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Speciation of Candida Isolates from Clinical Samples by using Conventional and Chromagar Method

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A B S T R A C T

Candida species have been reported from many countries worldwide and a significant morbidity & mortality in immunocompromised individuals and hospitalised patients. The *Candida* species are now among the four most common causes of hospital associated infections, catheter associated, urinary tract and blood stream infections. This was a cross sectional study in this the clinical samples received in the Microbiology laboratory for routine culture form the source. The study was conducted in the department of Microbiology JSSMC Mysore. A total of 75 isolates of Candia species recovered from urine, sputum, vaginal swab, ear swab and blood culture were used and identified by standard protocol. A total of 75 *Candida* species were collected from urine (72%), sputum (21%), swabs (2.6%) and Blood (1.3%) of patients. The goal of the study was to show there is an increase in the incidence of *Candida* non albicans. Majority of the isolates in our study were urine and throat samples, only few isolates were obtained from blood, vaginal swabs and ear swabs. So we obtained sensitivity and specificity of Chrom agar for *C. albicans* as 100%. As a result it can be concluded that the use of Chrom agar *Candida* is an easy reliable method for presumptive identification of most of the *Candida* species especially *Candida albicans, Candida tropicalis, Candida krusie, Candida parapsilosis* and *Candida dublineinsis*.

Introduction

Since the early 1980s there has been rise in the incidence and prevalence of fungal infections worldwide. The blood stream infections caused by various *Candida* species have been reported from many countries worldwide and a significant morbidity and mortality in immunocompromised individuals and hospitalised patients (Giri *et al.*, 2012).

The *Candida* species are now among the four most common causes of hospital associated infections, catheter associated, urinary tract and blood stream infections (Ahmad *et al.*, 2012).

The nosocomial *Candida* infections could be caused by the patient's own flora or could be acquired from exogenous sources or hands of health care workers. The most common pathogenic *Candida* species is *C. albicans* which is a major component of human microflora occurring normally on skin and mucosal surfaces. The vast majority of invasive *Candida* infections are associated by mainly four species which include *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and the role of other species is minor. However mini outbreaks caused by some species have occasionally been recorded among selected patient population (Sanjeev Kumar *et al.*, 2013; Elisa J Michael).

The importance of risk factors for analysis cannot be over emphasized for infections like Candidemia, so that the preventive measures and prophylactic therapy can be initiated for patients at risk. Many studies have been established independent risk factors for Candidemia on the basis of multivariate analysis. So we aimed at iidentification and speciation of *Candida* isolates from various clinical specimens by using simplified phenotypic identification scheme.

Materials and Methods

This was a cross sectional study in this the clinical samples received in the Microbiology laboratory for routine culture form the source. The study was conducted in the department of Microbiology JSSMC Mysore. A total of 75 isolates of *Candida* species recovered from urine, sputum, vaginal swab, ear swab and blood culture were used and identified by standard protocol. The ethical clearance was obtained from the institutional ethical committee.

The germ tube test (Reynolds-Broads phenomenon) is used as a presumptive test for identification of *Candida albicans*. The test was performed for identification of isolated cultures from clinical samples. A small amount of inoculum of test yeast cells was suspended in 0.5ml of human serum and incubated at 37^0C for 2 to 4 hrs. A wet mount preparation was made by using a Pasteur pipette, a drop of the suspension was placed on a clean dry glass slide and was covered with a clean cover glass. The slide was examined under a microscope for germ tube formation by using the 10X objective lens. A germ tube is a tube-like projection that extends from yeast cell known as germ tube. The 40X objective was used to confirm the presence or absence of germ tube. When the *Candida* with germ tube was seen, the culture was reported as *Candida albicans*. When they do not show germ tube, the culture was reported as non albicans *Candida* species.

The yeast and yeast like fungi has the ability to utilize the various carbohydrates as source of energy through assimilation and fermentation reactions. Sugar assimilation test is based on the ability of each *Candida* species that can assimilate a set of carbohydrates. It was performed by using Yeast Nitrogen Basal medium (YNB) and carbohydrate impregnated discs.

The suspected *Candida* isolates were inoculated on corn meal agar plate by making a 3 parallel cuts about half inches apart at a 45^0 angle to a culture medium and a sterile cover slip was added to one area. The inoculated Corn Meal Agar plates were incubated at 25^0C or at Room temperature for 48hours.

Chrom Agar *Candida* is a new differential culture medium intended for the isolation and rapid presumptive identification of the most common clinically important yeast species. The medium comprises per liter: Peptone-10grams, Glucose-20 grams, Chromogenic

mix-2 grams, Chloramphenicol-0.05 grams, Agar-15 grams) was prepared according to the manufactures instructions and dispensed 20ml to each 90mm petri plate and stored at 4^0C and used within 2 weeks.

