IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 4 Ver. V (Jul - Aug. 2015), PP 44-47
www.iosrjournals.org

Detection of enzymatic activities of Candida species isolated from hospitalized patients in Hilla

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Abstract: *To investigate some virulence factors in Candida species isolated from patients with suspected fungal infection in Hilla .*

A total of 200 Candida isolates were isolated ; Candida albicans was the predominant species 123 (61. 5%) , followed by C. tropicalis 38 (19 %) , C. glabrata 24 (12%) , C.parapsilosis 8 (4%) , C. krusei 5 (2.5 %) , C. guillermonidii 1 (0.5 %) , C . dubliniensis 1 (0.5 %) .

In the present study ,phospholipase activity of Candida species were demonstrated using egg yolk agar while casein agar was used for proteinase activity .

C. albicans and C.tropicalis were the most active in producing hydrolytic enzymes ,since the diameter of the reaction zones of C.albicans was(30,25)mm for phospholipase and protease respectively , and for C.tropicalis (27 ,30) mm for phospholipase and protease respectively.

Key words*: Candida species , protienase , phospholipase . virulence .*

I. Introduction

Candida species produce a wide spectrum of disease , ranging from superficial mucocutanous disease to invasive illness such as hepatosplenic candidiasis , peritonitis and systemic candidiasis (Odds ,1988 ; Pfaller et al , 2002 ; Laupland et al . , 2004 ; Pfaller &Diekema , 2007, Klis et al ., 2009) .

A number of putative virulence factors have been suggested in the enhancement of Candida species pathogenesis . These include yeast to – hyphal form transition , phenotypeswitching,molecular mimicry,adhesion factors or surface hydrophobicity , secretion of phospholipase and aspartyl protienase (Ghannoum , 2000 ; Abu- Elteen et al , 2001; Kuriyama et al , 2003 ,Tsang et al , 2007) . Among the most important hydrolytic enzymes are phospholipase and secreted aspartyl proteinase (Saps) that play an important role in adherence ,tissue penetration ,invasion and distraction of host tissues . (Schaller et al ., 2005 , Sliva et al ., 2011) .The aim of this study was to evaluate some virulence factors of Candida spp. isolated from clinical samples .

II. Materials and methods

Collection of samples

During the present study , a total of 330 samples were collected from patients suffering from candidiasis from December 2013- October 2014 , each sample was collected by disposable sterile swabs from skin ,vagina and oral cavity . Each specimen was inoculated on Sabourauds agar and CHROM agar plates and incubated at 37 C^0 for 24 – 48 hrs .

Identification

All isolates were identified by colony morphology , gram staining , germ tube formation , HiChrom Candida agar , and conventional assimilation reaction kit **(**Rapid ID Yeast Plus System **) .**

Extracellular protienase production

Detection of extracellular protienase was tested using Casien media, after inoculation of the medium with yeast isolates , and incubation for 24 – 48 hrs at 37 C^0.The positive result was read by observing the transparent area around the colony.

Phospholipase hydrolysis

Egg – yolk agar was used to detection of phospholipase producing by Candida , After inoculation of the medium agar, then plates incubated at 37C^0 for 24 – 48 hours .The positive results were represented by appearance of precipitation zones around the colonies (Cruickshank , 1975) .

Statistical analysis

Statistical analysis was conducted by LSD to determine significant differences in extracellular phospholipase production and proteinase production (Paulson , 2008).

III. Results

As shown in table (1) , 60 /123 (48.7 %) isolates of C.albicans were found to be positive for protienase production and 52 /123 (42 .2%) isolates were found to be positive for phospholipase production , 15 /38 (39.4 %) isolates of C.tropicalis were found to be positive for protienase production , 12 / 38(31.5 %) isolates were found to be positive for phospholipase production ,1/24 (4.1%) isolates of C.glabrata were found to be positive for protienase production , 2/ 24 (8.3 %) isolates were found to be positive for phospholipase production , 1/ 8 (12.5 %) isolates of C.parapsilosis were found to be positive for protienase production ,0/ 8(0%) isolates were found to be positive for phospholipase production ,0 /5 (0%) isolates of C.krusei were found to be positive for protienase production ,1/ 5 (20%) isolates were found to be positive for phospholipase production and one isolate of Candida guillermondii has ability to produce protienase and phospholipase 100% .Finally, C. dubliniensis has the ability to produce protienase 1/1 (100%) and has no ability to produce phospholipase (0%). Fig (1) , Fig (2) .

Table (1):Phospholipase and protienase detected in Candida spp. isolates .

