Original Research Article

Virulence factors and antifungal susceptibility profile of non *albicans Candida* species isolated from blood stream infections

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Received: 26-11-2020 / Revised: 18-12-2020 / Accepted: 02-01-2021

Abstract

Background: The spectrum of fungal infections, once only considered to be restricted only to cutaneous and mucocutaneous tissue has been changed. Invasive mycosis have emerged as an increase threat to mankind. Despite of the advent in both therapeutic and diagnostic modalities, invasive mycosis is associated with high mortality. Candida spp., is often the most important cause of blood stream infection. Although *C. albicans* is considered as the most pathogenic species from the genus, recent studies have documented the emergence of unusual, relatively uncommon and treatment resistant non albicans *Candida* (NAC) spp. **Material and methods:**NAC spp. isolated from blood cultures were included in the study. *Candida*isolates were identified up to species level by standard mycological protocol. NAC spp. were screened for production of virulence factor like extracellular hydrolytic enzymes, haemolysin and biofilm formation. The antifungal susceptibility profile of these isolates was studied by Ezy MIC Strip.**Results:**The rates of isolation of bacterial and fungal pathogens from blood cultures were 78.4% and 21.6% respectively. Candida spp. was the only fungal pathogen isolated from blood cultures. The isolation of NAC spp. was highly significantly compared to *C. albicans*. *C. tropicalis* followed by *C. glabrata* and *C. krusei* were most common isolates from NAC spp. ICU admission and fluconazole prophylaxis/treatment were significantly associated with BSI due to NAC spp.Fluconazole resistance was observed in 40.7% of NAC spp. All isolates of *C. krusei* were resistant to fluconazole. No isolates were resistant to voriconazole and echinocandins. **Conclusion**: Hitherto rare and new fungal species and fungi once considered to be non-pathogenic are increasingly implicated in human infections.NAC spp. have emerged as an important cause of infections including candidemia. These NAC spp., produce virulence factors once attributed to *C. albicans*. As NAC spp. differ widely in susceptibility to routine used antifungal agents, antifungal susceptibility testing plays an important role in evaluating therapy for *Candida* infections.

Keywords: Antifungal susceptibility testing, blood stream infections, *Candida*, candidemia, echinocandins, virulence factor.

Introduction

Globally, infectious diseases are one of the major challenges for healthcare setup. In developing countries, they are one of the most significant contributors to morbidity and mortality. Until the last century, majority of infectious diseases were attributed to bacterial, parasitic and viral pathogens whereas the role of fungi in human infections was less frequently recognized or documented[1]. However, in recent years, fungi are increasingly implicated in human infections. Risk factors that impacted increase in fungal infections include technological development in healthcare system, aging population, increased number of patients with chronic diseases and aggressive use of immunosuppressive drugs and antibiotics[2].The spectrum of fungal infections, once only considered to be restricted only to cutaneous and mucocutaneous tissue has been changed. Invasive mycosis have emerged as an increase threat to mankind[3]. Despite of the advent in both therapeutic and diagnostic modalities, invasive mycosis is associated with high mortality[3]. *Candida* spp., Cryptococococcus spp., and Aspergillus spp. are the most important etiological agents of invasive mycoses[4]. The incidence of invasive *Candida* infections is seven to fifteen fold higher than invasive aspergillosis[4].The opportunist *Candida* is unique among various pathogen is at least two aspects. First, it can exist both as commensal and pathogen. Second, it causes broad spectrum of clinical manifestations ranging from mere mucocutaneous overgrowth to life threatening disseminated infections [5]. Blood stream infections, medical device associated infections, intra-abdominal infections and urinary tract infections are examples of disseminated candidiasis. These infections are mostly seen in patients admitted to medical and surgical intensive care units[4].

