Original Article

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Comparison of Manual and Automated Method for Speciation and Antifungal Susceptibility of *Candida* Species Causing Blood Stream Infection in Critically ill Patients

Microbiology Section

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ABSTRACT

Introduction: Globally, the incidence Blood Stream Infection (BSI) by *Candida* species is increasing. It is associated with high mortality rates especially in immunocompromised critically ill patients. The epidemiology has been shifted from *Candida* species to *Non-albicans Candida* (NAC) in recent decades. Speciation of *Candida* spp. can help to improve the outcome of patients as few *Candida* species are intrinsically resistant to antifungal agents.

Aim: To identify the spectrum of *Candida* species causing BSI, study their sensitivity pattern to antifungal agents and comparison of conventional (germ tube test, Dalmau plate culture and Chromagar) and automated (Vitek-2) methods for the same.

Materials and Methods: Blood specimens of the clinically suspected cases of septicaemic patients were cultured by BacT/ALERT before administration of antibiotics or antifungals during the study period of one and half year. The isolated *Candida* were identified to the species level along with antifungal susceptibility testing done by both conventional and automated (Vitek-2) methods. Statistical analysis was done using Chi-square test.

Results: Of the total 100 *Candida* isolated, the most common *Candida* species was *C.parapsilosis* (26%), followed by *C.tropicalis* (23%), *C.albicans* (21%) and *C.auris* (15%). Total concordance between manual and Vitek-2 identification was 74%. Significant risk factors involved in patients with candidemia were malignancy, diabetes mellitus and chronic kidney disease. Antifungal susceptibility rate for amphotericin B, caspofungin, Fluconazole, Flucytosine, Micafungin and Voriconazole was 94%, 90.5%, 73.8%, 96.4%, 98.8% and 95.2%, respectively. Mortality rate was 45% due to sepsis and associated complications.

Conclusion: Early isolation, speciation and antifungal susceptibility is the key for management of candidemia cases to get the better outcome as *C.auris*, the important multidrug resistant variant has caused a major epidemiologic shift in candidemia. Molecular studies are required for accurate speciation of *Candida* species like *C. auris*, *C. haemulonii*, *C. famata*, etc.

Keywords: *Candida auris*, *Candida parapsilosis*, Candidemia, CHROMagar, Vitek-2

INTRODUCTION

A global increase in incidence of *Candida* infection has been reported by several authors over the past two decades showing remarkable shift in prevalence of different *Candida* species [1]. BSI by *Candida*, also known as candidemia, is defined as the isolation of *Candida* species from at least one blood culture in patients with symptoms or signs of a systemic infection [2]. Invasive candidiasis includes a spectrum of clinical conditions, the most frequent of which is candidemia, has become a remarkable threat to public health, a life-threatening infection which is associated with morbidity and mortality to be as high as 40% [3]. It represents 10% of nosocomial infections in hospitalised patients [4] and is the fourth most common blood stream pathogen [5]. The incidence of candidemia expressed as cases per 100,000 inhabitants has been reported to range from 1-8 cases [6] and the prevalence was found to be 6.9 per 1000 cases according to a survey of Intensive Care Units (ICUs) worldwide [7]. Risk factors associated with candidemia are premature birth, advanced age, long term hospitalisation, prolonged exposure to antimicrobial drugs, invasive procedures such as using intravascular catheters, multiple interventions and the immunosuppressive conditions like diabetes, HIV and malignancy [8]. Although *Candida albicans* continues to be the predominant species to cause candidemia, recent studies have detected a growing proportion of BSI by NAC species like *C. tropicalis, C. parapsilosis, C. glabrata, C. krusei* [9]. Ninety percent of all *Candida* BSI globally are attributed by these species [10]. Importantly, many NAC spp. Like C. krusei and *C. glabrata* are shown to have intrinsic resistance against Fluconazole [7]. There has been a recent epidemiologic shift in candidemia driven by *C.auris*, a novel *Candida* spp., which is a multidrug-resistant pathogen causing protracted healthcare-associated outbreaks [8,11]. It is now essential to do the species-level identification as antifungal agent can be selected by the clinicians for the better patient care [12,13]. Continuous surveillance is needed to know the incidence, species distribution and antifungal drug susceptibility profile of *Candida* causing BSI.

