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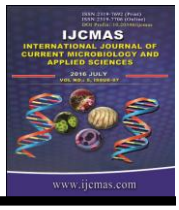

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Speciation of Candida Isolated from Various Clinical Samples and their Antifungal Susceptibility Profile in a Tertiary Care Hospital

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A B S T R A C T

Candida spp. can cause a wide variety of infections in immunosuppressed patients of long term broad – spectrum antibiotics, steroids or other immunosuppressive agents, diabetes mellitus, AIDS, Malignancy, Neutropenia. Although the majority of infections are caused by *Candida albicans*, Non-*C. albicans Candida* (NCAC) species are emerging as important pathogen in humans. Our study included 130 clinical isolates which showed gram positive budding yeasts cells with or without pseudohyphae. KOH mount, germ tube, Carbohydrate assimilation test and fermentation test and antifungal susceptibility by disc diffusion method was done. Most of the Candida isolates were from sputum (45%)samples followed by urine (24%), endotracheal tip(14%), vaginal swabs(9%),blood (4%)and pus(4%).Female (57.6%) more prone to *Candida* infections than male(42.3%).54 % of the patients from whom candida was isolated had been treated with >2 antibiotics, 15%treated with >3 antibiotics, 17%Diabetis Mellitus, 16% Neutropenia, 8 % Tuberculosis and Treatment with Steroids,6% Malignancy,2% HIV. Most common Candida species isolated belonged to *Candida albicans* (29.2%).*C parapsilosis (15.3%),C tropicalis (12.3%), C glabrata (11.5%),C krusei (9.2%), C lusitanae (9.2%), C guillermondi (8.46%), C dubliniensis (4.6%)*.65% of the total isolates were sensitive to Fluconazole and 82%of the total isolates were sensitive to Voriconazole.

Introduction

Candida is a part of the normal flora in healthy individuals, and is confined to the skin and mucosal surfaces of the oral cavity, gastrointestinal and urogenital tracts, and vagina. However, *Candida* spp. can cause a wide variety of infections in immuno-suppressed patients *Candida* species are responsible for around 80 % of fungal infections in the hospital environment (Sullivan, 1996; Vinaya and Sharma, 2013; Sumitra and Maheshwari, 2014). Majority of infections are caused by *Candida albicans*, non-*C. albicans Candida* (NCAC) species

like *C glabrata, C tropicalis, C parapsilosis, C krusei. C guillermondii, C kefyr, or lusitaniae* have been reported as cause of candidiasis (Sehal and Daniel, 2006; Chander, 2008; Kavitha, *et al.*, 2014).

Candida causes a variety of infections like Oropharnygeal candidiasis, vaginal candidiasis, urinary tract infections, single-organ infections including endophthalmitis, endocarditis, meningitis, pyelonephritis, septic arthritis; and disseminated infections with liver, kidney, spleen, and lung involvement (Chakrabarti and Shivprakash, 2005; Pfaller and Diekema, 2007; Manjunath, 2012).

Candida has emerged as the fourth most common cause of blood-borne infection. The mortality due to candida infections ranges from 14.5% to 49%, and *C albicans* is estimated to be responsible for 50-60% of the cases invasive candidiasis.

Materials and Methods

A total of 130 *Candida* species isolated from clinical samples like urine, pus, sputum, blood, oral swabs, vaginal swabs, endotracheal aspirates, endotracheal tips, were included in this study. The study was conducted from November 2012 to April 2014 in the department of Microbiology, ESIC MC PGI MSR Hospital Bangalore. Data of patients regarding the use of long term broad – spectrum antibiotics, steroids or other immunosuppressive agents, diabetes mellitus, AIDS, Malignancy, Neutropenia were collected at enrolment.

Examination of specimen was done by Gram staining,10% and 40 % KOH mount, Cultural characteristics were noted on SDA with chloramphenicol, Germ tube test was done to detect elongated daughter cells without constriction, chlamydospore formation was noted on cornmeal agar with 1% tween 80 (by Dalmau technique) (Fig 1), further speciation was done by Carbohydrate assimilation test by Auxanographic plate method (Haley and standard modification) using Yeast nitrogen base and 20 % sugars (Fig 2). Sugars used were glucose, sucrose, lactose, maltose, trehalose, cellobiose, raffinose, mellibiose, inositol, dulsitol, galactose, xylose. 3 ml of Yeast suspension to 12 ml of molten yeast nitrogen base agar was added and mixed by gently rotating the petriplate on the surface. With a sterile standard loop dipped in sugar is placed over the sterile disk and placed over the yeast suspension and agar medium and incubated at 37°C for 18-24 hours and then examined for growth around each disk. A positive reaction was indicated by the presence of growth in the medium

Sugar fermentation test with 6 % sugars was done for speciation.

