Original Research Paper

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Microbiology

"DETECTION OF VARIOUS VIRULENCE FACTORS OF CANDIDA SPECIES ISOLATED FROM DIFFERENT CLINICAL SPECIMENS"

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ABSTRACT **Background-** C. albicans is generally considered as most pathogenic member of the genus and most common cause of different types of candidiasis. However, many recent studies from various parts of world have documented a shift from 'pervasive' C. albicans to 'cryptic' Non albicans Candida (NAC) species. NAC spp. are closely related to C. albicans and cause similar clinical manifestations but differ with respect to epidemiology, virulence factors and most importantly the pattern of susceptibility to antifungal drugs. **Aim and objectives-** To detect phospholipase, lipase, hemolysin activity of Candida species isolated from different clinical specimens. **Material and methods-** From April 2021 to March 2022 at Govt. Medical College, Kota. A total of 100 Candida species isolated from different clinical specimens further identified using standard mycological procedures. Phospholipase, lipase and hemolysin activities were detected by growth on egg yolk agar, tween 80 agar and SDA with blood media respectively. **Result-** Out of 100 Candida isolates 38(38%) were C. albicans and 62(62%) were Non albicans Candida (NAC) including 42(42%) C. tropicalis, 10(10%) C. parapsilosis, 8(8%) C. glabrata, 2(2%) C. krusei. Phospholipase, lipase and hemolysin activities were detected 49(49%), 44(44%) and 68(68%) respectively. **Conclusion-** Detection of virulence factors might improve understanding of behaviour of Candida species and open doorways to management and prognosis of patients.

KEYWORDS : Candida, virulence factors, Non albicans Candida.

INTRODUCTION

The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in the large population of immunocompromised patients and those hospitalized with serious underlying diseases (Arendrup et al., 2005; Espinel-Ingroff et al., 2009).

Infections due to fungi belonging to the genus *Candida* are increasingly reported in recent years. *Candida* spp. is the only opportunistic fungi that exist both as a commensal and pathogen. It is also unique among mycotic pathogens as it causes a broad spectrum of clinical manifestations ranging from mere mucocutaneous overgrowth to life threatening systemic infections[1].

The severity of candidiasis ranges from moderate to fatal and is dependent on the site of infection, virulence of infecting strain and host's immune status[2]. *C. albicans* is generally considered as most pathogenic member of the genus and most common cause of different types of candidiasis[3,4]. However, many recent studies from various parts of world have documented a shift from 'pervasive' *C. albicans* to 'cryptic' Non *albicans Candida* (NAC) species[3,4]. NAC spp. are closely related to *C. albicans* and cause similar clinical manifestations but differ with respect to epidemiology, virulence factors and most importantly the pattern of susceptibility to antifungal drugs[3,4].

C. albicans is the most prevalent among *Candida* spp., which causes both superficial and systemic infections. Other pathogenic *Candida* species include *C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei* accounting for 25%, 8%, 7% and 4% of candidiasis, respectively[5].

Infections caused by *Candida* genus yeasts are known as candidiasis or candidosis[6]. Clinically manifested through lesions which can be classified as superficial with cutaneous and mucosal involvement or systemic, disseminated and of high severity[7]. This type of mycosis has mouth, throat, tongue, scalp, genitals, fingers, nails and internal organs as infection sites[6].

The main virulence factors of yeasts that induce infection are[8]:

a) secretion of extracellular enzymes such as phospholipases and proteinases which degrade the host tissue leading to tissue invasion[8]
b) Production of toxic substances that damage the cells[8]
c) Creation of biofilms on cells and inert surfaces[8]
d) Formation of pseudohyphae by certain species of *Candida* spp.[8]
e) Production of hemolysins[8]
f) The ability to adhere to medical and hospital materials and host cells[8]

The sum of these mechanisms with the weakness of the host response may lead to candidiasis[8].

Therefore the present study was taken up with an aim to study virulence factors of *Candida* species isolated from different clinical samples.

MATERIAL & METHODS

A total of 100 samples with isolated *Candida* were processed over a period of 12 months from April 2021 to March 2022. Various clinical samples included in present study were urine samples, vaginal swab, urine catheter, blood culture, pus, sputum, stool, throat swab, nail and ear swab etc. The specimens were subjected to preliminary tests like wet mount, Gram stain, culture on SDA. A total of 100 *Candida* isolates from various clinical samples were taken up for study. Species level identification of *Candida* isolates was done by various methods like conventional methods which includes germ tube formation, chlamydospore formation on cornmeal agar, CHROM agar, urease test, sugar fermentation and sugar assimilation.

Detection Of Virulence Factors

Detection Of Phospholipase Activity:-

The test medium contained 65g Sabouraud dextrose agar, 58.4g NaCl and 5.5g $CaCl_2$. All dissolved in 980ml distilled water and sterilized by autoclaving at 121^{0}C for 12 min. Egg yolk centrifuged at 5000rpm for 30 minutes. The supernatant was collected and added at rate of 2% to above medium, mixed and dispensed in plates. An aliquot (10µl) of yeasts suspension inoculated on center of test medium which then incubated at 37°C for 4 days. Then phospholipase activity was determined as clear zone.

