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Original Research Paper

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A CROSS SECTIONAL STUDY ON SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA ISOLATES FROM BLOOD STREAM INFECTIONS.

Dr Sukrutha Gopal Reddy Department of Microbiology, Osmania medical college, Hyderabd. Telengana, India.

Dr Udayasri. B* Assistant Professor, Department of Microbiology, Osmania medical college, Hyderabd. Telengana, India. *Corresponding Author

ABSTRACT

Background: The incidence of candidemia is on a rise worldwide. Non-albicans Candida (NAC) species have emerged as considerable causes of Candidemia in many countries. Antifungal drug resistance has become a major cause of concern in the management of Candidemia. **Materials And Methods:** In cross sectional study was conducted during the period of January 2016 and December 2017. Samples from clinically suspected cases of blood stream infections were collected and processed. Blood culture was performed using automated blood culture system (BacT/Alert 3D). positive blood cultures were processed according to standard protocol. The identification and antifungal susceptibility testing of the Candida isolates was performed by VITEK 2 compact system. **Results:** A total number of 8723 samples were tested, among them 1,475 (16.90%) were culture positive out of which 80 samples were positive Candida species (5.42%). Out of the 80 isolates, 51 (63.75 %) were C. tropicalis, 8 (10%) were C. albicans and 21 (26.25%) were other Candida spp. C. tropicalis, the most commonly isolated species, was susceptible to amphotericin B (96.07%), micafungin (94.11%), caspofungin (92.15%), voriconazole (82.35%) and fluconazole (80.39%). Susceptibility of C. albicans was better and other NAC was lesser when compared to C. tropicalis. **Conclusion:** In our study, non-albicans Candida bloodstream infections were more common than C. albicans. This highlights the change in epidemiology in the species distribution of Candida. Knowledge of the local species distribution of Candida along with their antifungal susceptibility is essential to initiate species directed therapy, especially in patients admitted to hospitals.

KEYWORDS : Non-albicans Candida (NAC), Candida spp, epidemiology, in-patients

INTRODUCTION:

Candida species can cause a wide range of infections from a simple cutaneous candidiasis to life threatening invasive candidiasis like blood steam infection. Blood stream infections (BSIs) due to candida spp have become a major concern in critical care medicine and associated with high mortality rate. 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[1]. Majority of Candida infections were due to Candida albicans till early 2000's. However NAC emerged thereafter. Growing population of immunocompromised patients, advances in medical and surgical managements, era of AIDS pandemic, use of broad spectrum of antibiotics, and introduction of fluconazole (1990) into market has contributed to this epidemiological shift.[8]

Most common NAC species include C.tropicalis, C.glabrata, C.parapsilosis, C.krusei, C.lusitaniae, C.guillermondii, C.dubliniensis, C.kefyr, C.famata etc. Virulence factors of candida species include expression of adhesions and invasiveness, proteinases secretion, morphological transition between yeast and hyphal forms, formation of biofilms, thigmotropism and phenotypic switching. Risk factors for disseminated candidiasis include AIDS, neutropenia, diabetes, broad spectrum antibiotics, chemotherapy and indwelling invasive devices. Clinical manifestations by NAC are usually indistinguishable from those due to C.albicans.

Factors attributed to anti fungal resistance involve properties of antifungal used, fungal pathogens and host factors. The problem of emergence of Non albicans candida has become more acute because different species of the same exhibit varying degrees of resistance either intrinsic or acquired or both, to commonly used antifungal drugs. Intrinsic resistance has been noted in C.krusei to imidazoles and acquired resistance observed in few species like C.tropicalis and C.dubiliensis to fluconazole. [3] Hence speciation and antifungal susceptibility of Candida isolates plays a major role in providing appropriate treatment. The current study, was aimed to study the prevalence of various Candida species and their susceptibility pattern causing blood stream infections.

MATERIALS AND METHODS:

The study was conducted between January 2016 and December 2017 in the department of Microbiology, at Krishna Institute of Medical Sciences, Secunderabad. Clinically suspected acses of blood stream infections were included in the study. Blood Samples from these patients were collected according to standard protocol under aseptic precautions. The samples were inoculated into BacT/ Alert blood culture bottles and processed by BacT/Alert3D (bioMerieux) automated blood culture system.