Data collected was entered in Microsoft excel 2007 and analysed using Epi Info 3.4.3. Descriptive statistics such as proportion, mean and SD were used.

Results and Discussion

A total of 75 *Candida* species were collected from urine (72%), sputum (21%), swabs (2.6%) and Blood (1.3%) of patients. The goal of the study was to show there is an increase in the incidence of *Candida* non albicans.

Candida species distribution shown in Table 1 and from various samples are shown in Table 2. *Candida albicans* isolates were 30% (23/75). Other *Candida* species isolated were 40% of *Candida tropicalis* (30/75), 22.66% of *Candida parapsilosis* (17/75), 2.66% of *Candida glabrata* (2/75), 2.66% of *Candida guilliermondii* (2/75), (1.33%) *Candida krusei* (1/75), thus the overall prevalence of non albican *Candida* species 69.33% (52/75).

In the recent years the number of serious opportunistic in yeast infections particularly in immunocompromised patients have increased significantly (Kangogo *et al.,* 2011). *Candida* species accounts for 80% of infections. This study investigated a total of 75 *Candida* isolates from clinical sources in Microbiology laboratory, JSSMC Hospital, Mysore. Isolates were recovered from urine, sputum, vaginal & ear swabs and blood.

Majority of the isolates in our study were urine and throat samples, only few isolates were obtained from blood, vaginal swabs and ear swabs. 23/75 The *Candida albicans* (30%) was isolated from urine, sputum and vaginal swabs. The other *Candida* species isolated include 30/75 *C. topicalis* (40%), 17/75 C. *parapsilosis* 22.66%, 2/75 *C. gabrata* (2.66%), 2/75 *C. guilliermondii* (2.66%) and 1/75 *C. krusie* (1.33%). Many previous studies have been reported the non-albican *Candida* emerging as significant pathogens (Ananthanarayan and Paniker 9th edition: Michael Gherna *et al.,* 2009)

The detection and identification of yeast and yeast like fungi depend on the availability of easy to perform screening and cost effective methods. The medium most widely used for the isolation for the *Candida* and other yeast species from clinical specimens is Sabouraud dextrose agar (SDA). It is not a differential medium on which colonies of different *Candida* species or different pathogenic species of yeast cannot be distinguished easily from each other. For that could be used biochemical tests, growth on Corn meal agar and Chrom agar *Candida* used. The Chrom agar is a differential culture medium, allows the presumptive differentiation of yeasts.

It contains various substrates for the enzymes of yeast species. It has been demonstrated that β-N-Acetylgalactoseaminidase which was produced by *Candida albicans* enables the chromogenic substrates into the medium and the isolates to be incorporated into the medium and the isolates of these species were seen as green coloured colonies (Shawn *et al.,* 2009; Mine Yucesoy *et al.,* 2003).

Chorm agar is reported to give green colonies of *Candida albicans* and blue colonies of *Candida tropicalis*. In this study 23/75 *Candida* isolates showed germ tube positive and grew as distinctive light green colonies on Chrom agar were reported as *Candida albicans*. We don't have any *Candida dubliensis* isolates, differentiation of this species from *Candida albicans* seems to be as dark green colour on Chrom agar.

Table.1 *Candida* species distribution

Candida species distribution	Number	Percentage
C.albicans	23	30%
C.*tropicalis*	30	40%
C.*parapsilosis*	17	22.66%
C.*glabrata*	2	2.66%
C.*guilliermondii*	2	2.66%
C.*krusei*	1	2.66%

