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Original Article

Microbiology Section

Antifungal Activity of Cinnamon Oil and Olive Oil against *Candida* Spp. Isolated from Blood Stream Infections

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ABSTRACT

Introduction: Recently non-*albicans Candida* has emerged as a major cause of morbidity and mortality in blood stream infections. Some species of the *Candida* are becoming increasingly resistant to first line and second line antifungals such as echinocandins and fluconazole. In view of increasing global antifungal resistance, role of alternative and better antifungals like natural plant products need to be explored. Essential oils are known to exhibit antimicrobial activity against various fungi. Hence, we evaluated the efficacy of cinnamon oil and olive oil against *Candida* spp.

Aim: To evaluate the invitro antifungal activity of olive oil and cinnamon oil against blood stream *Candida* isolates.

Materials and Methods: The present prospective observational study was conducted in the Department of Microbiology at a tertiary care teaching hospital during one year June 2011-July 2012. Blood samples were collected from 1376 patients clinically suspected to have fungal septicaemia, out of which 100 (7.2%) *Candida* isolates obtained, were speciated by conventional methods. Antifungal susceptibility testing of all the isolates was done against fluconazole, voriconazole as per NCCL (M27-A2) and against olive oil and cinnamon oil by agar well diffusion method.

Results: Prevalence of Candidemia was 7.26%. *C. albicans* (85.3%) and *C. parapsilosis* (85.7%) were most sensitive to fluconazole followed by *C. tropicalis* (67.4%). All isolates were 100% sensitive to voriconazole. Both oils were found to be effective against nearly 50% of the *Candida* isolates. About 55.5% of fluconazole resistant *C. krusei* strains were sensitive to olive and cinnamon oil.

Conclusion: Fluconazole resistant non-*albicans Candida* has emerged as major cause of *Candide*mia. Cinnamon and olive oil show marked sensitivity against *albicans* and non-*albicans* spp.

Keywords: Antifungal susceptibility, Candidemia, Essential oil, Fluconazole

INTRODUCTION

The dramatic rise in invasive fungal infections especially *Candide*mia has gained worldwide significance since last few decades. Compared to other microbial pathogens causing bloodstream infections, *albicans* and non- *albicans Candida* spp. are ranked fourth among the most common agents of bloodstream infections, and are associated with high mortality (30-40%) and morbidity [1]. There are mainly four major classes of antifungal drugs available to treat invasive fungal infections. They include polyenes, pyrimidine analogs, echinocandins, and triazoles. In medical practice, triazoles such as fluconazole and voriconazoles are still the most used antifungals. But, the use of antifungal drug therapy has led to the development of antifungal resistance to these common agents [1,2]. Hence, in view of increasing threats posed by drug resistant yeasts, we need to vigorously search for alternative and better antifungals, which are not only effective but also have lesser side effects. Traditional medicines have been known to play a major role in various health services. Various non antibiotic substances such as essential oils like olive oil and cinnamon oil have been reported to be effective against various yeasts and molds [3]. Essential oils are aromatic oily liquids which are obtained from various plant parts and are known to show wide spectrum of antimicrobial activity [3,4]. The use of these oils instead of antifungal drugs will be more preferable thus studies are needed to test the efficacy of these oils and hence with this purpose the present study was conducted.

MATERIALS AND METHODS

The present prospective observational study was conducted in the Department of Microbiology in association with Department of Pharmacy during one year period from June 2011-July 2012. A total of 1376 patients of all age groups and sex visiting the OPD and admitted to the hospital with clinical suspicion of fungal septicaemia were included in the study. Patients who were already on antifungals were excluded from the study. Demographic and clinical data such as age, sex, birth weight, history of pre-term birth, antibiotic prophylaxis history, presence of any co-morbid condition and presence of any intravenous lines were noted for all these patients. Consecutive blood samples in duplicate were collected from each patient under aseptic precautions in two sets of biphasic Brain Heart Infusion (BHI) medium. One BHI set was incubated at 25°C and other at 37°C and subcultures were performed after 24 hours, 48 hours and 72 hours up to 7 days. The colonies were identified by standard microbiological techniques. A total of 100 *Candida* isolates were obtained and identified up-to the species level on the basis of germ tube test, morphology on cornmeal agar, growth on Hi-Chrome *Candida* agar, carbohydrate fermentation and sugar assimilation test [5,6].

