Original Article

DOI: 10.7860/NJLM/2019/40198:2360

Microbiology Section

Clinico-microbiological Study of *Candida* Infections Focusing on Risk Factors, Species Identification, Virulence Factors and Antifungal Susceptibility Patterns

MV POORNIMA[1], K VIDYALAKSHMI[2]

ABSTRACT

Introduction: Infections caused by *Candida* species have been increasing along with the advancement in diagnostic and treatment interventions. Recently a shift from *Candida albicans* to non *albicans* species has also been observed. Infections by *Candida* species is aided by several virulence factors and prevalence of antifungal resistance.

Aim: To speciate and study the virulence factors and determine susceptibility pattern of *Candida* isolates obtained from various clinical specimens. To determine the risk factors for infection with *Candida* species in patients admitted to a tertiary care hospital at Mangalore, Karnataka, India.

Materials and Methods: This cross-sectional study was conducted at Department of Microbiology, Kasturba Medical College, Mangalore, Karnataka between October 2015 to September 2018. A total of 145 isolates from various clinical specimens were identified up to species level by germ tube test, chlamydospore formation, and growth characteristic on cornmeal agar, colour of colonies on chromogenic agar and carbohydrate fermentation tests. Virulence factors included were biofilm formation by microtitre plate method, phospholipase activity using egg yolk agar, proteinase activity using bovine serum albumin agar, haemolysin production, coagulase activity and production of true hyphae. Antifungal susceptibility test was performed as per CLSI 2009 M44-A2. The statistical analysis was done using statistical package version 16.0. The p-value less than 0.05 was considered as statistically significant.

Results: Out of 145 *Candida* isolates *C. tropicalis* were 59 (40.6%) followed by *C. albicans* and other species. Presence of an intravenous catheter 124 (85.5%), treatment with broad spectrum antibiotics 121 (83.4%) and admission to ICU 113 (77.9%) were the most common risk factors identified in this study. Biofilm formation was detected in 80 (55.2%) of isolates, among which *C. tropicalis* (42 out of 59 71.1% p= 0.02) showed the highest propensity for biofilm formation. Phospholipase activity was highest among *C. albicans* 42 (79.2%, p= 0.001) whereas proteinase activity was highest in *C. tropicalis* 50 (84.7%, p=0.001). Coagulase production was detected in 14 isolates and 39 of our isolates had produced true hyphae. Production of true hyphae was observed more among *C. tropicalis* 25 (64.1%, p= 0.04). Non *albicans* species showed highest proteinase activity and true hyphae production of 69 (75.6% p= 0.002) and 25 (27.2% p=0.04) respectively. Fluconazole resistance was detected in 13.8% of the isolates.

Conclusion: Study of risk factors and virulence factors will serve as a guideline for better understanding of the pathogenic mechanisms involved in fungal disease, which in turn will lead to better patient management and prognosis.

Keywords: Antifungal resistance, Biofilm, *Candida albicans*, Extracellular hydrolytic enzymes, Non *albicans Candida* species

INTRODUCTION

In the last few decades there has been a progressive upsurge in the incidence of fungal infections caused by *Candida* species [1,2]. This increase appears to run in parallel with the recent advances in medicine such as immunosuppressive therapy, increased use of surgical procedures, prostheses and broad-spectrum antibiotics [3]. Several host factors such as diabetic mellitus, chronic kidney disease and malignancy also account for the development of *Candida* infection [3].

Non-*albicans Candida* (NAC) species are now being recovered from the clinical samples with increasing frequency [3]. The clinical presentation of patients infected with *C. albicans* and NAC species are indistinguishable. Accurate identification of the species causing infection is important because antifungal treatment is different for *C. albicans* and NAC infections [2].

The transition of *Candida albicans* from commensal to a pathogen may be facilitated by a number of virulence factors like adhesion, biofilm production, extracellular hydrolytic enzyme activity and ability to form true hyphae as evidence by published literature [4]. But there are only few literatures [3,5] on virulence factors of NAC species, which also cause a significant number of infections. Study of virulence factors is important to understand pathogenesis and to explore new therapeutic options.

The last decade has witnessed the development of new antifungal agents, as well as the emergence of antifungal resistance [1]. Monitoring antifungal resistance among *Candida* species helps to detect the emergence of resistant strains; and also, to modify empirical treatment recommendations.

