

Correlation Of Biofilm Production Of *Candida* Species With *Candida* Score- A Futuristic Tool For Assessing Invasive Candidiasis

Chayanika Banerjee¹, Soumodip Dutta¹, Somnath Bhunia², Subhendu Sikdar^{2*}

¹Post Graduate Trainee, Department of Microbiology, Nil Ratan Sircar Medical College & Hospital, Kolkata, West Bengal, India

²Demonstrator, Department of Microbiology, Nil Ratan Sircar Medical College & Hospital, Kolkata, West Bengal, India

Received: 02-11-2020 / Revised: 25-12-2021 / Accepted: 03-02-2021

Abstract

Introduction: Invasive Candidiasis is associated with higher mortality rates. It may be due to multifocal colonization with more virulent and resistant *Candida* species. “*Candida* score” is a bedside scoring system to assess invasiveness and to start antifungal therapy early. **Aim and objectives:** The aim of this study is to determine biofilm formation of multidrug resistant *Candida* species isolated from patients with nosocomial invasive Candidiasis and its correlation with calculated *Candida* score of those cases. **Materials and methods:** *Candida* species were isolated and identified along with their antifungal susceptibility from blood cultures collected from critically ill patients having *Candida* associated blood stream infections. Biofilm production of those species was also examined. *Candida* score of those patients was calculated and it was correlated with biofilm productions. **Results:** A total of 150 critically ill cases were diagnosed as invasive *Candidiasis* and diagnosed *Candidaemia* microbiologically based on blood culture positivity or during their stay in ICU. Most of the patients in this study group were male (62.67%) than female (37.33 %) with male: female ratio being 1.68:1. 0-10 years of age group was more commonly (65.33%) affected, followed by age group >50 years (22%). 55 isolates (36.67%) were *C. albicans* and 95 (63.33%) were NAC species. Out of NAC species, 32 (21.33%) were *C. tropicalis*, 18 (12%) *C. glabrata*, 19 (12.67%) *C. guilliermondii*, 16 (10.67%) *C. parapsilosis* and 10 (6.67%) *C. auris*. 93 (62%) *Candida* isolates produced biofilm, out of which 37 (39.78%) were *C. albicans* and 56 (60.22%) were NAC species. Fluconazole was most resistant antifungal (46%) among the isolated *Candida* species. Cases with *Candida* score ≥ 3 were 68 (45.33%). Correlation between biofilm production and *Candida* score (invasiveness) was found to be strongly statistically significant. **Conclusion:** Higher *Candida* score points towards more virulent and resistant invasive Candidiasis with higher mortality rates, thus necessitating early and prompt anti-fungal therapy to reduce morbidity and mortality

Keywords: *Candida* Score, Biofilm, Virulent, Resistant

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Introduction

Candida has been recognized as one of the most important etiological agents of nosocomial infections over the past few years [1]. It is one of the most common cause of opportunistic fungal infections world-wide. [2] *Candida*, being a part of normal microbial flora of skin and mucous membrane in healthy individuals, may cause severe systemic infections in critically ill patients with underlying risk factors such as diabetes mellitus, prolonged duration of stay in ICU, or other factors suppressing the immunity. [3] The transition of *Candida* spp. from commensal to potent pathogen is facilitated by a number of virulence factors such as adherence to host tissues and medical devices, biofilm formation, and secretion of extracellular hydrolytic enzymes. [4] Biofilm formation is one of the most extensively investigated virulence factors of *Candida* species. [5] It was found from various studies that isolated *Candida* spp as nosocomial pathogen are more virulence factors producer and resistant to antifungals [6]. Prevalence of *Candidaemia* in our

country varies from 0.65 to 10.4 [7]. Invasive candidiasis is associated with high mortality, especially in ICUs. Prompt initiation of appropriate antifungal therapy is essential for the control of invasive *Candida* infections and has been shown to reduce mortality [8]. However, early diagnosis of invasive candidiasis is difficult, because they have variable and non-specific manifestations and the criteria for starting empirical antifungal therapy in ICU patients are poorly defined. Leon *et al.*, developed “*Candida* Score” a scoring system combining the risk factors and *Candida* colonization [9]. This score can differentiate patients who would benefit from early antifungal treatment from those for whom invasive candidiasis is highly improbable [10].

