INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 15, No. 4, p. 1-10, 2022

Open Access RESEARCH PAPER

Phenotypic identification of *Candida* species from various clinical samples in a resource limited setting

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Keywords: *Candida* speciation, CHROM agar, *Candida albicans,* Non-albicans *Candida,*

Hi Candida identification kit

Publication date: October 10, 2022

Abstract

Candidiasis is worldwide in distribution, and is one of the common fungal diseases isolated in man, which affects the skin, mucosa and various internal organs. It is caused by various species of *Candida,* which is a yeast-like fungi that produce pseudohyphae. Speciation helps to understand the epidemiology of *Candida* species particularly, the source and mode of transmission of resistant pathogens. Various commercially available chromogenic agar medium has been studied and evaluated for presumptive identification of various species of *Candida*. The present study was conducted for a duration of 12 months from the month of July 2019 to the month of July 2020, in the Department of Microbiology at a medical college in Siddipet, with prior approval of institutional ethics committee. The present study was aimed at isolating and identifying the *Candida* species from various clinical samples by using chromogenic media for easy and rapid speciation in addition to the time consuming and labour-intensive conventional methods. Among the *Candida* isolates the most frequently isolated species was found to be *Candida albicans*. In the present study *non-albicans Candida (NAC)* (50.91%) had predominance over *Candida albicans* (49.09%). In our study, an increase in the number of cases caused by *NAC* was noted though the most common species isolated was *Candida albicans. CHROMagar* was found to be a simple, easy and also a rapid method for *Candida* species detection. It considerably reduced the turn-around-time.

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Introduction

Candidiasis is worldwide in distribution, and is one of the common fungal diseases isolated in man, which affects the skin, mucosa and various internal organs. It is caused by various species of *Candida,* which is a yeast-like fungi that produce pseudohyphae. (Apurba S Sastry, Essentials of Medical Microbiology, 3rd Ed) The genus *Candida* belongs to Phylum: *Imperfectii*, the Order *Moniliales* and family *Cryptococcaceae*. (Chander J. Candidiasis. In: A textbook of Medical Mycology, 3rd Ed.) C*andida* is a human commensal, but it becomes an opportunistic pathogen because of any pre-disposing factors which impair, immune response to the micro-organisms.eg: AIDS, immunosuppressive chemotherapy, metabolic diseases, or cause an imbalance, in favour of fungal microflora e.g., Antibiotics, disrupt the integrity of the integument e.g., intravenous catheters, surgery. The source of infection is mostly endogenous, but in some cases, *Candida* can be introduced by exogenous sources too (Esther Segal and Daniel Elad. Candidiasis. Topley and Wilson's Microbiology and Microbial Infections. Medical Mycology. 10thEd.) Fungal infections, especially those caused by *Candida* species, has significantly increased, in the past decade, in immuno- compromised patients. (Dharmeswari T., 2014)

The conventional method of fungal culture on SDA is tedious and often isolation and detection are difficult in mixed cultures. This led to the use of many chromogenic agar which enables the detection based on colour of the colony. (Baradkar VP, 2010; Moyer GJ, 1995; Murray MP, 2005; Louwagie B, 1995; Odds FC, 1994; Pfaller MA, 1996; Raut SH, 2009). Speciation of various species of *Candida* is based on the colour of the colony along with other characteristic. This differentiation is facilitated by the presence of chromogenic substrates that produce different pigmentations based on specific enzymes produced by the specific candida species. (Lynn L., 2003)

CHROMagar *Candida* is of great use in clinical specimens suspected to contain yeast and hence can be used as a medium for primary isolation even in a resource limited setting as it requires less expertise. Moreover, it can act as a differential medium even if candida is isolated from other media like SDA or blood agar. (Odds FC, 1994) Though the biggest limitation of the medium could be the cost but the advantage in reducing the turn-around time can still be of great significance when compared to various conventional methods of identification.