We intend to study the common species of *Candida* causing infections in our patient population, their virulence factors and antifungal susceptibility patterns; as well as the risk factors which predispose patients to *Candida* infections.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Central Laboratory, Department of Microbiology, Kasturba Medical College, Mangalore, Karnataka, India, during the period of October 2015 to September 2018. The sample size was calculated using the formulae $4PQ/d^2$. From the previous study done by Kaur R et al., taking P as proportion of *Candida tropicalis* as 40.8%, Q as 100-P with 95% confidence

interval 80% power the calculated sample size was 145 [5]. The study protocol was submitted to the Institutional Ethics Committee and approval was obtained before commencement of study (letter no: KMC MLR 09-15/185 dated 16/09/2015).

A total of 145 consecutive isolates of *Candida* species from various clinical specimens were included in the study, isolates of *Candida* from exudate specimens were included based on the presence of inflammatory cells/ intracellular budding yeast cells/pseudohyphae in a Gram stained smear of the specimen. Isolates of *Candida* from urine specimens were included based on the presence of pus cells ≥5/HPF in a standard wet mount, along with oval budding yeast cells with pseudohyphae and a colony count of $\geq 10^5$ CFU/ml in culture. All isolates of *Candida* from blood cultures were included. Isolates from respiratory and urine specimens that showed numerous epithelial cells suggesting contamination with saliva or vaginal secretions, respectively, were excluded from the study. The clinical data were collected from Medical Record Department using a proforma **[Annexure 1]**.

Speciation was done by germ tube test [6], observing the morphology on cornmeal agar [7], CHRO Magar (Himedia laboratories Pvt., Ltd.,) [8] and carbohydrate fermentation test [2,7].

Assessment of Virulence Factors

Biofilm formation

Microtiter plate was used to evaluate biofilm formation by *Candida* isolates. Test isolates were grown in Saboraud's Dextrose broth with 8% glucose. The broth was then diluted 1:100 in fresh medium and 150µl of it was inoculated into microtiter plate wells. The negative control wells had the sterile broth and *Enterococcus faecalis* ATCC29212 was employed as a positive control strain. The plates were incubated at 37°C for 48 hrs. After incubation content well was discarded and were washed with sterile distilled water twice, excess water was eliminated by tapping the plates on to blotting paper. Then the plates were stained with 200µl of 0.5% crystal violet for 15 minutes. Excess stain was washed off and allowed to dry. The dye bound to the adherent yeast cells was resolubilized by adding 200µl of ethanol: acetone mixture (80:20 w/v). Optical density (OD) of the wells were read using an ELISA plate reader (BioTek instruments, Made in USA, ELx800) at wavelength 570 nm. OD values were recorded for each well. Four wells were used for each test strain and its average were used for analysis [9,10]. The cut-off Optical density (ODc) for the microtiter-plate is defined as three standard deviations above the mean OD of the negative control [11].

Phospholipase Activity

Phospholipase activity was determined by using egg yolk agar (65g Saboraud's Dextrose Agar with 1 M NaCl, 0.005 M $CaCl_2$ dissolved in 980ml distilled water and sterilized. A 20 mL of egg yolk was added to the cooled medium (45-50°C) mixed and dispensed in plates). Plates were inoculated with test isolates and incubated at 37°C for 5 days. The presence of phospholipase activity was determined by the formation of a precipitation zone around the yeast colonies [10]. Reference strain *C. albicans* ATCC 60193 and *C. krusei* ATCC 6258 served as positive control.

Proteinase Activity

The protease production was tested in agar containing bovine serum albumin (Yeast carbon base 11.7g, yeast extract 0.1g, and bovine serum albumin 2g and 16g of agar-agar in 800mL of distilled water). Plates were inoculated with test isolates and incubated for 10 days at 37°C. Diameter of the lytic area surrounding the growth area on the medium was measured as enzymatic activity [12]. Reference strain *C. tropicalis* ATCC 66029 and *C. krusei* ATCC 6258 served as positive control.

Haemolysin Activity

Haemolysin activity was assessed by using blood agar containing 7ml of human fresh blood in 100ml of SDA containing 3% glucose. Plates were inoculated with test isolates and incubated at 37°C. A transparent/semitransparent zone around the inoculation site after 48hrs of incubation was considered as positive haemolytic activity [10]. Reference strain *C. krusei* ATCC 6258 served as positive control.