Aim and objectives: The purpose of this study is to study biofilm formation *Candida* species isolated from patients with nosocomial invasive Candidiasis and its correlation with calculated *Candida* score of those cases.

Material and Methods

The present study was conducted in the Department of Microbiology, Nil Ratan Sircar Medical College and Hospital Kolkata for duration of one year from January 2019 to December 2019. All critically ill patients having sepsis or septic shock on admission and diagnosed *Candidaemia* microbiologically based on blood culture positivity or during their stay in ICU were included in the study. Blood samples were collected aseptically in blood culture bottle from suspected cases and put under BD-BACTEC blood culture system. Yeasts were

*Correspondence

Dr. Subhendu Sikdar

Demonstrator, Department of Microbiology, Nil Ratan Sircar Medical College & Hospital, Kolkata, West Bengal, India.

E-mail: subhendu.kolkata26@gmail.com

isolated from signal positive blood culture bottle in Blood agar and Sabouraud Dextrose agar and identified by VITEK II identification system. The culture was considered true *Candidaemia* only when *Candida* spp. was isolated from at least two blood culture samples or from a clinically significant single blood culture sample among hospitalized patients [11]. Demographic parameters of that cases were also recorded.

Antifungal susceptibility test:All the isolates were subjected to antifungal susceptibility testing via disc diffusion method using Mueller Hinton Agar supplemented with glucose and methylene blue. Antifungal discs used included Amphotericin B (10 µg), Fluconazole (25 µg), Itraconazole (10 µg), and Voriconazole (1 µg). The inhibition zone was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[12]. The control *C. albicans* ATCC 10231, *C. glabrata* ATCC 15126, and *C. tropicalis* ATCC 10610 strains were also used in this study.

Determination of biofilm production:Biofilm production was determined visually by the method described by Yigit *et al.* [13] with mild modification. Colonies from surface of SDA plate were

inoculated into each well of polystyrene micro titre plate tube containing 10 µl of Sabouraud dextrose broth (SDB) supplemented with glucose (Final concentration 8%). After incubation at 35°C for 48 hours, the broths in the wells were gently aspirated. The wells were washed with distilled water twice and then stained with crystal violet for 10 mins. They were then examined for the presence of an adherent layer and OD value were measured using filters of 492 and 540 nm. The OD values of positive and negative controls were 0.090 and 0.027 respectively. The biofilm producer *Staphylococcus epidermidis* ATCC 35984 was used as a positive control. [14,15]

Candida Scoring:Screening for *Candida* colonization was performed twice weekly by routine sampling from tracheal aspirates and urine. Other samples from vascular catheters, wound or drainage exudates, or other infected foci were obtained at the discretion of the attending physician. Isolation of *Candida* from one focus or site was defined as unifocal colonization and multi focal colonization was defined when it is isolated in more than one non-contiguous foci even with different species[9].

Table 1: The Candida Score

Variables	Score if present	Score if absent
Sepsis	2	0
Surgery	1	0
TPN (Total Parenteral Nutrition)	1	0
Multifocal colonization	1	0

In this study, components of “*Candida* Score” like severe sepsis, total parenteral nutrition, surgery, and multifocal *Candida* colonization were as per Leone *et al.* Bedside scoring was done for each patient. Clinical sepsis was given score of 2 if present and 0 if absent. All other variables were given score 01 if present and 0 if absent as shown in Table 1. Value more than 3 is considered significant. Samples from tracheal aspirates or urine were obtained at admission and the final *Candida* Score is only determined when cultures results are available. [9,16,17] As cases with *Candida* associated sepsis or septic shock are included only in this study, all cases had minimum 2 score.