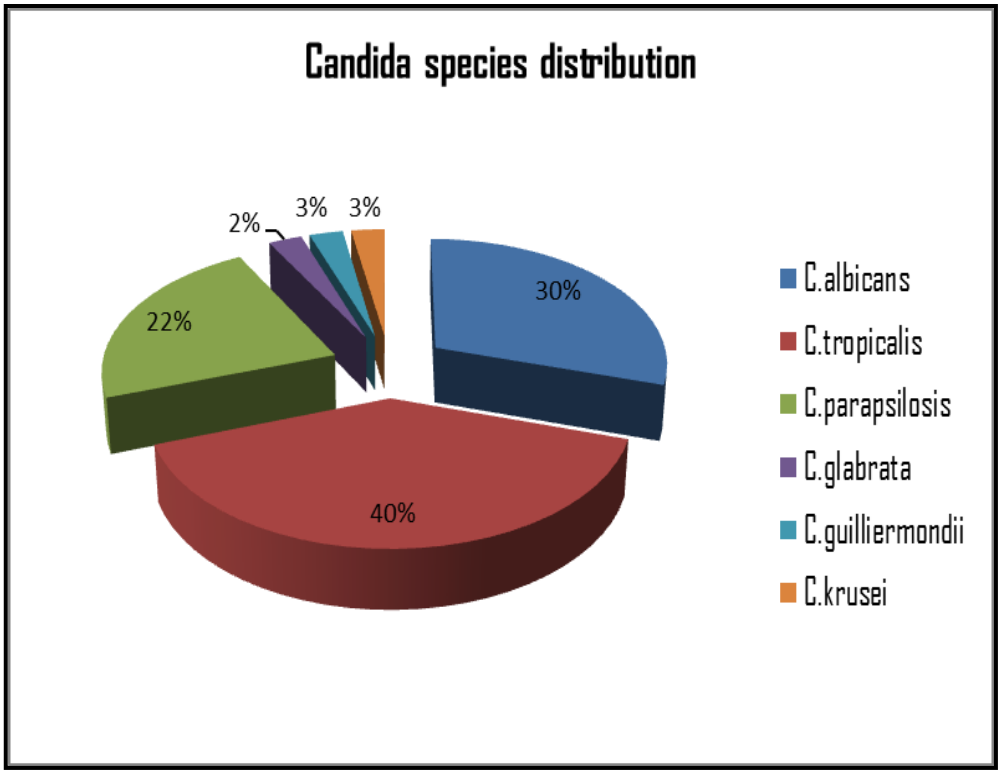
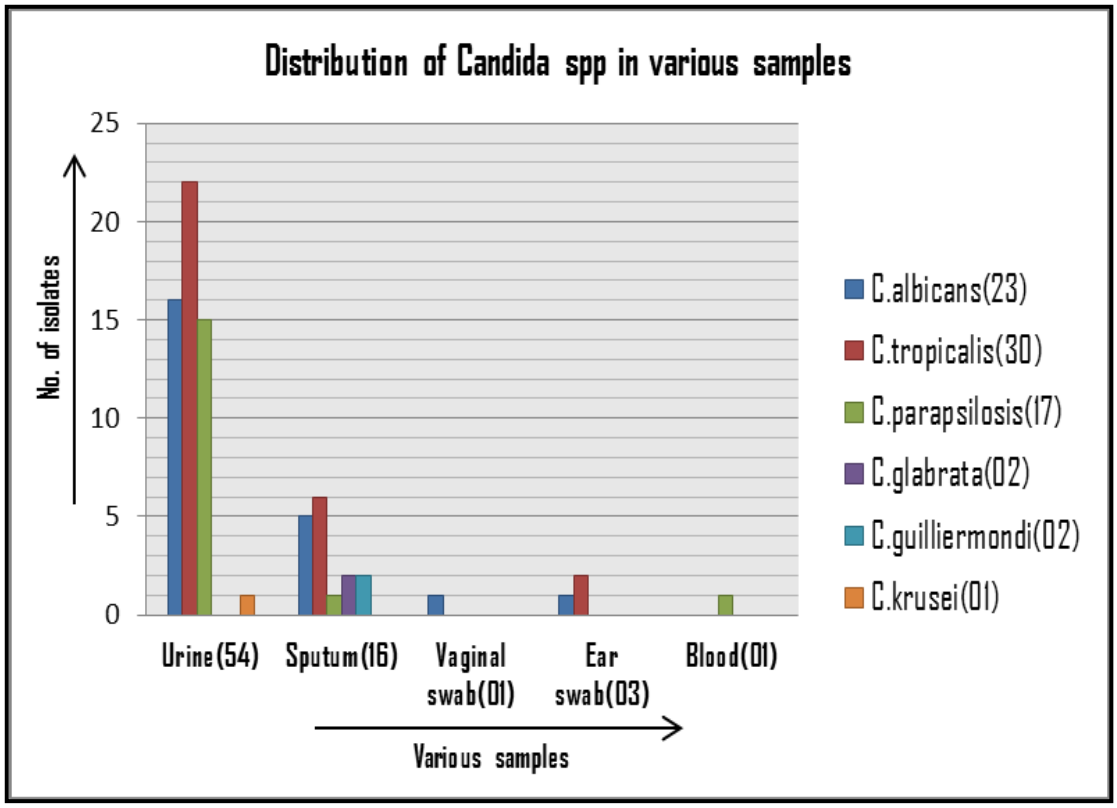


Table.2 Distribution of *Candida* species in various samples

Candida Species	Urine(54)	Sputum(16)	Vaginal swab(02)	Ear swab(03)	Blood(01)	Total (75)
C.albicans	16	05	01	01	00	**23**
C.tropicalis	22	06	00	02	00	**30**
C.parapsilosis	15	01	00	00	01	**17**
C.glabrata	00	02	00	00	00	**02**
C.guilliermondi	00	02	00	00	00	**02**
C.krusei	01	00	00	00	00	**01**



Tentelnot *et al.,* and willinger *et al.,* reported that some of *Candida dubliensis* isolates yielded a dark green colour. So we obtained sensitivity and specificity of Chrom agar for *C. albicans* as 100%. Our results are parallel with various studies at which these values are found to be 97 -100% and 100% (Pfaller *et al.,* 2007; Orazio Romea *et al.,* 2009; Mine Yucesoy *et al.,* 2003).

