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# Virulence Determinants of *Candida* Infections in High Risk Neonates and Infants in a Tertiary Hospital of North India

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**Abstract:** ***Introduction: Candida spp. are considered as opportunistic pathogens because they posses many virulence factors which contributes in the pathogenesis of Candida infections. The virulence of Candida spp. is attributed to certain factors like biofilm formation, and the production of hydrolytic enzymes. Objective: To study the virulence factors of Candida infections among high risk neonates and infants. Materials and methods: Samples were collected aseptically from 128 high risk neonates and infants.They were cultured and identified by standard microbiological techniques and their virulence factors such as biofilm formation was demonstrated by tube method, egg yolk agar for proteinase activity, bovine serum albumin agar for phospholipase activity and sabouraud dextrose agar with sheep blood for haemolytic activity. Results: All the virulence markers were significantly associated with the development of candidiasis among neonates and infants. However among all these factors biofilm and hemolysin production were found to be highly significant. Biofilm formation in this study was more often found in the non albicans Candida spp. (95%) as compared to Candida albicans. C. tropicalis produces stronger biofilms (40% were 3+ & 10% were 4+) than C. albicans (10.3% produced 3+ and 4+ biofilms). Phospholipase activity was noted in 34.7% of Candida spp., Maximum phospholipase production was seen in NAC spp. (65%) as compared to C. albicans (13.6%). Proteinase activity was seen 38.8% of Candida isolates. Conclusion: As non-albicans Candida infections are on the rise, microbiology laboratories should go for complete identification of all yeast isolates and their virulence.***

**Keywords:** Virulence factor; Non *Candida* species, Neonates and Infants

## 1. Introduction

Candidiasis is the commonest fungal disease found in human affecting mucosa, skin, nails and internal organs. Premature infants are a high risk group notably due to their undeveloped immune systems. *Candida* spp. may be acquired vertically from the mother, or horizontally in the neonatal intensive care unit (1).

*Candida* spp. are considered as opportunistic pathogens because they posses many virulence factors which contributes in the pathogenesis of *Candida* infections. The virulence of *Candida* spp. is attributed to certain factors like adherence, biofilm formation, and the production of tissue-damaging extracellular hydrolytic enzymes (2, 3). Extracellular hydrolytic enzymes like phospholipase and proteinase are important for colonization and invasion of host tissue (4). The most relevant virulence factors for *Candida* spp. are as follows:

1) Adhesion: Adherence of *Candida* spp. to a wide range of tissue types and inanimate surfaces is essential in the early stages of colonization and tissue invasion.
2) Hyphal dimorphism: Yeast hyphal dimorphism status of *Candida* plays an important role as a virulence factor.
3) The cell surface hydrophobicity: The hydrophobicity of the cell surface of *Candida albicans* plays an important role in the adhesion of the organism to eukaryotic cells and inert surfaces. The mannans ( glycoproteins present on the cell surface of *Candida albicans* ) contribute to the virulence of *Candida albicans* mainly by affecting the yeast cell surface hydrophobicity, leading to change in adherence to host tissues.
4) Enzymes: The extracellular hydrolytic enzymes including secreted aspartyl proteinase and phospholipases degrade immunoglobulins and proteins of the extracellular matrix; they also inhibit the phagocytosis of polymorphonuclear neutrophils and induce inflammatory reactions.
5) The phenotypic switching phenomena: *C. albicans* is capable of high frequency, reversible phenotypic switching. This property helps the yeast adapt to its diverse locations as a commensal or opportunistic pathogen (5) and to assists the fungus in evading the host's defence mechanisms (6).

## 2. Materials and Methods

The present study was carried out in the Department of Microbiology J. N. Medical College, AMU, on 128 high risk neonates and infants admitted in the NICU and in the HDU of Department of Paediatrics, during the period of one and half years from 2013 to 2014. Various clinical specimens including blood, tracheobronchial aspirate, oral swab, ear swab, CSF and urine were collected.

Specimens like endotracheal aspirate, urine, oral swab etc., were subjected to direct microscopy by making a lactophenol cotton blue(LCB) mount and /or a Gram stained smear. The samples were inoculated on to Sabouraud's dextrose agar as the main isolation medium. For blood samples, Approximately 1 to 2 ml of blood was collected under aseptic precautions and inoculated in biphasic brain heart infusion medium. The culture medium was incubated at 37°C for a week or longer if required. Subculture was done on third, fifth, and seventh day. All the *Candida*

isolates were subjected to germ tube test using normal human serum. Colonies were identified up to the species level on the basis of colony characteristics, morphology on Corn meal agar, growth on Hi- CHROME *Candida* agar, carbohydrate fermentation, and assimilation patterns (7, 8).

