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Speciation, Detection of Virulence Factors and Antifungal Susceptibility Testing of Candida Isolates in a Tertiary Care Hospital

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Abstract
Candida Species are normal flora of skin and mucosa of gastrointestinal tract (GIT) and genital region. They invade host tissue and cause diseases in patients whose cell mediated immunity (CMI) is impaired. The aim of the study is to identify species, detect virulence factors like phospholipase and esterase production and to determine antifungal susceptibility by E - test.
Materials & Methods*: A total of 60 candida isolates were taken from different clinical samples and speciated by growth on chrom agar medium. Phospholipase and esterase activities were detected by growth characteristics on egg yolk agar medium and Tween- 80 opacity test medium respectively. Antifungal susceptibility was detected by E test using Mueller- Hinton Agar (MHA).*
Results*: Out of 60 isolates, 34 (56.6%) were C.albicans, 12 (20%) were C.tropicalis, 13 (21.6%) were C. guilliermondii,1 (1.7%) was C. glabrata. Phospholipase and Esterase activities were detected in 32 (53.3%) and 33 (55%) respectively. The antibiotic susceptibility patterns revealed that all of them (100%) were sensitive to amphotericin-B and voriconazole, 96.7% to fluconazole , 93.3% to itraconazole, 93.3% to ketoconazole respectively.*
Keywords*: Antifungal susceptibility ,Candida, Chrom agar, Speciation, virulence factors.*

I. Introduction

Candida species form normal flora of skin, mucosa of GIT and genital regions. The rates of Candida infection are increasing worldwide for past two decades. (1) The carriage rate has reached to 80% in healthy individuals. Candida species that are part of normal flora of humans invade the host tissues and cause diseases in those whose cell mediated immunity is impaired. (2) The risk factors associated with the candida infections are prolonged stay in intensive care units (ICU's), diabetes mellitus, prolonged usage of antibiotics, immunosuppression and defects in CMI.

Many virulence factors were studied to correlate the pathogenic property of various candidal isolates. They are presence of hydrolytic enzymes, biofilm formation, phenotypic switching and adhesion. Studies differ in their observation of the presence of these factors in various species of Candida. Organisms secrete organic acids during infection and these acids activate the hydrolytic enzymes causing inflammation of the mucosa. In turn, the hydrolytic enzymes help candida in colonizing the host surfaces by increasing their adhesive property .These form adhesion and help the organisms to penetrate into deeper tissues by digesting host cell membrane and evading host defense mechanisms. The hydrolytic enzymes produced are phospholipase, esterase, proteinase, lipase etc. Phospholipase secretion takes place at the periphery of the yeast cell and at the tip of the pseudohyphae which destroy phospholipids in the host cell resulting in damage to cell membrane and cell lysis which facilitates tissue invasion. (3) Biofilms produced are essential not only for the adherence to host surface but also for metabolic co-operation, nutrient availability and acquisition of new genetic traits. (4) Phenotypic switching denotes the ability of organisms of single strain to switch reversibly at higher frequencies among different colony phenotypes and due to this ability; they can grow in a variety of morphological forms and evade the immune system. Adhesion controls binding of Candida to epithelial and endothelial cells that interact with receptors of host cells. Complement receptors (c3d and ic3b, cr2 and cr3) have ability to bind complement derived opsonins.

Species identification is important for treatment of infections as non albicans candida infections has been increasing. This study was aimed to identify species of candida, detect virulence factors (Phospholipase and esterase) and determination of susceptibility of candida species to amphotericin-B, fluconazole, itraconazole, ketoconazole and voriconazole by using E-test using Mueller Hinton agar medium (MHA).

II. Materials & Methods

A total of 60 candida isolates were collected from the different clinical samples in a tertiary care hospital .The samples collected include 28 from respiratory tract (sputum, gastric lavage, tracheal secretions,

oral secretions and endotracheal tube), 6 from blood,14 from urine, 9 from high vaginal swab (HVS),1 from nasal swab and 2 from wound swab.

