

Original Research Article

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Microbial Characterization of Yeast Isolates Recovered from Blood of Patient Admitted in ICU in a Tertiary Care Hospital

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ABSTRACT

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Fungal bloodstream infections (BSIs) are severe diseases that lengthen hospital stay, have elevated morbidity and mortality and increase medical care costs. Their incidence has recently increased, likely due to an increase in the number of susceptible hosts. The main aim of this study to characterization of *candida* isolated from blood from patient admitted in ICU. Our Objectives was to speciate *candida* isolated from the clinical sample & to do susceptibility testing of *candida* isolates using VITEK 2. The present study Prospective non-randomized, observational study was conduct in Department of Microbiology, medical college & hospital in Central India for a period of 1 year (April 01, 2016 – March 31, 2017). Non-albicans candida were the most common cause of blood stream infection by yeast. Risk factor for yeast infection found in our study were Head injury, Burn, Low birth weight, Birth asphyxia, Malignancy, Congenital heart defect. Out of 27 yeast isolates *Candida tropicalis* were 8 (29.62%), *Candida krusie* 7 (25.92%), *Candida albicans* 5 (18.52%), *Candida glabrata* 3 (11.11%), *Candida parapsilosis* 2 (7.41%), *Trichosporon asahii* 1 (3.7%), *Candida kefyr* 1 (3.7%).

Introduction

The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in the large population of immunocompromised patients and/or those hospitalized with serious underlying diseases (Michael *et al.*, 2011). *Candida* species belong to the normal microbial flora of an individual's mucosal oral cavity, gastrointestinal tract and vagina, and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections (Enoch *et al.*, 2006; Pfaller *et al.*, 2004). Fungal bloodstream infections (BSIs) are severe diseases that lengthen hospital stay, have elevated morbidity and

mortality and increase medical care costs. Their incidence has recently increased, likely due to an increase in the number of susceptible hosts (Laupland *et al.*, 2005; Carey *et al.*, 2008).

Candida spp. represent the principal aetiology of fungal BSIs and even though *Candida albicans* was reported as the most common species causing Candidaemia, an epidemiological shift towards non-albicans candida species has been reported in various geographical areas (Cugno *et al.*, 2012; Sardi *et al.*, 2013).

More than 17 different *Candida* species are known to be aetiological agents of human infection; however, more than 90 % of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*. The expanding population of immunocompromised patients that use intravenous catheters, total parenteral nutrition, invasive procedures and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections (Abi-Said *et al.*, 1997). Invasive *Candida* infections are also often seen after immunosuppressive treatments and transplantation procedures. The overall increase in nosocomial candidiasis observed worldwide in recent decades has been accompanied by a change in the epidemiology of these infections, characterized by a progressive shift from a predominance of *Candida albicans* to non-*C. albicans* *Candida* species that exhibit resistance to commonly used antifungal agents such as fluconazole (Asmundsdottir *et al.*, 2002; Mokaddas *et al.*, 2007; Poikonen *et al.*, 2007; Oberoi *et al.*, 1997).

Currently, an increase in the number of yeasts that are resistant to antifungal drugs is recognized worldwide; therefore, the use of in vitro laboratory tests may aid the doctor in choosing an appropriate therapy (Arunaloke *et al.*, 2008). The main aim and objectives of this study includes, characterization of *Candida* isolated from blood from patient admitted in ICU. And to speciate *Candida* isolated from the clinical sample. Also to do susceptibility testing of *Candida* isolates using VITEK 2

Materials and Methods

Settings: Department of Microbiology, medical college and hospital in Central India.

Study period

1 year (April 01, 2016 – March 31, 2017).

Study design

Prospective, non-randomized, observational study on patients visiting the hospital.

Inclusion criteria

Isolates of *Candida* recovered from blood samples from ICU.

Patients of all age groups.

Exclusion criteria

Isolation of *Candida* from samples other than blood.

Isolation of *Candida* other than ICU.

Procedure

Specimen

5-10 ml of Blood collected in Blood culture bottle.

Processing

Blood culture bottle were be place in the Incubator at 37⁰C within 30 min of collection.