Speciation helps to understand the epidemiology of *Candida* species particularly the source and mode of transmission of resistant pathogens. (Shaheen M.A, 2006) Conventional methods for speciation are more time consuming and laborious, therefore, in the present study CHROMagar has been used for rapid identification of various species of *Candida.*

The present study was aimed at isolating and identifying various *Candida* species from clinical samples by using chromogenic media for easy and rapid speciation in addition to the time consuming and labour-intensive conventional methods.

Material and methods

Source of data & Study period

The present study was conducted for a duration of 12 months from the month of July 2019 to the month of July 2020, in the Department of Mycology at a medical college in Siddipet, with prior approval of institutional ethics committee.

Sample size

A total of 950 clinical specimens were screened from patients visiting the OP department and patients admitted to various units.

Samples included in the study

The samples included high vaginal swabs (HVS), urine, blood, oral and throat swabs, wound swabs, sputum and Broncho alveolar lavage fluid (BAL).

Processing of specimens

The clinical specimens were directly examined by wet mount and also by gram staining. The clinical specimens which were positive for the presence of yeast cells on KOH wet mount and Gram stain smear positive for gram positive oval yeast-like budding cells (4-8µ) and/or pseudohyphae on microscopic examination were further processed for isolation, and species identification.

Isolation and species identification

Sabourauds Dextrose Agar (SDA) was used for fungal culture and was inoculated with the specimen. The medium was inoculated for 24-48 hours at 37^0C temperature. The growth of the *Candida* was seen as creamy paste-like colonies. Identification of all isolates was done to species level with the help of sugar assimilation test by *HiCandida* identification kit, demonstration of germ tube by germ tube test, chlamydospore formation on cornmeal agar and sugar fermentation test.

Growth on HiCrome Candida differential Agar

Simultaneously all the *Candida* isolates were inoculated on *HiCrome Candida* differential Agar. As per manufacturer's instructions based on the colour of the colonies, the species of *Candida* were identified as shown in table (1).

Table 1. Various colours of colonies of *Candida* species on CHROMagar.

Candida species	Colour of colonies
Candida albicans	Light –green
Candida tropicalis	Dark blue
Candida glabrata	Cream to white
Candida krusei	Purple and fuzzy
Candida parapsilosis	Cream to pale pink
Candida dubliniensis	Dark –green

The conventional methods were compared with CHROMagar. All the culture media were prepared in the lab using commercially available dehydrated media obtained from Himedia Laboratories Mumbai, India.

Carbohydrate assimilation test

Sugar assimilation was done using HiCandida Identification kit. This kit utilizes 12 biochemical Test and is a standardized colorimetric identification system. The first well contains medium for Urease detection test. Well 2-12 has medium for Carbohydrate Utilization test (with eleven different sugars in respective wells as Melibiose, Lactose, Maltose, Sucrose, Galactose, Cellobiose, Inositol, Xylose, Dulcitol, Raffinose, and Trehalose. The main principle of the test is based on substrate utility and P^H change. The colonies are identified by colour change in the media which occur due to metabolic changes of the organisms. Results were interpretated and candida species identified as per the manufacturer's instructions.

Results and discussion

A total of 950 clinical specimens were screened from patients visiting the OP department and patients admitted to various units. The number of candida species isolated from various specimens were 110 isolates in total. All the *Candida* isolates obtained were preliminarily screened by direct wet mount and Gram stain, later by other selection criteria were subjected to culture. The obtained isolates were processed further and speciated using germ tube test, growth on CMA, Carbohydrate fermentation and Carbohydrate assimilation and colour of the growth in CHROMagar.

Along with the conventional methods, HiCrome *Candida* Differential Agar (Himedia, India), a chromogenic medium has also been used for identification of *Candida* species. Quick speciation of various isolates was based on reactions between the chromogenic substrates present in the media with the specific enzymes produced by various species.

The highest number of isolates were from HVS constituting 43 (39.09%) followed by urine 32 (29.09%), sputum 21 (19.09%), blood 5 (4.54%), oral and throat swabs 5 (4.54%), wound swabs 2(1.81%) and BAL 2 (1.81%) as shown in Fig. 1. The clinical manifestations of the disease were found to be extremely varied ranging from simple mucocutaneous candidiasis to invasive candidiasis.