Detection Of Lipase Activity:-

The test medium contained 10g of peptone, 5g of NaCl, 0.1g of $CaCl_2$.$2H_2O$, 15g of agar and 1000ml of distilled water with pH adjusted to 6.5 sterilized by autoclaving at 121ºC for 12 min then it was cooled about 50ºC and 5ml of autoclaved Tween 80 added. An aliquot (10µl) of yeasts suspension was inoculated on the center of test medium then incubated at 37ºC for 5days. Then lipases activity was determined as precipitation zone around colonies.

Detection Of Hemolysin Activity:-

A Sabouraud dextrose agar (SDA) was prepared according to supplied company instruction and sterilized by autoclave. When the medium cooled down to 50-55°C, 7 % of human blood and 3% glucose was added with a final pH adjusted to 5.6 and dispensed into sterile petri dishes. An aliquot (10µl) of yeasts suspension inoculated on the center

of test medium which was incubated at 37ºC for 48h.This medium was used to detect ability of isolates to produce hemolysin.

Enzymes Assay:-
The activity expressed according to the Pz index, i.e. colony diameter (a) total diameter (b) of the colony plus the precipitation halo.

The following ranges of activity established according to the Pz index:

- Pz < 0.69- very strong (++++) ,
- Pz =0.70- 0.79- strong (+++),
- Pz = 0.80- 0.89- mild (++),
- Pz = 0.90- 0.99- weak (+),
- Pz = 1- Negative.

$$PZ = \frac{\text{Colony diameter}}{\text{Colony diameter + zone of precipitation}}$$

RESULT

During the study period a total of 100 *Candida* isolates were collected from different clinical specimens for species identification and detection of virulence factors. Out of these 100 *Candida* isolates 38(38%) were *C. albicans* and 62(62%) were Non *albicans Candida* (NAC) including *C. tropicalis* 42(42%), *C. parapsilosis* 10(10%), *C. glabrata* 8(8%), *C. krusei* 2(2%). Out of 100 *Candida* isolates 68% were hemolysin producer, 49% were phospholipase producer and 44% were lipase producer.

Table 1 - Distribution of Candida isolates among various samples

Type of sample	Number of Isolates N=100 (%)
Urine Catheter	33 (33%)
Urine	12 (12%)
Vaginal Swab	10 (10%)
Sputum	27 (27%)
Throat Swab	1 (1%)
Blood	5 (5%)
Pus	7 (7%)
Ear Swab	2 (2%)
Stool	2 (2%)
Nail	1 (1%)
Total	100

Table 2 - Species wise distribution of Candida species

Candida species isolated	Number of each Candida species isolated N=100(%)
Candida tropicalis	42 (42%)
Candida albicans	38 (38%)
Candida parapsilosis	10 (10%)
Candida glabrata	8 (8%)
Candida krusei	2 (2%)
Total	100

Table 3 - Distribution of Candida among heterogenous clinical samples

Name of the species of Candida	Urine catheterised	Urine	Vaginal Swab	Sputum	Throat Swab	Blood	Pus	Ear Swab	Stool	Nail	Total
C. tropicalis	13	3	6	10	1	2	4	1	2	0	42
C. albicans	9	5	3	13	0	3	3	1	0	1	38
C. parapsilosis	5	2	1	2	0	0	0	0	0	0	10
C. glabrata	4	2	0	2	0	0	0	0	0	0	8
C. krusei	2	0	0	0	0	0	0	0	0	0	2
Total	33	12	10	27	1	5	7	2	2	1	100

Table 4- Production of hemolysin, phospholipase and lipase by Candida spp.

Candida species	Hemolysin production (68%)	Phospholipase production (49%)	Lipase Production (44%)
C. albicans (38)	28 (73.7%)	31 (81.6%)	17 (44.7%)
C. tropicalis (42)	31 (73.8%)	16 (38.1%)	24 (57.1%)
C. parapsilosis (10)	4 (40%)	1 (10%)	2 (20%)
C. glabrata (8)	5 (62.5%)	1 (12.5%)	1 (12.5%)
C. krusei (2)	0 %	0 %	0 %
Total (100)	68 %	49 %	44 %