The present study was designed with an aim to identify the spectrum of *Candida* species causing BSI, study their sensitivity pattern to antifungal agents and comparison of conventional (germ tube, dalmau plate culture and CHROMagar) and automated (Vitek-2) methods for the same.

MATERIALS AND METHODS

This hospital based prospective study was carried out in the Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, Odisha, India over a period of one and half year (January 2018-June 2019) after obtaining ethical clearance from institutional ethical committee (Ref No/DMR/IMS-SH/SOA/16075). After taking informed consent, the blood samples from 8156 ICU patients, clinically suspected to have septicaemia, were collected following all the aseptic precautions and before administration of antibiotics or antifungals and were screened for candidemia. All the *Candida* isolates obtained as a single pathogen from blood cultures were included in the study. Patients other than those of ICU admitted and also those who were under antibiotics or antifungals even from ICU admission status were excluded from this study.

A total of 8156 specimens were inoculated into the BacT/ALERT (BioMerieux) blood culture bottles and incubated at 37°C for a maximum period of seven days in BacT/ALERT 3D system, a fully automated blood culture system for detection of aerobic growth in blood samples and if there was no growth, the result was read as negative. From culture bottles which were flagged positive in the system, Gram stain was performed. If yeast cells were observed on staining, blood was subcultured on two Sabouraud's Dextrose Agar (SDA) and blood agar plates and incubated at 37°C. SDA and blood agar plates were examined for white to cream coloured, smooth and pasty colonies after 24-48 hours of incubation [14].

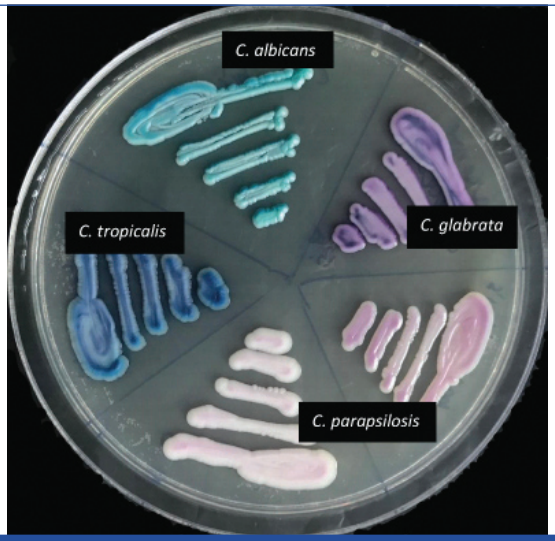
Gram staining was performed from the colony and the morphology of yeast cells was noted. All the isolates were processed by both conventional and automated methods in parallel.

Colonies from the SDA were then plated onto cornmeal agar with Tween 80 (Dalmau plate culture) for chlamydospores production [15]. Germ tube test was performed from colonies for presumptive identification of *C. albicans* [16].

Colonies from SDA were plated onto CHROM agar (HiCrome *Candida* differential agar- Himedia), a differential culture medium that is claimed to facilitate the isolation by colorimetric presumptive identification [17] and were incubated at 37°C for 48 hours. The results were interpreted according to the colour of the colony formed and as per manufacturer's guidelines [Table/Fig-1,2]. All the isolates that gave doubtful morphology or not identified by conventional methods were taken as "*Candida* spp."

Candida species	Morphology on CHROM agar
C. albicans	Apple green
C. dubliniensis	Dark green
C. tropicalis	Metallic blue with a pink halo
C. parapsilosis	White to pale pink
C. glabrata	Pale pink to violet
C. krusei	Fuzzy pink coloured colonies with matt surface and white edges

[Table/Fig-1]: Appearance of various *Candida* species on CHROMagar.



[Table/Fig-2]: Colour production of various *Candida* species on CHROMagar.

In parallel to conventional methods listed above, for all the isolates identification and antifungal susceptibility testing were also done by Vitek-2 (BioMerieux) with 0.5% McFarland suspension from the colonies from SDA using the Identification Card-Yeast (ID-YST) and Antifungal Susceptibility Testing card for yeast (AST YS01) as per Clinical and Laboratory Standards Institute (CLSI) guidelines and manufacturer's instructions [12]. Finally, the results of the two methods (conventional and Vitek-2) were compared with each other. *Candida albicans* ATCC 90028 strain was used as control for evaluation of various methods [18].