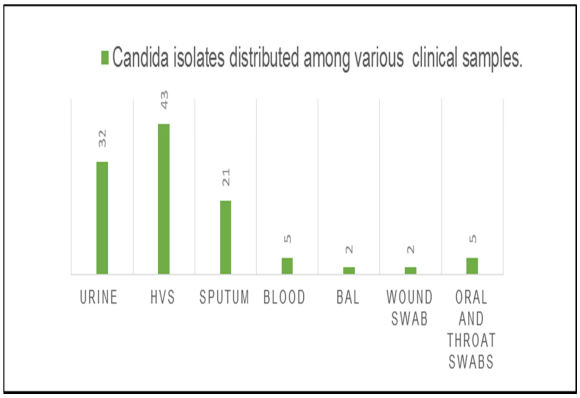

Fig. 1. Candida isolates distributed among various clinical samples

The major species isolated, was *Candida albicans* which accounted for 54 isolates (49.09%) amongst the total isolates, followed by *Candida tropicalis* 36(32.72%), *Candida parapsilosis* 8(7.27%), *Candida krusei* 6(5.45%), *Candida glabrata* 5(4.54%) and *Candida dubliniensis* 1 (0.90%) as shown in Fig. 2. Comparative studies on *Candida* species isolated by various researchers are shown in table (2) which can be compared with the present study. Growth of *Candida* species on CHROMagar and pattern of Candida species on HiCandida Identification kit for Carbohydrate assimilation test are shown in Fig.3 and Fig. 4 respectively.

Table 2. *Candida* species isolated by various workers.

Candida species isolated	Present study 2019	Rajeshwari Prabhakar Rao *et al* 2018	P.T. Rudrappa *et al* 2018	Kanna BV *et al* 2017	Shan 2016	Bhavana 2016	Pahwa 2014
Candida albicans	49.09%	54%	30%	51%	41%	35%	42.2%
Candida tropicalis	32.72%	24%	40%	26%	35%	39%	22.4%
Candida parapsilosis	7.27%	-	22.66%	-	3%	6%	6.3%
Candida krusei	5.45%	14%	1.33%	16%	-	13%	3.4%
Candida glabrata	4.54%	7%	2.66%	6%	14%	7%	3.8%
Candida dubliniensis	0.90%	1%	-	1%	6%	-	-