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Disseminated candidiasis often necessitates prolonged duration of mechanical ventilation and hospitalization. It is also associated with increased healthcare costs[4]. In United States, *Candida* spp. is fourth among leading cause of blood stream infections (BSI) whereas various studies from European countries have reported *Candida* as the sixth to the tenth cause of healthcare associated BSI[4]. However there is a dearth of information regarding *Candida* BSI from developing countries like India[5].Although *C. albicans* is considered as the most pathogenic species from the genus, recent studies have documented the emergence of unusual, relatively uncommon and treatment resistant non albicans *Candida* (NAC) spp[6]. NAC spp. though cause similar clinical manifestations like *C. albicans*, differ in respect to virulence factors, epidemiology and most important the pattern of susceptibility to commonly prescribed antifungal drugs [7].Therefore, the present study was conducted in tertiary care academic hospital of India with aim to study the virulence factors and antifungal susceptibility profile of NAC spp. isolated from BSI.

Materials and methods

The descriptive cross sectional study was conducted in Department of Microbiology of Government Medical College, Shivpuri, Madhya Pradesh, India. The study was conducted for a period of 2 years (October 2019 to 2020). The study sample comprised of NAC spp. isolated from blood cultures. A single isolation from blood culture was considered as significant. Patient's demographic features, underlying medical condition and associated risk factors were recorded and analyzed.

Identification of *Candida* spp.

Candida isolates from blood cultures were identified up to species level on the basis of colony characteristics, germ tube test, color on HiChrom *Candida* agar, colony morphology on corn meal agar and sugar assimilation test[8]. Hi*Candida* identification kit supplemented identification of isolates. HiChrom *Candida* agar and Hi*Candida* identification kit were procured from Himedia Laboratories Pvt Ltd., Mumbai.

Virulence factors

Candida isolates were screened for production of virulence factor like extracellular hydrolytic enzymes, haemolysin and biofilm formation. Phospholipase and proteinase were extracellular hydrolytic enzymes studied.

(i)**Phospholipase production**

Phospholipase produc-tion in *Candida* isolates was detected on egg yolk agar by the method suggested by Samaranayake *et al* (1984)[9]. Approximately 5µL of standard inoculum (10^8 yeast cells/mL) prepared from test strain was aseptically added onto the surface of egg yolk agar plate. The inoculum was allowed to dry on plate at room temperature and plates were then incubated at 35ºC for 72 h. After incubation, the plates were observed for the growth and zone of precipitate around the colonies.Presence of precipitate zone indicated phospholipase production by the isolate. Phospholipase activity (Pz) was expressed as the ratio of the colony to the diameter of the colony plus the precipitation zone. As a Pz value of 1 denoted no phospholipase production by the isolate whereas Pz<1 indicated phospholipase expression, the lower the Pz value, the higher the enzymatic activity. *C. albicans* ATCC 10231 and *C. kefyr* ATCC 25412 were used as positive and negative controls, respectively.

(ii) **Proteinase activity**

Proteinase activity in *Candida*spp. was determined using bovine serum albumin (BSA) agar by the method described by Aoki *et al* (1990) [10]. Approximately 10 µL of standard inoculum containing 10^8 yeast cells/mL was aseptically inoculated onto the surface of BSA agar plate. The plates were incubated at 37ºC for 7 days. After 7 days of incubation, further proteinase production was inhibited by adding 20% trichloroacetic acid and then 1.25% amidobalck was poured on to the media. The excess of stain was decanted and the plate was observed for presence of clear zone around the colonies. The diameter of *C. krusei* colonies was measured before staining whereas the diameter of clear zone around the colonies was measured after staining.

Proteinase activity (Prz) was measured in terms of the ratio of the colony to the diameter of unstained zone. A Prz value of 1 denoted no proteinase activity, whereas Prz<1 indicated proteinase production. The lower the Prz value, the higher the enzymatic activity. *C. albicans* ATCC 10231 and *C. kefyr* ATCC 25412 were used as positive and negative controls, respectively.

(iii) **Haemolysin production**

Haemolysin production in *Candida* isolates was screened sheep blood Sabouraud dextrose agar (SDA) plate as per the method described by Luo *et al* (2001)[11].

Approximately 10 µL of standard inoculum containing 10^8 yeast cells/mL was aseptically inoculated onto the surface of sheep blood SDA plate. The inoculated media were incubated at 37ºC in 5% $CO_2$for 48h. After incubation, the plates were examined for presence of a distinct translucent halo around the colony. The presence of a distinct translucent halo around the colony indicated haemolysin activity by the isolate. Haemolytic activity (Hz) was determined by calculating the ratio of the diameter of the colony to that of the translucent haemolytic zone. *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were used as positive and negative controls, respectively. Additional, one strain each of *Streptococcus pyogenes* (Lancefield A) and *Streptococcus sanguis* were used as controls for differentiation into β and α haemolysis.