## 3. Detection of Various Virulence Factors

**1) Detection of Biofilm formation**

Biofilm production of isolates was demonstrated with a slight modification method, as described by Hassan *et al.,*(9). Biofilm formation was positive when a blue visibly adherent layer lined the wall and bottom of the tube and was scored as weak (1+), moderate (2+ or 3+) or strong (4+), based on amount of biofilm production. Each isolate was tested at least three times and read independently by two different observers. Biofilm producer *Staphylococcus epidermidis* ATCC 35984 and non-biofilm producer *S. epidermidis* ATCC 12228 were used as the positive and negative controls, respectively.

**2) Detection of phospholipase, haemolysin and proteinase activity:**

**Preparation of the yeast suspensions:** Yeast suspensions were prepared from the yeast isolates included in the study to evaluate phospholipase, proteinase and hemolytic activity. First, a small amount of stock culture was inoculated on Sabouraud dextrose agar (SDA) (Oxoid) containing chloramphenicol by using a sterile loop and incubated at 37 °C for 24–48 h. Then the yeasts were harvested and suspended in sterile phosphate buffered solution (PBS) at turbidity equal to optical density (OD) of 0.5 McFarland. The final suspension was adjusted to contain $1 \times 10^7$ yeast cells/ml.

**Determination of phospholipase activity:** To determine phospholipase activity, the egg yolk agar method of Price *et al.,* (10) which was modified by Samaranayake *et al*., (11), was employed. The presence of enzyme activity was determined by the formation of a precipitation zone around the yeast colonies. Phospholipase activity (Pz) was calculated by dividing the diameter of the colony by the diameter of the colony plus precipitation zone. The Pz was scored as follows:A PZ of 1.0 was evaluated as negative (–), 0.99-0.9 as weak (+), 0.89-0.8 as mild (++), 0.79- 0.7 as relatively strong (+++) and<0.69 (++++) as very strong positive.

**Determination of hemolytic activity:** Haemolysin activity of *Candida* spp. was detected by blood agar plate assay as described by Manns *et al*., (12).

**Determination of proteinase activity:**To determine proteinase activity, bovine-serum albumin agar defined by Staib (13) was employed.

Enzyme activity (Prz) was calculated according to the method described by Price *et al.* Prz was scored as follows: Prz = 1, negative proteinase activity,Prz =0.99-0.9 as weak (+), Prz =0.89-0.8 as mild (++), Prz =0.79- 0.7 as relatively strong (+++) and Prz<0.69 (++++) as very strong positive.

**Detection of Pseudohyphae Formation:** The percentage of cells in pseudohyphae form, against blastospores, was determined by microscopy counting after 2 h of cell growth in a liquid medium containing equal volumes of RPMI 1640 (Sigma) and fetal bovine serum . In this, 100 cells per field were examined. Each experiment was conducted in triplicate (14).

## 4. Results

The present study was conducted to determine the virulence of *Candida* infections in high risk neonates and infants. One hundred and twenty eight high risk neonates and infants were included in the study.

**Table 1:** Prevalence of *Candida* infection among neonates and infants in the study group, (n =128)

| Isolate | No. of cases | % |
|---|---|---|
| *Candida* spp. isolated | 39 | 30.5 |
| No *Candida* spp. isolated | 89 | 69.5 |
| Total | 128 | 100 |

Table 1 show the prevalence of infection by *Candida* species in high risk neonates and infants included in the study. It can be seen that out of a total of 128 patients, *Candida* could be isolated in 39 (30.5%) cases.

