Original Article

Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India

Bhattacharjee P*

NH-Rabindranath Tagore International Institute of Cardiac Sciences 124, E. M. Bypass, Mukundapur, Kolkata,700099-West Bengal, India

*** Corresponding author: Partha Bhattacharjee**, NH-Rabindranath Tagore International Institute of Cardiac Sciences 124, E. M. Bypass, Mukundapur, Kolkata, 700099-West Bengal, India. Email: microbiology559@gmail.com

(Received: 21 July 2016; Revised: 12 September 2016; Accepted: 26 September 2016)

Abstract

Background and Purpose: The incidence of fungal infection as well as candidemia has increased significantly, contributing to morbidity and mortality in the developed countries. The alarming increase in infections with multidrug resistant bacteria is due to overuse of a broad spectrum antimicrobials, which leads to over growth of *Candida* spp.; thus, enhancing its opportunity to cause the disease. A shift has been observed in the relative frequency of each *Candida* spp. Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recent echinocandin class of molecules. In the past few decades, the incidence of resistance to antifungal treatment of *Candida* spp. has increased rapidly, which is of serious concern for healthcare professionals. Studies on prevalence of infections and antifungal susceptibility testing can help with deciding on clinical strategies to manage this problem. Herein, we aimed to identify the epidemiology of *Candida* spp. among blood culture isolates and to investigate the susceptibility pattern of these species to antifungal agents.

Materials and Methods: *Candida* spp. were isolated from blood cultures from 70 patients in a tertiary care hospital, Kolkata, India. The growth of *Candida* spp. on sabouraud dextrose agar was confirmed by Gram staining, where gram-positive budding fungal cells were observed. The species identification as well as antifungal susceptibility testing were performed with VITEK 2 compact automated system using VITEK-2 cards for identification of yeast and yeast-like organisms (ID-YST card). Antifungal susceptibility testing was carried out with VITEK 2 fungal susceptibility card (AST-YS07 kit).

Results: Out of 70 samples, *Candida albicans* were isolated from 34 (48.57%) samples. The remaining 36 (51.43%) were non-albicans *Candida* (NAC). Out of 34 *C. albicans*, antifungal susceptibility was detected in 28 isolates, all of which were sensitive to fluconazole (FLC). Resistance to amphotericine B (AMP), flucytosine (5FC), voriconazole (VRC), and itraconazole (ITC) was observed in 44.12%, 52.94%, 8.82%, and 17.65% of the cases, respectively. For other *Candida* spp. (other than *C. albicans*), antifungal susceptibility was evaluated for 36 isolates, among which resistance to AMP, FLC, 5FC, VRC, and ITC was found in 30.56%, 61.11%, 33.33%, 19.44%, and 38.89% cases, respectively.

Conclusion: Species-level identification of *Candida* and their antifungal sensitivity testing should to be performed to achieve better clinical result and to select an appropriate and effective antifungal therapy. High resistance to antifungal agents is an alarming sign to the healthcare professionals.

Keywords: *Candida albicans*, Fluconazole, Non-albicans *Candida*, Voriconazol, Itraconazole

➢ How to cite this paper:
Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016; 2(2): 20-27. DOI: 10.18869/acadpub.cmm.2.2.5

Introduction

Candida spp. are the normal flora of human skin and mucosa, but have been reported more frequently as pathogen due to risk factors such as excessive consumption of a broad spectrum of antibiotics, underlying malignant diseases, HIV infection, organ transplantation, prolonged hospital stay, and exposure to invasive procedures [1, 2].

Candida spp. can cause a wide range of infections including blood stream infections (BSIs) and disseminated candidiasis. In spite of advances in the diagnosis and treatment of candidiasis, among the pathogens involved in BSI, *Candida* ranks fourth in the United States and seventh in Europe [3-6]. Only few studies from India have reported candidemia rates (6-18%) [7-9] and increase in isolation of non-albicans *Candida* (NAC) from BSIs [10, 11]. In a recent study, the incidence rate of candidemia has been reported to be 6.9 per 1000 in intensive care unit (ICU) patients, and 7.5% of ICU patients receiving antifungal therapy [12, 13]. Candidemia increases mortality rate by 20-49%

Copyright© 2016, Published by Mazandaran University of Medical Sciences on behalf of Iranian Society of Medical Mycology and Invasive Fungi Research Center. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

[14, 15] and nosocomial candidiasis are associated with crude mortality rate of over 60%, while the attributable mortality rate may be as high as 49% [16, 17], but still there are many questions regarding management of candidiasis that remain unanswered.