(iv)**Biofilm formation**

Biofilm production in *Candida* spp. was detected by polystyrene test tube method as described by Branchini *et al* (1994)[12].

Briefly, a loopful of 24 h old *Candida* colonies from Sabouraud's Dextrose Agar (SDA) plate was inoculated into tube containing 10 ml Sabouraud dextrose broth supplemented with glucose (Final concentration 8%).The tubes were then incubated at 37^0C for 24 hrs without agitation. The broth was aspirated out with Pasteur pipette and the walls of the tubes were stained with 1% saffranin for 7 minutes.The tubes were examined for biofilm production.Presence of visible adherent film on the wall and the bottom of the tube indicated biofilm formation by the isolate.*C. albicans* ATCC 90028 and *C. albicans* ATCC

10231 were used as positive and negative control strains respectively.

Antifungal susceptibility testing

The antifungal susceptibility profile of *Candida* isolates from blood cultures was studied by Ezy MIC Strip. Ezy MIC Strip were procured from HiMedia Laboratories Pvt. Limited, Mumbai, India. It is a unique method for determination of minimum inhibitory concentration of *Candida* isolates, in which antifungal agent is coated on a single paper strip in a concentration gradient manner. All *Candida* isolates were tested against following antifungal agents.

Table 1:Range of different antifungal agents

Antifungal agent	Range (µg/mL)
Fluconazole	0.016-32
Itraconazole	0.002-32
Amphotericin B	0.002-32
Voriconazole	0.002-32
Anidulafungin	0.002-32
Caspofungin	0.002-32
Micafungin	0.002-32

For antifungal susceptibility testing, the inoculums was prepared by inoculating 3-4 colonies of the24 h old *Candida* isolate to be tested in saline.The turbidity of suspension was matched with 0.5 McFarl and standard. The suspension was inoculated by lawn culture method on the agar plate containing RPMI 1640 supplemented with 2% glucose by lawn culture method using tipped cotton swab. The manufacturer's instructions were adhered to throughout the test. The antifungal strips were aseptically placed on the media with the help of forceps and the plates were incubated at35°C for 24–48 h. *C.albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were used as quality control strains. The results of antifungal susceptibility test were interpreted as sensitive (*S*), dose-dependent susceptible (DDS), and resistant (*R*).Interpretative criteria for azoles were those recommended by the Clinical Laboratory Standard Institute (CLSI). Due to the lack of defined break points for amphotericin B arbitrary values based on the studies of other researchers were used.

Results

During the study period, a total 988 blood cultures were received from various inpatient departments of the hospital. Out of these 988 blood cultures, a total of 259 (26.2%) showed growth. The rates of isolation of bacterial and fungal pathogens from blood cultures were 78.4% (n=223) and 21.6% (n=36), respectively (Figure 1). *Candida* spp. was the only fungal pathogen isolated from blood cultures.

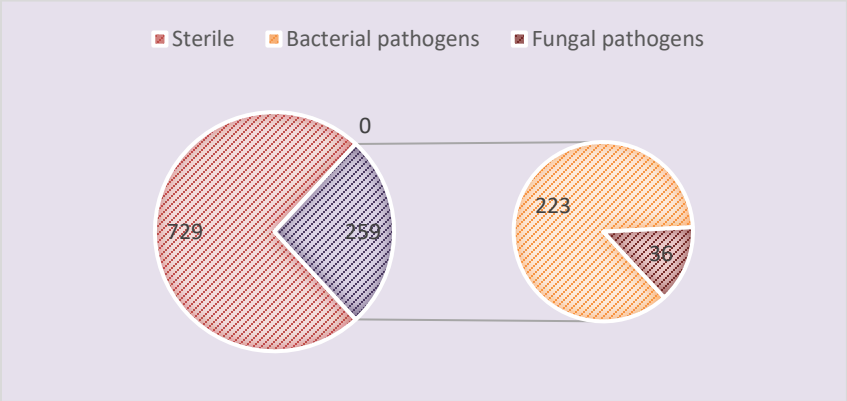

Fig1: Bacterial and fungal pathogens isolated from blood cultures

As shown in table 1, *Klebsiella* spp. followed by *Escherichia coli* and *Staphylococcus aureus* were common bacterial pathogens isolated from blood cultures. *Candida* spp. only fungal pathogen isolated from blood culture. *Candida* spp. was the fifth among various pathogens isolated in the present study.

