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Assessment of the antifungal susceptibility profile and virulence factors of non-albicans candida species obtained from the infection of the bloodstream

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Abstract---Background: Earlier the fungal infection spectrum was considered to be only restricted to mucocutaneous and cutaneous tissues. However, this consideration has now changed. The threatening rise in the incidence of invasive mycosis is seen recently. Despite advancements in diagnostic and therapeutic modalities, high mortality rates are linked to invasive mycosis. The most common cause of bloodstream infections is Candida spp. Among candida genus, the most pathogenic species is C. Albicans, recent literature data has shown the emergence of treatment-resistant, relatively uncommon, and unusual nonalbicans Candida (NAC) spp. Aim: The present study was conducted to assess the antifungal susceptibility profile and virulence factors of non-Albicans candida species obtained from the infection of the bloodstream. Material and methods: The present study included blood culture isolates of nonalbicans Candida (NAC) spp. which were identified to species level using the standard mycological protocol. This NAC spp. were screened for virulence factors production including biofilm formation, hemolysin, and extracellular hydrolytic enzymes. Ezy MIC strip was used for assessing

the antifungal susceptibility profile of these isolates. Results: 100% (n=1) C. rugosa NAC spp. showed hemolysin production. In C. gulliermondii, 50% (n=1) subject showed phospholipase production and biofilm formation each. For C. Krusei, 33.3% (n=1) showed phospholipase production and 66.6% (n=2) showed hemolysin production. In C. glabrata, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 16.6% (n=1), 16.6% (n=1), 66.6% (n=4), and 50% (n=3) subjects respectively. For C. tropicalis, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 77.7% (n=7), 77.7% (n=7), 88.8% (n=8), and 100% (n=9) subjects respectively. In total study subjects, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 52.38% (n=11), 38.09% (n=8), 71.42% (n=15), and 66.6% (n=14) subjects respectively For C. glabrata, for fluconazole 50% (n=3), 33.3% (n=2), and 16.6% (n=1) subject was S, DDS, and R respectively. for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=6) subject each. For C. tropicalis, in fluconazole, S, DDS, and R was seen in 55.5% (n=5), 11.1% (n=1), and 33.3% (n=3) subjects each. For itraconazole, S and R was seen in 88.8% (n=8) and 11.1% (n=1) subjects each. For voriconazole, 100% (n=9) subjects were sensitive, and for Amphotericin B, 95.23% (n=20) and 4.76% (n=1) subject. Conclusion: The present study concludes that new fungi and fungal species and Hitherto rates that were once considered non-pathogenic are seen in increasing the incidence of human infections. Also, NAC spp. has been seen as a rising and vital infection cause including candidemia. This non-albicans Candida spp. leads to the production of the virulence factors that were earlier linked to C. Albicans exclusively. The susceptibility to commonly used antifungal drugs for NAC spp. species is different, and testing of antifungal susceptibility has a vital role in assessing Candida infection therapy.

Keywords---antifungal therapy, susceptibility testing, bloodstream infections, *Candida,* candidemia, virulence factor.

Introduction

One of the major challenge in the healthcare sector worldwide is infectious diseases which also contributes to significant mortality and morbidity in developing countries. Until the recent past, infectious diseases were mainly linked to viral, parasitic, or bacterial etiology. However, the role of fungi in human infectious diseases is less documented and recognized. Recently, the role of fungi in causing human infections has been increasingly accepted. Risk factors that lead to increased incidence of fungal infections in humans include aggressive antibiotics and immunosuppressive drugs used, increased subjects with chronic systemic diseases, an aging population, and advancement in the healthcare system.[1] Earlier the fungal infection spectrum was considered to be only restricted to mucocutaneous and cutaneous tissues. However, this consideration has now changed. The threatening rise in the incidence of invasive mycosis is

seen recently. Despite advancements in diagnostic and therapeutic modalities, high mortality rates are linked to invasive mycosis. The most vital etiological factors associated with invasive mycoses are Aspergillus spp., Cryptococcus spp., and Candida spp. The incidence of invasive candida infections is nearly 15 folds higher when compared to invasive aspergillosis.[2]