Statistical analysis:All statistical analysis was done using Chi-square test and Fischer exact test. The software used for the statistical analysis was SPSS version 22.0 and Graph Pad Prism 7.

Results and Analysis

During the period of study, a total of 150 critically ill cases were diagnosed as invasive *Candidiasis* and diagnosed *Candidaemia* microbiologically based on blood culture positivity or during their stay in ICU. Most of the patients in this study group were male (62.67%) than female (37.33 %) with male: female ratio being 1.68:1 [Figure 1]. 0-10 years of age group was more commonly (65.33%) affected, followed by age group >50 years (22%). [Figure 2]

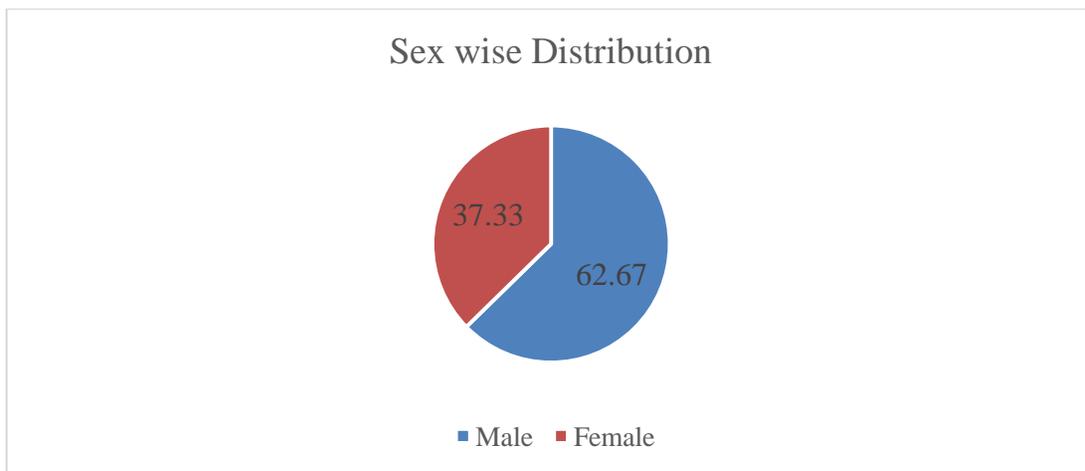


Fig 1: Sex wise distribution of cases of Invasive Candidiasis (n=150)

