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Antifungal sensitivity of *Candida Sp.* isolated from ICU patients at attending at a tertiary care hospital

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Abstract
Objective: In this study, we have found the prevalence of Candida Sp. In admitted patients at medicine Intensive Care Unit (ICU). We have also evaluated the drug sensitivity patterns of identified Candida sp.
Methods: This prospective, cross-sectional study was conducted at the IMS and SUM hospital from January 2017 to February 2018, and comprised the causative organisms of infections. Species differentiation was confirmed by specific culture medium methods. Antifungal susceptibility of isolated Candida species was evaluated with disc diffusion methods.
Results: Of the 219 Candida isolates, majority of them were isolated from the swab samples 78(35.6%) and from urine sample 59(26.9%). Moreover, 144(65.8%) samples were of females and 75(34.2%) were of males. *Candida albicans* 128(58.45%) was the most predominant species followed by *Candida glabrata* 30(13.69%), *Candida tropicalis* 26(11.87%), *Candida krusei* 17(7.76%), *Candida parapsilosis* 12(5.47%), *Candida dubliniensis* 3(1.37%) and *Candida lusitaniae* 3(1.37). All isolates were least susceptible to amphotericin B with a susceptibility rate of 213(97.26%). The highest resistance was found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%).
Conclusion: Candida species were resistant to many antifungals and in this study it is revealed that Amphotericin B is the most sensitive antifungal for skin infections.

Keywords: Candida albicans, Non-albicans (NAC), Antifungal sensitivity test, CHROMagar Candida.

1. Introduction
Fungal infections, a serious clinical condition causes substantial morbidity and mortality amongst the patients globally [1]. *Candida species* are the normal commensal of the oral cavity, GIT and the mucosal surfaces in the body as well as the pathogens leading to colonization and infection. In the recent times incidence of fungal infections has increased with the increased incidence of immunocompromised patients [2]. *Candida* species are frequently isolated from such patients as well as those who are diabetic [3], on immune-suppressants or neutropenic [4] with malignancy undergoing chemotherapy/ radiotherapy [5], long term steroid therapy, long term antibiotic therapy etc. [6]. It has been implicated as a cause of UTI, respiratory infections, septicaemia as well as cutaneous and mucocutaneous infections. The invasive fungal infections often lead to sepsis, severe sepsis and septic shock in critically ill patients in ICU with *Candida species* being the most common cause of fungal sepsis, especially in the hospital acquired infections [7]. More than 17 different *Candida species* are known to be aetiological agents of human infections. Though *Candida albicans* is the most commonly isolated fungal pathogen from clinical samples, gradually *non-albicans Candida species* are becoming predominant pathogens [6]. Moreover, the increased use of anti-fungal agents for treatment and also for prophylaxis especially in ICU patients has lead to development of resistance against commonly used anti-fungal agents in the treatment like various azoles [2, 4, 6]. However the *Candida species* have variable resistance towards various antifungal agents. Thus this study was carried out to identify different *Candida species* from specimens of clinically diagnosed sepsis patients and their antifungal susceptibility pattern which can be utilized for better management of sepsis patients in our set up. The objectives of this study were to isolate and identify the species of *Candida* from skin infection samples and to determine the susceptibility pattern of the *Candida species* isolates.

2. Materials and Methods

In this prospective study all the patients admitted at ICU were participated for a period of 1 year. Thus the specimens whose Gram stained smears showed presence of any yeast cells or yeast-like cells with budding and with or without pseudohyphae were processed for fungal culture and inoculated on Sabourad's Dextrose Agar (SDA). Plates were incubated aerobically at 37^0C for 24 hours. The colonies of *Candida species* were obtained after overnight incubation. The colonies were identified by colony morphology on SDA, colony colour on Candidal differential agar Media, germ tube test and chlamydospore formation as follows. The colonies were identified according to colour. In addition to the colour of the colonies on HiCrome, a germ tube test and observation of chlamydospore formation on cornmeal agar were carried out for identification of *Candida albicans*. For germ tube test, a well isolated colony from SDA was emulsified in 0.5 ml of human serum using sterile straight wire. The test tubes were incubated at 35^0C and no longer than 2 hours. A drop of serum sample was placed on a clean, grease free slide and a cover slip was placed over it. This slide was then observed first under 10X and then under 40X objective lens of microscope for the presence of germ tubes. Germ tube is a filamentous extension from yeast cell without constriction at the neck (true germ tube) and is seen in *C. albicans*. Antifungal Susceptibility test was carried out for *Candida species* according to CLSI guidelines for testing anti-fungal agents for yeasts