STATISTICAL ANALYSIS

It was done as per Chi-square test to compare the conventional and the automated method.

RESULTS

A total of 100 non-duplicate isolates of *Candida* species were obtained from 2202 positive blood culture cases as per inclusion criteria during the study period.

Male patients (55/100, 55%) predominated the female patients (45/100, 45%) in terms of isolation frequency. Maximum number of candidemia patients were in the elderly age group (>60 years, mean age- 71), i.e., 43%, followed by 41-60 years (mean age- 51.3) (33%); 14% and 10% patients were seen in the age groups of 21-40 years (mean age- 32) and up to 20 years (mean age- 8.4), respectively.

Overall 60% of cases had associated co-morbid conditions like malignancy (29/100), kidney disease (40/100), indwelling vascular catheters (47/100), surgical interventions (26/100) and diabetes mellitus (52/100), etc.

Candida parapsilosis (26/100, 26%) was the predominant species causing candidemia followed by *Candida tropicalis* (23/100, 23%), *Candida albicans* (21/100, 21%), *Candida auris* (15/100, 15%), *Candida glabrata* (5/100, 5%) and others (10/100, 10%) [Table/Fig-3].

	Vitek 2 identification											
Manual identification	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. auris*	*C. guillermondi*	*C. utilis*	*C. famata*	*C. pelliculosa*	*C. haemulonii*	*C. rugosa*	Total
C. albicans	21	0	0	0	0	0	0	0	0	0	0	21
C. tropicalis	0	23	0	0	0	0	0	0	0	0	0	23
C. parapsilosis	0	0	26	1	8	0	0	0	0	0	0	35
C. glabrata	0	0	0	4	0	0	0	0	0	0	0	4
Candida spp.	0	0	0	0	7	2	3	1	1	2	1	17
Total	21	23	26	5	15	2	3	1	1	2	1	100

[Table/Fig-3]: Comparison of *Candida* spp. identification by conventional and automated methods.
NB-concordance rate between two methods- 74/100

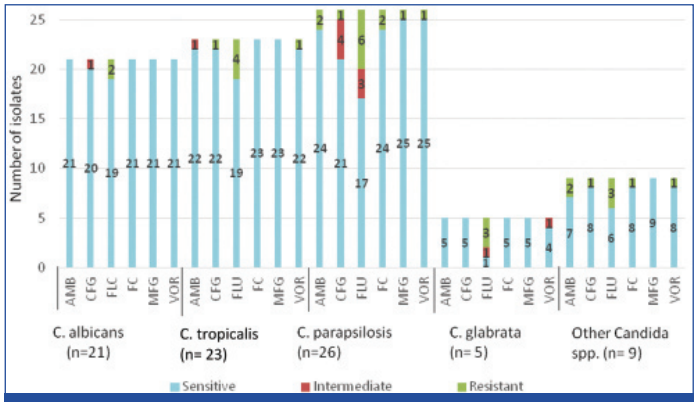
For speciation, of the total 100 Candida isolates concordance between conventional and Vitek-2 method was 74 (74%). Of the total *C.parapsilosis* identified by conventional method, 1 isolate as *C. glabrata* and 8 isolates as *C. auris* was established by Vitek-2 method. 17 isolates of *Candida* which could not be identified by conventional chromogenic method were interpreted as *Candida* spp. but final identification was considered as per Vitek-2 results. Vitek-2 is the better method ($p<0.001$) than the conventional method for *Candida* species identification.

Vitek-2 Compact system does not give Antifungal susceptibility test results for *C.auris* and *C.famata*, so Antifungal susceptibility rates were calculated for 84 isolates (n=84). Antifungal susceptibility rate for Amphotericin B, Caspofungin, Fluconazole, Flucytosine, Micafungin and Voriconazole was 94%, 90.5%, 73.8%, 96.4%, 98.8% and 95.2%, respectively [Table/Fig-4].

Antifungals	Sensitive	Percentage
Amphotericin B	79/84	94%
Caspofungin	76/84	90.5%
Fluconazole	62/84	73.8%
Flucytosine	81/84	96.4%
Micafungin	83/84	98.8%
Voriconazole	80/84	95.2%

[Table/Fig-4]: Antifungal susceptibility testing rate of various antifungals.