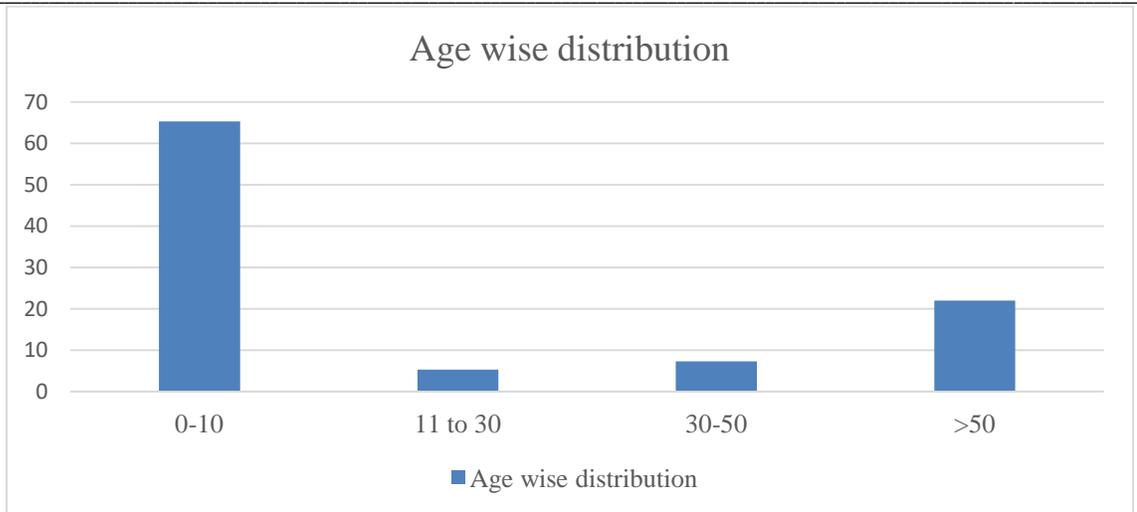


Fig 2: Age wise distribution of cases of Invasive *Candidiasis* (n=150)

In this study, 55 (36.67%) were *C. albicans* and 95 (63.33%) were NAC species. Out of NAC species, 32 (21.33%) were *C. tropicalis*, 18 (12%) *C. glabrata*, 19 (12.67%) *C. guilliermondii*, 16 (10.67%) *C. parapsilosis* and 10 (6.67%) *C. auris*. [Figure 3].

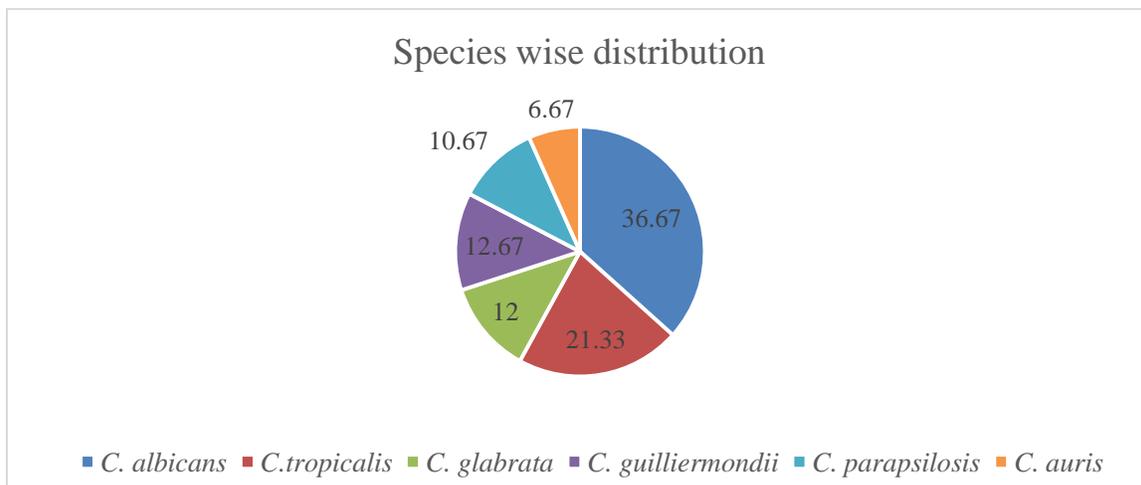


Fig 3: Showing species wise distribution of *Candida* isolated from blood cultures (n=150)

93 (62%) isolated *Candida* produced biofilm, out of which 37 (39.78%) were *C. albicans* and 56 (60.22%) were NAC species. Fluconazole was most resistant antifungal (46%) among the isolated *Candida* species, whereas Amphotericin B was the least one (8%). [Table 2]. Among the NAC species, *C. auris* was the most resistant isolate, followed by *C. glabrata* and *C. guilliermondii*. [Figure 4]

Table 2: Showing Antifungal resistance pattern of *Candida* isolates (n=150)

Antifungals tested	<i>C. albicans</i> (%) (n=55)	NAC (%) (n=95)	Total (%) (n=150)
Fluconazole (25 µg)	30 (10.96)	39 (24.66)	69 (35.62)
Itraconazole (10 µg)	19 (8.22)	31 (15.07)	50 (23.29)
Voriconazole (1 µg)	6 (1.37)	13 (6.85)	19 (8.22)
Amphotericin B (10 µg)	0 (0)	12 (4.11)	12 (4.11)