Fig.1 Gram Staining

Fig.2- Growth On SDA

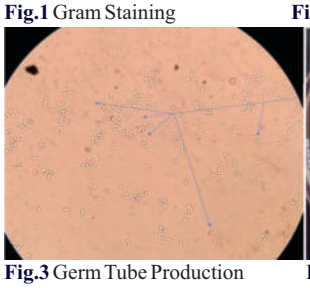

Fig.3 Germ Tube Production

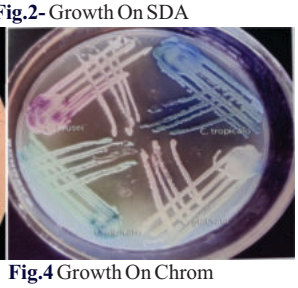

Fig.4 Growth On Chrom

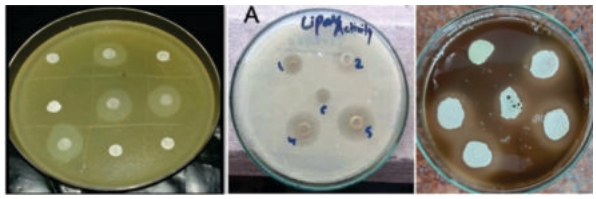

Fig.5 Production of virulence factors (A) Phospholipase (B) Lipase (C) Hemolysin]by *Candida* species

DISCUSSION

In present study NAC species 62(62%) were more isolated than *C. albicans* 38(38%) from all clinical specimens. A total of 100 *Candida* isolates were collected from different clinical specimens of which catheterized urine samples 33(33%) followed by sputum 27(27%), urine 12(12%), vaginal swab 10(10%), pus 7(7%), blood 5(5%), ear swab & stool 2(2%) each and nail & throat swab 1(1%) each. In this study isolates were identified as *C. tropicalis* 42(42%) followed by *C. albicans* 38(38%), *C. parapsilosis* 10(10%), *C. glabrata* 8(8%) and *C. krusei* 2(2%).

Phospholipase enzymes are an important virulence factor and these are associated with membrane damage to host cells, adherence and penetration. Invasion of host cells by microbes entails penetration and damage of outer cell envelope. Phospholipase production may be used as one of the parameters to distinguish virulent invasive strains from non-invasive colonizers. In our study out of 100 *Candida* isolates 49 (49%) produced phospholipase. Phospholipase production more common among *C. albicans* (63.26%) than Non *albicans Candida* (36.74%). Among Non *albicans Candida* phospholipase production more commonly observed among *C. tropicalis* (38.1%) followed by *C. glabrata* (12.5%) and *C. parapsilosis* (10%) while *C. albicans* (81.6%). While in study conducted by Sachin et al (2012)[9] (N=110) phospholipase production more common among *C. albicans* (53.73%) than Non *albicans Candida* (46.27%). Among Non *albicans Candida* phospholipase production commonly observed among *C. tropicalis* (76%) followed by *C. glabrata* (28.5%) while *C. albicans* (92.3%).

Lipase are enzyme that hydrolyse ester bonds of mono-, di-, and triglycerols to produce free fatty acids like monoacylglycerols and glycerols.[10] Lipase play role in adhesion and penetration of infection process in murine model of haemetogenously disseminated candidiasis and supporting a role for these extracellular hydrolases in *C. albicans* pathogenicity.[11] In our study, out of 100 *Candida* isolates 44(44%) produced lipase. Lipase production more common among Non *albicans Candida* (61.35%) than *C. albicans* (38.65%). The lipase production among Non *albicans Candida* is more commonly among (57.1%) *C. tropicalis* followed by *C. parapsilosis* (20%) and *C. glabrata* (12.5%). Lipase production was detected in 44.7% in *C. albicans*. While study done by Sikdar et al (2019)[12] (N=120) reported 68.33% *Candida* isolates produced lipase. Lipase production more common among Non *albicans Candida* (51.21%) than *C. albicans* (48.79%). Lipase produced by Non *albicans Candida* (60.86%) while *C. albicans* (78.43%). Another Study conducted by Jatta *et.al.*

(2009)[13] which showed that the highest enzyme activity detected in *Candida* albicans (51%) and in Non-*albicans Candida* (NAC) species (87%).

Hemolysin activity is known to be a putative virulence factor that contributes to dissemination of *Candida* spp. by facilitating iron acquisition from the host erythrocytes.[14] In our study, out of 100 *Candida* isolates 68(68%) produced hemolysin. The hemolysin production more common among Non *albicans Candida* (58.82%) than *C. albicans* (41.18%). Among Non *albicans Candida* hemolysin production more commonly observed in *C. tropicalis* (73.8%) followed by *C. glabrata* (62.5%) and *C. parapsilosis* (40%) while *C. albicans* (73.7%). While study done by Sachin et al (2012)[9] (N=110) observed hemolysin production more common among *C. albicans* (64.91%) than Non *albicans Candida* (35.09%). Among Non *albicans Candida* hemolysin production commonly observed among *C. tropicalis* (48%) followed by *C. glabrata* (21.4%) while *C. albicans* (94.8%). Sathiya *et al*. (2015)[15] reported that 100% of *C. albicans* and Non *albicans Candida* species isolates showed β-haemolytic activity.

CONCLUSION

Presence and absence of virulence factors and when present produced amount decides degree of pathogenicity of *Candida* species. Detection of virulence factors might improve understanding of behaviour of *Candida* spp. and open doorways to management and prognosis of patients.

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