Different *Candida* species also showed variable sensitivity result to different antifungal drugs used [Table/Fig-5].



[Table/Fig-5]: Antifungal susceptibility testing result for different *Candida* spp.

Mortality rate was found to be 45% due to sepsis and associated complications, 11% of patients were lost for follow-up and rest of the cases were treated successfully.

DISCUSSION

Candidemia is an emerging problem in healthcare settings, particularly among those in ICU's. Early isolation, speciation and antifungal susceptibility is essential for the choice of the best therapeutic approach for the patient, thereby, decreasing morbidity and mortality. Therefore, knowledge of epidemiology of candidemia can help to salvage patients. In this study, an overview of candidemia was obtained, including *Candida* species identification, antifungal susceptibility pattern, epidemiological characteristics and patient outcome.

In this study, candidemia was most commonly seen in patients >60 years of age (43%) which is in concordance with other studies [19,20]. On the contrary, a study by Thomas T and Dias M shows a higher preponderance in middle age groups (18-50 years) [9]. This was possibly because present study mainly focused on the critically ill patients in ICU's. In this study, the common risk factors for candidemia were malignancy, diabetes mellitus and chronic kidney disease which is similar to a study by Thomas T and Dias M [9].

The isolation rate of candidemia in the present study was 4.54% which is in concordance with many other studies from India [Table/Fig-6] [5,14,18,21-23].

Author	Year	Area	Candidemia rate
Verma AK et al., [5]	2003	North India	1.61%
Giri S et al., [18]	2013	South India	5.76%
Chander J et al., [21]	2013	North India	5.79%
Sudan SS et al., [22]	2016	J&K	4.48
Bhattacharjee P [23]	2016	Kolkata	4.03%
Gandham NR et al., [14]	2016	Pune	14.8%

[Table/Fig-6]: Studies showing incidence of candidemia from different parts of India [5,14,18,21-23].

In the present global scenario, there is a changing trend of *Candida* species, with predominance of NAC spp. In the present study *C. parapsilosis* (26%) was isolated as the predominant isolate which is similar to a study by Van Schalkwyk E et al., [8], while some other studies had *C. tropicalis* [9,24] or *C. albicans* [2] as the predominant pathogen.

C. auris has surpassed the number of cases caused by *C. albicans* or NAC for the last 10 years and is a major cause of candidemia. In this study also, it was the fourth most common isolated species comprising 15% of candidemia cases. It was identified by Vtek-2 method though which has certain limitation in identification. *C.auris* identification is confirmed if the initial identification of *C.auris* is made and *C.auris* identification is possible if the initial identification of *C.haemulonii, C.duobushaemulonii, C.famata* or *C.lusitaniae* is made [25]. There have been reports of *C. famata* being misdiagnosed by Vitek-2 [26]. So, the ideal method of identification of such species is by Matrix Assisted Laser Desorption Ionisation-Time Of Flight Mass Spectrometry (MALDI-TOFMS). This multidrug resistant pathogen is known to cause protracted healthcare associated outbreaks. This possibly happens due to its ability to persist on surfaces, form biofilms and resist routinely used environmental cleansing agents. Similarly, in this study, *C.auris* was associated with micro-outbreaks. Presently, disinfectants having sporicidal activity or hydrogen peroxide- based products has been recommended to clean the surfaces in rooms of patients infected or colonised with *C.auris* because of its high efficiency in eliminating this species from inert surfaces [27,28].

Candida species differ in their susceptibility to antifungal agents. For instance, *C.glabrata* is not very sensitive to Fluconazole, *C.krusei* is intrinsically resistance to Fluconazole and *C.lusitaniae* is resistant to amphotericin B [29]. In this study, all antifungal agents except Fluconazole demonstrated excellent activity against all *Candida* species. Fluconazole being a cheaper drug has been used as main drug against *Candida* infections. So, the use of this drug should be as per susceptibility result. Similar findings were published in a study from Southern India [30].

Death rate by candidemia is quite high. According to some studies, invasive infection with *Candida* is associated with mortality rates of 35-80% in ICU settings [31-33]. The mortality rate of patients with candidemia in this study was 45% which is well within the range reported by other studies and even more concern is about *auris* which showed 100% mortality.