Species were identified based on colour of the growth on Chrom- agar, germ tube formation and chlamydospores production on corn meal agar. Phospholipase activity was demonstrated by using egg yolk agar medium. Egg yolk agar medium consists of 6.63g Sabouraud Dextrose Agar (SDA), 5.95g NaCl, 0.56mg $CaCl_2$ dissolved in 100ml distilled water and sterilized at 121^{o}C for 15 minutes in autoclave. Egg yolk was centrifuged at 5000g for 30 min and 0.2ml of supernatant was added to the medium at 45-50^{o}c .10 microlitre of yeast suspension was spot inoculated on to this medium and incubated at 37^{o}c for 5-6 days. After incubation phospholipase activity was determined by measuring diameter of precipitation zone to colony diameter. Among the 60 isolates, 32 were positive for phospholipase activity and all showed intense phospholipase activity of < 0.70mm.[(5)]

Esterase activity was demonstrated on Tween 80 Opacity test medium. This medium consists of 1g peptone, 0.5g NaCl, 0.04g $CaCl_2$, 1.5g agar were dissolved in 100 ml of distilled water and autoclaved for 15 minutes at 121^{o}C and 0.5ml of Tween 80 was added to this medium at 50^{o}c. All the isolates were tested on this medium for esterase activity by spot inoculating a suspension for an area of about 10mm diameter and incubated at 37^{o}c for 4-5 days. The presence of halo around the inoculation site indicates esterase activity. [(6)] Antifungal susceptibility testing for all the isolates was determined using amphotericin-B, fluconazole, ketoconazole, itraconazole and voriconazole by E - test method (Hi-media) to know the Minimum Inhibitory Concentration (MIC's) on MHA medium.

III. Results

During the study period a total of 60 candida isolates were collected from different specimens for species identification, detection of virulence factors and antifungal susceptibility testing. Out of 60 candida isolates, 34(56.6%) were C.albicans, 12(20%) were C.tropicalis, 13(21.6%) were C. guilliermondii and 1(1.7%) was C.glabrata. (Table-1)

In the present study, out of 60 candida isolates , phospholipase activity was seen in 32 (53.3%) and esterase activity was seen in 33(55%). (Table-2)

All the 60 isolates are susceptible to amphotericin-B and voriconazole. All the 12 isolates of C. tropicalis and one isolate of C. glabrata are susceptible to all the antifungals tested. Further 97% of C. albicans were susceptible to fluconazole and itraconazole and 94% to ketoconazole. Similarly among C. guilliermondii 92.3% were susceptible to fluconazole, 84.6% to ketoconazole and 76.9% to itraconazole. 3% and 23.1% of C. albicans and C. guilliermondii were shown susceptible dose dependent to itraconazole.7.7% of C. guilliermondii were susceptible dose dependent to fluconazole. (Table-3)

Table-1: Distribution of Candida species identified from different clinical samples.

Type of Specimen	No.of Specimens	Species of candidal isolates			
		C. albicans	C. tropicalis	C. guilliermondii	C. glabrata
Oral	5	3	1	1	0
Sputum	16	10	4	2	0
Blood	6	0	0	6	0
Urine	14	7	4	2	1
Endo tracheal secretions	6	3	3	0	0
Nasal swab	1	0	0	1	0
Wound swab	2	2	0	0	0
HVS	9	8	0	1	0
Gastric lavage	1	1	0	0	0
Total	60	34 (56.6%)	12(20%)	13(21.6%)	1(1.7%)

Table-2: Phospholipase and Esterase activity exhibited by candida species

Species	Phospholipase activity	Esterase activity
C. albicans	28 (46.7%)	20 (33.3%)
C. tropicalis	1 (1.7%)	10 (16.7%)
C. guilliermondii	3 (5%)	3 (5%)
C. glabrata	0	0
Total (60)	32 (53.3%)	33 (55%)

Table-3:Antifungal susceptibility on candidal isolates (Figures shown in parentheses are in percentages.)