The opportunistic infections caused by the Candida species are unique as they lead to wide clinical manifestations ranging from mucocutaneous overgrowth to disseminated infections that may be life-threatening. Also, Candida infections exist as pathogens as well as commensals. Disseminated Candidiasis includes urinary tract infections, intra-abdominal infections, medical device-associated infections, and bloodstream infections that are common in subjects admitted to surgical or medical intensive care units. Disseminated Candidiasis usually leads to prolonged hospitalization and mechanical ventilation along with the increased cost of healthcare expenses.[3]

In developed countries BSI (bloodstream infections) caused by Candia spp. are high in the United States and European countries, Candida spp. is the fourth and sixth most common cause of Bloodstream infections seen in the healthcare profession. However, data assessing the bloodstream infections by Candida in developing countries like India are scarce. The most common cause of bloodstream infections is Candida spp. Among candida genus, the most pathogenic species is C. Albicans, recent literature data has shown the emergence of treatment-resistant, relatively uncommon, and unusual nonalbicans Candida (NAC) spp. Clinical manifestations of NAC spp. are similar to C. Albicans. The difference is seen in susceptibility patterns to antifungal drugs, epidemiology, and virulence factors.[4] Hence, the present study was conducted to assess the antifungal susceptibility profile and virulence factors of non-Albicans candida species obtained from the infection of the bloodstream.

Materials and Methods

The present cross-sectional study was conducted to assess the antifungal susceptibility profile and virulence factors of non-Albicans candida species obtained from the infection of the bloodstream. The study was carried out after obtaining clearance from the concerned Ethical committee. The study includes samples processed at the Department of Microbiology of the Institute. The study includes NAC spp. isolated from the cultures of the bloodstream. Up to the species level, from the blood culture, Candida isolates were identified based on the colony morphology on sugar assimilation test and corn meal agar, color on Hi Chrom *Candida* agar, germ tube test, and colony characteristics. Also, the HiCandida identification kit was used for isolates identification. Colony isolates were then screened for virulence factor production like biofilm formation, hemolysin, and extracellular hydrolytic enzymes. Extracellular hydrolytic enzymes assessed in the study were proteinase and phospholipase.

In Candida isolates, Phospholipase production was assessed on egg yolk agar using the Samarnayake et al[5] methods in 1984. 5µL made from the test strain was added to the egg yolk agar plate surface which was then dried at room temperature and incubated for 72 hours at 35^{o} C. The plates were then observed

for precipitate zone around colonies and growth that indicated Pz (Phospholipase activity) expressed as colony ratio to colony diameter added to the precipitation zone. Pz value of 1 showed no phospholipase production and Pz<1 showed expression of phospholipase. Higher enzymatic activity was denoted by a lower Pz value. Negative and positive controls were respectively denoted by *C. kefyr* ATCC 25412 and *C. Albicans* ATCC 10231. Activity in proteinase in the Candida spp. was assessed using BSA agar (bovine serum albumin) by Aoki et al[6] in 1990 where 10 µL of standard inoculum was inoculated on BSA agar plate surface which was then incubated for 7 days at 37ºC. After incubating for 7 days, proteinase production was inhibited by adding 20% trichloroacetic acid followed by 1.25% amidobalck. The decantation of excess stain was done and the plate was assessed for clear zone production around colonies. Before staining, *C. krusei* colonies' diameter was assessed. However, clear zone diameter was assessed after staining around colonies.

SDA (Sabouraud dextrose agar) from sheep blood was used to assess hemolysin production in Candida isolates following the method of Luo et al[7] in 2001. Nearly 10 µL of standard inoculum was inoculated on sheep blood SDA plate surface which was then incubated for 48 hours in 5% CO2 at 37° C. Following incubation, the distinct translucent halo around colonies was assessed indicating hemolysin activity in the isolates. Hz (Hemolytic activities) were assessed after the ratio calculation of colony diameter to the translucent hemolytic zone. Negative and positive controls were contributed by *C. parapsilosis* ATCC 22019 and *C. Albicans* ATCC 90028 respectively. In addition, one strain each of *Streptococcus sanguis and Streptococcus pyogenes* (Lancefield A) were taken as controls for differentiating β and α hemolysis.