Coagulase Activity

Inoculate 0.1 mL of overnight SDA broth suspension to 0.5 mL of human plasma and incubated at 35°C-45°C. The clot formation was observed at 2 hours, 4 hours, 6 hours, and 24 hours by gently tilting and shaking the tubes. The *S. aureus* ATCC 25923 was used as a positive control [13].

Hyphae Production

An inoculating loop was loaded with the test organism and a well was formed at the top of a plate of cornmeal agar supplemented with 1% Tween 80. Dilution strokes were then made by cutting into the agar with the edge of the sterilized loop, holding it at an angle of 45 degrees to the surface of the agar. A sterile cover slip was placed such that it covered a part of the well some of the dilution strokes. The plates were incubated at 22°C for 48-72 hrs. Then the lid was removed and plates were placed on the microscopic stage, and the area under the coverslip as well as the edges of cover slip were observed for filamentous structures under high power magnification [6,8]. The reference strains, *C. albicans* ATCC 60193, *C. tropicalis* ATCC 66029, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in each set of tests as quality control.

Antifungal Susceptibility Testing

Antifungal susceptibility was done on Muller Hinton Agar supplemented with 2% glucose and 0.5 µ/mL methylene blue to fluconazole (25 µg) and voriconazole (1 µg) (Hi-Media pvt., ltd.,) using disc diffusion method. Zone diameter was interpreted as per the M44/A2 protocol of CLSI guidelines 2009 [14]. *C. parapsilosis* ATCC 22019 was included in each test as quality control isolates.

STATISTICAL ANALYSIS

The statistical analysis was done using statistical package 16.0. The p-value less than 0.05 was considered as statistically significant.

RESULTS

The various clinical specimens from which *Candida* species were isolated are shown in [Table/Fig-1]. Overall, NAC species accounted for a higher number (92,63.4%) as compared to *Candida albicans*

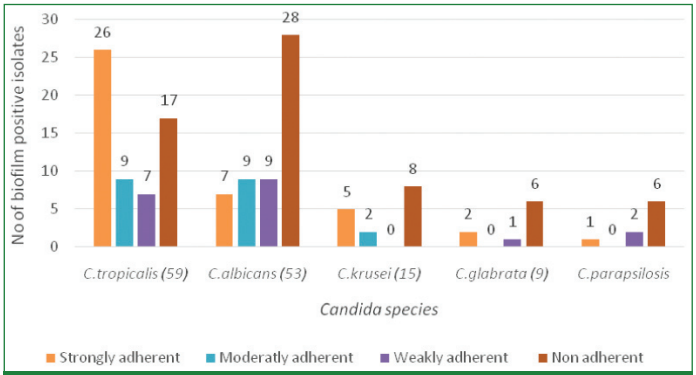
Sample	Number
Pulmonary isolates	38
Blood culture	31
Urine tract isolates	30
High Vaginal/ Swab	19
Pus	10
Deep tissue	5
Bile	2
Peritoneal fluid	2
Central line tip	2
Double J stent	2
Throat swab	2
Bone tissue	1
Oral swab	1
Total	145

[Table/Fig-1]: Specimen distribution of *Candida* isolates.

(53,36.5%). Among the individual species, *Candida tropicalis* accounted for the highest number 59 (40.7%) followed by *Candida albicans* 53 (36.5%) *C. krusei* 15 (10.3%), *C. glabrata* and *C. parapsilosis* 9 (6.2%). Presence of an intravenous catheter 124 (85.5%), treatment with broad spectrum antibiotics 121 (83.4%), admission to ICU 113 (77.9%), presence of urinary catheter 89 (61.4%), and diabetes mellitus 49 (33.8%) were the most common risk factors [Table/Fig-2].

Sl. no	Risk factors	Total number (n=145)	Percentage
1.	Intravenous catheter	124	85.5%
2.	Antibiotic usage	121	83.4%
3.	ICU admission	113	77.9%
4.	Urinary catheter	89	61.4%
5.	Diabetes mellitus	49	33.8%
6.	Steroid intake	43	29.7%
7.	Surgery	46	31.7%
8.	Endotracheal tube	34	23.4%
9.	Arterial catheter	37	25.5.1%
10.	Chronic Kidney Disease	23	15.9%
11.	Malignancy (solid organ and haematological)	23	15.9%
13.	Pregnancy	16	11.0%
14.	Lung disease	9	6.2%
15.	Total parenteral nutrition	7	4.8%
16.	Low birth weight	7	4.8%
17.	Neutropenia	2	1.4%
18.	HIV infection	1	0.7%

[Table/Fig-2]: Risk factors for *Candida* infection.