**Table 2**

| *Clinical diagnosis* | *No. of patients* | *Sample* | *No. of isolates* | *%* |
|---|---|---|---|---|
| Septicaemia | 25 | Blood(23) | 29 | 59.2 |
| | | blood (3)+urine(3)* | | |
| | | blood(3)+oral swab(3)* | | |
| Oral thrush | 5 | Oral swab | 5+3*=8 | 16.3 |
| UTI | 4 | Urine | 4+3*=7 | 14.3 |
| CSOM | 2 | Ear swab | 2 | 4.1 |
| Meningitis | 2 | CSF | 2 | 4.1 |
| Pneumonia | 1 | Endotracheal aspirate | 1 | 2 |
| Total | 39 | | 49 | 100 |

**Distribution of *Candida* isolates in relation to clinical diagnosis in neonates and infants with candidiasis**

**3 urine and 3 oral swab Candida isolates were from septicaemia patients.*

Table 2 shows the distribution of *Candida* isolates in relation to clinical diagnosis from neonates and infants with candidiasis. The maximum number of *Candida* isolates were found from patients with septicaemia (59.2%) followed by cases of oral thrush (16.3%) and cases of urinary tract infections (14.3%).

**Table 3:** *Candida* spp. isolated from patients in the study group, (n=49)

| Candida spp. | | No. of isolates | % | |
|---|---|---|---|---|
| *Candida albicans* (29) | *C.albicans* | 29 | 59.2 | |
| Nonalbicans *Candida* (20) | *C.tropicalis* | 7 | 14.3 | 40.8 |
| | *C.parapsilosis* | 6 | 12.2 | |
| | *C.guilliermondii* | 3 | 6.2 | |
| | *C.glabrata* | 2 | 4.1 | |
| | *C.dubliniensis* | 1 | 2 | |
| | *C.krusei* | 1 | 2 | |
| Total | | 49 | 100 | |

Table 3 depicts the distribution of various *Candida* spp. isolated from patients in the study group.

*Candida albicans* (59.2%) was the most common species isolated from neonates and infants suffering from candidiasis while non albicans *Candida* were 40.8%. In non albicans *Candida*, *Candida tropicalis* (14.3%) was most frequently isolated spp. followed by *Candida parapsilosis* (12.2%), *Candida guilliermondii* (6.2%), *Candida glabrata* (4%), *Candida krusei* (2%) and *Candida dubliniensis* (2%)

**Table 4:** Correlation of various virulence markers among different species isolated from patients, (n=39)

| *Candida* spp. | | No. of cases | Biofilm | Pseudohyphae | Hemolysin | Phospholipase | Proteinase |
|---|---|---|---|---|---|---|---|
| *C. albicans* | Non albicans *Candida* | 22 | 17 (77.3%) | 5 (22.7%) | 22 (100%) | 4 (18.2%) | 3 (13.6%) |
| *C. tropicalis* | | 5 | 5 | 2 | 5 | 3 | 3 |
| *C. parapsilosis* | | 5 | 3 | 2 | 5 | 2 | 3 |
| *C. guilliermondii* | | 3 | 3 | - | 3 | 2 | 2 |
| *C. glabrata* | | 2 | 2 | - | 2 | 2 | 2 |
| *C. krusei* | | 1 | 1 | 1 | 1 | 1 | 1 |
| *C. dubliniensis* | | 1 | 1 | - | 1 | 1 | 1 |
| Total | | 17 | 15 (88.2%) | 5 (29.4%) | 17 (100%) | 11 (64.7%) | 12 (70.6%) |
| | | 39 | 33 (84.6%) | 10 (25.6%) | 39 (100%) | 15 (38.5%) | 15 (15.8%) |

*Figures in parenthesis indicate percentage*

Table 4 exhibit the correlation of various virulence markers among different species of candidiasis patients.

It was observed that most common virulence factors were hemolysin (100%) and biofilm production 84.6%.
As it was evident from the table that proteinase (70.6%) and phospholipase production (64.7%) was most commonly produced in patients infected by non albicans *Candida* spp.as compared to *C.albicans.*

It was noted that all pseudohyphae producing strains also produced phospholipase.

**Table 5:** Presence of various virulence markers among *Candida* isolates

| *Virulence Factor* | *Positive* | *Negative* | *'P' value* |
|---|---|---|---|
| Biofilm formation | 43 | 6 | 0.0001(S) |
| Phospholipase production | 17 | 32 | 0.004(S) |
| Proteinase production | 19 | 30 | 0.042(S) |
| Hemolysin production | 49 | 0 | 0.0001(S) |
| Pseudohyphae formation | 10 | 39 | 0.0001(S) |

Table 5 depicts the various virulence markers studied among candidiasis patients.