Species	AMP-B			FLC			KET			ITR			VRC		
	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R
C. albicans	34 (100)	-	-	33 (97)	-	1 (3)	32 (94.1)	-	2 (5.9)	33 (97)	1 (3)	-	34 (100)	-	-
C. tropicalis	12 (100)	-	-	12 (100)	-	-	12 (100)	-	-	12 (100)	-	-	12 (100)	-	-
C. guilliermondii	13 (100)	-	-	12 (92.3)	1 (7.7)	-	11 (84.6)	-	2 (5.4)	10 (76.9)	3 (23.1)	-	13 (100)	-	-
C. glabrata	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-

AMP-B-Amphotericin B, FLC-Fluconazole, KET-Ketoconazole, ITR-Itraconazole, VRC-Voriconazole, S-Susceptible, R-Resistant, SDD-Susceptible dose dependent.

IV. Discussion

Candida is an asexual, dimorphic fungi present as normal flora in humans. A small number of candida species are pathogenic for humans. Candida infections may be primary or secondary. These organisms are capable of causing superficial and deep seated infections such as cutaneous, mucocutaneous, subcutaneous and systemic candidiasis. Candida is a commensal and it acts as pathogens when host defenses are interrupted. Risk factors of Candida infections include prolonged stay in ICU's, diabetes mellitus, prolonged usage of antibiotics, immunosuppression, defects in CMI etc. [(4)]

A total of 60 Candida isolates were collected from different clinical specimens, of which sputum samples showed the highest number of isolates (26.7%), followed by urine (23.3%) and high vaginal swabs (15%) respectively. The incidence of C. albicans was 56.6% where as C. tropicalis was 20%. C. guilliermondii also showed an incidence of 21.6% and C.glabrata was 1.7%. The six isolates out of 13 of C.guilliermondii are from blood and all are from pediatric age group. Other species were not encountered. Basu et al found an incidence of C. albicans of 45.8% where as C. tropicalis was 24.7%, C. guilliermondii was 3.5% and C.glabrata was 1.1%.[(5)] In an elaborate study on incidence of C. guilliermondii Pfaller et al found an incidence of 1.4% from over 75,761 isolates collected from 127 medical centres. [(6)]

The phospholipase activity which is thought to help the yeast in tissue invasion was found in 46.7% of C. albicans and 6.7% of non albicans candida. Others too found similar rates , but Fule et al noted an incidence of 81% C. albicans strains producing phospholipase activity .[(7)] The other hydrolytic enzyme namely esterase was found in 33.3% of C. albicans and 21.7% of non albicans strains. Aktas et al noted an incidence of 46.4% of C.albicans and 32.8% of non albicans strains producing esterase activity.[(8)]

The present study showed that all C. albicans isolates are susceptible to amphotericin B and voriconazole. Shivanand, Araj too got similar observations but Amina got a susceptibility of 78.3% only. Similarly against voriconazole too Amina found only 30.4% susceptibility.[(1,2,9)] Against the frequently used antifungal fluconazole, we noticed 97% susceptibility similar to Ogba and Araj.[(1,10)] However, Amina et al found only 30.4% susceptibility to fluconazole too which is quite less than the other studies.[(9)] The same difference persisted in the susceptibility pattern against ketoconazole and itraconazole. All 12 (100%) C. tropicalis isolates are susceptible to all the antifungals tested whereas Shivanand found 87.5% isolates being susceptible.[(2)] However Ogba found only 40% and 50% of their isolates being susceptible to ketoconazole and itraconazole respectively.[(10)] Pfaller have noticed C. guilliermondii to have decreased susceptibility to fluconazole over voriconazole. It is a rare cause of invasive fungal infections like septic arthritis, candiduria, endocarditis and osteomyelitis.[(6)] All isolates in the present study are from blood and are susceptible to voriconazole where as only 92.3% are susceptible to fluconazole.

V. Conclusion

Thus in the present study on 60 candidal isolates from various clinical specimens, C.albicans is found to be the highest and they also were susceptible to amphotericin-B and voriconazole. C.guilliermondii were isolated from 13 specimens and out of these six were from blood and they were all susceptible to amphotericin-B and voriconazole. Fluconazole susceptibility was 97% for C.albicans and 100% for C.tropicalis and 92.3% for C.guilliermondii.

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