[Table/Fig-3]: Species distribution of biofilm positive isolates.

Ability to form biofilm in vitro was detected in 80 (55.2%) isolates. The highest propensity for biofilm formation was shown by *Candida tropicalis* 71.1% (42 out of 59). For the purpose of comparative analysis of test results, the adherence capabilities of the test strains were classified into the following under four categories: non-adherent- OD≤ ODc (0), weakly-ODc< OD≤ 2x ODc (+), moderately-2 x ODc<OD≤ 4 x ODc(++), or strongly adherent-4 x ODc< OD (+++), based upon the ODs of fungal films. Out of the 80 biofilm producing isolates, strong adherent biofilm production was detected in 41(28.3%) [Table/Fig-3].

Phospholipase, proteinase and haemolysin activity were observed in 95 (65.5%), 95 (65.5%) and 116 (80%) respectively. Coagulase activity was observed in 14 (9.7 %) of isolates. True hyphae formation was observed in a total of 39 (26.9%) isolates. The comparison of virulence factors exhibited different *Candida* species, *C. albicans* and NAC species and their statistical significance are given in [Table/Fig-4,5].

Out of 145 isolates, 20 (13.8%) were resistant to fluconazole and 5 (3.4%) showed dose-dependent susceptibility to fluconazole. The susceptibility pattern among the different *Candida* species was also compared and given in [Table/Fig-6].

DISCUSSION

The last few decades have witnessed a rise in the infections caused by *Candida* species, particularly those caused by the NAC species. This study focuses on the identification of various species of *Candida* obtained from clinical specimens of in-patients admitted to our hospital, risk factors contributing to infections, study of their virulence factors and antifungal susceptibility patterns.

Among all isolates obtained in this study, NAC species accounted for the highest number and among them *Candida tropicalis* was the predominant species isolated (40.7%), followed by *Candida albicans* (36.5%). These findings were correlated with the studies done at different parts of India such as Kaur R et al., in which *C. tropicalis* (40.8%) and *C. albicans* (38.8 %) were the most common species isolated [5]. Bhatt M et al., had reported 39% *C. tropicalis*, 20% *C. parapsilosis*, 14.7% each of *C. krusei* and *C. albicans* and 5.4% *C. glabrata* [15]. *C. albicans* was found to be most common species isolated in studies conducted by Mnge P et al., in South Africa and Kiraz N and Oz Y in Turkey, but shift towards NAC species was reported [16,17]. The exact cause for the increase in NAC species is not known, however wide spread and inappropriate use of antifungal treatment could be contributing factor [15].

Candida albicans is a normal flora in the various anatomical sites in a healthy individual. But condition worses when host immunity

Virulence factors		Species					Total (145)	p-value
		C.albicans (53)	*C.tropicalis* (59)	*C.krusei* (15)	*C.glabrata* (9)	*C.parapsilosis* (9)		
Biofilm formation	Positive	25 (47.1%)	42 (71.1%)	7 (46.6%)	3 (33.3%)	3 (33.3%)	80	0.02
	Negative	28 (52.8%)	17 (28.8%)	8 (53.3%)	6 (66.6%)	6 (66.65)	65	
Phospholipase activity	Positive	42 (79.2%)	41 (69.4%)	6 (40%)	4 (44%)	2 (22.2%)	95	0.001
	Negative	11 (20.7%)	18 (30.5%)	9 (60%)	5 (55.5%)	7(77.8%)	50	
Proteinase activity	Positive	26 (49%)	50 (84.7%)	8 (53%)	5 (55%)	6 (66.6%)	95	0.001
	Negative	27 (50.9%)	9 (15.2%)	7 (46.6%)	4 (44.4%)	3 (33.3%	50	
Haemolytic activity	Present	44 (83%)	51 (86.4%)	11(73.3%)	6 (66.7%)	4 (44.4%)	116	0.067
	Absent	9 (16.9%)	8 (13.5%)	4 (26.7%)	3 (33.3%)	5 (55.6%)	29	
Coagulase activity	Positive	7 (13.2%)	6 (10.2%)	1 (6.7%)	0	0	14	0.32
	Negative	46 (86.7%)	53(89.8%)	14 (93.3%)	9 (100%)	9 (100%)	131	
True hyphae	Present	14 (26.4%)	25 (42.3%)	0	0	0	39	0.04
	Absent	39 (75.6%)	34 (57.6)	15 (100%)	9 (100%)	9 (100%)	106	

