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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.12.019

Emergence of non-*albicans Candida* species and antifungal resistance in intensive care unit patients

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ARTICLE INFO

Article history:
Received 17 Sep 2015
Received in revised form 18 Dec, 2nd revised form 21 Dec 2015
Accepted 28 Dec 2015
Available online 22 Mar 2016

Keywords:
Antifungal resistance
Candida species
Colonization
Intensive care unit patients

ABSTRACT

Objective: To evaluate the epidemiology of candidiasis and the antifungal susceptibility profile of *Candida* species isolated from the intensive care unit (ICU) patients.

Methods: The study used a qualitative descriptive design. Relevant samples depending on organ system involvement from 100 ICU patients were collected and processed. Identification and speciation of the isolates was conducted by the biochemical tests. Antifungal susceptibility testing was carried out as per CLSI-M27-A3 document.

Results: Ninety *Candida* isolates were isolated from the different clinical samples: urine (43.3%), tracheal aspirate (31.1%), urinary catheter (12.2%), endotracheal tube (7.8%), abdominal drains (3.3%), sputum (2.2%). The incidence of candidiasis caused by non-*albicans Candida* (NAC) species (63.3%) was higher than *Candida albicans* (36.7%). The various NAC species were isolated as: *Candida tropicalis* (41.1%), *Candida glabrata* (10%), *Candida parapsilosis* (6.7%), *Candida krusei* (3.3%) and *Candida kefyr* (2.2%). The overall isolation rate of *Candida* species from samples was 53.3%. Antifungal susceptibility indicated that 37.8% and 7.8% of the *Candida* isolates were resistant to fluconazole and amphotericin B, respectively.

Conclusions: Predominance of NAC species in ICU patients along with the increasing resistance being recorded to fluconazole which has a major bearing on the morbidity and management of these patients and needs to be further worked upon.

1. Introduction

Nosocomial infections constitute a serious public health problem and are among the major causes of morbidity and mortality leading to increased hospitalization time and consequently, generating high costs for patient treatment [1]. In the recent years, the incidence of nosocomial candidiasis has increased throughout the world, starting from tertiary care centers and spreading to community hospitals [2]. The epidemiological surveillance program in the United States has shown that 5%–10% of patients who admitted in the hospitals acquire nosocomial infection. Of these, about 80% of fungal infections were caused by *Candida* species. According to studies by Centers for Disease Control, *Candida albicans* (*C. albicans*) is the sixth most common cause of nosocomial infections [1].

The frequent use of broad-spectrum antibiotics, central venous catheters, urinary catheters, prosthetic devices and abdominal surgery in intensive care unit (ICU) patients requiring emergency care [3], put patients at a high risk of infection with *Candida* species [4]. Furthermore, ICU admission itself has

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The study protocol was performed according to the Helsinki declaration and approved by the Institutional Ethics Committee of Maulana Azad Medical College & Associated Hospitals, New Delhi, India. Informed written consent was obtained from the patients/guardians.

Foundation Project: Supported by the University Grants Commission (UGC), Government of India, New Delhi-110002, India [Grant: Rs. 908100/- and Grant number: F.31-260/2005(SR)].

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

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become an independent risk factor for the development of *Candida* species infection [5]. The most common species implicated was *C. albicans* recently, when the incidence of non-*albicans Candida* species (NAC) has risen dramatically [6]. In recent decades, several countries around the world have witnessed a change in the epidemiology of *Candida* infections, characterized by a progressive shift from a predominance of *C. albicans* to NAC species [7].

A worrisome trend is the increasing number of reports of fluconazole resistance among species that are typically fluconazole-susceptible, such as *C. albicans*. There are several reports regarding this phenomenon, but larger epidemiologic studies have failed to show a definite geographic or temporal trend toward fluconazole resistance, despite of heavy azoles usage. This epidemiologic shift has greatly impacted the therapeutic choices for initial and definite therapy for this disease [8]. Since there is scanty data on candidiasis in North India, the main objective of present study was to perform a study on the epidemiology of candidiasis and evaluate the antifungal susceptibility profile of the *Candida* species isolated from the ICU patients at our tertiary care hospital.

