Original Article

Characterization of *Candida* species from Intensive Care Unit Isolates in a Tertiary Care Centre in North-East India: A retrospective study

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Abstract

Background: Fungi have emerged as major causes of human diseases. Intensive Care Units (ICU), harbor almost all the risk factors for opportunistic fungal infections. Among these, *Candida* infections are very common with recent trends being rise in the non-*Candida albicans* (NCA) species along with an increase in resistance of these species to antifungal drugs. **Aims:** To characterize the *Candida* species from the clinical specimens of patients admitted in the ICU of Tertiary Care Centre in North-East India and to perform their antifungal susceptibility. **Settings and Design:** This retrospective study was conducted in the Department of Microbiology from January 2011 to December 2011. **Materials and Methods:** The following techniques were employed to characterize the isolates in the study – KOH mount, Gram's stain, India ink preparation, culture on Sabouraud's Dextrose Agar, Germ Tube test, Urea hydrolysis, morphology in Cornmeal Agar and chromogenic agar media, sugar fermentation and sugar assimilation tests and automated identification system, and the results were interpreted using standard protocols. **Statistical Analysis Used:** SPSS version 17.0 was used for all statistical computations and $P < 0.05$ was taken as significant. **Results:** Out of 85 *Candida* isolates, *Candida tropicalis* (38%) was the most common, in all age groups. Infections were more common in patients above 40 years and males were affected more than females. NCA species were more resistant to fluconazole than *C. albicans*. **Conclusions:** The study highlights the change in epidemiology in the species distribution of *Candida* and a rise in infections by NCA species as compared to those by *C. albicans*. Knowledge of the local species distribution of *Candida* along with their antifungal susceptibility is essential to initiate and optimize therapy and outcome, especially in an ICU setup, which harbors patients susceptible to fungal infections.

Key words: Antifungal susceptibility, *Candida* species, Intensive Care Unit, non-*Candida albicans* species, North-East India

INTRODUCTION

Fungi have emerged as major causes of human diseases and fungal infections have led to important public health problems since the early 1980s. Especially, among the immunocompromised and those hospitalized with serious underlying diseases, opportunistic fungal infections bring about a substantial increase in morbidity and mortality.[1] Among the various risk factors for developing

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fungal infections, the most important ones are an ever-expanding population with immuno-compromised states due to mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, and extremes of age. Other risk factors include sepsis, different surgical procedures, increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies, transplantations, parenteral nutrition, multiple-lumen catheter use, prior *Candida* species colonization, renal replacement therapy, mechanical ventilation, etc., which often involve the collateral damage of circumventing the body's normal defense mechanisms thus further increasing the risk for both common and uncommon opportunistic fungal infections.[2] Intensive Care Units (ICU), which though typically represent only about 5% of hospital beds, but are host to more than 20% of hospital infections and harbor almost all the risk factors for opportunistic fungal infections, provide such a suitable environment.[3]

Among the formidable list of opportunistic fungi, without question the single most important cause of opportunistic mycoses worldwide remains *Candida*. *Candida* species are responsible for up to 78% of nosocomial fungal infections. Overall, they are the sixth most common nosocomial pathogen, and fourth most common microorganism in nosocomial sepsis cases according to the National Nosocomial Infection Surveillance system.[4] Although worldwide increase in the incidence of invasive *Candida* infections has been witnessed since the 1980s, the recent trends demonstrate a gradual change in its species distribution, with many countries experiencing a relative rise in the proportion of non-*Candida albicans* (NCA) isolates. Moreover though *C. albicans* is generally susceptible to fluconazole, decreased *in vitro* susceptibility to fluconazole is more common among several NCA species; however, the clinical relevance of this fact is not well defined.[5,6]

India has a high prevalence of invasive candidiasis owing to the presence of a number of contributory factors including favorable climatic conditions, a large population of immuno-compromised hosts including people living with HIV/AIDS and diabetes mellitus, and widespread access to antibiotics and steroids without prescription.[7] *Candida* species is ranked fifth among nosocomial urinary pathogens and is also the eighth most common blood stream pathogen in India.[8,9] Despite the availability of a few studies from the North-East India,[10] lack of adequate diagnostic mycology laboratory procedures, precludes the availability of representative data on the epidemiological and mycological characteristics of invasive candidiasis occurring in vast stretches of the country. Nevertheless, because of the immense eco-geographical heterogeneity in the country and in view of the geographical and temporal variation often observed in the species distribution of *Candida*,[5] there is a need to investigate and monitor local epidemiological patterns of *Candida* infections in India.