Clinical isolates of Candida spp obtained from blood culture using the blood culture system were sub-cultured onto Sabouraud dextrose agar (SDA) and blood agar plates (HiMedia) after receiving Gram-positive budding yeast like cells 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,) using VITEK 2 cards for identification of yeast and yeast-like organisms (ID -YST cards) kits. Antifungal susceptibility testing was carried out with AST YS07 Kits on VITEK 2 Compact. Standard operative procedures and quality control as described by the manufacturer were followed.

Statistical Analysis:

The data was entered into an excel sheet and analyzed. Descriptive statistics such as graphs and tables were used to describe the data. Mean and percentage distribution were calculated wherever necessary.

RESULTS:

A total number of 8,723 samples were processed. Out of them 1,475 (16.90%) samples were culture positive, among them 80 (5.42%) were found to be positive for Candida species.

Out of 80 candidemia patients, 48(60%) were male and 32(40%) were females. Maximum number of Candida isolates were from 41-70 years age group. Among them maximum

number of C.albicans isolates were from 71-80 years age group patients, maximum number NAC were isolated from patients were from 51-60 years age group.

Table1: Age And Gender-wise Distribution Of Calbicans And Non-albicans Candida Species.

Age group	Candida albicans		Non-albicans Candida		Total (n=80)
	Male	Female	Male	Female	
0-10	1	1	4	1	7
11-20	0	0	5	2	7
21-30	0	0	1	2	3
31-40	0	0	3	0	3
41-50	0	2	6	5	13
51-60	0	0	9	12	21
61-70	0	1	9	3	13
71-80	2	1	3	2	8
>81	0	0	4	1	5

Out of 80 isolates, Candida albicans constituted 10% (8) of the isolates and the remaining 90% were Non-albicans Candida (NAC) species. Among NAC, Candida tropicalis 63.75 %(51) followed by C.haemulonii 8.75 %(7) were the predominant species.

Table 2: Frequency Of Isolation Of Various Species Of Candida Is Shown

S. No	Species of Candida	Number of isolates (%)
1.	Candida tropicalis	51(63.75)
2.	Candida albicans	8(10.0)
3.	Candida haemulonii	7(8.75)
4.	Candida parapsilosis	5(6.25)
5.	Candida glabrata	2(2.5)
6.	Candida gullermondii	2(2.5)
7.	Candida famata	2(2.5)
8.	Candida kefyr	1(1.25)
9.	Candida lusitaniae	1(1.25)

Table 3 : Susceptibility Pattern Of Various Candida Species Is Depicted.

Name of the organism	Antifungal drug														
	Fluconazole			Voriconazole			Amphotericin-B			Caspofungin			Micafungin		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
C.albicans (n=8)	8 (100)	0 (0.0)	0 (0.0)	8 (100)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)
C.tropicalis (n=51)	41 (80.39)	0 (0.0)	10 (19.60)	42 (82.35)	0 (0.00)	9 (17.64)	49 (96.07)	1 (1.96)	1 (1.96)	47 (92.15)	0 (0.0)	4 (7.84)	48 (94.11)	0 (0.0)	3 (5.88)
C.haemulonii (n=7)	1 (14.28)	0 (0.0)	6 (85.71)	3 (42.85)	2 (28.57)	2 (28.57)	2 (28.57)	0 (0.0)	5 (71.4)	5 (71.42)	0 (0.0)	2 (28.57)	6 (85.71)	0 (0.0)	1 (14.28)
C.parapsilosis (n=5)	4 (80.0)	0 (0.0)	1 (20.0)	4 (80.0)	0 (0.0)	1 (20.0)	3 (60.0)	1 (20.0)	1 (20.0)	5 (100)	0 (0.0)	0 (0.0)	5 (100)	0 (0.0)	0 (0.0)
C.glabrata (n=2)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	1 (50)	0 (0.0)	1 (50)	2 (100)	0 (0.0)	0 (0.0)
C.gullermondii (n=2)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)
C.famata (n=2)	0 (0.0)	0 (0.0)	2 (100)	1 (50)	0 (0.0)	1 (50)	2 (100)	0 (0.0)	0 (0.0)	1 (50)	0 (0.0)	1 (50)	1 (50)	0 (0.0)	1 (50)
C.dublinesis (n=1)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
C.kefyr (n=1)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
C.lusitaniae (n=1)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
Total (n=80)	59 (73.75)	0 (0.0)	21 (26.25)	63 (78.79)	2 (2.5)	15 (18.75)	69 (86.25)	2 (2.5)	9 (11.25)	70 (87.5)	0 (0.0)	10 (12.5)	73 (91.25)	0 (0.0)	7 (7.5)

C. tropicalis, the most commonly isolated species, was susceptible to amphotericin B (96.08%), micafungin (94.11%), caspofungin (92.15%), voriconazole (82.35%) and fluconazole (80.39%). Susceptibility of C. albicans was better and other NAC was lesser when compared to C. tropicalis.