The genus *Candida* is comprised of a heterogeneous group of organisms, and more than 17 different *Candida* spp. are known as etiological 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* [18].

A shift has been observed in the relative frequency of each *Candida* spp. isolated from blood. There are published data from various centers regarding the incidence and relative frequency of *Candida* spp. [19]. NAC are also implicated in the recent years [20-22].

Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recently developed echinocandin class of molecules [23, 24]. Fluconazole (FLC) is an antifungal agent most commonly used for prophylaxis as it can be administered orally and is comparatively cheaper than other antifungal agents. Nonetheless, selection of appropriate empiric therapy is complicated considering the increasing prevalence of NAC species [25]. Adverse side effects, toxicity, and emergence of drug resistance are the limitations for use of polyenes, allylamines, and azoles. Emergence of drug resistance in *C. albicans* is reported all over the world [23, 24].

Incidence of antifungal resistance to *Candida* spp. has been on a growing trend over the past decade [26, 27]. Studies on the prevalence rate of infections and antifungal susceptibility testing can help with deciding on clinical strategies [28]. The potential clinical importance of species-level identification has been recognized as *Candida* spp. differs in expression of putative virulence factors and antifungal susceptibility [29, 30]. Rapid identification of *Candida* spp. can also help with early management of antifungal therapy. In the current study, we aimed to identify the epidemiology of *Candida* spp. among blood culture isolates and to investigate the susceptibility pattern of these species to antifungal agents.

Materials and Methods

This retrospective, observational study was performed in the microbiology laboratory of a tertiary care hospital in Kolkata, India, during January 2011-March 2015. Ethics Committee permission was obtained prior to the study. From a total of 1735 positive blood cultures, only 70 *Candida* spp. were isolated from blood cultures.

Specimen collection

Specimens were collected under aseptic precaution, and blood culture was carried out by BacTAlert3D (Biomerieux, France) automated blood culture system.

Isolation and identification of Candida and antifungal susceptibility testing

Clinical isolates of *Candida* spp. obtained from blood culture using automated blood culture system were sub-cultured onto Sabouraud dextrose agar (HiMedia, India) and blood agar plates (HiMedia, India) after getting gram-positive budding yeasts on Gram stain of blood culture broth. Suspected colonies of *Candida* were confirmed through Gram stain and Germ tube test, and then were identified with VITEK 2 Compact (Biomerieux, France) using VITEK 2 cards for identification of yeast and yeast-like organisms (ID-YST cards) kits. Antifungal susceptibility testing was performed with AST YS07 Kits on VITEK 2 Compact. Standard operative procedures as described by the manufacturer were followed. Antifungal susceptibility testing was performed by NCCLS M44-A2 Disc diffusion method [31].

Results

All the 70 *Candida* spp. found to be microscopy and culture positive on both blood agar and SDA for Candida spp. were considered. Out of 70 samples, *C. albicans* were isolated from 34 (48.57%) and the remaining 36 (51.42%) samples were positive for NAC species. Among NAC, *Candida tropicalis* 17 (24.28%) followed by *Candida haemulonii* 6 (8.57%) had the highest frequency; the remaining NAC isolates are illustrated in Table 1 and Figure 1.