All the virulence markers were significantly present in the *Candida* isolates among neonates and infants. However, among all the factors, biofilm and hemolysin production were found to be highly significant ($p<0.01$).

**Table 6:** Different scores of biofilm production of various *Candida* species (n=49)

| *Candida* spp. | Non albicans-*Candida (n=20)* | Scores of biofilm formation | | | | | |
|---|---|---|---|---|---|---|---|
| | | – | + | ++ | +++ | ++++ | Total |
| *C.albicans (n=29)* | | 5(17.2) | 10(34.4) | 8(27.5) | 3(10.3) | 3(10.3) | 29 |
| *C.tropicalis* | | - | 3(42.8) | - | 3(42.8) | 1(14.1) | 7 |
| *C.parapsilosis* | | 1(16.6) | 1(16.6) | - | 4(66.7) | - | 6 |
| *C.guilliermondii* | | - | 1(33.3) | 2(66.7) | - | - | 3 |
| *C.glabrata* | | - | 1(50) | - | - | 1(50) | 2 |
| *C.dubliniensis* | | - | - | - | 1(100) | - | 1 |
| *C.krusei* | | - | 1(100) | - | - | - | 1 |
| Total | | 1(5) | 7(35) | 2(10) | 8(40) | 2(10) | 20 |
| Total | | 6(12.2) | 17(34.7) | 10(20.5) | 11(22.4) | 5(10.2) | 49 |

*Figures in parenthesis indicate percentage*

Table 6 shows the pattern of biofilm formation exhibited by different *Candida* isolates.

It was observed that the maximum number of isolates have score of 1+ (34.7%) followed by 3+ (22.4%) and 2+ (20.5%) score of biofilm formation.

Almost all the nonalbicans *Candida* spp. were positive for biofilm formation (95%) as compared to *C. albicans* (82.7%). In non albicans *Candida* spp. 8(40%) and 2(10%) showed 3+ and 4+ biofilm formation respectively. However only 3(10.3%) *C. albicans* produced 3+ and 4+ biofilm.

**Table 7:** Comparison of biofilm production by *Candida* spp. isolates obtained from the blood stream and from other sites in the study group

| *Candida* spp. | No. of isolates | Isolates positive for biofilm formation | | P value |
|---|---|---|---|---|
| | | Blood stream | Other sites | |
| *Candida albicans* | 29 | 12(41.4) | 17(58.6) | 0.29 (NS) |
| Non albicans *Candida* | 20 | 17(85) | 3(15) | 0.0001(S) |
| Total | 49 | 29 | 20 | |

*Figures in parenthesis indicate percentage*

Table 7 (figure 2) depicts the comparison of biofilm formation between *Candida albicans* with nonalbicans *Candida* isolates with respect to the site of collection either blood stream or from other sites.

As is evident from the table that biofilm formation among non albicans *Candida* isolates was more significantly associated with blood stream infections as compared to other site infections from where samples were collected.

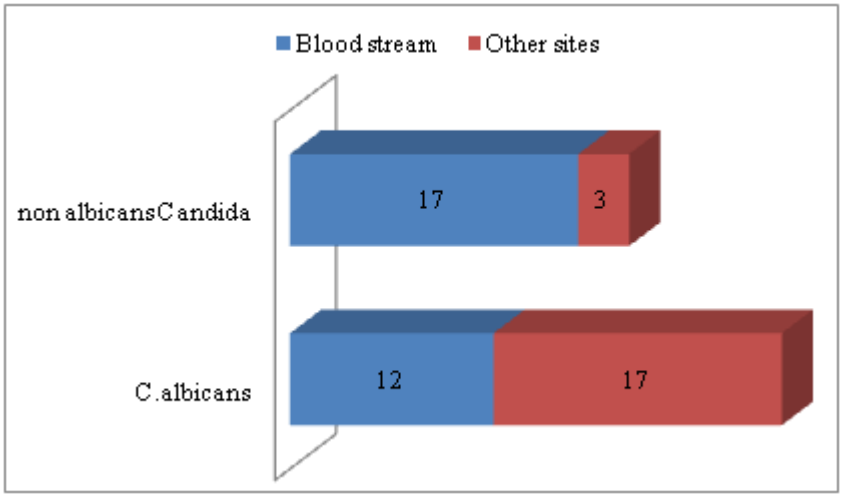


**Figure 1:** Comparison of biofilm production by *Candida* spp. isolates obtained from the blood stream and from other sites in the study group