30/75 *Candida tropicalis* strains produced blue violet, smooth colonies with halo diffusion into the surrounding agar on chrom agar. Mine Yucesoy *et al.,* (2003) emphasized that they did not observed this appearance in their study and also found that the false positive results due to *S. cervisiae* isolates. In our study two isolates of *C. glabarata* were produced pink glossy colonies with pale edges, identified and correlated with the study of Pfaller *et al.,* (2007). In 17/75 isolates of *C. parasiplosis* and 2/75 *C. guillermondii* had shown various tones of colour from off-white to pink on chrom agar. These all chrom agar results correlated with biochemical and growth on corn meal agar results and confirmed the identified patterns of *Candida* species.

As a result it can be concluded that the use of Chrom agar *Candida* is an easy reliable method for presumptive identification of most of the *Candida* species especially *Candida albicans*, *Candida tropicalis*, *Candida krusie*, *Candida parapsilosis* and *Candida dubliensis*.

References

Ahmad, S., Z Khan; Invasive candidiasis: A review of nonculture-based laboratory diagnostic methods; *Indian Journal of Medical Microbiology,* (2012) 30(3): 264-9.

Ananthanarayan and Paniker; Text Book of Microbiology; 9th Edition.

Elisa J, Michael R, Michael A; Text Book of Clinical Mycology; 2nd Edition, ELSEVIER publication.

Giri, S., AJ Kindo; A review of *Candida* species causing blood stream infection; *Indian Journal of Medical Microbiology, (2012) 30(3): 270-8.*

Kangogo MC, Wanyoike MW, Revathi G and Bii CC; Phenotypic characterization of *Candida albicans* from clinical sources in Nairobi, Kenya; Afr J Health Sci. 2011; 19:19-23.

Kangogo MC, Wanyoike MW, Revathi G and Bii CC; Phenotypic characterization of *Candida albicans* from clinical sources in Nairobi, Kenya; Afr J Health Sci. 2011; 19:19-23.

Michael Gherna and William G. Merz; Identification of *Candida albicans* and *Candida glabrata* within 1.5 Hours Directly from Positive Blood Culture Bottles with a Shortened Peptide Nucleic Acid Fluorescence In Situ Hybridization Protocol; Journal Of Clinical Microbiology, Jan. 2009, p. 247–248.

Mine Yucesoy; Serhat Marol; Performance of CHROMAGAR *Candida* and BIGGY agar for identification of yeast species; Annals of Clinical Microbiology and Antimicrobials 2003; 2:8.

Mine Yucesoy; Serhat Marol; Performance of CHROMAGAR *Candida* and BIGGY agar for identification of yeast species; Annals of Clinical Microbiology & Antimicrobials 2003; 2:8.

Orazio Romea *et al.,*; Molecular Epidemiology of *Candida albicans* and Its Closely related Yeasts *Candida* dubiniensis and *Candida* Africana; Journal Of Clinical Microbiology, Jan. 2009, p. 212–214.

Pfaller, M.A., D. J. Diekema, G. W. Procop; Multicenter Comparison of the VITEK 2 Antifungal Susceptibility Test with the CLSI Broth Microdilution Reference Method for Testing Amphotericin B, Flucytosine, and Voriconazole against *Candida* spp. Journal Of Clinical Microbiology, Nov. 2007, p. 3522–3528.

Pfaller, M.A., D. J. Diekema, G. W. Procop; Multicenter Comparison of the VITEK 2 Antifungal Susceptibility Test with the CLSI Broth Microdilution Reference Method for Testing Amphotericin B, Flucytosine, and Voriconazole against *Candida* spp. Journal Of Clinical Microbiology, Nov. 2007, p. 3522–3528.

Sanjeev Kumar, Anamika Vyas, Mrityunjay Kumar, S.K. Mehra; Application of CHROMagar *Candida* for Identification of Clinically Important *Candida* Species and their Antifungal Susceptibility Pattern; Int J Biol Med Res. 2013; 4(4): 3600-3606.

Shawn R, Shawn A, Michael A. Pfaller; Identification of *Candida* nivariensis and *Candida bracarensis* in Global Collection of *Candida glabrata* Isolates: Comparison of the Literature; Journal of Clinical Microbiology, Jan. 2009, p.1216-1217.

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