Table 1. Species distribution of *Candida*

Species	No. (%)
Candida albicans	34 (48.57%)
Candida tropicalis	17 (24.28%)
Candida haemulonii	6 (8.57%)
Candida glabrata	4 (5.71%)
Candida pelliculosa	2 (2.86%)
Candida sake	3 (4.29%)
Candida rugosa	2 (2.86%)
Candida famata	2 (2.86%)

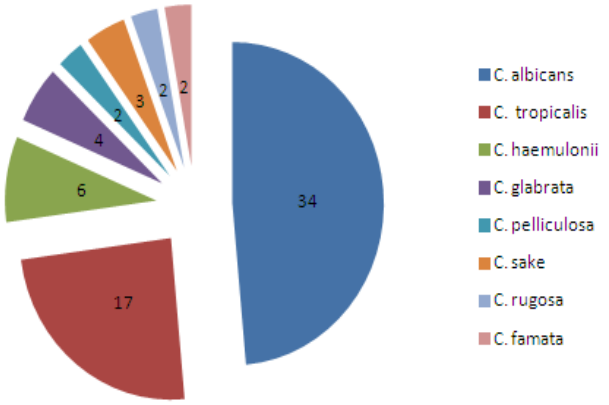

Figure 1. Species distribution of *Candida* isolates obtained from blood isolates

Table 2 demonstrates age and gender-wise distribution of patients. Out total 34 patients having isolates of *C. albicans*, 7 (20.59%) were female and 27 (79.41%) were male. Whereas, among the patients having NAC isolates, 19 (52.77%) were female and 17 (47.22%) were male.

Figure 2 shows *Candia albicans*; most of the patients were within the age group of 51-60 years, whereas for NAC, most of the patients were aged 31-60 years; it also shows that candidiasis is more prevalent in females than males.

Table 3 exhibits susceptibility pattern of *Candida* spp. to amphotericine B (AMP), FLC, flucytosine (5FC), voriconazole (VRC), and itraconazole (ITC). Out of all *Candida* spp, resistance to AMP, FLC, 5FC, VRC, and ITC was identified in 40.63%, 34.38% 46.88%, 18.75%, and 31.25% of the cases, respectively. *C. albicans* was found to be sensitive to FLC 100.0%, and resistance to AMP, 5FC, VRC, and ITC was detected in 53.6%, 64.3%, 10.7%, and 21.4% of the cases, respectively.

For NAC, resistance to AMP, FLC, 5FC, VRC, and ITC was found in 30.56%, 61.11%, 33.33%, 19.44%, and 38.89% of the cases, respectively. *C. albicans* showed maximum number of sensitive cases to FLC, whereas susceptibility pattern of few NAC species to caspofungin shows 100%

Table 2. Age and gender-wise distribution of *Candida albicans* and non-albicans *Candida* species

Age	*Candida albicans*		Non-albicans *Candida*		Total
	Male	Female	Male	Female	
0-10	1	1	4	2	8
11-20	2	2	2	0	6
21-30	6	0	2	1	9
31-40	0	1	0	7	8
41-50	1	0	2	5	8
51-60	7	2	5	2	16
61-70	7	1	0	2	10
71-80	3	0	2	0	5
Total	27	7	17	19	70

Table 3. Susceptibility of *Candida* species to antifungal drugs

Name of the organisms	Antifungal drug														
	Amphotericine B			Fluconazole			Flucytosine			Voriconazole			Itraconazole		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans (n=28) (%)	13 (46.4)	0 (0.0)	15 (53.6)	28 (100.0)	0 (0.0)	0 (0.0)	10 (35.7%)	0 (0.0)	18 (64.3)	25 (89.3)	0 (0.0)	3 (10.7)	22 (78.6)	0 (0.0)	6 (21.4)
C. tropicalis (n=17) (%)	12 (70.59)	0 (0.0)	5 (29.41)	7 (41.18)	1 (5.88)	9 (52.94)	8 (47.06)	0 (0.0)	9 (52.94)	10 (58.82)	2 (11.76)	5 (29.41)	9 (52.94)	2 (11.76)	6 (35.29)
C. haemulonii (n=6) (%)	2 (33.3)	0 (0.0)	4 (66.67)	2 (33.3)	0 (0.0)	4 (66.67)	6 (100.0)	0 (0.0)	0 (0.0)	4 (66.7)	0 (0.0)	2 (33.3)	2 (33.3)	0 (0.0)	4 (66.67)
C. glabrata (n=4) (%)	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)
C. pelliculosa (N=2) (%)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)
C. sake (n=3) (%)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)	0 (0.0)	0 (0.0)	1 (33.33)	2 (66.66)	0 (0.0)
C. rugosa (n=2) (%)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
C. famata (n=2) (%)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)
Total (n=64) (%)	38 (59.38)	0 (0.0)	26 (40.63)	41 (64.06)	1 (1.56)	22 (34.38)	34 (53.13)	0 (0.0)	30 (46.88)	50 (78.13)	2 (3.1)	12 (18.75)	40 (62.5)	4 (6.25)	20 (31.25)