The increasing population of immuno-compromised patients, together with the rising incidence of NCA species and the emergence of acquired antifungal resistance, necessitates the judicious administration of antifungal prophylaxis in at-risk patients and empirical antifungal therapy in patients suffering from candidiasis. Characterization and sensitivity profiles of the locally prevalent *Candida* strains and knowledge regarding risk factors relevant for the patient profile are utmost essential for the judicious administration of antifungal prophylaxis as well as antifungal therapy.

Hence, the current study was undertaken with the aim to characterize the *Candida* species obtained from the clinical specimens of patients admitted in the ICU of our hospital and to perform their antifungal susceptibility.

MATERIALS AND METHODS

A retrospective study was undertaken with the *Candida* isolates obtained from clinical specimens of patients admitted in the ICU for a period of 1-year from January 2011 to December 2011, in the Department of Microbiology in a tertiary care set-up in North-East India. It has a 32 bedded ICU catering to various patient profiles. *Candida* isolated from the samples collected from ICU were subjected to speciation using the following techniques according to standard protocols[11,12] – KOH mount, Gram's stain, India Ink preparation, culture on Sabouraud's Dextrose Agar, Germ Tube test, Urea hydrolysis, Cornmeal Agar (CMA) morphology (Dalmau technique), morphology in chromogenic agar media, Sugar fermentation test, Sugar assimilation test (Auxanographic Plate Method) and then finally subjected to automated identification system (Vitek 2 Compact, Biomerieux), using protocols prescribed by the manufacturer. Sugar assimilation test was taken as the gold standard for identification of the *Candida* species. The patient profile and associated risk factors were noted. The antifungal susceptibility was done according to Clinical Laboratory Standards Institute (CLSI) guideline, method for antifungal disc diffusion susceptibility for yeasts, with approved guideline M44-A.[13] We compared the sensitivity profile of the organisms to two antifungals – fluconazole (25 μg) and amphotericin B (10 μg). The inoculum was standardized to 0.5 McFarland units. Standard ATCC strains (*C. albicans*, ATCC 90028, *Candida parapsilosis,* ATCC 22019 and *Candida krusei,* ATCC 6258) were used as a control.

Statistical analysis

Differences in antifungal sensitivity between *C. albicans* and NCA species were also examined for statistical significance using Fisher's exact test. SPSS version 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) was used for all statistical computations and $P < 0.05$ was taken as significant.

RESULTS

A total of 266 samples from 237 patients were received, from the ICU of our hospital during the study period. Of these, a total of 85 *Candida* isolates were obtained from 74 patients.

Among the various co-morbid conditions observed in the patients, cerebro-vascular accidents (23%) was the most common, followed by tuberculosis (15%), respiratory distress (12%), patients in postoperative state (11%) and others [Table 1].

In this study, among the 85 *Candida* species isolated, NCA species were more in number than *C. albicans*. The most common among NCA species included *Candida tropicalis* (38%), *Candida lusitaniae* (13%), *Candida glabrata* (9%), *Candida parapsilosis* (7%), *Candida krusei* (6%) and *Candida guilliermondii* (4%). A single isolate each of *Candida kefyr* and *Candida zeylanoides* was also obtained. *C. albicans* accounted for 9% of the isolates. About 12% of the *Candida* species isolated could not be identified conclusively by any of the identification methods.

The highest number of samples were received in the age group of 41-60 years (49%), followed by the age groups of >60 years (27%), 21-40 years (15%) and <20 years (9%). Maximum isolates obtained were also from the age group of 41-60 years (45%), followed by the age groups of >60 years (26%), 21-40 years (18%) and <20 years (11%).

C. tropicalis was the most common isolate in all age groups. In the age group 41-60 years in which the maximum isolates were obtained, *C. tropicalis* (34%) was the predominant isolate, followed by *C. lusitaniae* (13%), *C. glabrata* (1%), *C. albicans* and *C. krusei* (8%, each), *C. parapsilosis* and *C. guilliermondii* (5%, each). The single isolates of *C. kefyr* and *C. zeylanoides* were obtained in the age groups of 41-60 years and >60 years, respectively.