[Table/Fig-4]: Comparison of virulence factors producers in different *Candida* species.
Fisher's-exact and chi-square test is used to calculate the p-value in this table

Virulence factors		Species		Total (145)	p-value
		C.albicans (53)	*NAC spp*, (92)		
Biofilm formation	Positive	25 (47.1%)	55 (59.7%)	80	0.14
	Negative	28 (52.8%)	37 (40.2%)	65	
Phospholipase activity	Positive	42 (79.2%)	53 (57.6%)	95	0.008
	Negative	11 (18.6%)	39 (42.3%)	50	
Proteinase activity	Positive	26 (49.1%)	69 (75%)	95	0.002
	Negative	27 (50.9%)	23 (25%)	50	
Haemolytic activity	Present	44 (83%)	72 (78.2%)	116	0.49
	Absent	9 (16.9%)	20 (21.7%)	29	
Coagulase activity	Positive	7% (13.2%)	7 (7.6%)	14	0.272
	Negative	46 (86.6%)	85 (92.3%)	131	
True hyphae	Present	14 (26.4%)	25 (27.2%)	39	0.04
	Absent	39 (75.6%)	67 (72.8%)	106	

[Table/Fig-5]: Comparison of virulence factors producers in *Candida albicans* and non *albicans* species.
Chi square test is used to calculate the p-value in this table

Species	Fluconazole (%)			Voriconazole (%)		
	Suscep-tible	S-DD	Resis-tant	Suscep-tible	S-DD	Resis-tant
Candida tropicalis	56 (94.9%)	1 (1.7%)	2 (3.4%)	58 (98.3%)	0	1 (1.7%)
Candida albicans	47 (88.7%)	1 (1.9%)	5 (9.4%)	49 (92.5%)	3 (5.7%)	1 (1.9%)
Candida krusei	0	3 (20%)	12 (80%)	10 (66.7%)	4 (26.7%)	1 (6.7%)
Candida parapsilosis	9 (100%)	0	0	9 (100%)	0	0
Candida glabrata	8 (88.9%)	0	1 (11.1%)	9 (100%)	0	0
Total	120 (82.8%)	5 (3.4%)	20 (13.8%)	135 (93.1%)	7 (4.8%)	3 (2%)
p-value	<0.001			0.02		

[Table/Fig-6]: Susceptibility/resistance among different *Candida* species to fluconazole and voriconazole.
Fisher's-exact is used to calculate the p-value in this table

weakens, it behaves as an opportunistic pathogen. *Candida* infection can also occur in patients who had a prolonged hospital stay. The mechanism of transmission of infection may be through the hands of health care workers, or health care materials such as catheters [1]. In a previous study done in our institution by Chowta MN et al., in 2007, 64% of patients with *Candida* infection had an intravenous catheter and 34.5% had prolonged exposure to antibiotics [18]. In Bhatt M et al., work 100% of the patients were on broad spectrum antibiotics, 89% had indwelling venous catheters and 65% were mechanically ventilated [15]. Thus, risk factors observed in our study is in accordance with another study. It is necessary to observe these risk factors extensively, and to start prophylaxis antifungal treatment according to *Candida* score in high-risk patients [19].

Candida species infect the medical implants, by adhering to the surfaces and forming a colony. These microbial colonies are enclosed in a self-produced polymeric matrix called biofilm and represent a common mode of microbial growth. In the present study 55.2% of isolates were biofilm positive. Bhatt M et al., reported 64.7% and Bansal R et al., reported 66% of biofilm positive isolates from the blood culture [15,20] and in the present study, it accounts for 54.8%. This shows blood culture isolates are more prone to develop biofilm and thus develops antifungal resistance which may lead to increase in mortality. In the current analysis *Candida tropicalis* was the highest biofilm producers (71.1%) when compared to other species. (p=0.02). The predominance of *C. tropicalis* to produce biofilm was reported by Bhatt M et al., [15].