S=Sensitive, R=Resistance, I=Intermediate

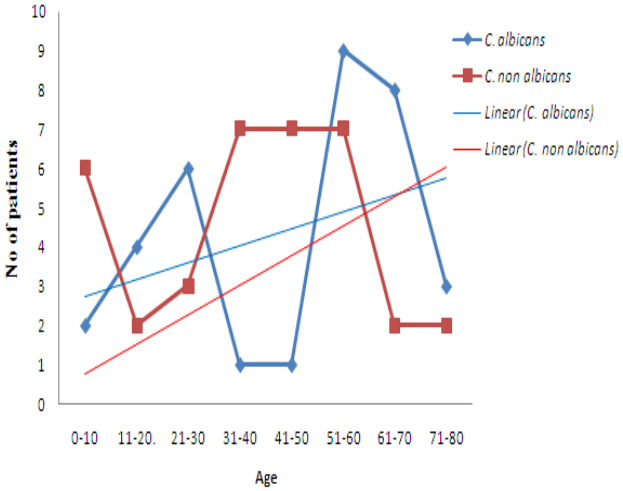

Figure 2. Male to female ratio of Candidiasis

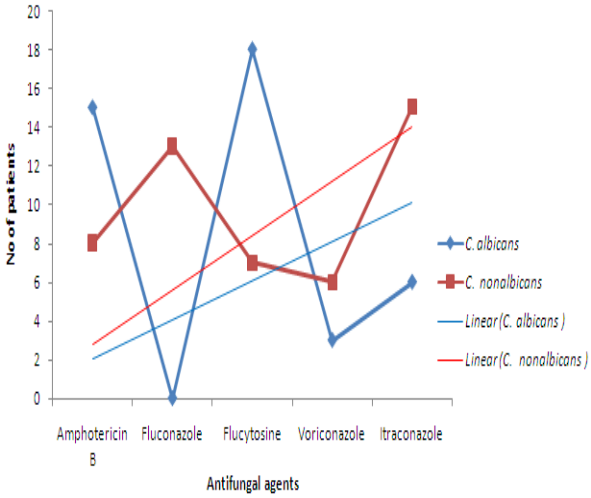

Figure 3. Resistance pattern of *Candida* species to different antifungal agents

NAC in the near future.

Figure 3 exhibits the resistance pattern of *Candida* spp. to different antifungal agents. According to this figure, NACs are more resistant to antifungal agents compared to *Candida albicans*.

Discussion

Infection represents a frequent complication among the patients admitted to tertiary care hospitals. *Candida* spp. infections have increased in the last few decades, particularly those caused by the NAC, indicating the importance of laboratory diagnosis for the correct identification of species involved and initiation of timely and adequate treatment [32].

In our study, the *Candida* spp. were isolated from 4.03% total positive blood culture for growth. Great variation in the prevalence and incidence of candidemia has been reported from India. Kumar et al. [33] from South India reported incidence of 5.7% for candidemia among children with hematological malignancy; Verma et al. [34] reported incidence rate of 1.61%, whereas Xess et al. [8] found a prevalence rate of 6% for candidemia. A study by Sahin et al. [35] performed in Moulana Azad Medical College, New Delhi, India, found incidence rate of 6.9% for *Candida* spp.

