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Extracellular hydrolytic enzyme activities of the different *Candida* spp. isolated from the blood of the Intensive Care Unit-admitted patients

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Abstract:

BACKGROUND: *Candida* spp. secretes various extracellular hydrolytic enzymes. These enzymes are the important virulence factor for the pathogenesis of *Candida*. We assessed four different enzymatic activities of *Candida* isolates obtained from bloodstream infections.

MATERIALS AND METHODS: We isolated 79 strains of different *Candida* species from the blood of the Intensive Care Unit-admitted patients. Species were identified by conventional methods including culture characteristic, germ tube, sugar assimilation, and Dalmou's culture technique. Phospholipase, proteinase, hemolysin, and esterase enzymatic activities were determined by the Plate method.

RESULTS: Non albicans candida were the most common isolates from the blood of the ICU admitted patient with a predominance of *Candida tropicalis*. Hemolytic activity was the most prominent enzyme activity followed by the proteinase activity. *Candida albicans* (89.86%) was the major proteinase producer, while 95.8% of *C. tropicalis* produced hemolysin. No esterase activity was shown by the *Candida glabrata* and *Candida krusei*.

CONCLUSION: No significant difference was observed between the two most common causative agents of candidemia: *C. albicans* and *C. tropicalis*.

Key words:

Candidemia, esterase, hemolysin, phospholipase, proteinase, virulence factor

Introduction

Candida infections are the leading cause of the morbidity and mortality in the hospitalized patients.^[1,2] *Candida* infections may disseminate hematogenously to the various organs. These disseminated *Candida* infections usually occur in patients with a history of prolonged antibiotic exposure, immunosuppressive treatment, and patients on parenteral nutrition.^[1,3] Different species of *Candida* are attributed as a cause of these disseminated infections, but four species, namely *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*, account for as much as 95% of these

infections.^[1] Previously, *C. albicans* was the most common cause of bloodstream fungal infections, but nowadays, non-*albicans* *Candida* has superseded the former.^[4,5]

Candida spp. has grown from successful commensalism to pathogens in various body sites. *Candida* spp. secrete different extracellular enzymes as phospholipase, protease, and esterase. These hydrolytic enzymes contribute the microbial pathogenicity by accentuating its adhesion, tissue damage, immune system evasion, as well as its dissemination. Hemolysin is another virulence factor that degrades the red blood cells, and iron is released which is taken up by the yeast cells.^[6,7] In this study,

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we have determined the extracellular enzyme activity of the *Candida* species, isolated from the blood of the Intensive Care Unit (ICU)-admitted patients.

Materials and Methods

Candida spp. was isolated from the blood of the ICU-admitted patients at a tertiary care center at Sir Sunderlal Hospital, Banaras Hindu University, Varanasi. *Candida* was identified based on microscopic examination, culture characteristics, germ tube formation, sugar fermentation, sugar assimilation, morphology on cornmeal agar, and color on CHROMagar.

The isolated *Candida* spp. was screened for the extracellular enzymatic activities, that is, phospholipase, proteinase, hemolysin, and esterase. Phospholipase activity was determined by the method described earlier by Price *et al.*,^[8] with little modification done by Costa *et al.*^[9] In this, Sabouraud's dextrose agar (SDA) medium was supplemented with 1 mol/L sodium chloride, 0.005 mol/L calcium chloride, and 8% sterile egg yolk suspension. Proteinase activity was determined using the bovine serum albumin agar.^[10] Tween 80 opacity medium was used to determine the esterase activity.^[10] For hemolysin activity, SDA plates supplemented with 3% glucose and 7% sheep blood were used.^[11] Ten microliters of a thick suspension of each strain were placed in the center of the plates which were incubated at 37°C for 5 days. The test was performed in triplicates. Average value of the colony diameter (a) and the diameter of the colony plus the precipitation zone (b) were measured. The enzyme activity was expressed as Pz value (a/b) as described by Price *et al.* All enzymatic activity index was classified as follows: Pz = 1, negative activity; 0.64 < Pz < 0.99, positive; and Pz ≤ 0.64, strongly positive.^[6]

Results

A total of 79 strains of *Candida* spp. were isolated from the blood of the patients, admitted in the ICU. Most of the patients were male with an M:F of 1.82:1. Majority of the *Candida* spp were isolated from the blood of neonates (n = 41), followed by patients below 18 years of age group (n = 16) [Table 1]. Out of these 79 isolates, in comparison to *C. albicans*, non-*albicans candida* (n = 63) were predominant. These non-*albicans* (n = 63) were categorized as follows: *C. tropicalis* (n = 24), *C. krusei* (n = 11), *C. parapsilosis* (n = 9), *Candida glabrata* (n = 5), and unidentified (n = 14) [Table 2].