The polystyrene test tube method by Branchini *et al*[8] in 1994 was used for assessing biofilm in Candida spp. From SDA, Candida colonies were inoculated into SDA supplemented with 8% glucose. For 24 hours without agitation, tube incubation was done at 37°C. Tube walls were stained for 7 minutes using 1% safranin. Biofilm formation by C. Albicans isolates ATCC and ATCC 90028 was indicated by the visible adherent film on the bottom and wall of the tube. For antifungal susceptibility testing, the inoculum was made by inoculating 4 colonies of 24 h old *Candida* isolates that were tested in saline. The suspension turbidity was matched with standard and .5 McFarl and inoculated on the agar plate using lawn culture method having RPMI 1640 with 2% glucose supplementation using tipped cotton swab using manufacturer's instructions Antifungal strips were placed on media followed by incubation for 24-48 hours at 35° C. Quality control strains were contributed by *C. parapsilosis* ATCC 22019 and *C.albicans* ATCC 90028. Antifungal susceptibility tests were assessed as resistant (R), dose-dependent susceptible (DDS), and sensitive (S). CLSI (Clinical Laboratory Standard Institute) interpretive criteria for azoles were used in the study. Amphotericin B arbitrary values as suggested by the previous studies were used owing to defined breakpoints lack.

Results

The present cross-sectional study was conducted to assess the antifungal susceptibility profile and virulence factors of non-Albicans candida species

obtained from the infection of the bloodstream. In the present study, a total of 494 blood cultures were taken from various departments of the Institute where the growth was seen in 25.91% (n=128) subjects where fungal and bacterial isolates were seen in 21.87% (n=28) and 78.12% (n=100) respectively. The demographic characteristics of the study subjects are listed in Table 1. The mean age of the study subjects was 38.5±12.4 years, Majority of study subjects were in the age range of 35.71% (n=10) subjects followed by 32.14% (n=9) subjects, 14.28% (n=4) subjects in the age of 2-12 years, and 17.85% (n=5) in ≤1 year. There were 67.85% (n=19) males and 32.14% (n=9) females in the study. Among risk factors, ICU admission was the most common risk factor seen in 85.71% (n=24) subjects followed by urinary catheterization and major surgery in 50% (n=14) subjects, Mechanical ventilator presence in 32.14% (n=9) subjects, total parenteral nutrition in 17.85% (n=5) subjects, central venous catheterization in 10.71% (n=3) subjects, and mean ICU stay the duration of 7.6±0.8 days. History of fluconazole treatment/ prophylaxis was seen in57.14% (n=16) study subjects. In comorbidities, most common was malignancy in 32.14% (n=9) subjects followed by diabetes in 25% (n=7) subjects, burn-in 17.85% (n=5) subjects, and Preterm low-birth-weight infants in 10.71% (n=3) subjects respectively (Table 1).

For assessment of the distribution of NAC species isolates from the bloodstream infections in the study subjects, it was seen that the most common species isolated was C. tropicalis has seen in 42.85% (n=9) subjects followed by C. glabrata in 28.57% (n=6) subjects, C. krusei in 14.28% (n=3) subjects, C. gulliermondii in 9.52% (n=2) subjects, and C. rugosa in 4.76% (n=1) subject respectively (Table 2). On comparing C. Albicans and NAC species based on risk factors and co-morbidities in subjects with BSI, it was seen that Fluconazole treatment/ prophylaxis history was seen in 12.5% (n=2) C. Albicans and 87.5% (n=14) NAC spp. This was statistically significant with p=0.001. Among risk factors, all the parameters showed statistically non-significant differences among C. Albicans and NAC spp. with 12.5% (n=3) and 87.5% (n=21) subjects respectively with p=0.02. For other parameters like major surgery, Central venous catheterization, Total parenteral nutrition, Urinary Catheterization, and Mechanical ventilator presence the respective p-values were 0.42, 1.0000, 0.7, 0.42, and 1.0000. In comorbidities, all comorbidities showed a non-significant difference between C. Albicans and NAC spp. with respective p-values of 0.18, 1.0000, 1.0000, and 1.0000 respectively (Table 3).

For Virulence factors of NAC spp. isolates seen in subjects with Blood stream infections, 100% (n=1) C. rugosa NAC spp. showed hemolysin production. In C. gulliermondii, 50% (n=1) subject showed phospholipase production and biofilm formation each. For C. Krusei, 33.3% (n=1) showed phospholipase production and 66.6% (n=2) showed hemolysin production. In C. glabrata, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 16.6% (n=1), 16.6% (n=1), 66.6% (n=4), and 50% (n=3) subjects respectively. For C. tropicalis, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 77.7% (n=7), 77.7% (n=7), 88.8% (n=8), and 100% (n=9) subjects respectively. In total study subjects, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 52.38% (n=11), 38.09% (n=8), 71.42% (n=15), and 66.6% (n=14) subjects respectively (Table 4).