The variables considered in this study include age, gender, and the hospital units (Figure 4), in which patients were infected. Furnaleto et al. [36] observed that infection caused by *Candida* spp. were more frequent in the elderly, those aged less than one year old, and ICU patients [37, 38]. The age and gender distribution of our study correlates the observation of other researchers in India [34, 39]. In our study, *C. albicans* has traditionally been the leading cause of candidemia worldwide, but this study shows that NAC infection is on a growing trend [40-42]. Although significant geographic variation is observed among cases of candidemia in different parts of the world, which appears to follow a specific pattern. In our study, BSIs caused by NAC were more common than those caused by *C. albicans*, more specifically in Asia, South Europe, South America, and the Indian subcontinent [43, 44].

In the present study, the incidence rate of BSIs caused by *C. albicans* was 48.57%, which is similar to the reports from United States, Europe, and Brazil [45-47]. Herein, we found *C. tropicalis* following *C. albicans* was the most predominant species, which is in line with other Indian studies showing *C. tropicalis* (35-45%) as the predominant isolates [8, 9, 11, 44, 48, 49]. We did not identify *C. parapsilosis*, which is the causative agent in the most cases of BSI and candidemia [8, 10, 41, 50-52], and *C. kruseri*, which has low prevalence in all settings and geographical regions [43].

C. glabrata has emerged as an important opportunistic pathogen worldwide. It is the second most common yeast isolated as part of normal flora

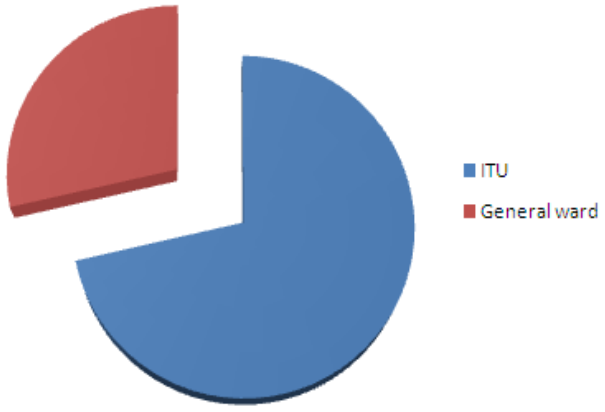

Figure 4. The Distribution of *Candida* spp. in different wards

and its role as a pathogen has only been recognized in the past few decades. Trick et al. [41] reported a remarkable increase in the incidence rate of isolation of *C. glabrata* from BSI patients, and there is concern over the increase in azole resistance among *C. glabrata* strains.

With the increased incidence of candidemia and the growing number of antifungal agents, laboratory aids with the accurate selection of antifungal therapy. The standardized broth microdilution method is expensive, laborious, and cumbersome for use in clinical microbiology laboratories. Recently, disc diffusion method has been approved by the Clinical and Laboratory Standards Institute (CLSI) for antifungal susceptibility testing of yeasts. We have confirmed the susceptibility pattern by VITEK 2 automated system since VITEK 2 system is a reliable technique for antifungal susceptibility testing of yeast species; this technique can be clinically useful for determining susceptibility of *Candida* spp. and other yeast species. It is also a reliable technique for in-vitro identification of azole and AMP resistance. It has the advantage of being more rapid and easier than the alternative procedures developed by either the CLSI or the EUCAST [53] and the disk diffusion method [31].

Even though CHROM agar helps with identification at a lower cost compared to VITEK 2, which is useful in low resource countries, its main drawback is the long duration that it takes for complete identification. As early and rapid instillation of treatment can save the life of patients with fungal infections, rapid diagnosis is of utmost importance. Accordingly, VITEK 2 can be applied for rapid identification [54].

In our study, FLC resistance rate of *Candida* spp. was 34.8%, which is in line with other Indian studies [9, 33]. Resistance to FLC is of great concern as it is the most common azole used for the treatment of disseminated candidiasis including candidemia. FLC is available in both intravenous and oral formulations with high bioavailability and is more cost-effective than other antifungal agents. Although AMP is effective against most strains of *Candida* spp., it is not the first-line treatment for candidemia due to the nephron toxicity associated with it. ITC is used for the treatment of mucosal candidiasis [55]; studies regarding its role in the treatment of candidemia are sparse. In a study by Kothari et al. [9], 24% of *Candida* isolates were resistant to ITC, which is consistent with our result.