Candida species were isolated more in males (62%) than females in the present study. *C. tropicalis*, the most isolated species, was also more common in the males (72%) than females [Table 2].

Candida species were isolated mainly from urine samples (37%), followed by sputum and Endo-tracheal secretions (21%), throat swab (16%), Ryle's tube aspirate (12%), pus and exudates (8%), blood (4%) and cerebrospinal fluid (CSF; 2%) [Table 3].

Out of 85 samples, 55 samples showed consistent identification results by the entire range of diagnostic tests used but 30 samples exhibited discordant results on CMA and Vitek 2 Compact tests. The result of the sugar assimilation test was considered as the confirmatory finding for these 30 samples. 18 samples identified by Vitek 2 Compact showed concordance with sugar assimilation test; 23 samples identified by CMA showed concordance with sugar assimilation test. The difference was however not statistically significant ($P = 0.27$). The *Candida* isolates were also cultured in chromogenic agar media for identification.

Table 1: Co-morbid conditions associated with *Candida* infections in ICU

Co-morbid conditions	Number of patients
Respiratory distress	9
Acute renal failure (with/without sepsis)	3
Tuberculosis (pulmonary/extra pulmonary)	11
Diabetes mellitus (with complications)	5
Cerebro-vascular accident	17
Multiple infarct	3
Chronic obstructive pulmonary disease	7
Cerebral malaria	6
Alcoholic liver disease/hepatic encephalopathy	2
Postoperative state	8
Seizures	3
Respiratory distress	9
Total	74

ICU = Intensive care unit

Table 2: Age-wise and sex-wise distribution of *Candida* species isolated from ICU

Candida isolates	Age-groups (in years)				Total (male/female)
	<20	21-40	41-60	>60	
Candida albicans	1	1	3	3	8 (5/3)
Candida tropicalis	3	7	13	9	32 (23/9)
Candida krusei	0	1	3	1	5 (3/2)
Candida parapsilosis	1	1	2	2	6 (3/3)
Candida lusitaniae	2	2	5	2	11 (7/4)
Candida glabrata	1	2	4	1	8 (6/2)
Candida guilliermondii	0	0	2	1	3 (1/2)
Candida kefyr	0	0	1	0	1 (0/1)
Candida zeylanoides	0	0	0	1	1 (1/0)
Unidentified	2	1	5	2	10 (4/6)
Total	10	15	38	22	85 (53/32)

ICU = Intensive care unit

Table 3: Sample-wise distribution of *Candida* species isolated from ICU

Candida isolates	Urine	Sputum/Endo-tracheal secretion	Blood	Throat swab	Ryle's tube aspirate	CSF	Pus/exudate	Total
Candida albicans	1	3	0	2	2	0	0	8
Candida tropicalis	11	7	1	6	4	1	2	32
Candida krusei	2	1	0	1	1	0	0	5
Candida parapsilosis	3	1	0	1	0	0	1	6
Candida lusitaniae	6	2	1	0	1	0	1	11
Candida glabrata	5	1	0	1	1	0	0	8
Candida gullerimondi	0	1	0	1	0	0	1	3
Candida kefyr	0	0	0	0	0	0	1	1
Candida zeylanoides	0	0	0	1	0	0	0	1
Unidentified	3	2	1	1	1	1	1	10
Total	31	18	3	14	10	2	7	85

CSF = Cerebrospinal fluid, ICU = Intensive care unit

In this study, none of the isolates showed resistance to amphotericin B (10 µg). As far as resistance to fluconazole (25 µg) is concerned, *C. glabrata* showed highest resistance (72%) followed by *C. tropicalis* (43%), *C. parapsilosis* (37%), *C. albicans* (23%) and *C. lusitaniae* (13%). The difference in resistance of NCA species and *C. albicans* to fluconazole was found to be statistically significant (P = 0.0087) [Figure 1].