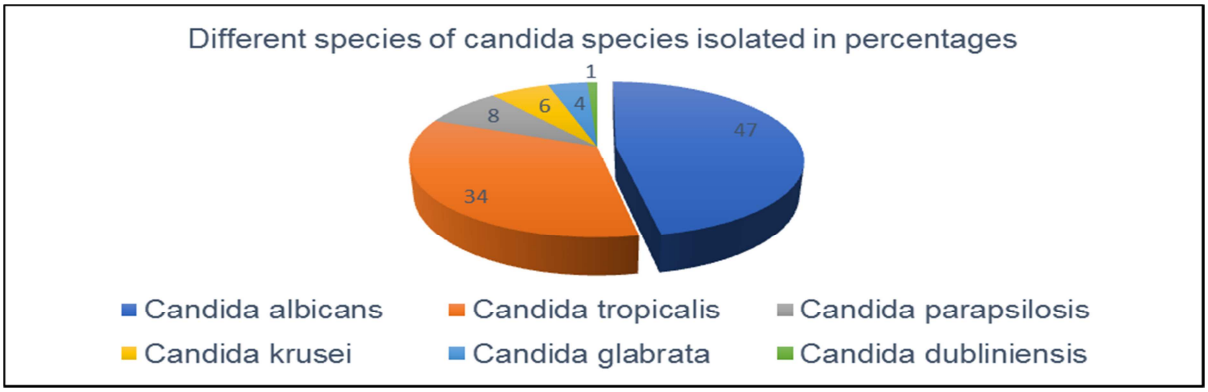

Fig.2. Different species of candida species isolated in percentages

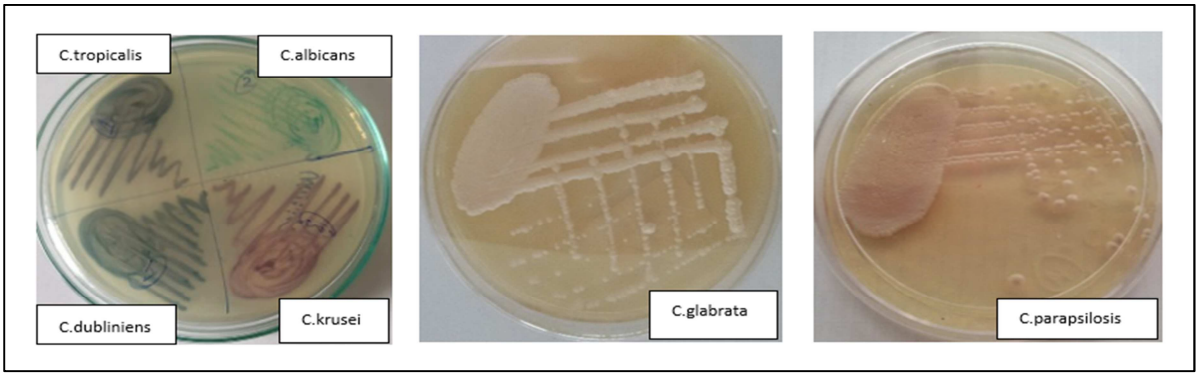

Fig. 3. *Candida* species on CHROMagar

Fig. 4. *Candida* species on HiCandida Identification kit for Carbohydrate assimilation test. CA=*Candida albicans,* CT=*Candida tropicalis,* CP=*Candida parapsilosis,* CK=*Candida krusei,* CG=*Candida glabrata,* CD=*Candida dubliniensis*

In the present study *NAC* (50.91%) had predominance over *Candida albicans* (49.09%) as shown in Fig. 5. It is in concordance with the other similar studies reported from various regions as represented in table (3). *Candida albicans* was the most frequently isolated species from all clinical samples, except Blood and BAL where the most predominantly isolated species was *Candida tropicalis* as shown in Fig. 6.

Table 3. Comparison of *Candida albicans* and *Non-albicans Candida isolated* by various workers.

SN	Work done by	Year	*Candida albicans*	Non-albicans Candida
1	Present study	2019	49.09%	50.91%
2	Rudrappa *et al*	2018	30.66%	69.33%
3	Bhavana *et al*	2016	35%	65%
4	Shah *et al*	2016	41%	59%
5	Jaggi *et al*	2014	44%	56%

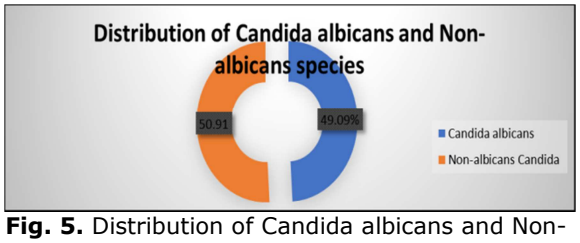

Fig. 5. Distribution of Candida albicans and Non-albicans species

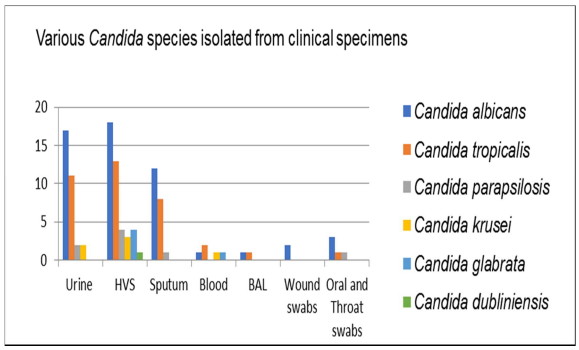

Fig. 6. Various *Candida* species isolated from clinical specimens

A correlation of risk factors among the positive cases for *Candida* species was done. It was observed that a history of prolonged usage of corticosteroids, broad-spectrum antibiotics and chemotherapeutic agents was the most frequently associated risk factor as shown in Fig.7. Corticosteroids and chemotherapeutic agents result in a state of immune suppression.