The decrease in susceptibility of *Candida* isolates to FLC (75%) is a matter of concern although VRC, AMP, and 5FC continue to show good efficacy. Western data have revealed that *Candida* species are reliably susceptible to polyenes, azoles, and echinocandins, but Indian studies demonstrate very high resistance to FLC for all *Candida* isolates although AMP susceptibility is high [56], which is in agreement with our findings.

In this present study, caspofungin shows 100% sensitivity pattern to few NAC species, which may be useful for healthcare professional to treat the *Candida* infection caused by NAC. Widespread use of FLC in various clinical conditions is the major cause of NAC dominance over *C. albicans* [27].

With various types of antifungal agents available in the market, performing antifungal susceptibility testing and reporting their therapeutic outcome seems to be necessary. Evaluation of the recent antifungal agents is required, as well.

Our study is limited to a single institution′s experience, other shortcomings of this study include retrospective design and scarcity of data on the costs associated with *Candida* infection and virulence among *Candida* species in different age groups. Additionally, the small number of isolates of *Candida* spp. has limited our ability to evaluate the relative significance of these specific non-albicans species.

Further studies with richer clinical data and larger samples are required to evaluate the costs associated with albicans and NAC species. In summary, the prevalence of *Candida* BSI has shifted dramatically from *C. albicans* to NAC spp. Therefore, early and accurate diagnosis of *Candida* infection is essential since each species varies significantly in susceptibility to the currently used antifungal drugs. Conducting antifungal susceptibility testing in the laboratories can aid clinicians with timely administration of the appropriate and accurate antifungal agents, it may also restrict the empirical use of the current antifungal agents.

Conclusion

The changing epidemiology of candidemia highlights the need for close monitoring of *Candida* species distribution and susceptibility to optimize treatment and outcome. We should also develop guidelines for empiric therapy based on the disease epidemiology in India.

Acknowledgments

The author would like to thank Dr. (Col) Tapas Ray (HOD. Dept. of Microbiology), Dr. Anjuli Barai (Manager, Dept. of Clinical Research), and NH-Rabindranath Tagore International Institute of Cardiac Sciences, Kolkata, for giving me the permission to perform this study. Also, the author

extends his gratitude to Mr. Tanmoy Mondal and Ms. Sumati Mahato for their help with data collection. The study was not funded by any funding agencies.

Author's contribution

P. B. designed the study, and drafted and prepared the manuscript.

Conflicts of interest

None declared.

Financial disclosure

There was no financial interest related to the materials of the manuscript.