Antifungal susceptibility tests were assessed as resistant (R), dose-dependent susceptible (DDS), and sensitive (S). Concerning the Antifungal susceptibility pattern of NAC spp. isolates from the bloodstream infections, in C. rugosa, susceptibility to fluconazole was seen as sensitive (S) in 100% (n=1) subjects and resistant (R) in 100% (n=1) subjects, for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=1) subject each, and no resistant or dose-dependent susceptible (DDS) case was seen. For c. gulliermondii, for fluconazole, S and R were seen in 50% (n=1) subject each, for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=2) subject each. In C. Krusei, for fluconazole, 100% (n=3) subjects were resistant, for itraconazole and Amphotericin B, S and R was seen in 66.6% (n=2) and 33.3% (n=1) subject each, whereas, for Voriconazole, it was sensitive and dose-dependent susceptible (DDS) in 66.6% (n=2) and 33.3% (n=1) subject each. For C. glabrata, for fluconazole 50% (n=3), 33.3% (n=2), and 16.6% (n=1) subject was S, DDS, and R respectively. for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=6) subject each. For C. tropicalis, in fluconazole, S, DDS, and R was seen in 55.5% (n=5), 11.1% (n=1), and 33.3% (n=3) subjects each. For itraconazole, S and R was seen in 88.8% (n=8) and 11.1% (n=1) subjects each. For voriconazole, 100% (n=9) subjects were sensitive, and for Amphotericin B, 95.23% (n=20) and 4.76% (n=1) subject was resistant (Table 5).

Discussion

The present cross-sectional study was conducted to assess the antifungal susceptibility profile and virulence factors of non-Albicans candida species obtained from the infection of the bloodstream. In the present study, a total of 494 blood cultures were taken from various departments of the Institute where the growth was seen in 25.91% (n=128) subjects where fungal and bacterial isolates were seen in 21.87% (n=28) and 78.12% (n=100) respectively. The mean age of the study subjects was 38.5±12.4 years, Majority of study subjects were in the age range of 35.71% (n=10) subjects followed by 32.14% (n=9) subjects, 14.28% (n=4) subjects in the age of 2-12 years, and 17.85% (n=5) in ≤1 year. There were 67.85% (n=19) males and 32.14% (n=9) females in the study. Among risk factors, ICU admission was the most common risk factor seen in 85.71% (n=24) subjects followed by urinary catheterization and major surgery in 50% (n=14) subjects, Mechanical ventilator presence in 32.14% (n=9) subjects, total parenteral nutrition in 17.85% (n=5) subjects, central venous catheterization in 10.71% (n=3) subjects, and mean ICU stay the duration of 7.6±0.8 days. History of fluconazole treatment/ prophylaxis was seen in57.14% (n=16) study subjects. In comorbidities, most common was malignancy in 32.14% (n=9) subjects followed by diabetes in 25% (n=7) subjects, burn-in 17.85% (n=5) subjects, and Preterm low-birth-weight infants in 10.71% (n=3) subjects respectively. These results were consistent with the studies of Kauffman C[9] in 2006 and Maertens J[10] in 2004 where authors assessed subjects with demographics comparable to the present study.

For assessment of the distribution of NAC species isolates from the bloodstream infections in the study subjects, it was seen that the most common species isolated was C. tropicalis has seen in 42.85% (n=9) subjects followed by C. glabrata in 28.57% (n=6) subjects, C. krusei in 14.28% (n=3) subjects, C.

gulliermondii in 9.52% (n=2) subjects, and C. rugosa in 4.76% (n=1) subject respectively. On comparing C. Albicans and NAC species based on risk factors and co-morbidities in subjects with BSI, it was seen that Fluconazole treatment/ prophylaxis history was seen in 12.5% (n=2) C. Albicans and 87.5% (n=14) NAC spp. This was statistically significant with p=0.001. Among risk factors, all the parameters showed statistically non-significant differences among C. Albicans and NAC spp. with 12.5% (n=3) and 87.5% (n=21) subjects respectively with p=0.02. For other parameters like major surgery, Central venous catheterization, Total parenteral nutrition, Urinary Catheterization, and Mechanical ventilator presence the respective p-values were 0.42, 1.0000, 0.7, 0.42, and 1.0000. In comorbidities, all comorbidities showed a non-significant difference between C. Albicans and NAC spp. with respective p-values of 0.18, 1.0000, 1.0000, and 1.0000 respectively. These results were in agreement with the findings of Kathiravan M et al[11] in 2012 and Mane A et al[12] in 2011 where authors reported comparable NAC and Candida spp. distribution and risk factors and comorbidities as of present study in their studies.