Antifungal susceptibility test was done by Kirby- Bauer's disc diffusion method on glucose methylene blue Mueller Hinton agar (GM-MH) using commercially available antifungalcdiscs [Hi Media, Mumbai, India] fluconazole (25µg), Voriconazole 1 µg.(Fig 3). Zone diameters were interpreted as per the approved NCCLS (M44-A) guidelines. The quality control test was performed by using *C. parapsilosis* (ATCC 22019), and *C. albicans* (ATCC 90028).

Zone Diameter Interpretive Standards

Antifungal agent	Disk content	Zone Diameter, Nearest whole R S	S-DD
Fluconazole	25 µg	≤14 ≥19	15-18
Voriconazole	1 µg	≤13 ≥17	14-16

Results and Discussion

Out of 130 *Candida* species isolated, *Candida albicans* (29.2%) was the most common species isolated from the various clinical specimens. The *Non Candida albicans* species isolated were *C parapsilosis (15.3%), C tropicalis (12.3%), C glabrata(11.5%),C krusei (9.2%), C lusitanae (9.2%), C guillermondi (8.46%),C dubliniensis(4.6%).*

Candida species were isolated more commonly from sputum (45%) followed by urine specimen (24%), endotracheal tip (14%), vaginal swabs (9%), blood (4%), and pus samples (4%). Identification of risk factors is important way for prevention of diseases. In the present study, 54 % of the patients from whom candida was isolated had been treated with >2 antibiotics,15% had been treated with >3 antibiotics, 17% had Diabetis Mellitus,16% had Neutropenia,8 % had Tuberculosis and 8%in those Treated with Steroids,6% had Malignancy,2% had HIV. In a study conducted by Kao AS *et al.*, risk factors associated were antibacterial therapy (68%), steroid therapy (40%), neutropenia (13%) while a study conducted by A.K. Verma showed association with broad-spectrum antibiotics (42.8%) immunosuppressive therapy (23.8%), neutropenia (14.3%) which was similar to our present study. isolation of *C albicans* was 16% among HIV patients. *C tropicalis* (32%) and *C glabrata* (32%)most commonly isolated *Candida spp* in HIV infected individuals.

Fig.1 Demonstration of Single terminal chlamydospore of *Candida* in corn meal agar