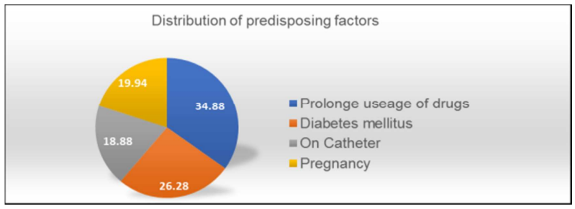

Fig. 7. Distribution of predisposing factors

According to Rippon (Rippon JW. Candidiasis and the pathogenic yeasts. Medical Mycology, 3rd.Ed.) prolonged usage of antibiotics predisposes host tissue to invasion by organism, and the antibiotic itself stimulates the growth of *Candida*. The most important effect of antibiotics is the elimination and alteration of the bacterial flora that holds the population of *Candida* in check. Use of antibiotics was also associated with prolonged hospital stay.

The next important associated risk factor was Diabetes mellitus. (Khandari KC, 1969) It has been seen in various experimental studies that hyperglycaemia significantly increased the *Candida* growth (Warnock DW, 1979). This may probably be true in humans also that an increase in the concentration of glucose in the tissues, blood & urine promotes the growth of *Candida.* Uncontrolled glycaemic index is known to increase the glucose concentration in the tissues, urine and blood which leads to colonization of *Candida* and hence there is an increased risk of infections. Sobel & Colleagues reported a fucose (6-deoxy-galactose) vaginal epithelial cell receptor that aids in adhesion of *Candida* to vaginal epithelial cells. (Sobel JD, 1981)

About 60-70% women are affected by vaginal candidiasis in their reproductive age. (Jacqueline M. Achkar, 2010) Vaginal candidiasis was common in occurrence amongst women of child - bearing age group. The most common predisposing factor in the present study was found to be pregnancy. Our findings show agreement with other similar study. (Francisca I. Okungbowa, 2003) Factors favouring candidiasis in pregnant women is attributed to increased vaginal glycogen content and also due to an increase levels of reproductive hormones

Though *Candida albicans* was predominant spp. causing vulvovaginitis, (Vijaya D, 2014; Shivanand Dharwad, 2011), it was observed that the frequency of non-*albicans Candida* species as potential causes of the vaginal candidiasis has increased, which can also be compared with other studies. (Mondal S, 2013; Deepa Babin , 2013) Most frequently isolated *NAC* was *Candida tropicalis.* (Deepa Babin , 2013)

An important cause of nosocomial UTIs especially in elderly age group can be attributed to the various species of *Candida. Candida* is more commonly isolated from urine samples in elderly especially during prolonged ICU admission. *(Jacqueline* M. Achkar, 2010) Most of the cases having Candiduria were catheterized ICU patients. Catheterization even for a short duration enables the organisms from the peri-urethral surface to enter the bladder. This explains the increased risk of UTIs. Nayman Alpat *et al* had believed that long duration of ICU and hospital stay increase the incidence of Candiduria in patients. Candiduria is relatively frequent in patients with prolonged hospitalization and is rare in healthy people (N. Jain, *et al.,* 2007; Weinberger M *et al.,* 2003)

The commonest isolate was *Candida albicans* followed by *NAC* species. According to Behzadi *et al* the three *Candida* species of importance are *Candida albicans, Candida glabrata, and Candida tropicalis*. Despite the increase in number of UTIs caused by *NAC* species, *Candida albicans* still ranks first for fungal UTIs. (Behzadi P *et al.,* 2015)

