constituting 9% each, prolonged hospitalization (7%), usage of intra uterine contraceptive device (5%) and HIV infection accounting up to 3% (Table 4).

100 *Candida* isolates were also subjected to identification using chromogenic agar. There was an agreement in identification by chromogenic agar method in 98% strains. Two strains which were identified as *Candida glabrata* by the conventional methods were identified as *Candida albicans* by chromogenic agar, however conventional method identification was taken into final consideration. All the isolates of *Candida* tropicalis and *Candida* krusei were correctly identified by chromogenic agar.

Candida species colonise the mucosal surfaces of all the humans soon after birth. Increasing incidence of iatrogenic *Candida* infections and the infections in immune-compromised and immunosuppressed individuals are due to the collective role of the fungal virulence factors and host susceptibility. In the immunocompetent several host conditions predispose to fungal infections like prolonged antibacterial therapy, corticosteroid use, integumentary breach as in intravenous or intra arterial catheters, surgical procedures, poor nutritional status and metabolic derangements. The extensive use of antimycotic drugs for prolonged therapeutic courses has led to a change in the relative prevalence of various species of *Candida*. Past two decades have seen a significant rise in mycotic infections (Jayalakshmi *et al.*, 2014).

A total of 100 *Candida* isolates from various clinical specimens were included in our study, of which 31 isolates of high vaginal swab yielded *Candida* such a finding was seen by Shivanand Dharwad and Saldanha Dominic (2011) and Sumitra Devi and Megha Maheshwari (2014) where in rate of isolation of *Candida* was 38% and 33.3% respectively. *Candida* may be either a commensal or a pathogen of the vagina, a fact which indicates, that changes in the vaginal microenvironment by hormones, antibiotics or metabolic disorders are generally necessary for *Candida* to induce pathological changes associated with clinical symptoms.

24 isolates of *Candida* were obtained from urine. Candiduria is becoming an important nosocomial infection as also observed by previous authors. The major contributing factors are indwelling catheters, prior antibiotic usage, advanced age, pregnancy and diabetes mellitus. Candiduria is rarely seen as a community acquired in a structurally normal urinary tract and in healthy individuals. Catheterization increases chances of UTIs by allowing migration of the organisms into the bladder from external periurethral surface. Use of broad spectrum antibiotics favours *Candida* colonization by suppressing commensal bacterial flora. Diabetes increases Candidial colonization by promoting stasis of urine in neurogenic bladder (Abhijit Awari, 2012; Deorukhkar and Saini, 2014; Jain and Dogra, 2011). 17 isolates of sputum were culture positive for *Candida*. The isolation of *Candida* from culture of sputum, endotracheal aspirates, bronchoscopic

samples, percutaneous lung needle aspirates and even from lung tissue may only represent colonization of the tracheobronchial tree. Despite the debate about the diagnosis of pulmonary Candidiasis, the definite diagnosis of pulmonary Candidiasis still rests on the histological demonstration of the yeast in lung tissue with associated inflammatory changes (Jha *et al.,* 2006). Isolation of *Candida* from wound swabs, indwelling catheters, aspirated fluids, blood and BAL reinforces the importance of these fungi in causation of nosocomial infections (Neetu Jain *et al.,* 2012).

100 culture positive isolates, were subjected to further tests for the characterization of the species which revealed that *Candida albicans* was the most frequent etiological agent which accounted for 46% of the isolates, similar findings have been reported by Shivanand Dharwad and Saldanha Dominic *et al.* (2011) and Tavleen Jaggi *et al.* (2014) where in the rate of isolation was 47% and 44% respectively. Higher incidence of non albicans *Candida* ranging from 50 to 74% have been seen in various studies. Among the non albicans species, *Candida* tropicalis and *Candida glabrata* were reported to be the most predominant species. In the present study *Candida glabrata* was more commonly isolated followed by *Candida* tropicalis (Shivanand Dharwad and Saldanha Dominic *et al.,* 2011; Sumitra Devi and Megha Maheshwari, 2014; Tavleen Jaggi *et al.,* 2014; Vijaya *et al.,* 2011).

These non albicans yeasts are relatively non pathogenic but ultimately get selected and start appearing more frequently because of the widespread abuse of over the counter antifungals, use of single dose oral or topical azole regimens and long term maintenance regimens of oral azoles. *Candida albicans* eradication by these means causes a selection of non albicans that are resistant to commonly used drugs. Therefore culture is valuable for identifying the species of *Candida* and to monitor the changing trends in the microbiology of systemic Candidiasis. Though Candidiasis can occur at all ages, studies by several authors [8. 15, 17] showed the highest incidence of Candidiasis to be in the age group of 21-40 years. These findings were in concurrence with the present study, where the age group of 16-30 years followed by 31 -45 years had the highest incidence of Candidiasis.

In the present study, there was a female preponderance, 64% of the females were affected by *Candida* infection as compared to 36% of the males, such a observation has been documented by many authors around the world (Shivanand Dharwad and Saldanha Dominic *et al.,* 2011; Abhijit Awari, 2012; Amar C. Sajjan and Mahalakshmi, 2014).