**Table 8:** Phospholipase (Pz) value of different *Candida* spp.

| *Candida* spp. | | Phospholipase (Pz) value | | | | | |
|---|---|---|---|---|---|---|---|
| | | – | + | ++ | +++ | ++++ | **Total** |
| ***C.albicans (n=29)*** | | 25(86.2) | 2(6.8) | 2(6.8) | - | - | 29 |
| ***C.tropicalis*** | *Non albicans Candida* | 3(42.8) | - | 1(14.2) | 1(14.2) | 2(28.4) | 7 |
| ***C.parapsilosis*** | | 4(66.7) | 1(16.6) | - | 1(16.6) | - | 6 |
| ***C.guilliermondii*** | | - | 1(33.3) | 2(66.7) | - | - | 3 |
| ***C.glabrata*** | | - | - | - | 1(50) | 1(50) | 2 |
| ***C.dubliniensis*** | | - | 1(100) | - | - | - | 1 |
| ***C.krusei*** | | - | - | - | 1(100) | - | 1 |
| **Total** | | 7(35) | 3(15) | 3(15) | 4(20) | 3(15) | 20 |
| **Total** | | 32(65.3) | 5(10.2) | 5(10.2) | 4(8.1) | 3(6.2) | 49 |

*Figures in parenthesis indicate percentage*

Table 8 depicts phospholipase production by different *Candida* species causing infection among neonates and infants.

Most of the isolates showed lower range (i.e. 1+ and 2+) production of phospholipase

Out of 20 non albicans *Candida* isolates, 13(65%) showed phospholipase production while only 4 (13.6%) of *C. albicans* were phospholipase producer. In nonalbicans *Candida* isolates, higher range (i.e. 3+ and 4+) of phospholipase production was observed, vis –a-vis lower range phospholipase (i.e. 1+ & 2+) production was noted among *C. albicans* isolates.

**Table 9:** Proteinase production among different *Candida* species.

| *Candida* spp. | | Proteinase score | | | | | |
|---|---|---|---|---|---|---|---|
| | | – | + | ++ | +++ | ++++ | Total |
| *C.albicans (n=29)* | | 24 | 1 | 2 | 2 | - | 29 |
| *C.tropicalis* | Non albicans *Candida* | 3 | - | 1 | 2 | 1 | 7 |
| *C.parapsilosis* | | 3 | 1 | 1 | 1 | - | 6 |
| *C.guilliermondii* | | - | 2 | 1 | - | - | 3 |
| *C.glabrata* | | - | - | 1 | 1 | - | 2 |
| *C.dubliniensis* | | - | 1 | - | - | - | 1 |
| *C.krusei* | | - | - | - | 1 | - | 1 |
| Total | | 6(30) | 4(20) | 4(20) | 5(25) | 1(5) | 20 |
| Total | | 30(61.2) | 5(10.2) | 6(12.2) | 7(14.3) | 1(2.1) | 49 |

*Figures in parenthesis indicate percentage*

Table 9 depicts the proteinase production by different *Candida* spp. isolated from neonates and infants. It was observed that the maximum number of isolates had a score of 3+ followed by 2+ and 1+ proteinase production.

Only 17.2% *C.albicans* isolates showed proteinase production. However among non albicans *Candida* isolates 70% isolates showed proteinase production.

**Table 10:** Hemolysin production of various *Candida* species

| *Candida* spp. | | Hemolysin production | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| *C.albicans* | | 29(100) | - | 29 |
| *C.tropicalis* | Non albicans *Candida* | 7(100) | - | 7 |
| *C.parapsilosis* | | 6(100) | - | 6 |
| *C.guilliermondii* | | 3(100) | - | 3 |
| *C.glabrata* | | 2(100) | - | 2 |
| *C.dubliniensis* | | 1(100) | - | 1 |
| *C.krusei* | | 1(100) | - | 1 |
| Total | | 49 | - | 49 |

*Figures in parenthesis indicate percentage*

Table 10 shows the production of hemolysin by various *Candida* species among neonates and infants.

100 % of *Candida albicans* as well as other non albicans *Candida* isolates produced hemolysin.

## 5. Discussion

The present study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh from February 2013 to October 2014. A total of 128 children suffering from various clinical diseases, categorized into different predefined high risk groups were included in the study to determine the profile of *Candida* infections with respect to the predominant species, pathogenic characteristics in high risk neonates and infants.