Phospholipases enables the invasion of the host cell by cleaving the phospholipid bonds [11]. Thus, phospholipase enzyme can cause cell lysis and allow the hyphae to penetrate the cytoplasm [21]. In our study, 65.5% were positive for phospholipase production. Highest phospholipase activity in this study was detected in *C. albicans* (p-value 0.001) [Table/Fig-2] which is in harmony with a study done by Das VM et al., [22]. During our study the phospholipase activity of *C. tropicalis* was 69.4%. There is a wide variation in published reports of phospholipase production among *C. tropicalis*. Inci M et al., from Turkey reported that NAC species do not exhibit phospholipase activity, in either aerobic or anaerobic condition [9]. However, Sachin CD et al., reported that phospholipase was the major virulence factor in *C. tropicalis*, which is in agreement with our study [21]. Proteinases are capable of invading hosts epithelial and mucosal barrier proteins by hydrolyzing the peptide bond. Proteinase enzymes can disturb the significant proteins responsible host immune mechanism such as antibodies, complement, and cytokines [12]. The proteinase activity of blood culture isolates in our study was 71%. These results are in accordance with Das VM et al., study which shows 74.56% of proteinase activity in blood culture isolates [22].

Haemolysin production is also a virulence factor contributing to *Candida* pathogenesis. Secretion of haemolysin helps the organism to acquire iron and produce hyphae which facilitate invasion in disseminated candidiasis [23]. In the recent study 80% of isolates were haemolysin positive which is in accordance with Inci M et al., (91.1%) [10] and Sachin CD et al., (51.8%) [21]. Sachin CD et al., had reported maximum haemolytic activity from *C. albicans* in his work, and in the present study maximum haemolytic activity was observed in *C. tropicalis* (86.4%) and *C. albicans* (83%) [21].

The ability of the *Candida* isolates to produce coagulase by using human plasma was analysed by Padmajakshi. G et al., in 2014 and they reported 4.3% of isolates producing coagulase activity in which three were *C. albicans* and one being *C. tropicalis* [13]. None of the isolates produced coagulase by human plasma in another study done by Yigit N et al., [24]. In our study, 14 isolates had produced coagulase using human plasma. Thompson D S et al., studied the virulence factors of *Candida* species and reported *C. albicans*, *C. tropicalis* and *C. dubliniensis* were major species of *Candida* that can produce true hyphae, which is in accordance with our study [25]. Hyphae is capable of anchoring the cell layer and penetrate into the endothelial cells by which it can cause invasive infection.

The *Candida* isolates showed 13.8% of resistance to fluconazole and 2.1% to voriconazole. But the percentage of resistance reported from Korea by Won EJ et al., was 2.6% to fluconazole and all the isolates were susceptible to voriconazole [26], Whereas the percentage resistance to fluconazole and voriconazole was more in two Indian studies done by Battacharjee P and Gupta S et al., [27,28]. In our study, 80% of the isolates of *C. krusei* were resistance to fluconazole (20% intermediate) which is consistent with other studies [18,20,22,28]. This shows antifungal resistance is becoming more prevalent. So, it is important to perform routine antifungal susceptibility testing for better therapeutic outcome.

LIMITATION

Since this an academic study: duration was only 18 months and confined to an institution so our sample size was only 145, this is a major limitation of this study. Accuracy of the study can be increased by incorporating more institution and more duration. Financial support from our institution was not sufficient to perform sugar assimilation test for speciation.

CONCLUSION

A total of 145 consecutive isolates of *Candida* species were obtained from various clinical specimens. NAC species accounted for a higher number as compared to *Candida albicans*. Among the individual

species, *Candida tropicalis* accounted for the highest number. Considering all virulence factors tested among all the isolates in this study, isolates of *Candida tropicalis* showed higher tendency to produce biofilm, proteinase, haemolysin, coagulase and true hyphae, compared to *C albicans* which showed higher tendency to produce phospholipase. Fluconazole resistance was detected in 13.8% of the isolates and 3.4% showed dose-dependent susceptibility. Three of the isolates were resistant to voriconazole and 7 isolates showed dose-dependent susceptibility. Presence of an intravenous catheter, treatment with broad spectrum antibiotics and admission to ICU were the most common risk factors.