Isolates	Protienase (no%)	Phospolipase (no%)
C.albicans	60 (48.7%)	52 (42.2)%
C.tropicalis	15 (39.4%)	12(31.5 %)
C.glabarta	1 (4.1 %)	2 (8.3 %)
C. parapsilosis	1 (12.5%)	0 (0%)
C. krusei	0 (0%)	1 (20%)
C. guillermondii	1 (100%)	1(100%)
C.dubliniensis	1 (100%)	0 (0% **)**
Total	79 / 200 (39 .5%)	68 /200(34 %)

Results shown in table (2) indicate that the Candida spp. have the ability to produce phospholipase and protienase . It appears that the two species of Candida : Candida albicans and Candida tropicalis were more efficiency in producing phospholipase and protienase with diameter zones of hydrolysis (30,25mm), respectively, and for the second (27 ,30 mm) respectively , followed by the isolates of Candida krusei and Candida glabrata with diameter zones of hydrolysis for phospholipase , (10,3mm) respectively .Whereas no positive reaction in hydrolysis of phospholipase for the three species of Candida:, C.dubliniensis ,C.parapsilosi and C. guillermondii ,C. glabrata , C. dubliniensis and C. parapsilosis appeared positive reactions for protienase with diameter (3 , 3, 10) respectively , whereas no positive reactions for hydrolysis (protienase) for isolates Candida krusei, and C. guillermondii .

Table (2):Diameter (mm) of hydrolysis zone of protienase and phospholipase for isolated Candida sp.

Candida spp. (no)	Phospholipase (mm)	Protienase (mm)
C. albicans	30	25
C. tropicalis	27	30
C. krusei	10	-
C.glabrata	3	3
C.dubliniensis	-	3
C. parapsilosis	-	10
C .guillermondii.	-	-
LSD (0.05) for protienase = 1.214 , (significant) **LSD (0.05) for phospholipase = 1.086 , (significant)**		

Egg yolk agar : for production phospholipase
Casien agar : for production protienase

Fig (1): Protienase production , Hdrolysis zone (arrow)

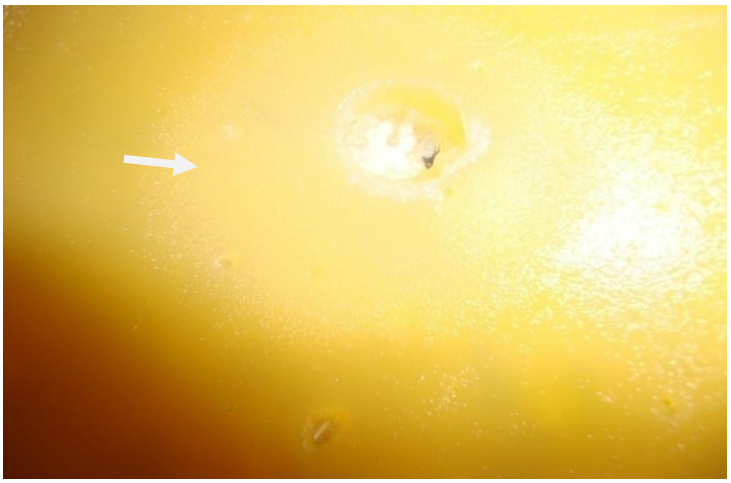

Fig (2) Phospholipase production , precipitation zone , arrow

IV. Discussion

In the present study, different percentage of secretion for protienase and phospholipase from Candida species were detected , as shown in table (1). Many reports demonstrated that the enzymatic production of C. albicans and other species isolated from different clinical conditions and anatomical sites indicated a variation of (62.5 –100%) for protienase activity which is consistent with a number of studies (Ruchel et al ,1982 ; Samaranayke et al ,1984 ; Maffei et al ,1997 ; Pichova et al , 2001 , Fotedar &AL- Hedaithy , 2005 , Oksuz et al ., 2007).

Variations in different hydrolytic zones of protienase and different phospholipase were statistically significant :LSD (0.05) for protienase = 1.214 , LSD (0.05) for phospholipase = 1.086) in Candida sp. due to various anatomical sites isolated from their as shown in table (2) .These findings are in agreement with Sukru et al. 2007 who found that phospholipase activity of Candida spp. was found to be higher in oral (59.0%)and fecal (42.8%) isolates and that protienase activity of Candida spp. was found to be higher in urogenital (55.1%) and skin (58.8%) isolates .

Some studies (Price et al,1982 ;Wu et al , 1996) have reported that phospholipase activity detected in 30 to 100 % of Candida species isolated from various groupsof patients,also Samaranake et al .(2006) showed that phospholipase gene expression has been affected by growth conditions .

In contrast Kantarcioglu &Yucel (2002) could not found any differences in phospholipase or protease activity between the isolates of Candida spp. from various anatomically sites .

Lower virulence of C. dubliniensis compared to the virulence of C. albicans was found in this study . It has been suggested that the reason for the comparatively low virulence is its lower capacity to form hyphae compared to C. albicans . This result was consistent with study of Stokes et al., 2007 .

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