Table 1: Pathogens isolated from blood cultures

Pathogen	Number (%)
Klebsiella spp.	51 (19.7)
Escherichiacoli	48 (18.5)
Staphylococcus aureus	38 (14.7)
Pseudomonas spp.	37 (14.3)
Candida spp.	36 (13.9)
Coagulase negative Staphylococci	21 (8.1)
Acinetobacter spp.	12 (4.6)

Enterobacter spp.	09 (3.5)
Enterococcus spp.	07 (2.7)
Total	259

As shown in figure 2, out of 36 *Candida* spp., 9 isolates were identified as *C. albicans*, whereas 27 isolates belonged to NAC spp. In the present study, the isolation of NAC spp. was significantly high compared to *C. albicans* (Fisher's Exact Test, *P* value <0.0001).

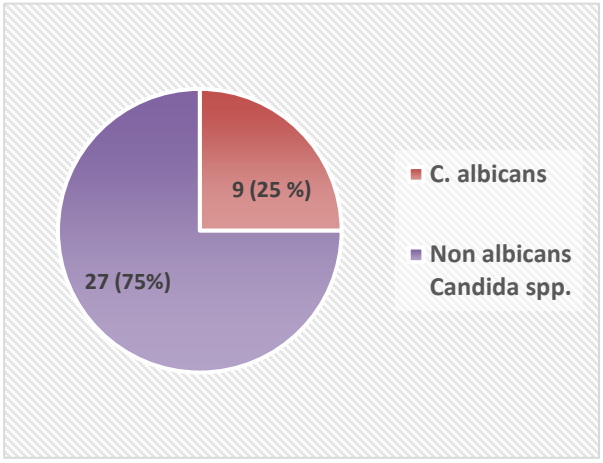

Fig 2: Distribution of *C. albicans* and NAC spp.

The demographic and clinical features of patients with *Candida* BSI is shown in table 2. Out of 36 patients, 24 (66.7%) were males whereas 12 (33.3%) were females. The mean age of patients with *Candida* BSI was 38.7±12.2. Admission to ICU (86.1%) was the major factor for *Candida* BSI. A total of 21 (58.3%) patients had history of fluconazole prophylaxis/treatment.

Table 2: Demographic and clinical features of patients with *Candida* BSI

Demographic/clinical feature	Number of cases (%)
Gender	
Male	24 (66.7)
Female	12 (33.3)
Age group in years	
≤ 1	06 (16.7)
2-12	05 (13.9)
13-64	12 (33.3)
≥ 65	13 (36.1)
Mean age in years ± SD	38.7±12.2
Co-morbidities	
Malignancy	11 (30.6)
Preterm infants low birth weight	04 (11.1)
Burn	06 (16.7)
Diabetes	09 (25)
Risk factors	
ICU admission	31 (86.1)
Duration of ICU stay Mean± SD	7.8±0.6
Presence of mechanical ventilator	12 (33.3)
Urinary catheterization	18 (50)
Total parenteral nutrition	06 (16.7)
Central venous catheterization	04 (11.1)
Major surgery	12 (33.3)
History of Fluconazole prophylaxis/ treatment	21 (58.3)

C. tropicalis(44.5%)followed by *C. glabrata* (22.3%) and *C. krusei*(14.8%)were most common isolates from NAC spp (Table 3). *C. rugosa* was isolated from 2(7.4%) blood cultures. In the present study, isolation of *C. tropicalis* was significantly high as compared to other *Candida* spp (Fisher's Exact Test, P value <0.0001).