References

1. Messer SA, Jones RN, Fritsche TR. International surveillance of Candida spp. and *Aspergillus* spp.: report from the SENTRY antimicrobial surveillance program (2003). J Clin Microbiol. 2006; 44(5):1782–7.
2. Richardson M, Lass-Flörl C. Changing epidemiology of systemic fungal infections. Clin Microbial Infect. 2008; 14(Suppl 4):5–24.
3. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004; 39(3):309-17.
4. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidaemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clin Infect Dis. 2004; 38(3):311-20.
5. Yapar N. Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk Manag. 2014; 10:95-105.
6. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2009–2010. Infect Control Hosp Epidemiol. 2013; 34(1):1-14.
7. Magill SS, Shields C, Sears CL, Choti M, Merz WG. Triazole cross-resistance among *Candida* spp.: case report, occurrence among bloodstream isolates, and implications for antifungal therapy. J Clin Microbiol. 2006; 44(2):529-35.
8. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care centre of north India: 5-year study. Infection. 2007; 35(4):256-9.
9. Kothari A, Sagar V. Epidemiology of *Candida* bloodstream infections in a tertiary care institute in India. Indian J Med Microbiol. 2009; 27(2):171-2.
10. Shivaprakasha S, Radhakrishnan K, Karim PM. *Candida* spp. other than *Candida albicans*: a major cause of fungaemia in a tertiary care centre. Indian J Med Microbiol. 2007; 25(4):405-7.
11. 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(4):275-84.
12. Kett DH, Azoulay E, Echeverria PM, Vincent JL; Extended Prevalence of Infection in ICU Study (EPIC II) Group of Investigators. *Candida* bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med. 2011; 39(4):665-70.
13. Azoulay E, Dupont H, Tabah A, Lortholary O, Stahl JP, Francais A, et al. Systemic antifungal therapy in critically ill patients without invasive fungal infection. Crit Care Med. 2012; 40(3):813-22.
14. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis. 2003; 37(9):1172-7.
15. Arendrup MC, Sulim S, Holm A, Nielsen L, Nielsen SD, Knudsen JD, et al. Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. J Clin Microbiol. 2011; 49(9):3300-8.
16. Lark RL, Chenoweth C, Saint S, Zemencuk JK, Lipsky BA, Plorde JJ. Four year prospective evaluation of nosocomial bacteremia: epidemiology, microbiology, and patient outcome. Diagn Microbiol Infect Dis. 2000; 38(3):131-40.
17. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidaemia, revisited. Clin Infect Dis. 2003; 37(9):1172-7.
18. Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against Candida spp. J Clin Microbiol. 2007; 45(11):3522-8.
19. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur J Clin Microbiol Infect Dis. 2006; 25(7):419-25.
20. Fidel PL Jr, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin Microbiol Re. 1999; 12(1):80-96.
21. Pappas PG, Rex JH, Sobel JD, Filler SG, Dismukes WE, Walsh TJ, et al. Guidelines for treatment of candidiasis. Clin Infect Dis. 2004; 38(2):161–89.
22. Fadda ME, Podda GS, Pisano MB, Deplano M, Cosentino S. Prevalence of *Candida* species in different hospital wards and their susceptibility to antifungal agents: results of a three year survey. J Prev Med Hyg. 2008, 49(2):69-74.
23. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, et al. Efflux-mediated antifungal drug resistance. Clin Microbiol Rev. 2009; 22(2):291-321.
24. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev. 1998;