2. Materials and methods

2.1. Design and setting

A prospective study was focused on epidemiology of candidiasis in ICU patients and to evaluate the antifungal susceptibility profile of *Candida* species isolated from the ICU patients. Relevant samples depending on organ system involvement were collected from the ICU patients of a 1 500 bedded tertiary care hospital and processed in the Department of Microbiology in an Indian medical college.

2.2. Ethics approval

Ethics committee approval was granted by the Institutional Ethics Committee of the College & Associated Hospitals, India. Written consent was taken from the patients and they were informed that their participation was voluntary and that they withdraw from the study at any stage without incurring any penalty.

2.3. Participants

All hospitalized medical/post-operative patients admitted to ICU for > 48 h undergoing therapy for one or more acute organ system failure or requiring intensive post-operative monitoring was studied. Detailed clinical history and investigations of each patient was recorded prospectively and analyzed.

2.4. Collection of samples

Relevant clinical samples depending on organ system involvement were collected as: urine, sputum, tracheal aspirate, urinary catheter, endotracheal tube, abdominal drains. All samples were inoculated on Sabouraud dextrose agar slants containing gentamicin (0.02 mg/mL) and cycloheximide (0.5%). One set of inoculated slants was incubated at 25 °C and the other at 37 °C, and they were examined every other day for growth up to 4–6 weeks.

2.5. Microscopy, culture and identification

Identification and speciation of the isolates were done by colony morphology, Gram's staining, germ tube formation, corn meal agar with Tween 80, HiCrome *Candida* agar and enzymatic triphenyltetrazolium chloride reduction test. For further characterization each isolate was subjected to carbohydrate assimilation and fermentation tests as per standard recommended procedures [9].

2.6. Antifungal susceptibility test

The *in vitro* minimal inhibitory concentrations (MICs) of the fluconazole and amphotericin B was done by the broth microdilution method as per Clinical and Laboratory Standards Institute (CLSI) M27-A3 document using Roswell Park Memorial Institute medium and 3-(N-morpholino) propane sulfonic acid buffer. The concentration ranges tested were 0.125–128.000 μg/mL for fluconazole and 0.016–16.000 μg/mL for amphotericin B. *Candida parapsilosis* (ATCC 22019) (*C. parapsilosis*) and *Candida krusei* (ATCC 6258) (*C. krusei*) from the American Type Culture Collection (ATCC) used as quality control with each batch of clinical isolates [10]. The MIC breakpoints recommended by CLSI guidelines were followed [10]. For fluconazole, MIC breakpoints were as follows: sensitive (MIC 8 μg/mL); susceptible-dose dependent (MIC 16–32 μg/mL); resistant (MIC ≥ 64 μg/mL). For amphotericin B, isolates with MICs of 1 μg/mL were categorized as resistant [10].

3. Results

The age (mean ± SD) of the patients was 37.90 ± 17.20 years (range 8–81 years). *Candida* colonization was seen in 57.3% patients, with 90 *Candida* isolates from different samples (Figure 1).

The majority of patients (77%) had one or more risk factors at the time of the diagnosis of candidiasis (Table 1). The mean age of patients with candidiasis (case group) was higher than the patients admitted to the same ICU with no candidiasis (control group). Case group was divided into two sub-groups: first (CG1) – patients with *C. albicans* colonization and second (CG2) – patients with NAC colonization. The time of hospital stay (mean) in CG2 (19.5 days) > CG1 (9.8 days) > controls (6.3 days) with no statistically significant difference. In control, CG1 and CG2 group, the percentage of patients with an indwelling device and prolonged antibiotic therapy was significantly higher than control and CG1 group