Table 3: Species distribution NAC isolates from BSI

NAC spp.	Number (%)
C. tropicalis	12 (44.5)
C. glabrata	06 (22.3)
C. krusei	04 (14.8)
C. gulliermondii	03 (11.2)
C. rugosa	02 (7.4)
Total	27

Comparison of underlying co-morbid conditions and risk factors for BSI due to *C. albicans* and NAC spp. is shown in table 4. In the present study, ICU admission (Chi square test P value 0.03) and fluconazole prophylaxis / treatment (Chi square test P value 0.01) were significantly associated with BSI due to NAC spp. There was no significant difference observed in other co-morbid conditions and risk factors for BSI due to *C. albicans* and NAC spp. (table 4).

Table 4: Comparison of underlying co-morbid conditions and risk factors for BSI due to *C. albicans* and NAC spp

Feature	Total	*C. albicans* (%)	NAC spp. (%)	Chi square test *P* value
Co-morbidities				
Malignancy	11	02 (18.2)	09 (81.8)	1.0000
Preterm infants low birth weight	04	01 (25)	03 (75)	1.0000
Burn	06	01 (16.7)	05 (83.3)	1.0000
Diabetes	09	04 (44.4)	05 (55.6)	0.16
Risk factors				
ICU admission	31	05 (16.1)	26 (83.9)	0.03*
Presence of mechanical ventilator	12	03 (25)	09 (75)	1.0000
Urinary catheterization	18	03 (16.7)	15 (83.3)	0.44
Total parenteral nutrition	06	02 (33.3)	04 (66.7)	0.6
Central venous catheterization	04	01 (25)	03 (75)	1.0000
Major surgery	12	04 (33.3)	08 (66.7)	0.40
Fluconazole prophylaxis/ treatment	21	02 (9.5)	19 (90.5)	0.01*

Virulence factors produced by NAC spp. isolated from BSI is shown in table 5. Haemolysin production (66.7%) was the major virulence factor produced by NAC spp. Haemolysin production was not seen in *C. guilliermondii* isolates. A total of 83.3% of *C. tropicalis* and *C. glabrata* isolates showed haemolysin production. Biofilm formation was seen in 62.9% of NAC spp. All isolates of *C. tropicalis* demonstrated ability to form biofilm. No isolate of *C. krusei* and *C. rugosa* showed biofilm formation. Phospholipase production was seen in 48.1% of NAC spp. A total of 75% of *C. tropicalis* isolates showed phospholipase production. Phospholipase production was not seen in *C. rugosa* isolates. Proteinase activity was noted 37.1% of NAC spp. Proteinase activity was not seen in *C. krusei*, *C. guilliermondii* and *C. rugosa*.

Table 5: Virulence factors of NAC spp. isolated from BSI

NAC spp. (N)	Phospholipase production (%)	Proteinase activity (%)	Haemolysin production (%)	Biofilm formation (%)
C. tropicalis (12)	09 (75)	09 (75)	10 (83.3)	12 (100)
C. glabrata (6)	02 (33.3)	01 (16.7)	05 (83.3)	04 (66.7)
C. krusei(4)	01 (25)	-	02 (50)	-
C. gulliermondii (3)	01 (33.3)	-	-	01(33.3)
C. rugosa (2)	-	-	01 (50)	-
Total (27)	13 (48.1)	10 (37.1)	18 (66.7)	17 (62.9)

Antifungal susceptibility pattern of NAC spp. isolated from BSI is shown in table 6. Fluconazole resistance was observed in 40.7% of NAC spp. isolated from BSI. All isolates of *C. krusei* were resistant to fluconazole. A total of 33.3% of *C. tropicalis* isolates were resistant to fluconazole. Itraconazole resistance was seen in only 2 (7.4%) NAC spp. These included single isolate each of *C. tropicalis* and *C. krusei*. No isolates were resistant to voriconazole. Amphotericin B resistance was seen in only 2 (7.4%) isolates. These included single isolate each of *C. tropicalis* and *C. krusei*.