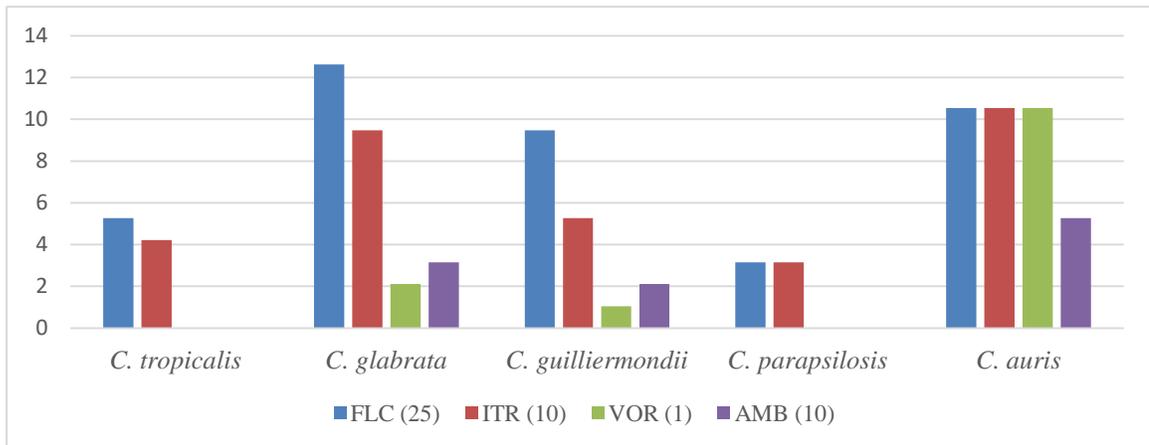


Fig 4: showing anti-fungal resistance pattern of isolated NAC species

The percentage of patients with *Candida* infection showing risk factors were mentioned in the Table 3. As cases with *Candida* associated sepsis or septic shock are included only in this study, all cases had minimum 2 score. 49 (32.67%) cases had multifocal colonization, patients on total parenteral nutrition (17, 11.33%) and surgery (22, 14.67%). As per Figure 5, the patients with the *Candida* score ≥ 3 were 68 (45.33%).

Table 3:Percentage (%) of patients with *Candida* infection showing risk factors.

Variables	Number	%
Sepsis	150	100
Surgery	22	14.67
TPN (Total Parenteral Nutrition)	17	11.33
Multifocal colonization	49	32.67

Table 4: Showing correlation between biofilm production and *Candida* score (n=150)

	Score ≥ 3	Score < 3	Total
Biofilm (+)	56	37	93
Biofilm (-)	12	45	57
Total	68	82	150

Correlation between biofilm production and *Candida* score (invasiveness) was found to be strongly statistically significant [Table 4]. $\chi^2 = 20.319$, $df = 1$, $p = <0.0001$

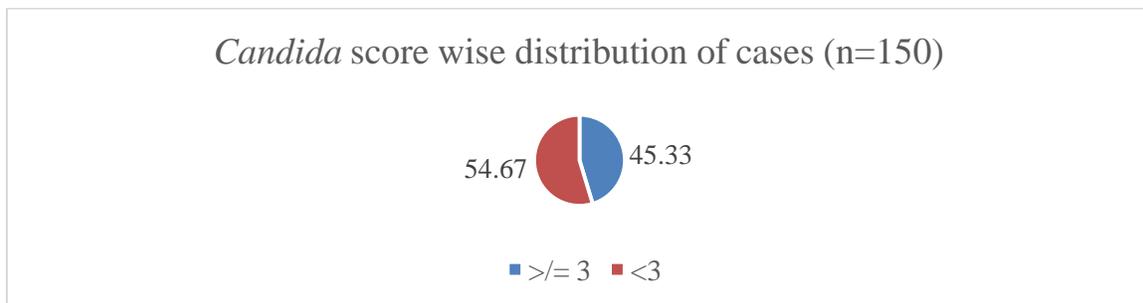


Fig 5: *Candida* score wise distribution of cases (n=150)