11(2):382-402.
25. Davis SL, Vazquez JA, McKinnon PS. Epidemiology, risk factors, and outcomes of Candida albicans versus non-albicans candidemia in nonneutropenic patients. Ann Pharmacother. 2007; 41(4):568-73.
26. Skrodeniene E, Dambrauskiene A, Vitkauskiene A. Susceptibility of yeast to antifungal agents in Kaunas University of Medicina Hospital. Medicina. 2006; 42(4):294-9.
27. Kothavade RJ, Kura MM, Yaland AG, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing to fluconazole. J Med Microbiol. 2010; 59(Pt 8):873-80.
28. Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum M, et al. Antifungal susceptibility testing: practical aspects and current challenges. Clin Microbiol Rev. 2001; 14(4):643-58.
29. Murray MP, Zinchuk R, Larone DH. CHROMagar *Candida* as the sole primary medium for isolation of yeasts and as a source medium for the rapid-assimilation-of-trehalose test. J Clin Microbiol. 2005; 43(3):1210-2.
30. Baillie GS, Douglas LJ. Iron-limited biofilms of *Candida albicans* and their susceptibility to amphotericin B. Antimicrob Agents Chemother. 1998; 42(8):2146-9.
31. Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of Yeasts. Approved guideline. 2nd ed, M44-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
32. Colombo AL, Guimarães T. Epidemiologia das infecções hematogênicas por *Candida* spp. Rev Soc Bras Med Trop. 2003; 36:599-607.
33. Kumar CP, Sundarajan T, Menon T, Venkatadesikalu M. Candidosis in children with onco-hematological studies in Chennai, South India. Jpn J Infect Dis. 2005; 58(4):218-21.
34. Verma AK, Prasad KN, Singh M, Dixit AK, Ayyagari A. Candidaemia in patients of a tertiary health care hospital from north India. Indian J Med Res. 2003; 117:122-8.
35. Sahni V, Agarwal 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.
36. Furnaleto MC, Rota JF, Quesada RM, Furnaleto-Maia L, Rodrigues R, Oda S, et al. Species distribution and in vitro fluconazole susceptibility of clinical *Candida* isolates in a Brazilian tertiary-care hospital over a 3-year period. Rev Soc Bras Med Trop. 2011; 44(5):595-9.
37. Chang MR, Correia FP, Costa LC, Xavier PC, Palhares DB, Taira DL, et al. *Candida* bloodstream infection: data from a teaching hospital in Mato Grosso do Sul, Brazil. Rev Inst Med Trop Sao Paulo. 2008; 50(5):265-8.
38. Akeme Yamamoto AC, de Paula CR, Dias LB, Tadano T, Martins ER, Amadio JV, et al. Epidemiological and clinical characteristics of nosocomial candidiasis in university hospitals in Cuiabá--Mato Grosso, Brazil. Rev Iberoam Micol. 2012; 29(3):164-8.
39. Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J Clin Microbiol. 2004; 42(4):1519-27.
40. Pfaller MA, Messer SA, Hollis RJ, Jones RN, Doern GV, Brandt ME, et al. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. Diagn Microbiol Infect Dis. 1999; 33(4):217-22.
41. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trends of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. Clin Infect Dis. 2002; 35(5):627-30.
42. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1):133-63.
43. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans *Candida* spp. among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis. 2010; 14(11):e954-66.
44. Chakrabarti A, Mohan B, Shrivastava SK, Marak RS, Ghosh A, Ray P. Change in distribution and antifungal susceptibility of *Candida* species isolated from candidaemia cases in a tertiary care centre during 1996-2000. Indian J Med Res. 2002; 116:5-12.
45. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin Infect Dis. 1999; 29(2):239-44.
46. Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, et al. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis. 2004; 23(4):317-22.
47. Colombo AL, Nucci M, Park BJ, Nouér SA, Arthington-Skagg B, da Matta DA, et al. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol. 2006; 44(8):2816-23.
48. Singh RI, Xess I, Mathur P, Behera B, Gupta B, Misra MC. Epidemiology of candidemia in critically ill trauma patients: experiences of a level I trauma center in North India. J Med Microbiol. 2011; 60(Pt 3):342–8.
49. Rani R, Mohapatra NP, Mehta G, Randhawa VS. Changing trends of *Candida* species in neonatal septicemia in a tertiary north Indian hospital. Indian J Med Microbiol. 2002; 20(1):42-4.
50. Levy I, Rubin LG, Vasishtha S, Tucci V, Sood SK. Emergence of *Candida parapsilosis* as the predominant species causing candidemia in children. Clin Infect Dis. 1998; 26(5):1086-8.

51. Saha R, Das Das S, Kumar A, Kaur IR. Pattern of *Candida* isolates in hospitalized children. Indian J Pediatr. 2008; 75(8):858-60.
52. Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of non-albicans *Candida* species and antifungal resistance in a tertiary care hospital. Jpn J Infect Dis. 2005; 58(6):344-8.
53. Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, Bernal-Martinez L, Cuesta I, Buitrago MJ, et al. Comparison of the Vitek 2 antifungal susceptibility system with the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference methods and with the Sensititre Yeast One and Etest techniques for in vitro detection of antifungal resistance in yeast isolates. J Clin Microbiol. 2010; 48(5):1782-6.
54. Rajkumari N, Mathur P, Xess I, Misra MC. Distribution of different yeasts isolates among trauma patients and comparison of accuracy in identification of yeasts by automated method versus conventional methods for better use in low resource countries. Indian J Med Microbiol. 2014; 32(4):391-7.
55. Pappas PG, Kauffman CA, Andens D, Benjamin DK Jr, Calandra TF, Edwards JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by infectious diseases society of America. Clin Infect Dis. 2009; 48(5):503-35.
56. Adhikary R, Joshi S. Species distribution and anti-fungal susceptibility of Candidaemia at a multi super-specialty center in Southern India. Indian J Med Microbiol. 2011; 29(3):309-11.