Table 6: Antifungal susceptibility pattern of NAC spp. isolated from BSI

***Candida* spp (N)**	**Fluconazole**			**Itraconazole**			**Voriconazole**			**Amphotericin B**		
	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)
C.tropicalis (12)	6 (50)	2 (16.7)	4 (33.3)	11 (91.7)	-	1(8.3)	12 (100)	-	-	11(91.7)	-	1 (8.3)
C. glabrata (6)	3 (50)	2	1(16.7)	6	-	-	6	-	-	6 (100)	-	-

		(33.3)		(100)			(100)					
C. krusei (4)	-	-	4 (100)	3 (75)		1 (25)	3 (75)	1 (25)	-	3 (75)	-	1 (25)
C.gulliermondii (3)	2 (66.7)	-	1 (33.3)	3 (100)	-	-	3 (100)	-	-	3 (100)	-	-
C. rugosa (2)	1(50)	-	1(50)	2 (100)	-	-	2 (100)	-	-	2 (100)	-	-
Total (27)	12(44.4)	4 (14.8)	11 (40.7)	25(92.6)	-	2(7.4)	26 (96.3)	1 (3.7)	-	25(92.6)	-	2 (7.4)

Echinocandin susceptibility profile of NAC spp. isolated from BSI is shown in table 7. In the present study, no isolates were resistant to echinocandins.

Table 7: Echinocandin susceptibility profile of NAC spp. isolated from BSI

***Candida* spp (N)**	**Anidulafungin**			**Caspofungin**			**Micafungin**		
	S (%)	SDD(%)	R (%)	S (%)	SDD(%)	R (%)	S (%)	SDD(%)	R (%)
C. tropicalis (12)	12 (100)	-	-	12(100)	-	-	12 (100)	-	-
C. glabrata (6)	6 (100)		-	6 (100)	-	-	6 (100)	-	-
C. krusei (4)	4 (100)	-	-	4 (100)		-	4 (100)	1 (25)	-
C. gulliermondii (3)	3 (100)	-	-	3 (100)	-	-	3 (100)	-	-
C. rugosa (2)	2 (100)	-	-	2 (100)	-	-	2 (100)	-	-
Total (27)	27 (100)	-	-	27(100)	-	-	27 (96.3)	1 (3.7)	-

Discussion

Blood stream infection (BSI) is one of the most devastating but preventable complication seen in patientsadmitted to Critical Care Units[13]. It represents as one of the important adverse iatrogenic events experienced by hospitalized patients. Although rare community acquired BSI do occur.Globally, the exact rate of BSI vary markedly[13]. In the United States, the rate of BSI is estimated to be around 19.8 per 1000 catheter associated days with approximately 100,000 episodes per year[13]. Within the country, the rate of BSI differ as per the region, the type of healthcare setup, the types of patients care for, compliance with infection prevention and control practices, and rational use of antibiotics. In the present study rate of isolation of pathogens from BSI was 26.2%. In a recent study from South India, Rukadikaret al (2019) reported blood cultures collected from clinically diagnosed cases of BSI[14]. Vasudeva and colleagues (2016) from Jaipur reported culture positivity in 31.2% of cases by using automated blood culture system[15]. Dash et al (2016) from reported isolation of pathogens from 17.2% of blood cultures collected from clinically suspected cases of BSI[16].Predominance of bacterial isolates (n= 223, 78.4%) was noted in the present study. Similar observation was noted by various researchers. Klebsiella spp. (19.7%) followed by E. coli (18.5%), S. aureus (14.3%) were common bacterial pathogens isolated from blood cultures. Various international and national studies have reported E. coli, Klebsiella spp. and Serratia spp. arethe commonest among Enterobacteriaceae. However in the present study, Serratia spp. was not isolated. Pseudomonas spp. and Acinetobacter spp.are the commonest amongst the non-fermenter Gram negative organisms[13]. In the present study, Acinetobacter spp. was isolated from a total of 12 (4.6%) cases. In recent years, Acinetobacter spp. has emerged as an important cause of health care associated BSI especially in terminally ill patients.In the present study, a total of 36 (21.6%) blood cultures showed growth of Candida spp. Very few researchers have reported rate of candidemia in their studies. The reported rates of Candida BSI from India varies from 6% to 18%.[5]Although, as compared to bacterial counterparts the incidence of Candida BSI is less, it is usually associated with increased morbidity and mortality. The outcome of Candida BSI depends on various factors like immune status of patient, co-morbidities, standard of healthcare facilitates available, distribution of Candida spp, and antifungal resistance pattern of infecting strain[17].Various studies mostly from developed part of world have reported either increase or decrease or no change in incidence of Candida BSI. Among Indian studies, Verma et al (2003) from North India reported Candida as the 8th common pathogen isolated from blood cultures[18]. In the study from Maharashtra, Deorukhkar et al (2017) reported Candidaspp. as the 5th common pathogen isolated from BSI[5].In the present study predominance of NAC spp. (75%) over C. albicans (25%) was noted.Our finding is accordance to many recent studies. Emergence of NAC spp. is mostly implicated to increased empirical use of azoles as therapeutic or prophylactic agents. However, role of advancement in mycological techniques like use of chromogenic media, commercially available identification kit and molecular diagnosis cannot be overlooked[7].C. tropicalis was the predominant Candida spp. isolated from BSI. Various Indian researchers have reported predominance of C. tropicalis. [7]It is reported in as many as 67-90% of candidemia cases. C. tropicalis infection is usually seen in patients admitted to critical care units[5,7]. Patients with prolonged indwelling medical devices and broad spectrum antibiotic treatment are at high risk.In the present study, C. glabrata(22.3%) was the second common NAC spp. isolated from BSI. Similar to our observation, Deorukhkar et al