ACKNOWLEDGEMENTS

We are grateful to Manipal University for the financial support in conducting this study.

REFERENCES

[1] Giri S, Kindo AJ. A review of *Candida* species causing blood stream infection. Indian J Med Microbiol. 2012;30(3):270-78.
[2] Cheng M-F, Yang Y-L, Yao T-J, Lin CY, Liu JS, Tang RB, et al. Risk factors for fatal candidemia caused by *Candida albicans* and non-albicans *Candida* species. BMC Infect Dis. 2005;5(22):1-5.
[3] Deorukhkar SC, Saini S, Mathew S. Non-albicans *candida* infection: An emerging threat. Interdisciplinary Perspectives on Infectious Diseases. 2014:1-7.
[4] Edwards JE, JR. *Candida* Species. In: Mandell, Douglas and Bennett's. Principle and Practice of Infectious Diseases, 7th edition. United States: Elsevier, 2010: 3225-3240.
[5] Kaur R, Goyal R, Dhakad MS, Bhalla P, Kumar R. Epidemiology and virulence determinants including biofilm profile of *candida* infections in an ICU in a tertiary hospital in India. Journal of Mycology. 2014;2014:303491. 8 pages.
[6] Segal E, Elad D. Mycoses caused by Opportunistic Fungi. In:WilliamMerz and Roderick J Hay eds. Topley and Wilson's Microbiology and Microbial Infection Medical Mycology,10th edition; London; Hodder Arnold publishers; 2005:579-623.
[7] Milne LJ R. Fungi. In: Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie &MaCartney Practical medical microbiology, 14 edition. Elsevier, 1996: 695-717.
[8] Vijaya D, Harsha TR, Nagaratnamma T. *Candida* speciation using chromagar. Journal of Clinical and Diagnostic Research. 2011;5(4):755-57.
[9] Inci M, Atalay MA, Koç AN, Yula E, Evirgen O, Durmaz S, et al. Investigating virulence factors of clinical *Candida* isolates in relation to atmospheric conditions and genotype. Turk J Med Sci. 2012;42(Sup.2):1476-83.
[10] Jasim ST, Flayyih MT, Hassan AAK. Biofilm formation and susceptibility to itraconazole in *Candida albicans*. World Journal of Pharmacy and Pharmaceutical Sciences. 2016;5(8):1704-16.
[11] Dheepa M, Rashme VL, Appalaraju B. Comparison of biofilm production and multiple drug resistance in clinical isolates of *Acinetobacter baumanii* from a tertiary carehospital in South India. Int J Pharm Biomed Sci. 2011;2(4):103-07.
[12] Mattei AS, Alves SH, Severo CB, Guazzelli LDS, Oliveira FdM, Severo LC. Determination of germ tube, phospholipase, and proteinase production by bloodstream isolates of *Candida albicans.* Revista da Sociedade Brasileira de Medicina Tropical. 2013;46(3):340-42.
[13] Padmajakshi. G, Saini S, Deorukhkar S, Ramana KV. Coagulase activity of *candida* Spp isolated from HIV seropositive patients using different animal plasma. American Journal of Microbiological Research. 2014;2(2):57-59.
[14] CLSI. Method for antifungal Disc diffusion susceptibility testing of yeast: Approved guidelines. Second edition CLSI document M44-A2 Wayne, PA. Clinical laboratory standard institute, 2009.
[15] Bhatt M, Sarangi G, Paty BP, Mohapatra D, Chayani N, Mahapatra A, et al. Biofilm as a virulence marker in *Candida* species in Nosocomial blood stream infection and its correlation with antifungal resistance. Indian Journal of Medical Microbiol. 2015;33 Suppl:112-14.
[16] Mnge P, Okeleye BI, Vasaikar SD, Apalata T. Species distribution and antifungal susceptibility patterns of *Candida* isolates from a public tertiary teaching hospital in the Eastern Cape Province, South Africa. Brazilian Journal of Medical and Biological Research. 2017;50(6):1-7.
[17] Kiraz N, Oz Y. Species distribution and in vitro antifungal susceptibility of clinical *Candida* isolates from a university hospital in Turkey over a 5-year period. Med Mycol. 2011;49(2):126-31.
[18] Chowta MN, Adhikari P, Rajeev A, Shenoy A Study of risk factors and prevention of invasive Candidiasis in tertiary care hospital. Indian Journal of Critical Care Medicine. 2007;11(2):67-73.
[19] Gaspar GG, Menegueti MG, Auxiliadora-Martins M, Basile-Filho A, Martinez R. Evaluation of the predictive indices for candidemia in an adult intensive care unit. Rev Soc Bras Med Trop. 2015;48(1):77-82.
[20] Bansal R, Oberoi L, Singh K, Devi P. Species distribution, biofilm formation and antifungal susceptibility of *candida* isolates in blood samples of NICU patients at tertiary care centre, Amritsar, India. Int J Curr Microbiol App Sci. 2016;5(12):628-34.
[21] Sachin CD, Ruchi K, Santosh S. In vitro evaluation of proteinase, phospholipase and haemolysin activities of *Candida* species isolated from clinical specimens. Int J Med Biomed Res. 2012;1(2):153-57.
[22] Das VM, Ballal M. Proteinase and phospholipase activity as virulence factors in *Candida* species isolated from blood. Rev Iberoam Micol. 2008;25:208-10.
[23] Linares CE, de Loreto ES, Silveira CP, Pozzatti P, Scheid LA, Santurio JM, et al. Enzymatic and hemolytic activities of *Candida dubliniensis* strains. Rev Inst Med Trop Sao Paulo. 2007;49(4):203-06.
[24] Yigit N, Aktas AE, Ayyildiz A. Detection of coagulase activity in pathogenic *Candida* species. J Int Med Res. 2008;36(6):1378-82.
[25] Thompson DS, Carlisle PL, Kadosh D. Coevolution of morphology and virulence in *candida* species. Eukaryotic Cell. 2011;10(9):1173-82.
[26] Won EJ, Shin JH, Choi MJ, Lee WG, Park Y J, Uh Y, et al. Antifungal susceptibilities of bloodstream isolates of *Candida* species from nine hospitals in Korea: application of new antifungal breakpoints and relationship to antifungal usage. PLoS One. 2015;10(2):1-9.
[27] Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Current Medical Mycology. 2016;2(2):20-27.
[28] Gupta S, Goyal RK. Species distribution and antifungal drug susceptibility of *candida* in clinical isolates from a tertiary care centre at Bareilly. IOSR-JDMS. 2017;1(2):57-61.