Candida speciation by conventional methods has long been used as benchmark identification procedures. However, these methods are time consuming, laborious and not reliable in identifying broad spectrum of species and require additional tests like assimilation and fermentation. Several commercial systems were developed which can produce rapid yeast identification and their antifungal susceptibility in at least 15 hour. Their use is limited because these systems are not reliable for identification of some species and some are also misidentified. So, now-a-days, molecular strategies, PCR or non-PCR based methods, are being used as complementary to conventional methods for providing more accurate results in less time.

Limitation(s)

Limitation of present study is that we have not done fermentation and assimilation tests which are the crucial means of species identification by conventional method, also considered as reference method. Although Vitek-2 is a reliable method for identification of common fungal species, for accurate speciation of *C.auris*, *C.guilliermondii, C.haemulonii, C.famata*, etc., molecular studies like nucleic acid sequencing may be required as researchers are now claiming Vitek-2 as the inaccurate method for identification of such species.

CONCLUSION(S)

Even though CHROMagar helps with identification at a lower cost as compared to Vitek-2, which is useful in countries having low resources, but it takes longer duration for complete identification, which is the main drawback of this method. Vitek-2 is considered as a reliable technique for antifungal susceptibility of yeast species, it also has the added advantage of being more rapid and easier than the alternative procedure developed by CLSI, broth microdilution method which is cumbersome and expensive. So, a fast and accurate technique for yeast identification is very important for microbiological laboratories. Accordingly, Vitek-2 can be applied for early identification and antifungal susceptibility testing. Local

epidemiological data and antifungal susceptibility profile should be taken into consideration when establishing antifungal treatment strategies. Infection control measures like hand and personal hygiene by healthcare workers, proper catheter care, frequent clinical examination of patients who are weaned off invasive device to be practiced to reduce nosocomial transmission as candidemia represents 10% of nosocomial infections in hospitalised patients and also antibiotic stewardship must be emphasised.