Extracellular enzyme activities of these isolated *Candida* spp. were determined by the Plate method. Proteinase, phospholipase, and hemolysin enzymes were produced by most of the *Candida* species although

strains of *C. krusei* and *C. glabrata* failed to produce the esterase enzyme [Figure 1]. Hemolysin activity was shown by 89.86% of the isolated *Candida* spp., whereas other extracellular enzymes such as proteinase, phospholipase, and esterase were produced only by 84.72%, 55.69%, and 37.97% of the strains, respectively [Table 3 and Figure 2]. Strong proteinase activity was shown by the *C. albicans* (93.75%), followed by the *C. tropicalis* (87.5%). Strong hemolytic activity was shown by 95.85% of the *C. tropicalis*, followed by the *C. albicans* (81.25%). About 56.25% of *C. albicans* strains followed by 54.16% *C. tropicalis* were strong esterase producers. Only one *C. parapsilosis* isolate was able to strong esterase producer. No statistical analysis could be performed for esterase activity, as the number of isolates producing esterase enzyme was considerably small. No significant difference between the enzyme production by the *C. albicans* and non-*albicans Candida* was observed. Similarly, no significant difference was observed between the two most common causes of candidemia, *C. albicans*, and *C. tropicalis* [Table 3].

Discussion

Bloodstream *Candida* infections in ICU-admitted patients are the life-threatening affliction with an incidence of 0.24–34.3 patients/1000 ICU admissions.^[12] These disseminated *Candida* infections may be dependent on various host defense mechanisms and fungal virulence attributes. These *Candida* infections are common in the ICU patients, taking parenteral nutrition, prolonged antibiotics, and immunocompromised conditions.

Table 1: Demographic characteristics of patients with bloodstream infection

Characteristics	Patients (%)
Sex	
Male	51 (64.56)
Female	28 (35.44)
Age (years)	
Neonates	41 (51.89)
≤ 1-18	16 (20.25)
19-35	7 (8.86)
35-60	9 (11.39)
≥ 60	7 (8.86)

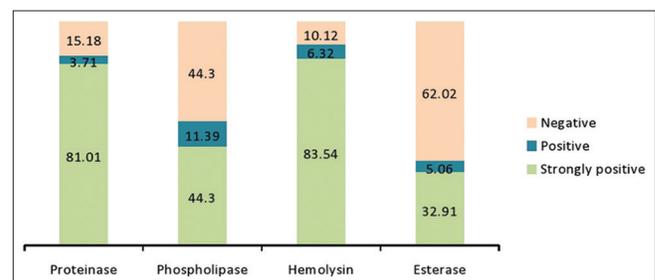


Figure 1: Percentage of *Candida* strain with extracellular enzyme production

Table 2: Extracellular enzyme production by *Candida* spp.

<i>Candida</i> spp.	Degree of variance*	Proteinase	Phospholipase	Hemolysin	Esterase
<i>Candida albicans</i> (n=16)	Negative	0	4	2	7
	Positive	1	3	1	0
	Strongly positive	15	9	13	9
<i>Candida tropicalis</i> (n=24)	Negative	1	9	1	9
	Positive	2	2	0	2
	Strongly positive	21	13	23	13
<i>Candida krusei</i> (n=11)	Negative	4	6	1	11
	Positive	0	0	1	0
	Strongly positive	7	5	9	0
<i>Candida parapsilosis</i> (n=9)	Negative	3	5	2	6
	Positive	0	2	2	2
	Strongly positive	6	2	5	1
<i>Candida glabrata</i> (n=5)	Negative	1	2	2	5
	Positive	0	1	0	0
	Strongly positive	4	2	3	0
Others (n=14)	Negative	3	9	0	11
	Positive	0	1	1	0
	Strongly positive	11	4	13	3

Table 3: Distribution of *Candida* spp. showing different virulence attributes

<i>Candida</i> spp.	Virulence factor	Range	Mean	SD
<i>Candida albicans</i> (n=16)	Proteinase	0.2-0.77	0.3025	0.1308
	Phospholipase	0.28-1	0.595625	0.3061
	Hemolysin	0.29-1	0.466875	0.2287
	Esterase	0.18-1	0.670625	0.3112
<i>Candida tropicalis</i> (n=24)	Proteinase	0.18-1	0.349167	0.1951
	Phospholipase	0.26-1	0.629167	0.3109
	Hemolysin	0.2-1	0.3675	0.1463
	Esterase	0.18-1	0.633333	0.3231
<i>Candida krusei</i> (n=11)	Proteinase	0.2-1	0.519	0.3823
	Phospholipase	0.31-1	0.7109	0.3334
	Hemolysin	0.33-1	0.47	0.2033
	Esterase	1	1	0
<i>Candida parapsilosis</i> (n=9)	Proteinase	0.2-1	0.511111	0.3697
	Phospholipase	0.22-1	0.801111	0.297
	Hemolysin	0.28-1	0.568889	0.2954
	Esterase	0.41-1	0.891111	0.2036
<i>Candida glabrata</i> (n=5)	Proteinase	0.27-1	0.464	0.3071
	Phospholipase	0.28-1	0.696	0.36
	Hemolysin	0.28-1	0.626	0.3469
	Esterase	1	1	0
Others (n=14)	Proteinase	0.22-1	0.410714	0.3213
	Phospholipase	0.37-1	0.802143	0.287
	Hemolysin	0.29-1	0.381429	0.1081
	Esterase	0.35-1	0.867143	0.2645