All bottles were be subcultured on solid media after 1,2,3,7 days of incubation/ examine macroscopically for evidence of growth after similar incubation, make a smear and subculture on solid media [Blood agar and Sabouraud's Dextrose agar (SDA)] (Arunaloke *et al.*, 2008).

Identification of *Candida* spp. will be done using standard manual methods as described by Jagdish chander (2011) and the identification will be done as follows:

Colony characteristics

Smooth, white or cream colored colonies will be identified as those of *Candida*.

Gram's staining

Round to oval Gram positive budding cells with or without pseudohyphae will be considered as positive for *Candida*.

Germ tube test (Arunaloke *et al.*, 2008; Chander *et al.*, 2011)

The observation of germ tube production (Reynolds-Braude phenomenon) has been used as a method of presumptive identification of *Candida albicans* isolates from clinical samples. *C. albicans* (ATCC90028) used as a Positive control and *C. parapsilosis* (ATCC22019) as Negative control.

Chlamyospore formation (Arunaloke *et al.*, 2008; Chander *et al.*, 2011)

Morphological characters on cornmeal agar including the pattern of growth and presence or absence of chlamyospores will be observed for identification of *Candida* species. Terminal chlamydoconidia seen in *C. albicans* and *C. dubliniensis*, in *C. tropicalis* abundant pseudohyphae, pine forest arrangement, blastoconidia formed at or in between septa, in *C. parapsilosis* giant hyphae, blastospores at nodes, in *C. krusei* elongated yeasts, abundant pseudohyphae (matchstick-like appearance), in *C. glabrata* and *C. famata* yeasts only, in *C. guilliermondii* scant pseudohyphae with chains of blastoconidia in *C. lusitaniae*—short, distinctly curved pseudohyphae with occasional blastoconidia at septa. *C. albicans* (ATCC90028) used as a Positive control and *C. parapsilosis* (ATCC22019) as Negative control.

Sugar fermentation test (Arunaloke *et al.*, 2008)

Candida species ferment carbohydrates anaerobically. Liquid fermentation medium containing peptone (1%), sodium chloride (0.5%), Andrade's indicator (0.005%) was used. It will be sterilized by autoclaving at 120°C for 15 min. at 15 pounds pressure. Filter sterilized carbohydrates (glucose, lactose, sucrose, maltose, galactose and trehalose from HiMedia, Mumbai) at concentration of 2% will be added to the medium. Sterile Durham's tube, to capture gas, will be placed in test tube with 5ml of the medium. Inoculum will be prepared by suspending yeast grown on sugar free medium.

Each carbohydrate broth will be inoculated with approximately 0.1 ml of heavy inoculum of the *Candida*. The tubes will be incubated at 25°C upto 1 week. Tubes will be examined every 48 – 72 hrs interval to see the production of acid (pink color) and gas (in Durham's tube). Fermentation will be determined by observing change in colour and / or production of gas in Durham's tube. Interpretation of results will be done as described by Arunaloke Chakrabarti *et al.*, (2008).

Sugar assimilation test (Arunaloke *et al.*, 2008)

It is an aerobic process in which carbon and nitrogen compounds are utilized with oxygen as final electron acceptor. Carbohydrates used for assimilation test will be glucose, lactose, galactose, maltose, sucrose and trehalose, (HiMedia, Mumbai). The Disc impregnation – Pour plate Auxanographic method of Wickerham and colleagues which has withstood the test of time and easier to perform will be followed.

Preparation of YNB and agar separately will be done as described. 24-48 hrs growth of *Candida* will be used to prepare a suspension and 2ml of this will be added to 18 ml of molten agar (cooled to 45⁰C) and mixed well. The entire volume will be poured into a 90mm petri plate. The various carbohydrate impregnated discs will be placed onto the surface of the agar plate. The plates will be incubated at 37⁰C for 3 – 4 days. The presence of growth around the disc will be considered as positive for that particular carbohydrate. Growth around glucose disc will be recorded first which will serve as positive control (viability of yeast). Interpretation of carbohydrate assimilation and species identification will be done as detailed by Arunaloke Chakrabarti *et al.*, (2008).