Fig.2 Carbohydrate assimilation test

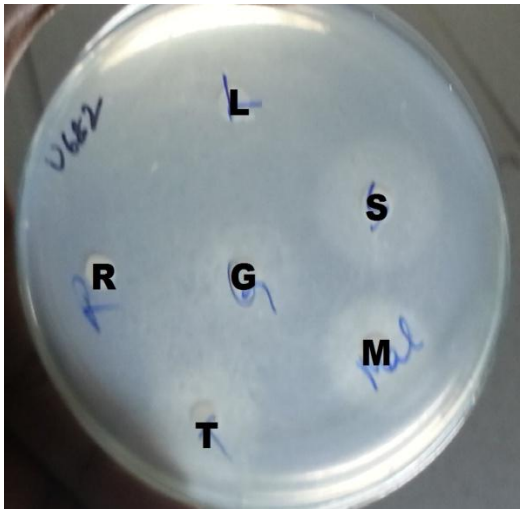


Fig.3 Antifungal Susceptibility test

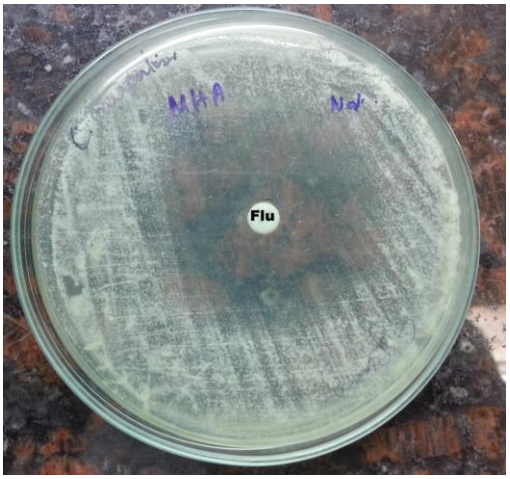


Fig.4 Gender Wise Distribution of the samples

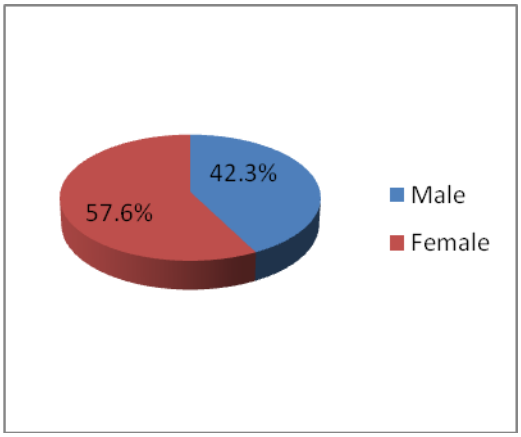


Fig.5 Age wise distribution of patients

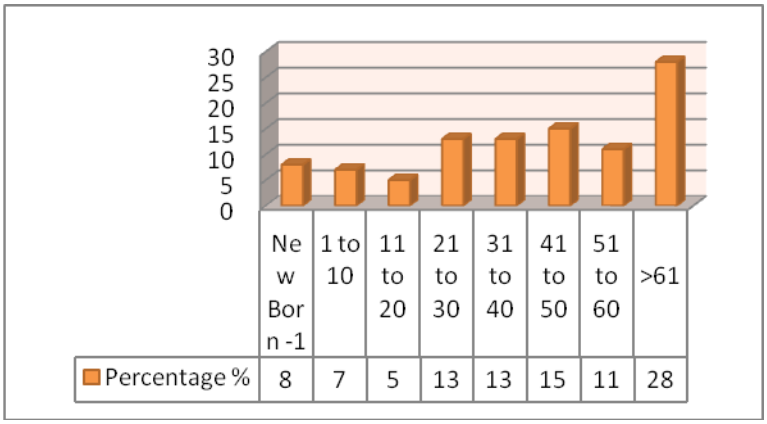


Fig.6 Ward Wise Distribution of samples

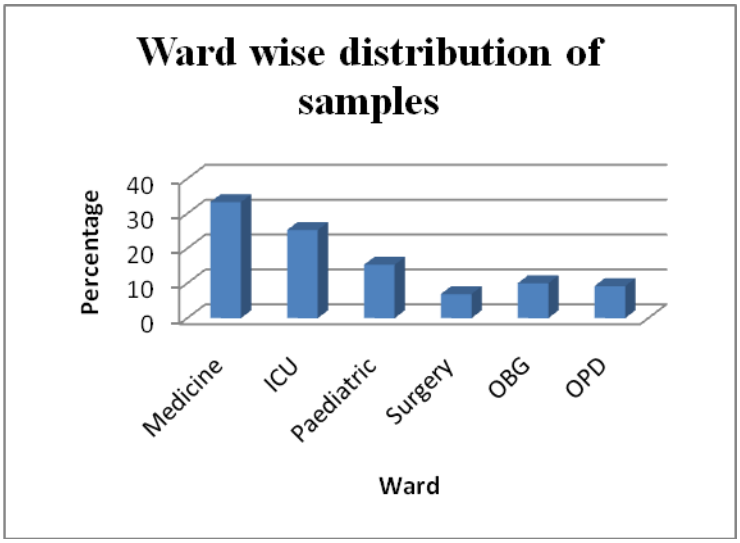


Fig.7 Sample wise distribution of *Candida* isolates

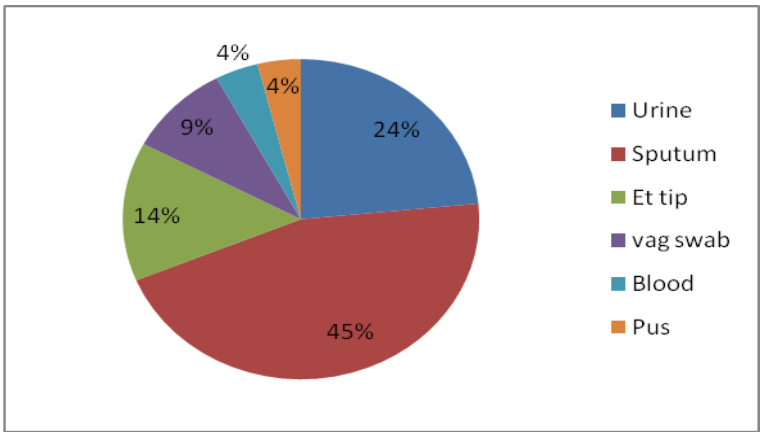


Fig.8 Distribution of different species of *Candida*

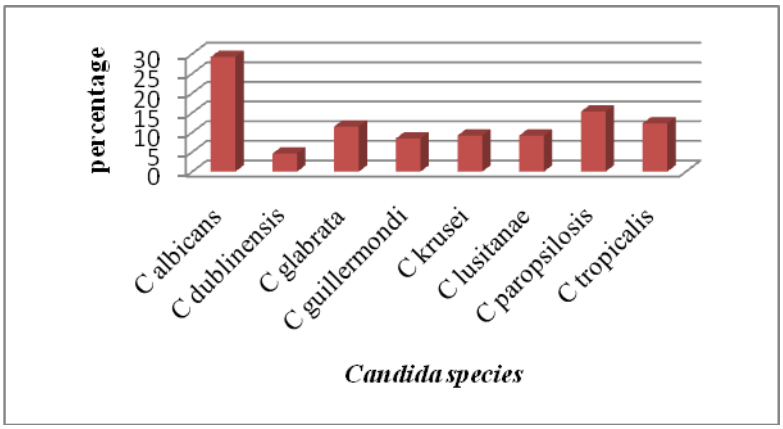
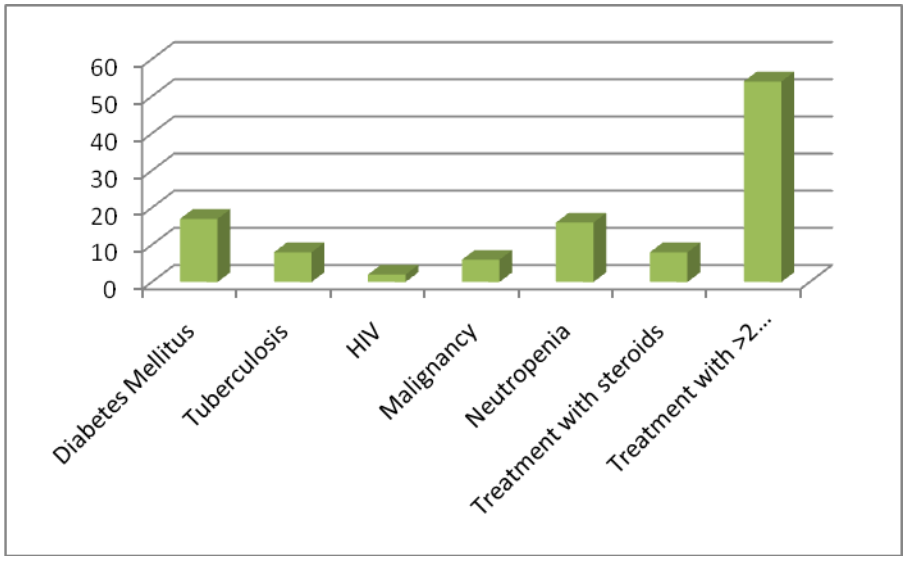


Fig.9 Risk Factors associated with *Candida* infections



Age wise distribution of candida showed that 28% were >60 years, followed by 41-50 years (15%)**,** 21-30(13%),31-40 (13%) and 51-60 (11%), NB-1 yr(8%) ,1-10(7%) which was similar to a study conducted by Larone, (2002) who showed >60 (52.4%) , 51-60 (23.8%), 31-40 (14.3%), 41-50 (4.8%), 21-30 (4.8%).

The study showed that female (57.6%)were more prone to *Candida* infections than male (42.3%). Antifungal susceptibility testing showed 65 % of the isolates sensitive to Fluconazole and 35% resistant. 82% were sensitive and 18% resistant to Voriconazole.

100% of *C dubliniensis* isolates were sensitive to fluconazole and Voriconazole in the present study. 6 (4.6%) of the total isolates were resistant to both fluconazole and Voriconazole.4 out of 6 isloates belonged to *C glabrata* and 2 belonged to *C tropicalis.*

6 (4.6%) isolates of the total 130 isolates were resistant to fluconazole but sensitive to Voriconazole. Of which 1 isolate was *C. albicans, C. guillermondi*(1each), *C. glabrata* and *C. krusei(2 each).*

In conclusion, the present study showed that *C albicans* was the predominant species isolated. Among the *non Candida albicans C parapsilosis* was predominantly isolated followed by *C tropicalis, C glabrata,C krusei,C lusitanae, C guillermondi, C dubliniensis*.

Voriconazole seemed to be superior to fluconazole with a better susceptibility in the fluconazole resistant strains also. The triazoles remain active against many of isolates and voriconazole remains sensitive to the fluconazole resistant strains, also Fluconazole can be continued as the first line antifungal agent for treating suspected cases of uncomplicated candidiasis.

Voriconazole can be reserved for refractory cases of candidiasis. But whenever there is lapses in infection control precautions coupled with broad use of fluconazole, more fluconazole-resistant strain of endemic species may emerge

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