The most commonly identified patient group in this study were neonates and infants with septicaemia (69.6%), followed by patients with oral thrush (10.9%), urinary tract infection (9.4%).The other group included patient with pneumonia (3.1%), meningitis (3.1%) and ear infection (3.9%). Thus, the study group compromised of a varied patient population with different clinical diagnosis.

Multiple samples from different sites were also collected. The most frequently collected specimens were blood (72.8%), followed by urine (9.4%). The next most common sample collected was oral swab (9.4%). Other relevant specimens included the endotracheal aspirate, CSF and ear swab.

Overall the rate of *Candida* isolation from various specimens in our study group was 30.5%. *C.albicans* formed the largest group (59.2%) of *Candida* species isolated in this study. Jarvis (15) and Pfaller (16) had reported 50 to 70% *Candida albicans* isolation, Roilides *et al* (17) 65%, Ariff S (18) 55% of isolation in their respective studies. Indian studies which reported almost similar findings were S Narain (53.3%)(19), Kaur R (50%)(20). However, Kotwal A *et al* (21) noted a much higher prevalence of *C.albicans* (78.1%).

Although *C. albicans* was the most commonly isolated species (59.2%) in our study non albicans *Candida* (NAC) also substantially caused candidiasis. The next most common isolate, *C. tropicalis* formed 14.3% of the total isolates.

*Candida* spp. have various virulence factors that facilitate proliferation, they may result in adhesion to the epithelium and invasion of the host tissue. Biofilm formation, hemolysin production and pseudohyphae formation also plays an essential role in the pathogenicity of *Candida* spp. The extracellular hydrolytic enzymes including secreted aspartyl proteinase and phospholipases also play an important role in candidal growth. Early detection of virulence factors by *Candida* is useful in clinical decision making.

In the present study, we observed that all the isolates (100%) produced hemolysin, 43 isolates (87.8%) showed biofilm formation, 19 isolates (38.8%) showed proteinase production, phospholipase production was formed in 17 (34.7%) and pseudohyphae formation by 10 (20.4%) *Candida* isolates.

All the virulence markers were significantly associated with the development of candidiasis among neonates and infants. However among all these factors biofilm and hemolysin production were found to be highly significant ($p<0.001$).

Biofilm is a community of microorganisms and their extracellular polymers that are attached to a surface. The ability to form biofilms is associated with the pathogenecity and should be considered as an important virulence determinant during candidiasis.

Biofilm formation in this study was more often found in the non albicans *Candida* spp. (95%) as compared to *Candida albicans*. It was also observed that the maximum no. of isolates have scored 1+ biofilm formation followed by 3+ score. While among non *albicans Candida* spp. 3+ grade was more frequent, this suggest that in high risk neonates and infants, non *albicans Candida* spp. were more virulent. This finding was in accordance with the study done by Shin JH *et al* (22).

We observed the combined biofilm positivity of bloodstream isolates of all the non-*albicans Candida* species was significantly higher than for isolates from other sites. This finding was similar to the study done by Kaur R *et al* (20), who observed that 60.78% of isolates of NAC spp. produced biofilm in comparison to 39.21% of *C. albicans* producing lower slime.

Our data also suggests that *C. tropicalis* produces stronger biofilms (40% were 3+ & 10% were 4+) than *C. albicans* (10.3% produced 3+ and 4+ biofilms).

The production of haemolysin plays an important role in virulence. Haemolysins are proteins produced by micro-organisms to destroy red blood cells. Iron, an inorganic element, is essential for the development of micro-organisms, including yeasts, and the ability to obtain this element is essential for the establishment of an infectious process (12). In our study, all the isolates were able to express hemolytic activity. Manns *et al*. (12), demonstrated that *C. albicans* produced hemolytic activity and Luo *et al*. (23) observed that NAC species are capable of producing one or more types of hemolysins in vitro with differences among species.

The other important hydrolytic enzymes are proteinase and phospholipases. The aspartic proteinase (Sap) isoenzymes are also responsible for the proteinase activity of *C. albicans*. Sap proteins have also been described in *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii* (24). Several studies have demonstrated a relationship between an increase in the synthesis and activity of extracellular hydrolytic enzymes and an increase in the pathogenic potential of the yeasts, leading to clinical signs of severe candidiasis (25,26).