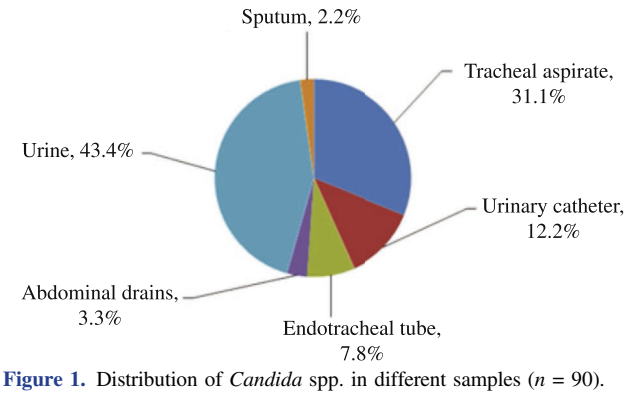

Figure 1. Distribution of *Candida* spp. in different samples (n = 90).

Table 1
Risk factors for candidiasis among the ICU patients.

Variables		Controls[a] (n = 50)	CG1 (n = 19)	P value	CG2 (n = 31)	P value
Continuous variables (mean ± SD)	Age (years)	34.50 ± 17.76	40.00 ± 17.76	0.86	36.60 ± 16.87	0.66
	Length of hospital stay (days)	6.30 ± 2.83	9.80 ± 4.79	0.95	19.50 ± 31.83	0.76
Categorical variables [n (%)]	Surgery (n = 47)	18 (36.0)	10 (52.6)	0.32	19 (61.3)	0.04*
	Trauma (n = 14)	5 (10.0)	4 (21.1)	0.41	5 (16.1)	0.64
	Pneumonia (n = 12)	4 (8.0)	1 (5.3)	0.69	7 (22.3)	0.12
	Neurological (n = 9)	4 (8.0)	2 (10.5)	0.73	3 (9.7)	0.79
	Malignancy (n = 6)	1 (2.0)	2 (10.5)	0.37	3 (9.7)	0.30
	Tuberculosis (n = 9)	2 (4.0)	1 (5.3)	0.31	6 (19.4)	0.06
	Renal failure (n = 4)	1 (2.0)	1 (5.3)	0.47	2 (6.5)	0.67
	Heart disease (n = 5)	3 (6.0)	1 (5.3)	0.90	1 (3.2)	0.97
	Anemia (n = 4)	2 (4.0)	0 (0.0)	NA	0 (0.0)	NA
	Peritonitis (n = 16)	4 (8.0)	5 (26.3)	0.10	7 (22.3)	0.12
	Multiple organ system involvement (n = 17)	2 (4.0)	4 (21.1)	0.07	11 (35.5)	0.0006*
	Indwelling devices (n = 77)	28 (56.0)	19 (100)	NA	30 (96.8)	0.0002*
	Prolonged antibiotic therapy (n = 55)	23 (46.0)	8 (42.1)	0.98	24 (77.4)	0.01*
	Duration of stay > 1 week (n = 25)	4 (8.0)	7 (36.8)	0.01*	14 (45.2)	0.0003*
	Immunosuppressant (n = 20)	2 (4.0)	6 (31.6)	0.005*	12 (38.7)	0.0002*
	Diabetes mellitus (n = 21)	5 (10.0)	5 (26.3)	0.18	11 (35.5)	0.01*
	Smoking (n = 12)	6 (12.0)	2 (10.5)	0.86	4 (12.9)	0.90
	Previous surgery (n = 11)	6 (12.0)	1 (5.3)	0.70	4 (12.9)	0.90

*: Indication of significant P values ($P < 0.05$); NA: Not applicable.
[a]: Patients admitted to the same ICU with no candidiasis; CG1: Patients with *C. albicans* infection; CG2: Patients with NAC infection.

while prolonged antibiotic therapy and surgery in CG2 group (Table 1).