(2017) reported the rate of isolation of this Candida spp as 25.3%[5,6].The rate of isolation of C. glabrata varies from 8% to 37%. C. glabrata is the only haploid species of the genus Candida.It lacks the ability to form both hyphae and pseudohyphae.C. rugosa was isolated from 2 cases. This NAC spp. is primarily an animal pathogen. However in recent years it is implicated in human infections especially nosocomial BSI in burn and critically. Oberoi and his coworkers from New Delhi reported isolation of C. rugosa from 9 cases of BSI[19]. Deorukhkar et al. (2017) reported, C. rugosa from 6 cases, out which 3 were from burn patients. In the present study, both the isolates of C. rugosa were from burn patients[5].As candidiasis is usually encountered as a secondary infections, candidiasis can be called as "disease of diseased". Various factors are known to increase the host's susceptibility to invasive Candida infections. These include altered immune status, total parenteral nutrition and presence indwelling medical devices[20]. Prior to 1990, malignancy and neutropenia were considered as risk factors for Candida BSI, however in recent years admission to critical care unit especially surgical intensive care unit (SICU) is considered as important[20]. In the present study, admission to ICU (86.1%) was the major factor for Candida BSI. Similar finding was noted in report of National Epidemiology of Mycoses Survey (NEMIS) group. Investigators like Verma et al (2003)[18] Wu et al (2014)[21] and Deorukhkar et al (2017)[5] also reported candidemia to be high in patients admitted to ICU. High incidence of Candida BSI in ICU might be due to admission of more severely ill patients with most of them being on life support systems.In the present study, ICU admission and fluconazole prophylaxis/treatment were significantly associated with BSI due to NAC spp. Similar findings were also reported by Verma et al (2003)[18] and Deorukhkar et al (2017)[5]. As NAC spp. are intrinsically less susceptible to fluconazole, its widespread use is one of the major factors responsible for emergence of NAC spp[22]. As fluconazole is a fungistatic drug, many Candida isolates may acquire resistance during course of treatment[23]. Numerous virulence factors like adherence to host tissues, biofilm formation, the secretion of extracellular hydrolases, thigotrophism, polymorphism and phenotypic switching mediates pathogenicity of Candida spp[24]. These virulence factors are all traits produced by an infecting strain for establishment and progression of infection in a susceptible host[25]. Virulence factors directly interact with host cells and damage them[25]. In the present study, phospholipase production was seen in 48.1% of NAC spp. Phospholipases are group of hydrolytic enzymes that lyse phospholipids into fatty acid and expose receptors on the host cell membrane to facilitate adherence of Candida spp[26]. The quantity of phospholipase production also varies with the species of Candida, infecting strain and the site of infection[27]. Isolates from BSI are known to have high phospholipase activity compared to those from wound or urine[27]. In Candida spp., proteinases facilitate invasion and colonization of host tissues by degrading important cellular and immunological defense proteins.[28]In this study,proteinase activity was noted 37.1% of NAC spp.Proteinase activity was not seen in C. krusei, C. guilliermondii and C. rugosa. Proteinases ofC. albicans and other medically important NAC spp. has been extensively studied. Less pathogenic or non pathogenic NACspp. doesn't produce significant proteinase activity, even though they may possess secreted aspartyl proteinases (SAP) gene[29].Hemolysin proteins are considered as one of the important virulence attributes of Candida spp. These proteins aid in Candida survival and its persistence in the host tissues.Hemolysinsdegrade hemoglobin and facilitates recovery of the elemental iron from host erythrocytes[25] which is essentialfor establishment and progress of processof infection initiated by Candida spp[11,25].In the present study, haemolysin