The respiratory tract secretions (sputum, induced sputum, bronchial washings, BAL, and tracheal aspirations) are common specimens collected for fungal culture. (Bailey and Scott's, Diagnostic Microbiology. 13th ed) Various chronic lung conditions like Bronchiectasis, cavitary lesions of TB increase the risk of colonization of fungal organism including *Candida* species. In our study, the most common species isolated from sputum sample was *Candida albicans*, which is similar to other studies. (Pendleton *et al.,* 2017; Jha B.K. *et al.,* 2006)

Though *Candida* is part of normal flora of upper respiratory tract, when immunity is low it acts as

an opportunistic pathogen (M. Bharathi and A. Usha Rani, 2011) Patients with chronic lung pathology, provide a suitable nidus for fungal colonization. (Debasis Biswas *et al.,* 2010)

The need for clinical correlation is of great importance in asserting the clinical significance of pulmonary Candidiasis as demonstration of *Candida* doesn't necessarily imply to infection. (Esther Segal and Daniel Elad. Candidiasis. Topley and Wilson's Microbiology and Microbial Infections. Medical Mycology. 10thEd.)

The main limitation in the diagnosis of infections caused by *Candida* is attributed to the frequent contamination of samples like sputum with various commensal organisms. Hence isolation of *Candida* species from lung biopsy or translaryngeal aspirate could be of some significance.

Of the five *Candida* species isolated from blood, 1 was *Candida albicans* and the remaining were *NAC.* Among the *NAC, Candida tropicalis* was found to be predominant and, this was found in correlation with other studies. (N. Jain *et al.,* 2007; Shivaprakasha S *et al.,* 2007) The isolates were obtained from ICU patient who were on IV catheters. The ability of *Candida* species to adhere to various prosthetic devices and inanimate surfaces along with biofilm formation makes them resistant to various antifungal agents. (Yang YL *et al.,* 2003) Oral candidiasis produced by colonization of *Candida* species is also known as oral thrush. In our study, various species of *Candida* were isolated from elderly patients with dentures, patients with diabetes and individuals on cancer chemotherapy. Oropharyngeal Candidiasis can occur in individuals with diabetes mellitus, those receiving antibiotics, in infants, patients infected with HIV and chemotherapy. (Chander J. Candidiasis. A textbook of Medical Mycology, 3rd Ed.)

Immunosuppressed, burn patients receiving antibiotics for suppression of bacterial infection are ideal host for opportunistic fungi. (Desai MH *et al.,* 1987) In the present study *Candida* was isolated from wound swabs recovered from patients with burns on antimicrobial therapy.

The conventional methods were compared with CHROMagar, the sensitivity and specificity of CHROMagar for *Candida* isolates were in total agreement with that of the conventional methods. It was observed that CHROMagar helped in quick identification of *Candida* i.e., as quickly as 24 to 48 hours. The direct identification of species helps in determining the appropriate antifungal drug. The conventional methods require more technical expertise, it is labour- intensive when compared to the cost-effective CHROMagar used in our study.

Conclusion

Fungal infections, especially those caused by *Candida* species, has significantly increased, in the past decade, in immuno-compromised patients. (Dharmeswari T *et al.,* 2014) Prolonged administration of drugs secondary to underlying disease was the commonest predisposing factor. In our study, most common species of *Candida* causing infections was found to be *Candida albicans*. CHROMagar was found to be of great convenience in a resource -limited setting. It reduced the turn-around-time and was also found to be a sensitive and specific method of identification. HiCandida Identification Kit (used for sugar assimilation) can differentiate between various *Candida* species

Acknowledgements

All the author(s) are thankful to the clinical and technical staff of the hospital for extending their cooperation.

Conflict of interest

All the contributing author(s) declare no Conflict of interest.

Author's contribution

All the listed author(s) have made substantial, direct and intellectual contribution to the work and approved it for publication.

Funding

None.

Ethics statement

The study has been conducted with prior approval of institutional ethics committee.

Availability of data

All the data generated or analysed during the study are included in the manuscript.

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