SD = Standard deviation. *Pz=1, negative activity; 0.64<Pz<0.99, positive; and Pz≤0.64, strongly positive

Previously, *C. albicans* was considered as the most common cause of bloodstream infection, but in the present study, *C. tropicalis* outnumber the cases. Similar results of *C. tropicalis* as the most common etiology of *Candida* infection in ICU patients have been reported by Costa et al. and Chakrabarti et al.^[4,12] In comparison

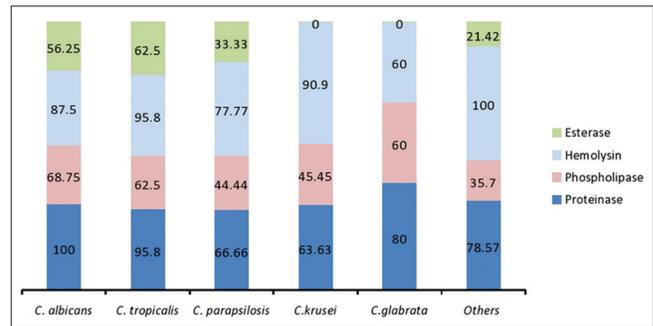


Figure 2: Percentage of extracellular enzymes produced by isolated *Candida* spp on the basis of score value

to studies performed in European and South American countries, *C. parapsilosis* is considered as the most common cause of *Candida* bloodstream infection. This variation may be attributed to the environmental factors, use of central lines, life-supporting devices, and antibiotics usages. In our study, *C. tropicalis* was the most common isolated *Candida* followed by the *C. albicans* (n = 16), *C. krusei* (n = 11), and *C. parapsilosis* (n = 9). High *C. krusei* strains (n = 9) isolation may be due to the frequent use of fluconazole either as prophylactic or therapeutic.^[13]

Candida secretes various extracellular hydrolytic enzymes which act as important virulence factors.^[6,2] These hydrolytic enzymes degrade the cellular component of tissues and facilitate their survival, adhesion, invasion, and dissemination. Phospholipase, proteinase, lipase, esterase, hemolysin, etc., are the common hydrolytic enzymes. Phospholipase and proteinase hydrolytic enzymes are considered as the two most common virulence factors as they contribute to the *Candida*-host interaction. Proteinase enzyme degrades the surface protein and disrupts the local immunity, resulting

in tissue invasion. Phospholipase enzyme degrades the phospholipid component of the cell membrane, resulting in cell damage and lysis which accentuates its dissemination. In our study, higher phospholipase and proteinase enzyme production was shown by the *C. albicans* strains. Similar results of higher enzyme production have been reported by Canela *et al.* and Mutlu Sariguzel *et al.*^[6,15,14] In our study, in comparison to the *C. tropicalis*, phospholipase and proteinase were produced by a higher number of the *C. albicans* strain, whereas hemolysin and esterase enzymes were produced by a higher number of the *C. tropicalis* strains.

Hemolysin is the enzyme which degrades the cellular component of the blood. This virulence property of *Candida* spp. helps to acquire iron by degrading hemoglobin from the host and further help pathogen to persist and survive.^[6,15,16] Hemolysin activity was shown by 95.8% of *C. tropicalis* and 90.9% strains of the *C. krusei* strains. Only 87.5% strain of *C. albicans* produced hemolysin activity. Hemolysin and proteinase enzymes were produced by >80% of the isolated *Candida* spp. Similar results of higher hemolysin production have been reported by Riceto *et al.*^[17] Esterase enzyme is also an important virulence factor which degrades the ester bonds and accentuates its tissue invasion. About 56% of *C. albicans* showed the esterase activity in comparison to the *C. tropicalis* where only 54% strain showed the esterase activity. This result is concordant with a study conducted by Kumar *et al.*, where the author reported *C. albicans* and *C. tropicalis* species with the highest rate of esterase activity.^[18] No activity was observed in the isolated strains of the *C. glabrata* and *C. krusei*, which are considered as the intrinsic fluconazole resistant. These results are contradictory to the study published by the Fatahinia *et al.*, where they reported the 3+ level esterase activities in *C. glabrata* isolated from diabetic patients suffering from oral candidiasis.^[10] On the other hand, they have also reported *C. glabrata* as the lowest esterase producer in vulvovaginal candidiasis patients.^[19] Although esterase activity was low, yet its mechanism in virulence due to cytotoxic effects in host tissue has been reported by Williams and Lewis^[20] In our study, these enzymes were produced by the both common *C. albicans* and *C. tropicalis* ($P > 0.05$) that necessitate the need for search of other factors responsible for their virulence. Our study has a limitation as a considerable number of isolates remain unidentified which needs to be done for proper assessment.

Conclusion

Non-*albicans candida* with a preponderance of *C. tropicalis* is the most common isolated yeast from the blood of the ICU-admitted patients. No statistically significant

difference for enzymatic activity was observed among the *C. albicans* and *C. tropicalis*. Intrinsic fluconazole-resistant *C. krusei* and *C. glabrata* failed to produce any esterase activity which needs to be evaluated further.

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Conflicts of interest

There are no conflicts of interest.

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