Among the isolates, *C. albicans* accounted for 36.7% and NAC species for 63.3% of all the *Candida* colonization cases. Among the 63.3% NAC species isolates, the most common was *Candida tropicalis* (*C. tropicalis*) (41.1%) followed by *Candida glabrata* (*C. glabrata*) (10%), *C. parapsilosis* (6.7%), *C. krusei* (3.3%) and *Candida kefyr* (*C. kefyr*) (2.2%).

Predominance of *C. albicans* was seen in urine (16.7%) followed by tracheal aspirate (14.4%), catheter (3.3%), endotracheal and abdominal drains (1.1% in each). NAC was also predominant in urine (26.7%) followed by tracheal aspirate (16.7%), catheter (8.9%), endotracheal (6.7%) and sputum (2.2%). Among NAC spp.: *C. tropicalis* (15.6%), *C. glabrata* (5.6%) and *C. krusei* (2.2%) were dominant in urine samples while *C. parapsilosis* (2.2%) and *C. kefyr* (1.1%) were predominant in urine and tracheal aspirate samples.

Candida colonization rate was highest in 41–50 years (75%) age group, followed by 0–10 years (66.67%), > 60 years (55.6%), 31–40 years (54.5%), 21–30 years (46.2%), 51–60 years (46.2%) and 11–20 years (36.8%) age group (Table 2).

Table 2
Age wise distribution of patients and *Candida* species isolated from the patients.

Age group (years)	Total patients (n)	Colonized patients [n (%)]	Colonization rate (%)	*C. albicans* [n (%)]	NAC species [n (%)]	Total isolates [n (%)]
0–10	3	2 (4)	66.7	0 (0.0)	3 (3.3)	3 (3.3)
11–20	19	7 (14)	36.8	6 (6.6)	7 (7.7)	13 (14.5)
21–30	26	12 (24)	46.2	7 (7.8)	15 (16.6)	22 (24.5)
31–40	22	12 (24)	54.5	8 (8.9)	12 (13.3)	20 (22.2)
41–50	8	6 (12)	75.0	1 (1.1)	9 (10.0)	10 (11.1)
51–60	13	6 (12)	46.2	5 (5.7)	6 (6.7)	11 (12.2)
> 60	9	5 (10)	55.6	6 (6.6)	5 (5.7)	11 (12.2)
Total	100	50 (100)	–	33 (36.7)	57 (63.3)	90 (100.0)

Table 3
Susceptibility to fluconazole and amphotericin B of colonized isolates of *Candida* species. n (%).

Isolates (number)	Urine		Sputum		Catheter		Endotracheal		Tracheal		Abdominal drains	
	FLU	AMB	FLU	AMB	FLU	AMB	FLU	AMB	FLU	AMB	FLU	AMB
C. albicans (n = 33)	6 (40.0)	2 (13.3)	0 (0.0)	0 (0.0)	2 (66.6)	0 (0.0)	0 (0.0)	0 (0.0)	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)
C. tropicalis (n = 37)	5 (35.7)	1 (7.1)	0 (0.0)	0 (0.0)	3 (42.8)	0 (0.0)	3 (60.0)	1 (20.0)	3 (30.0)	1 (10.0)	1 (100.0)	0 (0.0)
C. krusei (n = 3)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)
C. parapsilosis (n = 6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
C. kefyr (n = 2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C. glabrata (n = 9)	2 (40.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (n = 90)	15 (38.5)	4 (10.2)	1 (50.0)	0 (0.0)	5 (45.5)	0 (0.0)	3 (42.8)	1 (14.3)	8 (28.6)	2 (7.1)	2 (66.6)	0 (0.0)

FLU: Fluconazole drug, for fluconazole, isolates with MICs of 64 μg/mL were categorized as resistant; AMB: Amphotericin B drug, for amphotericin B, isolates with MICs of 1 μg/mL were categorized as resistant.