REFERENCES

[1] Antinori S, Milazzo L, Sollima S, Galli M, Corbellino M. Candidemia and invasive candidiasis in adults: A narrative review. Europ J Int Med. 2016;34:21-28.
[2] Xiao Z, Wang Q, Zhu F, An Y. Epidemiology, species distribution, antifungal susceptibility and mortality risk factors of candidemia among critically ill patients: Aretrospective study from 2011 to 2017 in a teaching hospital in China. Antimicrob Resist Infect Control. 2019;8:89.
[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 Off Publ Infect Dis Soc Am. 2004;39(3):309-17.
[4] Ghrenassia E, Mokart D, Mayaux J, Demoule A, Rezine I, Kerhuel L, et al. Candidemia in critically ill immunocompromised patients: Report of a retrospective multicenter cohort study. Ann Intensive Care. 2019;9:62.
[5] Verma AK, Prasad KN, Singh M, Dixit AK, Ayyagari A. Candidemia in patients of a tertiary health care hospital from North India. Ind J Med Res. 2003;177:122-28.
[6] Neofytos D, Lu K, Hatfield-Seung A, Blackford A, Marr KA, Treadway S, et al. Epidemiology, outcomes, and risk factors of invasive fungal infections in adult patients with acute myelogenous leukemia after induction chemotherapy. Diagn Microbiol Infect Dis. 2013;75(2):144-49.
[7] 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.
[8] Van Schalkwyk E, Mpembe RS, Thomas J, Shuping L, Ismail H, Lowman W, et al. Epidemiologic shift in candidemia driven by *Candida auris*, South Africa, 2016-2017. Emerg Infect Dis. 2019;25(9):1698-707.
[9] Thomas T, Dias M. Epidemiological profile of *Candida* isolated from septicaemic patients. Indian J Microbiol Res. 2018;5(4):508-11.
[10] Giri S, Kindo AJ. A review of *Candida* species causing blood stream infection. Indian J Med Microbiol. 2012;30(3):270-78.
[11] Ruiz-Gaitan A, Moret AM, Tasias-Pitarch M, Aleixandre-Lopez AI, Martinez-Morel H, Calabuig E, et al. An outbreak due to *Candida auris* with prolonged colonisation and candidaemia in a tertiary care European hospital. Mycoses. 2018;61:498-505.
[12] Pahwa N, Kumar R, Nirkhiwale S, Bandi A. Species distribution and drug susceptibility of *Candida* in clinical isolates from a tertiary care centre at Indore. Indian J Med Microbiol. 2014;32(1):44-48.
[13] Souza MN, Ortiz SO, Mello MM, Oliveira FM, Severo LC, Goebel CS. Comparison between four usual methods of identification of *Candida* species. Rev Inst Med Trop Sao Paulo. 2015;57(4):281-87.
[14] Gandham NR, Vyawahare CR, Jadhav SV, Misra RN. Candidemia: Speciation and antifungal susceptibility testing from a tertiary care hospital in Maharashtra, India. Med J DY Patil Univ. 2016;9:596-99.
[15] Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for Isolation, Phenotypic Characterization and Maintenance of Yeasts. In: Kurtzman, C.P., Fell, J.W. and Boekhout, T., Eds., The Yeasts, 5th Edition, Elsevier, Amsterdam, 2011;87-110.
[16] Taschdjian CL, Burchall JJ, Kozinn PJ. Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. AMA Am J Dis Child. 1960;99:212-15.
[17] Vijaya D, Harsha TR, Nagaratamma T. *Candida* speciation using Chrom agar. J Clin Diag Res. 2011;5(4):755-58.
[18] Giri S, Kindo AJ, Kalyani J. Candidemia in intensive care unit patients: A one year study from a tertiary care center in South India. J Postgrad Med. 2013;59(3):190-95.
[19] Shivaprakasha S, Radhakrishnan K, Karim PMS. *Candida* spp. other than *Candida albicans*: A major cause of fungaemia in a tertiary care centre. Indian J Med Microbiol. 2007;25(4):405-07.
[20] Dewan E, Biswas D, Kakati B, Verma S, Kotwal A, Oberoi A. Epidemiological and mycological characteristics of candidemia in patients with hematological malignancies attending a tertiary-care center in India. Hematol Oncol Stem Cell Therapy. 2015;8(3):99-105.
[21] 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):670-75.
[22] Sudan SS, Sharma P, Sharma M, Sambyal SS. Candidemia in a tertiary care hospital in Jammu (J&K)- A comparison of conventional methods and chromagar techniques for speciation. IJSR. 2016;5(8):451-54.
[23] Bhattacharjee P. Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016;2(2):20-27.
[24] Jose LR, Savio J. Candidemia isolates, identification and antifungal susceptibility testing: A study from a tertiary care centre. Indian J Microbiol Res. 2017;4(4):464-67.
[25] Algorithm to identify Candida auris based on phenotypic laboratory method and initial species identification. National center for emerging and zoonotic infectious diseases. CDC 2019. https://www.cdc.gov/fungal/diseases/candidiasis/pdf/Testing-algorithm-by-Method-temp.pdf.
[26] Rajkumari N, Mathur P, Xess I, Misra MC. Distribution of different yeasts isolates among trauma patients andcomparison 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):391-97.
[27] Cadnum JL, Shaikh AA, Piedrahita CT, Sankar T, Jencson AL, Larkin EL, et al. Effectiveness of disinfectants against *Candida auris* and other *Candida* species. Infect Control Hosp Epidemiol. 2017;38(10):1240-43.
[28] Biswal M, Rudramurthy SM, Jain N, Shamanth AS, Sharma D, Jain K, et al. Controlling a possible outbreak of *Candida auris* infection: Lessons learnt from multiple interventions. J Hosp Infect. 2017;97(4):363-70.
[29] Ruiz L, Sugizaki M, Montelli A, Matsumoto F, Pires M, da Silva B, et al. Fungemia by yeasts in Brazil: Occurrence and phenotypic study of strains isolated at the Public Hospital, Botucatu, São Paulo. J Mycol Med. 2005;15(1):13-21.
[30] Adhikary R, Joshi S. Species distribution and antifungal susceptibility of candidemia at a multi super specialty centre in Southern India. Indian J Med Microbiol. 2011;29(3):309-11.
[31] Mean M, Marchetti O, Calandra T. Bench-to-bedside review: *Candida* infections in the intensive care unit. Crit Care. 2008;12:204.
[32] 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):954-66.
[33] Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: Data from the prospective antifungal therapy alliance registry. Clin Infect Dis. 2009;48(12):1695-703.

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