CHROM agar *Candida* medium

It is a selective and differential chromogenic medium used for identification of various *Candida* species. The following procedure will be followed: Suspected *Candida* colonies will be inoculated onto CHROM agar (HiMedia). Plates will be incubated at 30⁰C for 48 to 72 hours. Plates will be examined for the colour of colonies for presumptive identification. The following colour of colonies may be seen: *C. albicans* Light green, *C. dubliniensis* Dark green, *C. glabrata* pink to purple, *C. krusei* Pink, *C. parapsilosis*

cream to pale pink, *C. tropicalis* blue with pink halo

Antifungal susceptibility testing by VITEK 2 (Pfaller *et al.*, 2007)

Antifungal susceptibility testing will be determine by Vitek 2 for all isolates against Amphotericin B (AMB), Fluconazole (FLC) Voriconazole (VRC), Capsfungin, Micofungin, Flucytocine, as described by the manufacturer.

Results and Discussion

Non-albicans candida were the most common cause of Blood stream infection by yeast. Yeast infection were slightly higher prevalence in male (55.56%) as compare to female (44.44%). 15 yeast strains were isolated from Medicine ICU, 6 from Pediatrics ICU, 6 from Surgical ICU as shown in Table 1.

Percentage positivity for yeast isolates in clinical samples was 2.25% (27 yeast isolates /1200 blood samples). Out of 27 yeast isolates *Candida tropicalis* were 8 (29.62%), *Candida krusei* 7 (25.92%), *Candida albicans* 5 (18.52%), *Candida glabrata* 3 (11.11%), *Candida parapsilosis* 2 (7.41%), *Trichosporon asahii* 1 (3.7%), *Candida kefyr* 1 (3.7%). Antifungal susceptibility for each isolates shown in table 2.

Table.1 Distribution of candida according to gender & ward

	Male	Female	Total
MICU	10	5	15
SICU	2	4	6
PICU	3	3	6
Total	15(55.56%)	12(44.44%)	27

Table.2 Percentage positivity of yeast isolates and their antifungal susceptibility testing

Species of <i>Candida</i>	Total no. (27)	Percentage	Antifungal susceptibility test (in percentage)					
			FLUCO-NAZOLE	VORICO-NAZOLE	CAPSO-FUNGIN	MICA-FUNGIN	Ampho-tericin B	FLUCYTO-SINE
<i>Candida tropicalis</i>	08	29.62	71.43	85.71	71.43	71.43	85.71	71.43
<i>Candida krusie</i>	07	25.92	14.28	85.71	28.57	57.14	57.14	57.14
<i>Candida albicans</i>	05	18.52	80	80	60	60	60	60
<i>Candida glabrata</i>	03	11.11	33.33	100	33.33	66.67	100	100
<i>Candida parapsilosis</i>	02	7.41	50	50	50	50	50	100
<i>Trichosporon asahii</i>	01	3.7	0	100	0	100	100	100
<i>Candida kefyr</i>	01	3.7	100	100	100	100	100	0

Candida species are the fourth leading cause of Hospital acquired bloodstream infection (BSI) in the United States, accounting for 8% to 10% of all BSIs acquired in the hospital (Wisplinghoff *et al.*, 2004). These yeasts are commensal in healthy humans and may cause systemic infection in immunocompromised situations due to their great adaptability to different host niches. Bassetti *et al.*, (2006) also find increase prevalence of non albicans *Candida* in their study. *C. tropicalis* was the most predominant yeast infection found in our study as observed by Rajendra Kothavade *et al.*, (2010).

Candida krusie was found to most resistant (85.62%) to Fluconazole in our study. *C. tropicalis* show 71.43% *C. albicans* 80%, *C. parapsilosis* 50%, *C. glabrata* 33.33% susceptibility to fluconazole. We also isolate a *Trichosporon asahii* strain that is resistant to Fluconazole.

Candida tropicalis was most susceptible to Amphotericin B and Voriconazole. *Candida krusie* was most susceptible to Voriconazole. *Candida albicans* was most susceptible to Fluconazole and Voriconazole. *Candida glabrata* was most susceptible to Amphotericin B, Voriconazole and Flucytosine.

In conclusion, *Candida tropicalis* & *krusie* were the leading cause of blood stream

infection in the presence of risk factor in our study. Resistance to Fluconazole was high for treatment of candida infection. Amphotericin B & Voriconazole can be used as a good option for the treatment of candida infection.

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