The antifungal susceptibility testing showed that 34 *Candida* isolates (37.8%) were resistant to fluconazole. Out of the 34 resistant *Candida* isolates, 12 (35.3%) were *C. albicans* and 22 (64.7%) were NAC species as: *C. tropicalis* 15 (68.2%), *C. krusei* and *C. glabrata* 3 (13.6%) each and *C. parapsilosis* 1 (4.5%). However, 7 (7.8%) *Candida* isolates showed resistance to amphotericin B; 2 (28.6%) were *C. albicans* and 5 (71.4%) were NAC species as: *C. tropicalis* 3 (60%), *C. krusei* 2 (40%) as shown in Table 3.

4. Discussion

In recent years, there had been a remarkable increase in the use of indwelling devices in hospitals. *Candida* species are important nosocomial pathogens. Modern medical procedures including implantation of various kinds of devices contribute to the risk factors for developing candidiasis [11].

In present study, the majority of patients (77%) had one or more risk factors at the time of the diagnosis of candidiasis. The most common risk factors in these patients were indwelling devices accounting for 98%, followed by prolonged antibiotic therapy (64%), surgery (58%), duration of stay > 1 week (42%), immunosuppressants use (36%), diabetes mellitus (32%), multiple organ system involvement (30%), peritonitis and trauma (18%) each, pneumonia (16%), tuberculosis (14%), smoking (12%), previous surgery, neurological involvement and malignancy (10%) each, renal failure (6%), heart disease (4%) of patients with *Candida* colonization.

However, a study at USA reported that the presence of prior intravenous antibiotics (95.8%), prior surgical procedure (73.9%), diabetes mellitus (37.3%), cancer (21.1%), alcoholism (16.9%), neutropenia (0.7%), renal failure (6.3%), corticosteroid therapy (5.6%), parenteral nutrition (9.2%) were the common risk factors in adult intensive care patients [12]. According to Arslankoylu *et al.* on symptomatic and asymptomatic candidiasis in a pediatric ICU, reported the use of broad spectrum antibiotics and parenteral nutrition were the most common risk factors followed by central venous catheter (96.8%), blood transfusion (96.8%), surgery (45.2%) and use of corticosteroids (35.5%) [13].

In India, a study by Singh *et al.* reported that the most common risk factor was presence of urinary catheter (63.2%), mechanical ventilation (63.2%), peritoneal dialysis (63.2%), followed by central line insertion (47.4%), diabetes mellitus (26.3%) and use of corticosteroids (21.1%) of patients with fungal colonization of ICU patients [14]. In another Indian study by Jain *et al.*, prolonged antibiotic therapy (100%), diabetes mellitus (38.6%) and previous surgery (52.9%) were the universal risk factors reported, where 100% of catheterized ICUs patients had previous history of antibiotics. Antibiotics increase the risk of colonization of *Candida* spp. by suppressing endogenous flora [15]. Likewise, Sahni *et al.* reported that the antibiotics use, mechanical ventilation and central catheters significantly related to candidaemia [16]. High rate of colonization has been reported in critically ill surgical patients [13]. Urinary catheterization and broad-spectrum antibiotics use were the most common risk factors. On the other hand, Harvey and Myers observed that the most common risk factors in adult patients were central catheterization and blood transfusion [17].

In this study, CG1 (patients with *C. albicans* colonization) had risk factors of duration of stay > 1 week in ICU (36.8%) and immunosuppressant (31.6%), and CG2 (patients with NAC colonization) had risk factors of multiple organ system involvement (35.5%), indwelling devices (96.8%), prolonged antibiotic therapy (77.4%), duration of stay > 1 week (45.2%), use of immunosuppressant (38.7%) and diabetes mellitus (35.5%) which were significantly higher and statistically significant ($P < 0.05$) than the control group. The number of patients with central line insertion (81.3%) and urinary catheter (85.7%) were significantly more in fungal infection group, whereas those with peritoneal dialysis (63.2%) were significantly more in fungal colonization group. However, there was no significant difference in the mean duration of presence of these risk factors in both the groups [14].