In our study phospholipase activity was noted in 34.7% of *Candida* spp., Maximum phospholipase production was seen in NAC spp. (65%) as compared to *C. albicans* (13.6%). Among NAC *C. tropicalis* (57.1%) showed maximum phospholipase production, which was similar to the observation of Thangam *et al* (27) and Deorukhkar *et al*.,(4). Changdeo S. Aher, (28) studied phospholipase activity in (58.1) isolates and found phospholipase production was maximum in *C. albicans* followed by *C. glabrata* and *C. tropicalis*. Samaranayake *et al*, (11) reported no production of phospholipase by *C. tropicalis*. The variation in different strains or the difference in the method of media preparation may be the reason for discrepancy observed by different workers in the phospholipase activity of the NAC spp. Also, Phospholipase production was observed to be at higher range by NAC as compared to by *C. albicans* isolates. As phospholipase helps in host tissue invasion, it may be one of the important virulence factor utilized by NAC to cause BSIs.

Proteinase activity was seen 38.8% of *Candida* isolates in our study. This finding was in accordance with Changdeo S. Aher (28), who observed 37.8% of proteinase production in *Candida* isolates. We observed that only 17.2% *C. albicans* isolates showed proteinase production. However among non albicans *Candida* isolates 70% isolates showed proteinase production. Our findings were similar to Mohandas V (29). Who also reported that non-albicans *Candida* spp. produced more proteinase than *C. albicans* strains.

*C. albicans* reversibly converts from unicellular yeast cells to either pseudohyphal or hyphal growth, a morphogenesis phenomenon (creating a transition between unicellular yeast cells and a filamentous growth form). *C. albicans* and *C. dubliniensis* form both types of filamentous growth, indicating that these yeasts are capable of growing isotropically (yeast) or apically (hyphal and pseudohyphal). The growth of hyphae, a virulence mechanism, plays an important function in tissue invasion and resistance to phagocytosis (30). We found 22.4% isolates showed pseudohyphae formation. The morphogenetic change from the yeast to hyphal form is involved in fungal tissue invasion and penetration.

## 6. Conclusion

The incidence of *Candida* infection among a total of 128 patients in the present study was 30.5%. *C.albicans* (59.2%) was the most common species isolated from all the specimens. The second most common *Candida spp.* after *C.albicans* to cause candiasis was *C.tropicalis* (1.43%) followed by *C.parapsilosis* (12.2%), *C.guilliermondii* (16.2%), *C.glabrata* (4%), *C.dubliniensis* (2%) and *C.kursei* (2%).

On studying the various virulence factors produced by the *Candida* isolates, we observed that all the isolates (100%) produced hemolysin, 87.8% formed biofilm, 38.8% produced proteinase enzyme, 34.7% produced phospholipase enzyme while 20.4% showed pseudohyphae formation.

All the virulence markers were significantly associated with the development of candidiasis. However, biofilm formation and hemolysin production were found to be highly significant ($p<0.01$).

We found biofilm formation was more related to NAC then *C.albicans*. We observed that the biofilm formation among NAC was more significantly associated with blood stream infections as compared to other site infections. Among NAC, *C.tropicalis* was found to produce stronger biofilms as compared to *C.albicans*. *C.tropicalis* was the commonest NAC producing biofilms.

All the *Candida* isolates expressed hemolytic activity. Hemolysin production by *C.albicans* had positive correlation with hemolysin production by NAC species and was statistically significant ($p<0.01$).

In this study phospholipase activity was noted in 34.7% of *Candida* species. Phospholipase production was more commonly seen in NAC (65%) as compared to *C.albicans* (13.6%) also phospholipase production was observed to be in higher range in NAC and in lower range in *C.albicans* isolates.

While observing proteinase enzyme production, we found 38.8% of *Candida* isolates to be proteinase producers. Also, more NAC (70%) expressed proteinase activity as compared to *C.albicans* (17.2%). In this work we found 22.4% of *Candida* isolates were forming Pseudohyphae.

As non-albicans *candida* infections are on the rise, microbiology laboratories should go for complete identification of all yeast isolates.

*Candida* is significantly rising. Non albicans candidiasis should be considered when initiating antifungal prophylaxis as they possess a different antifungal susceptibility spectrum from *C. albicans.*

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