For Virulence factors of NAC spp. isolates were seen in subjects with Bloodstream infections, 100% (n=1) C. rugosa NAC spp. showed hemolysin production. In C. gulliermondii, 50% (n=1) of subjects showed phospholipase production and biofilm formation each. For C. Krusei, 33.3% (n=1) showed phospholipase production and 66.6% (n=2) showed hemolysin production. In C. glabrata, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 16.6% (n=1), 16.6% (n=1), 66.6% (n=4), and 50% (n=3) subjects respectively. For C. tropicalis, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 77.7% (n=7), 77.7% (n=7), 88.8% (n=8), and 100% (n=9) subjects respectively. In total study subjects, Phospholipase production, proteinase activity, hemolysin production, and biofilm formation was seen in 52.38% (n=11), 38.09% (n=8), 71.42% (n=15), and 66.6% (n=14) subjects respectively. These results were comparable to the results of Kumar V et al[13] in 2009 and Silva S et al[14] in 2012 where authors have reported comparable Phospholipase production, proteinase activity, hemolysin production, and biofilm formation in their study subjects as in the present study.

Antifungal susceptibility tests were assessed as resistant (R), dose-dependent susceptible (DDS), and sensitive (S). Concerning the Antifungal susceptibility pattern of NAC spp. isolates from the bloodstream infections, in C. rugosa, susceptibility to fluconazole was seen as sensitive (S) in 100% (n=1) subjects and resistant (R) in 100% (n=1) subjects, for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=1) subject each, and no resistant or dose-dependent susceptible (DDS) case was seen. For c. gulliermondii, for fluconazole, S and R were seen in 50% (n=1) subject each, for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=2) subject each. In C. Krusei, for fluconazole, 100% (n=3) subjects were resistant, for itraconazole and Amphotericin B, S and R was seen in 66.6% (n=2) and 33.3% (n=1) subject each, whereas, for Voriconazole, it was sensitive and dose-dependent susceptible (DDS) in 66.6% (n=2) and 33.3% (n=1) subject each. For C. glabrata, for fluconazole 50% (n=3), 33.3% (n=2), and 16.6% (n=1) subject was S, DDS, and R respectively. for itraconazole, Voriconazole, and Amphotericin B, it was sensitive in 100% (n=6) subject each. For C. tropicalis, in fluconazole, S, DDS, and R was

seen in 55.5% (n=5), 11.1% (n=1), and 33.3% (n=3) subjects each. For itraconazole, S and R was seen in 88.8% (n=8) and 11.1% (n=1) subjects each. For voriconazole, 100% (n=9) subjects were sensitive, and for Amphotericin B, 95.23% (n=20) and 4.76% (n=1) subject was resistant. These results were consistent with the studies of Sardi JCO et al[15] in 2013 and Berkow E et al[16] in 2017 where antifungal susceptibility comparable to the present study was reported by the authors in their studies.

Conclusion

Within its limitations, the present study concludes that new fungi and fungal species and Hitherto rates that were once considered non-pathogenic are seen in increasing the incidence of human infections. Also, NAC spp. has been seen as a rising and vital infection cause including candidemia. This non-albicans Candida spp. leads to the production of the virulence factors that were earlier linked to C. Albicans exclusively. The susceptibility to commonly used antifungal drugs for NAC spp. species is different, and testing of antifungal susceptibility has a vital role in assessing Candida infection therapy. The present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