3. Results

Of all, 219(7.28%) samples were positive for candida infections, including 78(35.62%) from the swab samples, and rest from the urine samples. All these positive samples produced cream to white, smooth and glossy colonies - characteristic of Candida species on the SDA. These Candida-positive colonies were gram stained and only those which were round to oval with purple-coloured budding yeast cells were further processed for germ tube (GT) test. A total of 131(59.82%) strains produced germ tubes, hence were categorised as either *C. albicans* or *C. dubliniensis*, while 88(40.18%) strains which were GT negative and were designated as *Candida* species. Species level identification was performed by using CHROM agar Candida and corn meal agar. On the basis of growth on both the media, out of all the positive isolates *C. albicans* 128(58.45%) was the most predominant species followed by *C. glabrata* 30(13.69%), *C. tropicalis* 26(11.87%), *C. krusei* 17(7.76%), *C. parapsilosis* 12(5.47%), *C. dubliniensis* 3(1.37%) and C. lusitaniae 3(1.37). Among NACs, C. glabrata was the most abundant species. The Candida species were also identified through various biochemical tests and the results confirmed microscopic and morphological observations. Moreover, 139(63.5%) of the infections were acquired in hospitals compared to 80(36.5%) community-acquired infections. *C. albicans* was the most abundant species in ICU, followed by *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*. *C. krusei* was more prevalent, while other species were abundant in ICU. Highest prevalence of Candida species was in Swab samples (Table-1). It was observed that the number of *C. albicans* and all the NAC species was high in females as compared to males. Among the NACs, *C. tropicalis*, *C. glabrata* and *C. krusei* were the predominant species in females. In case of males, *C. tropicalis*, *C. glabrata* were high in number after *C. albicans* (Table-2). Patients were divided into six age groups. The highest rate of Candida species was obtained from the patients aged above 60 years with highest prevalence of *C. albicans* followed by *C. glabrata*, *C. tropicalis* and *C. krusei*. In the age group 26-40 and 41-60 years, *C. glabrata*, and *C. tropicalis* were prevalent. *C. krusei* was most abundant within the middle-aged group, i.e. 41-60 years (Table-3). In our study, amphotericin B was the most effective antifungal against all the Candida species with a susceptibility rate of 213(97.26%). Resistance towards amphotericin B was noted for 3(2.34%) *C. albicans*, 1(3.33%) *C. glabrata* and 1(5.88*%) C. krusei* species. Interestingly, the highest resistance was found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%). *C. krusei* 4(23.5%) were the most resistant Candida species to fluconazole followed by *C. albicans* 24(18.75%), *C. glabrata* 3(10%) and *C. parapsilosis* 1(8.3%). However, *C. parapsilosis* was the most resistant to voriconazole 4(33.3%), followed by *C. krusei* 4(23.5%), *C. albicans* 26(20.3%), *C. glabrata* 4(13.3%) and *C. tropicalis* 2(7.7%). A 100% susceptibility rate was noted in *C. dubliniensis* and *C. lusitaniae* for both the azole antifungals (Table-4). According to the antifungal resistance data of this study, cross-resistance between fluconazole and voriconazole was found among 18(8.2%) of the isolates. Of them, 16(88.9%) were *C. albicans* while 2(11.1%) were *C. glabrata*. Both the *C. glabrata* isolates were cross-resistant to fluconazole and voriconazole. Among *C. albicans*, 14(87.5%) isolates were cross-resistant to fluconazole and voriconazole, 1(6.25%) isolate was resistant against amphotericin B and voriconazole while 1(6.25%) C. albicans isolate was resistant to all the three antifungals i.e., amphotericin B, fluconazole and voriconazole.