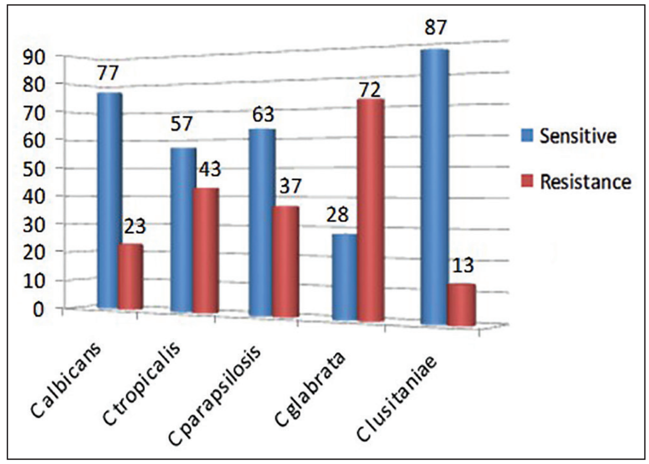

Figure 1: Susceptibility pattern of *Candida* species to fluconazole, 25 µg (%)

DISCUSSION

Candida species are the most common agents of fungal infection in humans affecting skin, nails, mucosa and internal organs of the body.[14] The species are endogenous in nature and are usually responsible for opportunistic infections.

In the present study, we observed that NCA species were more frequently encountered than *C. albicans*. In different studies around the globe[5,15-18] and in India,[6,9,19-23] it was observed that, NCA species were predominant (30-90%) among the isolated *Candida* species. Some authors have attributed this emergence of NCA species to the prophylactic use of fluconazole, in the species distribution of *Candida*, but it is not the factor in our study. Various co-morbid conditions of the patients, stay in ICU, and use of invasive monitoring devices and administration of broad-spectrum antibiotics as combination therapy were a few notable risk factors that could have led to the current spectrum of NCA species prevalence in the present study. Further studies with larger sample size are needed to verify our findings.

C. tropicalis was the most common among NCA species in this study. The isolation of more NCA species as compared to *C. albicans* is in agreement with various other studies on *Candida* infection.[23-28]

In the present study, males were more affected than females with an overall male:female ratio of 1.55. The preponderance of male patients suffering from candidiasis in this study correlates with those of Kashid *et al.* and Singh *et al.*[23,29] Infections were more common in the older age groups in our study. This can be attributed to the various co-morbid conditions and the health issues pertaining to the particular age-groups, as is relevant in our study. However, further studies have to be carried out to justify the significance of the fact.

The disk diffusion method, according to CLSI guidelines, was used for antifungal susceptibility testing as it is simple and easy to perform, but it is only a preliminary screening procedure. To confirm resistance of any strain, broth dilution tests should be used to determine the minimum inhibitory concentrations (MIC) of the drugs. The isolates in the study showed no resistance to amphotericin B. The NCA species showed more resistance than *C. albicans* to fluconazole and this finding was found to be statistically significant (P = 0.0087). These findings corelates with studies by Binesh and Kalyani and Kashid *et al.*[22,23]

Our study is limited by a retrospective design, relatively smaller sample size, a lesser group of antifungals tested, a lack of information regarding the MIC values of the antifungals tested and clinical outcome of the patients. The isolation of a smaller numbers of some of the NCA species like *C. krusei, C. gullerimondii, C. kefyr, C. zeylanoides* etc., precludes our ability to evaluate the clinical significance of these species, although an overall increase in resistance was significantly observed in the NCA species. Therefore, further studies with a larger sample size, elaborate clinical data with treatment details and outcome of the patients and testing of different groups of antifungals along with their MIC values are required for a better understanding of the sure and steady rise of fungal infections, and especially by the NCA species.

The present study highlights the change in epidemiology in the species distribution of *Candida* and also highlights a rise in the infections by NCA species as compared to those by *C. albicans*, along with an increase in resistance of these NCA species to the routinely used antifungals. Therefore knowledge of the local species distribution of *Candida* through presumptive identification, followed by confirmation, is essential to initiate early empirical therapy, especially in an ICU setup, which harbors a lot of immuno-compromised patients and other patients susceptible for acquiring various fungal infections. Moreover, antifungal susceptibility testing, though not as routinely performed as antibacterial susceptibility testing, should be carried out, along with MIC values of the isolates for the various groups of antifungals, in order to optimize therapy and outcome.

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Conflicts of interest

There are no conflicts of interest.

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