Discussion

Candida species are now the fourth most common cause of nosocomial BSIs worldwide. [18]It ranks among the infections with

highest mortality rates[19].The impact of Candidaemia on mortality suggested a compelling need for clinical prediction rules to identify subsets of hospitalized patients at particularly high risk for it[20].It was found to be more common in male in our study (M: F= 1.68:1). Similar findings were also noted in Gonzalez de Molina et al,(1.7:1) [21],Leon et al, (1.89:1) [22], Pallavi S Tatte 2018 (1.8:1) [23] and Vicky Gandhi 2017 (2.03:1) [7]. Neonatal and paediatric age group was found to be more susceptible for invasive Candidiasis in this study, followed by age group >50 years, which were consistent with the study of Pallavi S Tatte 2018 (1.8:1) [23] and Vicky Gandhi 2017 (2.03:1) [7]. Candidaemia is well-known for affecting the extremes of age, possibly due to the immaturity of the immune system in children, and the waning of the immune response in the elderly [24]. In the present study, NAC species (63.33%) were isolated more than *C. albicans* (36.67%). Out of NAC species, 32 (21.33%) were *C. tropicalis*,18(12%) *C.glabrata*, 19(12.67%) *C.guilliermondii*, 16 (10.67%) *C. parapsilosis* and 10 (6.67%) *C. auris*. This type of similar findings was also found in the study of S. Deorukhkar 2012 [25],Vicky Gandhi 2017 [7],Pallavi S Tatte 2018[23], P Bhattacharjee 2016 [26], and Jagdish Chander et al[27]. Shiva-prakash et al.,2007 (35.6%) [28], Adhikary et al., 2011 (39.7%) [29], Pallavi S Tatte 2018 [23] and Xess et al 2007 [30]also found in their study that *C. tropicalis* was the major isolate from Candida associated blood stream infection, which was also similar with this study. NAC species were found to be more resistant than *C. albicans* in this study and Fluconazole was found to be most resistant antifungal, which was also similar with the study of Sikdar S et al 2019 [6], Priyanka Gupta et al, 2015[24],Pallavi S Tatte 2018 [23], P Bhattacharjee 2016 [26], D. Sabhapandit et al 2017 [31] and Deepthi.T et al [32]. Azole resistance has been found to be higher among *C. glabrata* in Priyanka Gupta et al, 2015 [24] and Tan et al [33], as of this study, although *C. auris* was found to be most resistant species in this study.In the present study, NAC (60.22%) was the major biofilm producer than *Candida albicans* (39.78%), which is consistent with other studies also. [34]A linear and significant association between increasing values of the “Candida score” and the rate of invasive candidiasis was observed in the study of Leon’s et al[10]. In this study, biofilm formation of *Candida* spp and higher *Candida* score (>3) was found to be significantly associated, thus higher *Candida* score depictinginvasive candidiasis with more virulent and resistant *Candida* spp, necessitating early anti-fungal therapy to reduce morbidity and mortality.

Conclusion

Increased incidence of candidiasis along with antifungal resistance has become an important healthcare issue worldwide now a days[6]. The identification of virulence attributes specific for each species and their correlation with each other will aid in the understanding of the pathogenesis of infection [35]. Early identification of invasive candidiasis with the use of “Candida Score” in critically ill patients may help to initiate antifungal interventions and even help the treating physician or intensivist to formulate the more effective treatment algorithms. This may help in decreasing the mortality associated with invasive *Candida* infection [9].

Acknowledgement

I am very grateful to my Head of the Department, Prof (Dr.) Swagata Ganguly Bhattacharjee for helping and supporting my work. Without her continuous support, this work could not be possible.

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Conflict of Interest: Nil

Source of support: Nil