production (66.7%) was the major virulence factor produced by NAC spp. A total of 83.3% of C. tropicalis and C. glabrata isolates showed haemolysin production. Luo et al (2001)[11] and Kumar et al (2009)[30]reported similar findings.Ability to form biofilm enables Candida adherence to variety of surfaces including medical devices. Candida spp. is capable of forming biofilm on most, if not all, medical devices. Biofilms are specific and organized surface–associated communities of microbial cells that are embedded within extracellular matrix[31]. In the present study, biofilm formation was seen in 62.9% of NAC spp. All isolates of C. tropicalis demonstrated ability to form biofilm. Researchers like Kumar et al (2009)[30] and Mane et al (2011)[32]. also reported high biofilm forming ability in C. tropicalis.Only few decades back, antifungal resistance was rarely encountered in Candida spp. As there is no or very minimal person to person transmission of Candida isolates, the secondary resistance is very rare event.[20] Antifungal resistance in Candida spp. often arises during course of therapy. However, some NAC spp. are inherently less susceptible to commonly prescribed antifungal drugs[20]. In the present study, fluconazole resistance was observed in 40.7% of NAC spp. isolated from BSI. In clinical practice, fluconazole resistance is of great concern because it is one of the most widely used first line antifungal agents for treatment and prophylaxis of almost all types of candidiasis.[33]It is available in variety of formulation like a tablet, an oral suspension and an intravenous formulation. As compared to other antifungals, fluconazole is better tolerated has and has high bioavailability. It has appreciable water solubility, low degree of protein binding and wide volume of dissemination into body tissues and fluids[33].In this study, all isolates of C. krusei were resistant to fluconazole.Various national and international studies have reported total fluconazole resistance in C. krusei isolates[7].A total of 33.3% of C. tropicalis isolates were resistant to fluconazole.C. tropicalis was initially regarded as fluconazole susceptible species however the scenario has changed drastically in last few years[7].In the present study, no NAC spp. demonstrated resistance to voriconazole. Voriconazole is the 2nd generation synthetic triazole. It structurally resembles to fluconazole and has broad spectrum activity against Candida spp. including fluconazole resistant or less susceptible species like C. krusei and C. glabrata[34]. Only 2 (7.4%) NAC spp. were resistant to amphotericin B. Amphotericin B, the polyene group of antifungal agents has broad spectrum activity against both C. albicans and NAC spp[34]. Echinocandins are

the most recent addition to antifungal armamentarium. As mammalian cells lack the target of activity (1-6-β-D-glucan), the toxicity of echinocandins is considered to be minimal in humans[35].Now a days, echinocandins are increasingly used as first line drugs for treatment and management of candidemia among patients who are terminally ill, clinically unstable or having recent history of azole exposure or are colonized /infected with fluconazole resistant Candida spp[36]. In the present study, no NAC spp. demonstratedin vitro resistance to echinocandins. All echinocandins (caspofungin, anidulafungin and micafungin) are approved for treatment of candidemia and other forms of disseminated candidiasis[35].

Conclusion

The very nature of mycotic infections has changed drastically. Hitherto rare and new fungal species and fungi once considered to be non-pathogenic are increasingly implicated in human infections.NAC spp. have emerged as an important cause of infections including candidemia. These NAC spp., produce virulence factors once attributed to C. albicans. As NAC spp. differ widely in susceptibility to routine used antifungal agents, antifungal susceptibility testing plays an important role in evaluating therapy for Candida infections.

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Conflict of Interest: Nil
Source of support:Nil