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Tables

Table 1
Demographic and disease characteristics of the study subjects

S. No	Characteristics	Percentage (%)	Number (n=28)
1.	Mean age (years)	38.5±12.4	
2.	Age range (years)		
a)	≤1	17.85	5
b)	2-12	14.28	4
c)	13-65	32.14	9
d)	≥65	35.71	10
3.	Gender		
a)	Males	67.85	19
b)	Females	32.14	9
4.	Risk factors		
a)	Major surgery	50	14
b)	Central venous catheterization	10.71	3
c)	Total parenteral nutrition	17.85	5
d)	Urinary Catheterization	50	14
e)	Mechanical ventilator presence	32.14	9
f)	ICU stay duration (Mean± S. D)		7.6±0.8
g)	ICU admission	85.71	24
5.	Co-morbidities		
a)	Diabetes	25	7
b)	Burn	17.85	5
c)	Preterm low-birth-weight	10.71	3

	infants		
d)	Malignancy	32.14	9
6.	Fluconazole treatment/ prophylaxis history	57.14	16

Table 2
Distribution of species of NAC isolates from BSI (blood-stream infections)

S. No	Non-albicans candida spp.	Percentage (%)	Number (n)
1.	C. rugosa	4.76	1
2.	C. gulliermondii	9.52	2
3.	C. krusei	14.28	3
4.	C. glabrata	28.57	6
5.	C. tropicalis	42.85	9
6.	Total	100	21

Table 3
Comparison of C. Albicans and NAC species based on risk factors and co-morbidities in subjects with BSI

S. No	Parameter	Total (n)	C. Albicans n (%)	NAC spp. n (%)	p-value
1.	Fluconazole treatment/ prophylaxis history	16	2 (12.5)	14 (87.5)	0.001
2.	Risk factors				
a)	Major surgery	14	5 (35.71)	9 (64.28)	0.42
b)	Central venous catheterization	3	1 (33.3)	2 (66.6)	1.0000
c)	Total parenteral nutrition	5	1 (20)	4 (80)	0.7
d)	Urinary Catheterization	14	3 (21.42)	11 (78.57)	0.42
e)	Mechanical ventilator presence	9	2 (22.2)	7 (77.7)	1.0000
f)	ICU admission	24	3 (12.5)	21 (87.5)	0.02
3.	Co-morbidities				
a)	Diabetes	7	2 (28.57)	5 (71.42)	0.18
b)	Burn	5	1 (20)	4 (80)	1.0000
c)	Preterm low-birth-weight infants	3	1 (33.3)	2 (66.6)	1.0000
d)	Malignancy	9	2 (22.2)	7 (77.7)	1.0000

Table 4
Virulence factors of NAC spp. isolates were seen in subjects with Bloodstream infections

S. No	NAC spp. (n)	Phospholipase production n (%)	Proteinase activity n (%)	Haemolysin production n (%)	Biofilm formation n (%)
1.	C. rugosa (1)	-	-	1 (100)	-
2.	C. gulliermondii	1 (50)	-	-	1 (50)

	(2)				
3.	C. krusei (3)	1 (33.3)	-	2 (66.6)	-
4.	C. glabrata (6)	1 (16.6)	1 (16.6)	4 (66.6)	3 (50)
5.	C. tropicalis (9)	7 (77.7)	7 (77.7)	8 (88.8)	9 (100)
6.	Total (21)	11 (52.38)	8 (38.09)	15 (71.42)	14 (66.6)

Table 5
Antifungal susceptibility pattern of NAC spp. isolates from the bloodstream infections

NAC spp. (n)	Fluconazole			Itraconazole			Voriconazole			Amphotericin B		
	S	DDS	R	S	DDS	R	S	DDS	R	S	DDS	R
C. rugosa (1)	1 (100)	-	1 (100)	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-
C. gulliermondii (2)	1 (50)	-	1 (50)	2 (100)	-	-	2 (100)	-	-	2 (100)	-	-
C. krusei (3)	-	-	3 (100)	2 (66.6)	-	1 (33.3)	2 (66.6)	1 (33.3)	-	2 (66.6)	-	1 (33.3)
C. glabrata (6)	3 (50)	2 (33.3)	1 (16.6)	6 (100)	-	-	6 (100)	-	-	6 (100)	-	-
C. tropicalis (9)	5 (55.5)	1 (11.1)	3 (33.3)	8 (88.8)	-	1 (11.1)	9 (100)	-	-	8 (88.8)	-	1 (11.1)
Total (21)	9 (42.85)	3 (14.28)	8 (38.09)	-	1 (4.76)	20 (95.23)	1 (4.76)	20 (95.23)	1 (4.76)	20 (95.23)	-	1 (4.76)