Table 1: Candida Species isolated from the study

C. albicans	*C. glabrata*	*C. tropicals*	*C. krusel*	*C. parapsllosis*	*C. dubllnieniensis*	*C. lusitaniae*	**Total**
47	9	10	5	7	7	0	78 (35.6%)
31	9	6	7	3	3	1	59(26.9%)
20	6	4	1	1	1	0	32(14.6%)
13	1	3	2	0	0	0	20(9.1%)
5	2	0	2	0	0	1	10(4.6%)
5	1	3	0	0	0	1	10(4.6%)
7	2	0	0	1	1	0	10(4.6%)
43	11	11	10	4	4	0	80(63.5%)
85	19	15	7	8	8	3	139(14.6%
128	30	26	17	12	12	3	219(100%)

Table 2: Gender wise distribution of Candida albicans and Non albicans (Nacs) species

Gender	C.Albicans (128)	C.Glabrata (30)	C.tropicals (26)	C.krusel (17)	C.parapsllosis (12)	C.dubllnieniensis (3)	C.lusitaniae (3)	Total	Total Nacs	Total isolates
Female	81	21	17	12	8	3	2	63	63	144(65)%
Male	47	9	9	5	4	0	1	28	28	75(34.2%)

Table 3: Age wise Candida species distribution.

Gender	Children (0-1)	Teenagers (12-18)	Young adults (19-25)	Age group Adults (26-40)	Middle aged (41-60)	Senior citizen (>60)	Total
Female	4	2	13	42	35	48	144(65.8%)
Male	3	2	3	9	20	38	75(34.2%)
Total	7	4	16	51	55	86	219(100%)

Fig 1: Colony of Candida Species on SDA

Fig 2 Antifungal susceptible of Candida species

4. Discussion

The virulence factors and antifungal susceptibility profile of *C. albicans* and NACs vary which has necessitated correct and rapid species identification as this has a direct impact on the choice of treatment. [7] In our study, *C. albicans* (58.4%) was the leading pathogen as compared to NACs similar to earlier reports.[8-11] Nucci *et al*. [12] also reported *C. albicans* (37.6%) as major contributor of Candida infection followed by *C. parapsilosis* and *C. tropicalis*. The order of prevalence of NACs in our study was *C. glabrata* (13.7%), *C. tropicalis* (11.9%), *C. krusei* (7.8%), *C. parapsilosis* (5.5%), *C. dubliniensis* (1.4%) and *C. usitaniae* (1.4%). A significant finding of our study is *C. glabrata* among NACs being the most common species in clinical samples. This could be a perturbing threat due to high incidence of increased resistance of this species to the routinely used antifungal agents. Patel *et al*. [10] isolated highest number of Candida isolates from urine and sputum, which is similar to our work where urine 78(35.6%), vagina 59(26.9%) and sputum 32(14.6%) had predominant Candida species. Farooqi *et al*. [13] reported a different epidemiological trend where C. tropicalis was the most common organism followed by *C. parapsilosis* and *C. glabrata*. Candida infection was higher in females 144(65.8%) as compared to males (30.9%) in our study, which is in accordance with findings of Nardin *et al*. [14] The reason of high distribution and virulence of Candida species in females is that it has a receptor for female reproductive hormones. Rashwas *et al*. [15] observed candiduria in 34.4% females and 14.9% in males. Aslam *et al*. [16] also reported nosocomial candidiasis more frequent in female patients (56%) as compared to male patients (44%). In our results, high percentage of female patients visiting the QIH may be due to problem in personal hygienic conditions. In this study, Candida infection was most prevalent within the age group of >60 years and middle aged-group, which is in accordance with studies of Furnaleto *et al*. [17] and Al-Hussaini *et al*. [18]

In the present study, Candida infection rate was high in swab samples of ICU admitted patients. However, other studies reported that Candida infection was more common in ICU and surgical ward. [19] Amphotericin B was found to be highly effective against all tested species except for *C. albicans*, *C. glabrata* and *C. krusei*, which is similar to report of De Almeida *et al*. [20] Antifungal susceptibility data of this study also observed marked rise in azole resistance in NACs as compared to *C. albicans*. *C. krusei* was the most resistant species among all the isolates followed by *C. albicans*, *C. glabrata* and *C. parapsilosis*. Oberoi *et al*. [21] reported high fluconazole sensitivity in *C. tropicalis*, high resistance in *C. glabrata* and less resistance in *C. parapsilosis*. All tested *C. tropicalis* local isolates were fluconazole sensitivity in contrast to *C. parapsilosis* and *C. glabrata*. Badiee and Alborzi [22] report 89.5% susceptibility of *C. albicans* to fluconazole; which is quite similar to our results. Fluconazole resistance was 18.8% similar to the Sojakova *et al*. [23] which reported 13% fluconazole resistance in 227 Candida isolates. Kaya *et al*. reported an alarming increased fluconazole resistance in *C. albicans* (68.7%) and NACs (63.2%). [24]

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