Susceptibility testing

The isolated *Candida* isolates were further subjected to antifungal susceptibility testing against fluconazole (Hi-media), voriconazole (Pfizer), cinnamon oil and olive oil. Susceptibility testing for fluconazole and voriconazole was performed by the broth microdilution minimum inhibitory concentration (BMD-MIC) method using RPMI (Roswell Park Memorial Institute) medium and MOPS (3-(N-morpholino) propane sulphonic acid) buffer. MIC results were interpreted as per NCCL (M-27-A2) guidelines. The quality control test was performed using ATCC strain, *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *Candida* tropicalisATCC750 and *Candida krusei* ATCC 6258 [7]. All the 100 isolates were subjected to susceptibility testing against cinnamon oil and olive oil by agar well diffusion method. *Candida* strain to be tested was inoculated on sterilized Sabouraud's Dextrose Agar plate. A hole was punched aseptically by sterile cork borer of 6-mm diameter on

the agar surface. A 50 µl of cinnamon and olive oil was introduced into each of the peripheral wells with sterilized DMSO as negative control. The plates were incubated at 28ºC. Antimicrobial activity of essential oils was analysed by observing the zone of inhibition. The results were interpreted as <9 mm- inactive; 9-12 mm- partially active; 13-18 mm- active; >18 mm- very active [8,9]. Statistical analysis was performed by chi-square test using SPSS version-16.0 software.

RESULTS

During the study period, a total of 100 (7.26%) isolates of *Candida* spp. were isolated from 1376 patients. Rate of *Candida* isolation was higher in paediatric (1-15years) age group (89%) as compared to adults (> 15 years) (11%).

Majority of *Candida* strains were isolated from neonates (36%) followed by infants (28%), 1-15 years (25%) and adults (11%) [Table/Fig-1]. The male to female ratio was 3:1. The rate of *Candida* isolation was maximum from PICU (Paediatric Intensive Care Unit) patients (72%) followed by wards (21%) and OPD's (7%) [Table/Fig-2].

Out of the 100 *Candida* isolates, *C. tropicalis* (43%) was the most common isolate followed by *C. albicans* (41%), *C. krusei* (9%) and *C. parapsilosis* (7%). *C. albicans* was more frequently isolated from the infants (0-1month) whereas *C. tropicalis* was more common in the neonates (1-12 months) [Table/Fig-1]. All species were predominant in males than females. The most common risk factor among paediatric age group was I/V line (92%) followed by premature birth (73.03%), low birth weight (65.17%) and use of prior antibiotics (60.67%) [Table/Fig-3,4]. In adults, the most common risk factor was central venous line (90.9%), followed by ICU admission (63.64%), prior use of antibiotics (45.4%), patients on ventilator (36.4%), and immune-compromised status (18.18%). Antifungal susceptibility results revealed that *C. albicans* (85.3%) and *C. parapsilosis* (85.7%) were found to be most sensitive to fluconazole followed by *C. tropicalis* (67.4%). All *Candida* isolates were 100%sensitive to voriconazole. For olive oil, sensitivity of *C. albicans* was 53.65%, *C. tropicalis* was 48.83%, *C. krusei*was 55.55% and *C. parapsilosis* was 57.12%. Sensitivity to Cinnamon oil was 58.83% for *C. albicans*, 46.51% for *C. tropicalis*, 44.45% for *C. krusei* and 57.12% for *C. parapsilosis*. The resistance to fluconazole as well as olive oil and cinnamon oil was significantly higher (p-value > 0.05) in non *albicans Candida* spp. as compared to *C. albicans* [Table/Fig-5-7].