We observed a predominance of NAC species (63.3%) however *C. albicans* was the most frequently isolated species (36.7%). This is also consistent with the emergence of predominance of NAC species worldwide [14]. The preponderance of *C. tropicalis* in our study, is consistent with a prospective study by Chakrabarti *et al.*, where *C. tropicalis* was the most common (42.1%) yeast isolated [18].

We report a lower rate of *C. glabrata* (10%) and *C. parapsilosis* (6.7%) almost similar to a study among adult diabetes mellitus patients by Al-Attas and Amro, in which they also reported a lower rate of *C. glabrata* (11.1%) and *C. parapsilosis* (6.7%) [19]. Traditionally, *C. tropicalis* has been the second and *C. glabrata* the third or fourth most common *Candida* species isolated [20]. Our study clearly shows that *C. krusei* (3.3%) and *C. kefyr* (2.2%) are relatively rare as seen in rest of India [18,21,22]. In last few decades, there have been numerous reports documenting the rare isolation of *C. kefyr* and *C. krusei* [21–24].

In this study, *Candida* colonization rate was 50%, which is similar to the colonization rate of 51% found in a previous international multicentric study from 75 countries [25]. *Candida* colonization rate due to NAC isolates (63.3%) was higher than *C. albicans* isolates (36.7%). In our study, urinary tract *Candida* colonization was the most common.

In our study, highest rate of *Candida* colonization was seen in 41–50 years (75.00%) followed by 0–10 years (66.67%), > 60 years (55.60%), could be due to lowered host defenses at extremes of age. This finding is also supported by many other researchers'. Prolonged antibiotic use increases the risk of colonization of *Candida* species by suppressing the endogenous flora [15]. Higher rate of *Candida* colonization in 0–9 years could be understood as they are more susceptible to infections due to various reasons including weak immune systems and in the > 60 years age group this could correlate with prevalence of debilitating conditions, decreased immune status and aging [26].

The most common site of *Candida* isolation was urine (43.3%), followed by tracheal aspirate (31.1%), urinary catheter (12.2%), endotracheal tube (7.8%), abdominal drains (3.3%) and sputum (2.2%). Singh *et al.*, reported that in medical and surgical critical care section patients, the most common site or specimen of fungus isolation in patients was also urine (74.7%), followed by blood (20.8%) [14]. In a study by Jain *et al.*, *C. tropicalis* was predominant in catheterized ICU patients [15] similar to our study where *C. tropicalis* was most common isolate from catheterized and endotracheal patients.

In this study, *C. albicans* was most commonly isolated from urine and tracheal samples. Most observational studies of candiduria have reported *C. albicans* to be the predominant species isolated [27]. However, Paul et *al.*, reported *C. tropicalis* as the most prevalent NAC species causing candiduria [28].

In our study, colonization of ICU patients with *Candida* species was common, occurring at least once in 73% of patients, a figure not dissimilar to that found in prior studies [12]. As the length of stay in ICUs and the frequency of invasive procedures increases, the incidence of *Candida* colonization and *Candida* infection increases [13]. Most patients suffer no ill effects due to *Candida* colonization because of its low-level virulence; however, in some patients with suppressed defenses, the organisms invade and cause illness [13]. Stamos and Rowley [29] reported that *Candida* colonization was observed after 7 days of hospitalization, which is similar to our results.

In our study 37.8% *Candida* isolates demonstrated resistance to fluconazole, concordant to a study by Deorukhkar *et al.* [27]. In our study, the resistance rate for fluconazole (37.8%) was more compared to amphotericin B (7.8%). In the present study, *C. tropicalis* isolates were found to be more resistant to fluconazole (38.5%). The increase in the rate of fluconazole resistance in *C. tropicalis* is of concern because this species is one of the most commonly isolated NAC species and fluconazole is the most common antifungal agent used for the treatment of various types of candidiasis [27]. In this study, *C. albicans* (36.4%) isolates was also found to be resistant to fluconazole, compared to the different resistance rates reported in other studies (10.5–21%) [30].