The association of the risk factors in all the 100 patients from whom the *Candida* species were isolated were studied. diabetes mellitus was the most frequently associated risk factor. Experimental evidence in vitro shows that a glucose concentration of 150mg/100ml increases the growth of *Candida*, an increase in the concentration of glucose in the tissues, blood and urine promotes the growth of *Candida* [18]. Shivanand Dharwad and Saldanha Dominic *et al.* (2011) and Amar C. Sajjan and Mahalakshmi *et al.* (2014) found the incidence of Candidiasis among diabetics to be high as compared to other factors where in the rate of isolation was 32% and 33% respectively. The findings (28%) of the present study correlated well with other studies, diabetes mellitus being the commonest predisposing feature. A history of drug usage was the second most frequently associated risk factor (26%) as

also seen by Shivanand Dharwad and Saldanha Dominic *et al.* (2011) and Dr Amar C. Sajjan and Mahalakshmi *et al.* (2014). The drugs implicated were corticosteroids, antibiotics and antifungal agents. Antibiotics suppress the bacterial flora, which allows the colonization by *Candida* species, however the risk of yeast infection increases with the duration of treatment.

OCPs leads to increased colonization by *Candida*, the infection rate is high among the pill users and this might be because of similarity between the mechanism operating during pregnancy and high estrogen OCP. The increased carriage is thought to result from the effects of hormones on epithelial cell adherence or from the glycogen and substrates available to the microorganisms as well as the direct effect of OCP on the yeast virulence (Joharah M. Al Quaiz., 2000). Nine patients on oral contraceptives had vulvovaginitis.

Table.1 Distribution of *Candida* species in various clinical isolates

S.No	Sample	Total no	C. albicans	C. glabrata	C. tropicalis	C. krusei	C. kefyr	C. parapsilosis
1	High vaginal swab	31	15	4	11	-	1	-
2	Urine	24	10	12	2	-	-	-
3	Sputum	17	9	3	5	-	-	-
4	Wound swabs	10	5	2	2	-	1	-
5	In situ catheters	5	-	2	1	1	-	1
6	Aspirated fluids	4	1	2	-	1	-	-
7	Blood	3	2	-	-	1	-	-
8	BAL	3	1	1	1	-	-	-
9	Skin scraping	2	2	-	-	-	-	-
10	Nail clippings	1	1	-	-	-	-	-
	Total	**100**	**46**	**26**	**22**	**3**	**2**	**1**

Higher incidence of *Candida albicans* found in the high vaginal swabs (15/46, 33%) followed by urine (10/46, 22%) and sputum (9/46, 19.5%). *Candida glabrata* was more frequently isolated from urine samples (12/26, 46%). *Candida* tropicalis was found in high vaginal swab (11/22, 50%). *Candida* krusei was isolated from blood (33%), indwelling catheters (33%) and aspirated fluids (33%).

Table.2 Distribution of different species of *Candida*

Sl. no	*Candida* species	Number
1	*Candida albicans*	46
2	*Candida glabrata*	26
3	*Candida* tropicalis	22
4	*Candida* krusei	3
5	*Candida* kefyr	2
6	*Candida* parapsilosis	1
	Total	**100**

Candida albicans was the predominant species (46%). The following non-albicans species were isolated – *Candida glabrata* (26%), *Candida* tropicalis (22%), *Candida* krusei (3%), *Candida* kefyr (2%) and *Candida* parapsilosis (1%). The overall isolation of non- albicans species was 54%.

Table.3 Age and sex wise distribution of patients with Candidiasis

Sl. no	Age of patients	Males	Females
1	0-15 years	4	3
2	16-30 years	8	27
3	31-45 years	10	19
4	46-60 years	6	11
5	>60years	8	4
	Total	36	64

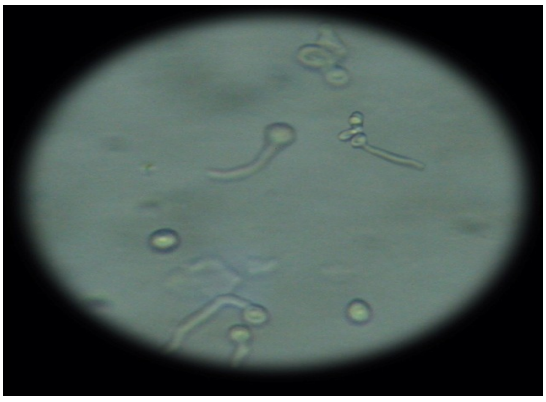
Age wise distribution showed 35 % prevalence of Candidiasis in the age group of 16-30 years and 29 % and 17% in the age group of 31-45 years and 46-60 years respectively. The rate of isolation of the *Candida* species was more in females (64%) than in males (36%)

Table.4 Distribution of predisposing factors in patients with Candidiasis

Sl. No	Predisposing factors	No. of patients
1	Diabetes mellitus	28
2	History of antibiotics/steroids intake	26
3	Oral contraceptive pills	9
4	Pregnancy	9
5	Prolonged Hospitalization	7
6	Use of Intrauterine contraceptive devices	5
7	In dwelling catheters	13
8	HIV positive	3
	Total	**100**