DISCUSSION:

Prevalence of Candida species in BSI has increased in the last few decades. In present study, the overall prevalence of Candida species isolates from BSI were found to be 5.42%(n=80). Great similarity of prevalence of candidemia were reported from a study of Sibin P.S et al.[10] from Kerala reported 5.8% candidemia from patients underlying various clinical conditions.

Study of Kumar et al. [28] from south India reported that incidence of candidemia among childrens with hematological malignancy was 5.7%. Bhattacharrjee.P et al. [1] from Kolkata reported incidence rate of 4.03%. A study conducted by Xess et al.[14] reported that prevalence rate of candidemia is 6%.whereas Sachin c et al.[8] conducted a study from Maharashtra reported prevalence rate of candidemia 3.9%.Overall similar prevalence rate noted from different studies done from different parts of India, prevalence of candidemia ranging from 3.9-6%.

Ours study show 10% of C.albicans and 90% NAC were isolated from candidemia patients. Few studies have shown an increase incidence of NAC candidemia with isolation range from 60-90% [5,6] Several factors are implicated for emergence of NAC spp.over C.albicans .These include empirical prophylactic and therapeutic use of azoles and use of chromogenic media.

Available literature on species distribution of Candida has pointed out the significant variation with respect to frequency of isolation of NAC spp. from BSI, in the present study, C. tropicalis (63.7%) was the predominant Candida spp. This finding is in consistent to that of other researchers from India [8,13,14]. C.haemulonii (8.75%) was the second predominant NAC isolate .Great variation in species distribution observed in other studies ,it can be due to geographical areas and health care set up.

Several classes of antifungal drugs (azoles, echinocandins and polyenes) are available for treatment of candidemia. The choice of antifungal drug depends on various factors the local epidemiology and the patient's co-morbidities. The emergence of NAC spp. has initiated the need of antifungal susceptibility testing of Candida isolates. C.tropicalis, which

is predominant isolate in our study showed susceptibility to Amphotericin (96.15%), micafungin(94.11%), caspofungin(92.15%), voriconazole (82.35%) and fluconazole(80.39%).

Azole drugs still remain a safe and effective choice for treatment for candidemia, an increase trend of azoles resistance was significantly higher in NAC spp. compared to C.albicans. Advantage of fluconazole is that it is available in both intravenously administered and oral formulations with high bioavailability. Extended prophylactic use of azoles in high risk patients and empirical azoles therapy in patients suffering from candidasis would be a probable cause for high resistance pattern and major cause of NAC spp. dominance over C.albicans. Few NAC spp. showed resistance to amphotericin –B in our study. Amphotericin is not a first choice for treating candidemia ,because of nephrotoxicity associated with it. In present study susceptibility to micafungin is high compared to caspofungin in few NAC spp. Echinocandins are highly expensive and cannot be afforded by a majority of the Indian population.

Novel findings in present study include C.albicans, C.parapsilosis, C.dubienesis, C.kefyer are susceptible to all azoles, polyenes and echinocandins tested in our study and C.guillemondii is resistant to all drugs tested. These findings were observed in small number isolates of candida spp., hence limited ability to evaluate significance of these findings.

Our study is limited to a single institute's data, with small number of isolates, More number of studies should be done in a larger population at different health settings. Risk factors and outcome of the patient were not included in the study. This antifungal susceptibility pattern may not applicable to all hospital setting due to variation in population, health settings and distribution of species. henceforth antifungal stewardship should be followed to know local species distribution and fungal susceptibility of Candida spp.

CONCLUSION:

In conclusion, there is a significant epidemiological shift of candidemia cases due to. NAC species, especially C. tropicalis is the most frequent pathogen isolated in our tertiary care center. Based on the present results, it is evident that routine identification of Candida isolates to the species level, and the detection of resistant strains by antifungal susceptibility test is essential. Furthermore, there is a continued need for surveillance of candidemia to monitor changes in the epidemiological features and antifungal susceptibility and also to develop and evaluate prevention strategies.

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