Age	Total (n=100)	*C.albicans* (n=41)	*C.tropicalis* (n=43)	*C.krusei* (n=9)	*C. parapsilosis* (n=7)
< 1 month	36	19 (52.78%)	10 (27.78%)	4 (11.11%)	3 (8.33%)
1-12 months	28	8 (28.6%)	16 (57.14%)	2 (7.14%)	2 (7.14%)
1-15 years	25	11 (44%)	12 (48%)	1 (4%)	1 (4%)
Adults	11	3 (27.27%)	5 (45.45%)	2 (18.18%)	1 (9.05%)

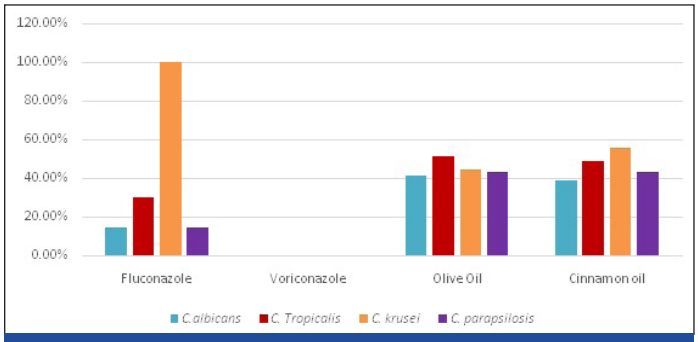
[Table/Fig-1]: Age wise distribution of various *candida* species.

Clinical Settings	Number of *Candida* isolates n=100
PICU	72
Ward	21
OPD	07
Total	100

[Table/Fig-2]: Distribution of isolates according to clinical settings.

Predisposing factors	*C. albicans* (n= 38)	*C. tropicalis* (n= 38)	*C. krusei* (n= 7)	*C. parapsilosis* (n= 6)	Total (89)
Intravenous line (82)	36 (43.9%)	35 (42.7%)	6(7.3%)	5(6.1%)	82 (92.13%)
Prior antibiotics (54)	25 (46.3%)	22(40.7%)	4(7.4%)	3(5.56%)	54 (60.67%)
Prematurity (65)	27 (41.5%)	30(46.15%)	5(7.7%)	3(4.6%)	65 (73.03%)
Low birth weight (58)	26 (44.8%)	25(43.1%)	5(8.6%)	2(3.4%)	58 (65.17%)

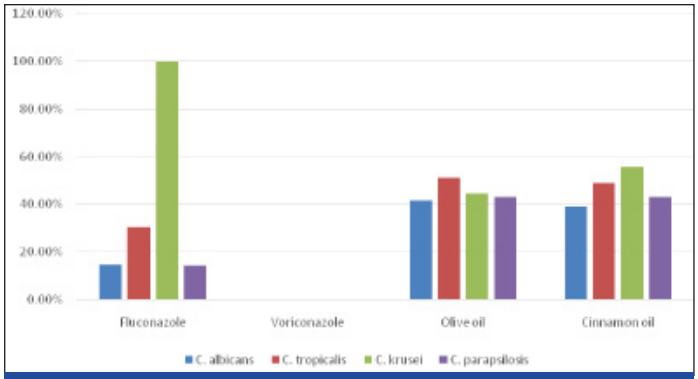
[Table/Fig-3]: Major predisposing factors among paediatric patients with *Candide*mia.



[Table/Fig-4]: Predisposing risk factors among pediatric patients.

Drugs	*C. albicans* (n=41)	*C. tropicalis* (n=43)	*C. krusei* (n=9)	*C. parapsilosis* (n=7)
Fluconazole	6 (14.6%)	13 (30.3%)	9 (100%)	1 (14.3%)
Voriconazole	0	0	0	0
Olive oil	17 (41.46%)	22 (51.16%)	4 (44.44%)	3 (42.85%)
Cinnamon oil	16 (39.02%)	21(48.83%)	5 (55.55%)	3(42.85%)

[Table/Fig-5]: Antifungal resistance pattern against fluconazole, voriconazole, olive oil and cinnamon oil among various *Candida* species.



[Table/Fig-6]: Resistance pattern of various condida isolates against antifungal drugs and oils.