A hundred percent resistance was reported for *C. krusei*, with its greatest potential to acquire resistance to fluconazole because of its intrinsic resistance toward azoles and poor susceptibility to all other antifungals, including amphotericin B [31,32]. Also, the resistance of *C. glabrata* to fluconazole was consistently higher (16.7%) with increased resistance (19.2%) reported by Bassetti *et al.* [20]. A reduced antifungal susceptibility in NAC species and a correlation with routine fluconazole prophylactic use has been suggested [20]. Extensive fluconazole use is one of the possible causes, for the increased resistance to the drug as well as for the progressive substitution of *albicans* species with non-*albicans* drug resistant strains as principal etiologic agent of infection [33]. The sensitivity pattern of *Candida* species as revealed in this study shows that amphotericin B to be suitable drug for empirical therapy unlike fluconazole with a high resistance in *Candida* species [30].

The outcomes of our study, highlight the predominance of NAC species colonization in ICU patients similar to the trends in the western countries, a cause of concern in our country as reported by other workers too [14]. The major risk factors were use of indwelling devices, prolonged antibiotic therapy and surgery. The increase in resistance being recorded to fluconazole has a major bearing on the morbidity and management of these patients and needs to be further worked upon. Identification and antifungal susceptibility of *Candida* isolates from ICU patients will help in building a data center of the prevalent *Candida* species along with their antifungal resistance profile and will go a long way in the management of these serious patients.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] de Cássia Orlandi Sardi J, de Souza Pitangui N, Gullo FP, e Maria José Soares Mendes Giannini AMFA. A mini review of *Candida* species in hospital infection: epidemiology, virulence factor and drugs resistance and prophylaxis. *Trop Med Surg* 2013; http://dx.doi.org/10.4172/2329-9088.1000141.

[2] Singhi S, Deep A. Invasive candidiasis in pediatric intensive care units. *Indian J Pediatr* 2009; **76**(10): 1033-44.

[3] Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 503-35.

[4] Chander J, Singla N, Sidhu SK, Gombar S. Epidemiology of *Candida* blood stream infections: experience of a tertiary care centre in North India. *J Infect Dev Ctries* 2013; **7**(9): 670-5.

[5] Zaragoza R, Pemán J. The diagnostic and therapeutic approach to fungal infections in critical care settings. *J Invasive Fungal Infect* 2008; **6**(3): 90-8.

[6] Bajwa S, Kulshrestha A. Fungal infections in intensive care unit: challenges in diagnosis and management. *Ann Med Health Sci Res* 2013; **3**: 238-44.

[7] Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K. Non-*albicans Candida* species in blood stream infections in a tertiary care hospital at New Delhi, India. *Indian J Med Res* 2012; **136**: 997-1003.

[8] Ostrosky-Zeichner L, Pappas PG. Invasive candidiasis in the intensive care unit. *Crit Care Med* 2006; **34**(3): 857-63.

[9] Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Mycology. In: *Color atlas and textbook of diagnostic microbiology*. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 1997, p. 983-1057.

[10] Clinical and Laboratory Standards Institute. *MM27–A3: reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard*. 3rd ed. Wayne: Clinical and Laboratory Standards Institute; 2008.

[11] Punithavathy PM, Nalina K, Menon T. Antifungal susceptibility testing of *Candida tropicalis* biofilms against fluconazole using calorimetric indicator resazurin. *Indian J Pathol Microbiol* 2012; **55**: 72-4.

[12] Hedderwick SA, Lyons MJ, Liu M, Vazquez JA, Kauffman CA. Epidemiology of yeast colonization in the intensive care unit. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 663-70.

[13] Arslankoylu AE, Kuyucu N, Yilmaz BS, Erdogan S. Symptomatic and asymptomatic candidiasis in a pediatric intensive care unit. *Ital J Pediatr* 2011; http://dx.doi.org/10.1186/1824-7288-37-56.