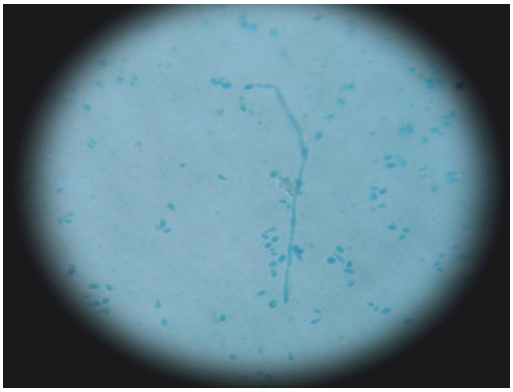
Diabetes mellitus was the commonest underlying predisposing factor accounting up to 28% followed by previous use of antibiotics/steroids (26%), in dwelling catheters (13%), usage of oral contraceptive pills (OCPs) and pregnancy constituting 9% each, prolonged hospitalization (7%), usage of intra uterine contraceptive device (5%) and HIV infection accounting up to 3%.

Figure.1 Germ tube test



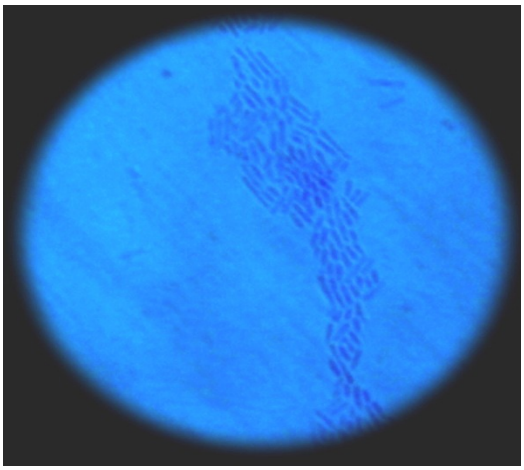
Germ tube test in *Candida albicans*

Figure.2 Morphology on corn meal agar (CMA) of *Candida albicans*



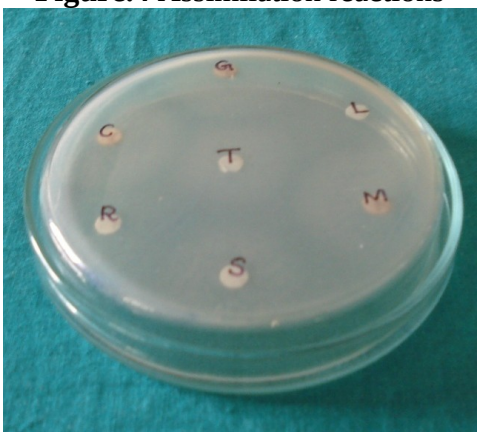
Terminal chlamydospores, yeast cells and pseudohyphae of *Candida albicans* on CMA

Figure.3 Morphology on corn meal agar (CMA) of *Candida* kefyr



"Logs in stream appearance of *Candida* kefyr on CMA.

Figure.4 Assimilation reactions



Assimilation reactions of *Candida* tropicalis
G- glucose, L- lactose, M- maltose,
S- sucrose, R- raffinose, C-cellobiose, T- trehalose

It has been established in literature that the prevalence of genital Candidiasis increases in pregnancy. The high hormone level leads to increase in the glycogen content of the vagina, thus producing a favourable environment for the growth of *Candida*. The reduced glucose tolerance and the increased incidence of glycosuria render some patients more susceptible to Candidiasis (Warnock *et al.*, 1979; Joharah M. Al Quaiz, 2000). In the present study, nine pregnant women had Candidiasis. Candidial vulvovaginitis in mother can also result in oral thrush in the new born. Intrauterine contraceptive devices (IUD) are commonly associated with an endogenous infection 5 women who were using IUDs had vaginal Candidiasis.

The other significant risk factors were the presence of indwelling catheters (13%), prolonged hospitalization (7%) and HIV infection (3%). Patients with these underlying risk factors are at an increased risk of developing Candidemia and invasive mycosis (Jha *et al.*, 2006).

Chromogenic agar has the advantage of rapid identification of *Candida* species, technically simple, rapid and cost effective compared to technically demanding time consuming and laborious conventional method. Though the results on chromogenic agar paralled that of conventional methods, 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 fungi and this capability will also enable clinicians to choose appropriate anti fungal agents, thus decreasing patient's morbidity and mortality (Birgit Willinger and Manafi, 1999).

Dawn of 21st century has seen the emergence of non albicans *Candida* in causation of spectrum of infections. This shift has generated the concern as these species are either inherently resistant or has acquired resistance to commonly used antifungal agents as compared to *Candida albicans*. Changing epidemiology, nonspecific risk factors, clinical presentation and late diagnosis with culture-based methods are major challenges in the management of invasive Candidiasis. The present study recommends the use of CHROM agar for speciation of *Candida*. Continuous monitoring in trends of species distribution is essential to control and optimize the therapy of *Candida* infection which will eventually have an impact on patient care and society in large.

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