Antifungal agents	*C. albicans*	Non *albicans Candida*	p-value
Fluconazole	6 (14.63%)	23 (38.93%)	0.238
Voriconazole	0	0	
Olive oil	17 (41.46%)	29 (49.15%)	0.083
Cinnamon oil	16 (39.03%)	29 (49.15%)	0.593

[Table/Fig-7]: Comparison of antifungal resistance pattern of *Candida albicans* and non-*albicans Candida*.

DISCUSSION

*Candide*mia has emerged as an alarming problem in healthcare settings since last few decades causing increased morbidity and mortality [10]. In our study, the rate of isolation of *Candida* spp. from blood stream infections was 7.25%. Our study co-relates with previous studies conducted in India and Thailand, which reported rate of *Candide*mia as 6.9% and 6.14% respectively [11,12]. In contrast to our study, few studies from Pennsylvania and Saudi Arabia showed isolation rates as low as 3.5% and 2.8% respectively [13,14]. The difference in isolation rates in these studies could be due to the difference in antibiotic policies in different hospitals, as injudicious use of antibiotics leads to suppression of normal flora making the patients prone to develop *Candide*mia. The various risk factors for acquisition of *Candide*mia can be ICU settings, extremes of age and previous antibiotic therapy [15]. Our study showed a higher rate of *Candide*mia (72%) in ICU settings as well as in neonates (36%). Various studies across the globe have also observed a higher incidence of *Candide*mia in ICUs as compared

to non ICU locations [1,15]. Ours is a tertiary care hospital and higher rate of *Candide*mia in our ICU can be explained by the fact that most patients were already on antibiotics, moreover about 48% of our study population consisted of neonates who generally have a suppressed immune system. Higher rates of *Candide*mia in neonates have been documented by various studies done earlier also [16,17]. In our study, the most common species was *C. tropicalis* followed by *C. albicans*, *C. krusei* and *C. parapsilosis* co-inciding with the observations from various other studies, which also depict a shifting trend from *albicans* to non-*albicans Candida* spp [1,18,19]. The rate of fluconazole resistance in this study is higher than the previous study done at our center as well as at some other parts of North India [20,21]. There is growing evidence that increasing use of azole agents may be associated with increasing fluconazole resistance as well as emergence of non-*albicans Candida* spp [22]. The high rate of resistance in the present study may be due to the irrational and liberal use of fluconazole or acquisition of resistance by previously susceptible strains of *C. albicans* following long term azole exposure. Some non- *albicans Candida* spp like *C. krusei* and *C. glabrata* exhibit resistance to traditional triazole antifungals like fluconazole. Voriconazole can be used effectively for the patients with infections due to *C. kruesi* and fluconazole resistant voriconazole susceptible *C. glabrata,* however, these spp may also demonstrate cross resistance to voriconazole resulting in treatment failure [23,24]. Such reports have encouraged looking for the alternative new approaches with low potential for development of resistance. We studied the efficacy of cinnamon oil and olive oil against *Candida* spp including *C. krusei* which is intrinsically resistant to fluconazole. Besides having antimicrobial properties, these compounds have other beneficial health effects including antioxidant and anti-inflammatory properties and most importantly free from any systemic side effects [25]. In our study although 50% of the isolates were resistant to both the oils but encouraging finding was that about 55.5% of *C. krusei* strains were sensitive to olive oil and cinnamon oil. Similar study, has also demonstrated that cinnamon oil and olive oil have marked antimicrobial activity against the fluconazole resistant *C. albicans* strain (ATCC-10231) [26]. In the present study all the *C. krusei* strains from *Candide*mia patients were sensitive to voriconazole but cross resistance may lead to voriconazole resistance resulting in treatment failure [23,24]. Thus these plant oils can find use as anti-*Candida* agents in future against azole resistant strains.

LIMITATION

One of the potential limitations of the study is that we did not perform in vivo study. In vitro and in vivo results may not correspond with each other.

CONCLUSION

We conclude that fluconazole resistant non-*albicans Candida* has emerged as a major cause of *Candide*mia especially in neonates and ICU patients. Voriconazole still continues to be a promising drug at our center. Cinnamon oil and olive oil showed marked sensitivity against the fluconazole resistant *C. krusei*.

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