[14] Singh T, Kashyap AK, Ahluwalia G, Chinna D, Sidhu SS. Epidemiology of fungal infections in critical care setting of a tertiary care teaching hospital in North India: a prospective surveillance study. *J Clin Sci Res* 2014; **3**: 14-25.

[15] Jain M, Dogra V, Mishra B, Thakur A, Loomba PS, Bhargava A. Candiduria in catheterized intensive care unit patients: emerging microbiological trends. *Indian J Pathol Microbiol* 2011; **54**: 552-5.

[16] Sahni V, Aggarwal SK, Singh NP, Anuradha S, Sikdar S, Wadhwa A, et al. Candidemia—an under-recognized nosocomial infection in Indian hospitals. *J Assoc Physicians India* 2005; **53**: 607-11.

[17] Harvey RL, Myers JP. Nosocomial fungemia in a large community teaching hospital. *Arch Intern Med* 1987; **147**: 2117-20.

[18] Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, et al. Recent experience with fungaemia: change in species distribution and azole resistance. *Scand J Infect Dis* 2009; **41**: 275-84.

[19] Al-Attas SA, Amro SO. Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients. *Ann Saudi Med* 2010; **30**(2): 101-8.

[20] Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, Viscoli C. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. *PLoS One* 2011; **6**(9): e24198.

[21] Prasad KN, Agarwal J, Dixit AK, Tiwari DP, Dhole TN, Ayyagari A. Role of yeasts as nosocomial pathogen and their susceptibility to fluconazole and amphotericin B. *Indian J Med Res* 1999; **110**: 11-7.

[22] Kaur R, Goyal R, Dhakad MS, Bhalla P, Kumar R. Epidemiology and virulence determinants including biofilm profile of *Candida* infections in an ICU in a tertiary hospital in India. *J Mycol* 2014; http://dx.doi.org/10.1155/2014/303491.

[23] Sengupta P, Ohri VC. Study of yeast species isolated from clinical specimens. *Med J Armed Forces India* 1999; **55**: 319-21.

[24] Jain N, Mathur P, Misra MC, Behera B, Xess I, Sharma SP. Rapid identification of yeast isolates from clinical specimens in critically ill trauma ICU patients. *J Lab Physicians* 2012; **4**: 30-4.

[25] Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; **302**(21): 2323-9.

[26] Raval PN, Patel PG, Patel BV, Soni ST, Bhatt SK, Vegad MM, et al. Microbiological surveillance of intensive care units in a tertiary care teaching hospital-Western India. *Int J Microbiol Res* 2012; **4**(7): 270-4.

[27] Deorukhkar SC, Saini S, Mathew S. Virulence factors contributing to pathogenicity of *Candida tropicalis* and its antifungal susceptibility profile. *Int J Microbiol* 2014; http://dx.doi.org/10.1155/2014/456878.

[28] Paul N, Mathai E, Abraham OC, Mathai D. Emerging microbiological trends in candiduria. *Clin Infect Dis* 2004; **39**: 1743-4.

[29] Stamos JK, Rowley AH. Candidemia in a pediatric population. *Clin Infect Dis* 1995; **20**: 571-5.

[30] Badiee P, Alborzi A. Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: a five year study. *Iran J Microbiol* 2011; **3**(4): 183-8.

[31] Ernst JF, Schmidt A, editors. *Dimorphism in human pathogenic and apathogenic yeasts*. Basel: S. Karger AG; 2000.

[32] Wissing H, Ballus J, Bingold TM, Nocea G, Krobot KJ, Kaskel P, et al. Intensive care unit-related fluconazole use in Spain and Germany: patient characteristics and outcomes of a prospective multicenter longitudinal observational study. *Infect Drug Resist* 2013; **6**: 15-25.

[33] Musu M, Evangelista M, Mura P, Cossu A, Carta M, Aru GN, et al. Fluconazole therapy for treatment of invasive candidiasis in intensive care patients. Is it still valid from a pharmacological point of view? *J Pediatr Neonatal Individ Med* 2014; **3**(1): e030120.