PARTICULARS OF CONTRIBUTORS:
1. Junior Consultant, Department of Microbiology, Baby Memorial Hospital, Calicut, Kerala, India.
2. Professor and Head, Department of Microbiology, Kasturba Medical College, Mangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. MV Poornima,
Sisiram, Kolaithazham, Karanthur, Kozhikode-673571a, Kerala, India.
E-mail: poornimamv@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: As declared above.

Date of Submission: **Nov 12, 2018**
Date of Peer Review: **Dec 13, 2018**
Date of Acceptance: **May 30, 2019**
Date of Publishing: **Jul 01, 2019**

ANNEXURE 1.

PROFOMA

Name: ……. Hospital no: ……. Age: …….. sex: ……

Lab no Address: ……. Ward/unit no: ……

Religion: ……. Marital status: …….. Date of admission: ……

CLINICAL HISTORY

Presenting complaints: ……

Risk factors:

- History and duration of Diabetes: ……
- Steroid therapy/ immunosuppressive therapy: ……
- Antibiotic (broad spectrum) therapy, and its duration: ……
- HIV status: Positive Negative

- Malignancy (haematological/ any other)……..
- Chronic kidney disease:
- Chronic lung disease
- Organ failure
- Organ transplant recipient
- Use of invasive devices:

Indwelling Catheters: urinary/intravenous/arterial/shunts;

Endotracheal tube/ mechanical ventilation

- Indication of any invasive procedures, surgery or fracture.
- Total Parenteral Nutrition
- Admission to ICU……..
- Neutropenia and its duration…….
- Neonates: low birth weight/ prematurity; developmental history
- Primary reason for hospitalization……

Clinical Diagnosis

Microbiology Report:

Nature of specimen: …….. Gram stain: …….

Wet mount/KOH mount: Culture: …….

Virulence factors: ……

Antifungal susceptibility test result: