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Virulence Compartment between Clinical and Environmental Candida Albicans Isolates

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Abstract:
The phospholipases, biofilm and germ tube of Candida albicans are considered to play a significant role in the pathogenesis of human infections. The aim of this study detection of virulence factors of Candida ssp.Therefore 60 clinical isolates of C. albicans from human and 28 isolates from environmental habitat were collected. 48 clinical isolates and 12 soil isolates were screened for phospholipase using an egg yolk agar medium production, biofilm and germ tube formation in vitro . 55% of clinical C.albicans isolates and 11.6% environmental isolates was high phospholipase producer where 1.66% of clinical isolates was low phospholipase producers isolates .

Keywords: Clinical, Environmental isolate, Candida albicans ,Phospholipase, Biofilm .Iraq.

1.Introduction:

Candida spp important opportunistic yeast (McCullough, et al., 1996) .Extensive researches on these virulence factors were focused on clinical isolates of *C. albicans*, which is considered the most pathogenic member of the genus (Luo et al.,2001;Sardi et al.,2013) .However, quite no research articles refer to virulence factor production in environmental *C.albicans*. *C.albicans* associated in particular the secretion of phospholipases is considered a key attribute that aids invasion of the host mucosal epithelia (Leidich et al., 1998). The phospholipases in general catalyse the hydrolysis of phospholipids, which are major components of all cell membranes (Banno et al., 1985; Salyers & Witt, 1994).

It is also known that in clinical isolates of *C. albicans* higher levels of extracellular phospholipase activity correlate well with a number of other pathogenic attributes of the yeast, invasion of host tissues as in vaginal candidiasis (Ribeiro et al., 2004). Hence the aim of the current study were to screen 60 clinical and environmental isolates of *C. albicans* from individuals for phospholipase activity, and correlate enzyme production in vitro with phospholipase expression in egg-yolk agar.

2.Materials and methods

One hundred forty eight samples were collected ,88 clinical sample(61 buckle swabs,27 vaginal swabs) and 60 soil sample from different regions

the higher the phospholipase activity (Deorukhkar and S. Saini 2014).To minimize experimental error, the assay was conducted in duplicate on three separate occasions for each isolate.

Isolates of *C. albicans* were inoculated in triplicate. Each culture was incubated at 37°C for 5-6 days. Calculation of the zone of phospholipase activity was performed according to Price et al. (1982) with some modifications Method. Phospholipase activity was measured by dividing colony diameter by the diameter of precipitation zone (Pz) around the colony formed on the plate.

$$Pz = \frac{\text{Colony Diamtere}}{\text{Colony Diameter + Zone of preciptation}}$$

Five classes were described for phospholipase activity including; Pz value = 1 means that the test strain is negative for phospholipase, while a value of Pz <0.90- 1 = nigative phospholipase activity, 0.8-.89= low phospholipase activity, 0.70-0.79= moderate phospholipase activity and <0.69 = strong phospholipase activity

Germ-tube production.

Germ-tube production in *C. albicans* was measured by using a slightly modified method described by Ibrahim et al. (1995). Briefly, yeast grown overnight in an SDA plate were harvested and a cell suspension of $1x10^7$ cell/ml .loop full of suspension was added to 500 µL of human serum and incubated at 37 ºC for 3 h in 0.2ml tube. The tube was then vortexes gently , 20 µL of suspension was mixed with blue cotton stain and examined under field microscope .Yeast cells with a germ tube that had no constriction at the junction between the cells were considered as germ tube-positive. Any remaining yeast cells clumped with germ tubes and pseudohyphae were excluded.

2.3.Biofilm Formation

The ability of *C.albicans* isolates to form biofilms was assessed by the tube method described by

(arid soils, hospital gardens, field soils) were randomly collected

buckle and virginal swab were streaked on the surface of Sabouraud dextrose agar (SDA) plate and incubated for 48 h at 37 ºC. The yeasts were maintained in eppindroff tubes and subcultured monthly on SDA and maintained at 4 ºC during the experimental period.

2.1.Culturing on CHROMagar Candida.

The phenotypic characteristic of *Candida* isolates were further defined by culture on CHROMagar Candida plates. The CHROMagar Candida differential agar is a rapid contains chromogenic substrates that react with enzymes secreted by the target Candida spp. to yield colonies of varying colors., single colonies were re -cultured on CHROMagar and incubation for 24-48h at 30 ºC. only colonies showed green colors identified as *C.albicans* based on Nadeem et al ., (2010).

2.2.Determination of phospholipase activity

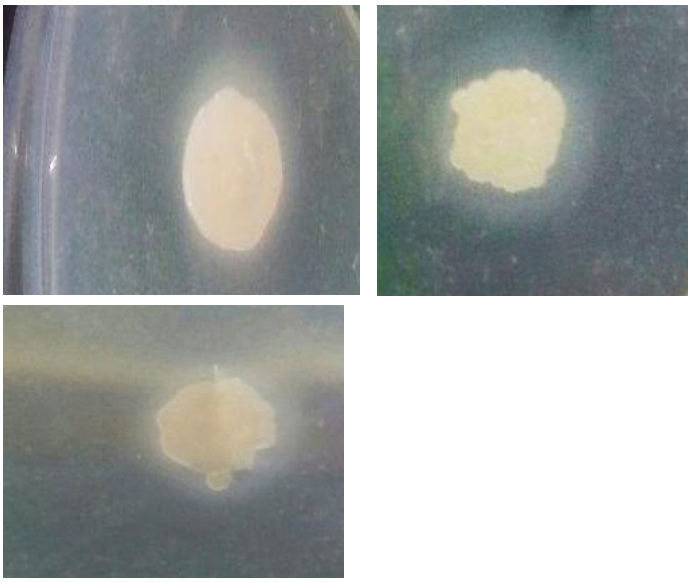
The phospholipase activity of *C.albicans* was detected by the method of Samaranayake et al. (1984). Approximately 5 µL of standard inoculum of test strain containing 10^8 Candida cells/mL was aseptically inoculated onto egg yolk agar. The plates were dried at room temperature and then incubated at 37°C for 48 h. The plates were examined for the presence of precipitation zone around the colony. The presence of precipitation zone indicated expression of phospholipase enzyme.

The phospholipase index (Pz) was defined as the ratio of the diameter of the colony to the total diameter of the colony plus the precipitation zone. A Pz value of 1 denoted no phospholipase activity; Pz < 1 indicated phospholipase production by the isolate. The lower the Pz value,

Figure 1:Colony appearance of *Candida albicans*. On CHROMagar.

3.1.Phospholipase production by *C. albicans*.

Phospholipase activity was detected in 100 % of the *C. albicans* isolates in this study. Previous studies have reported phospholipase activity in 30 to 100 % of *Candidal* isolates from various groups of patients and from various sites. Price et al., (1982) reported to the phospholipase activity has been found in 55, 50 and 30 % of the Candida species isolated from blood, wound infections and urine, respectively . Our results showed a marginally higher phospholipase positivity (Figure 2) .similar results was obtained by Kothavade & Panthaki (1998), while Mahmoudabadi (2010) observed that 56 of the 60 (93·3 %) clinical *C. albicans* strains isolated from the oral cavity, respiratory tract, blood and urogenital systems of non-immunocompromised individuals were phospholipase producers(Calderone & Fonzi (2001)). These data imply that a vast majority of clinical *C. albicans* isolates are phospholipase producers (Tsang et al.,2007).



Yigit et al.,2008 Colonies of *C.albicans* from Sabouraud dextrose agar were inoculated in saline and incubated overnight at 37°C. 0.5 mL of this saline suspension was added into screw capped conical polystyrene tubes containing 5 mL of Sabouraud dextrose broth supplemented with glucose (final concentration of 8%). The tubes were incubated at 35°C for 48 h without agitation.

After incubation the broth from the tubes was aspirated gently using Pasteur pipette. The tubes were washed twice with distilled water and stained with 2% safranin. The stain was decanted after 10 min. The tubes were rinsed with distilled water to remove excess stain. Presence of visible adherent film on the wall and at the bottom of the tube indicated biofilm formation.

3.Results and discussion

A total of 148 *Candida* were isolated, of which 60 were classified as *C.albicans* showed green color on CHROMagar medium, whereas the remaining were classified as non-albicans showed white or pink to white pink color on CHROMagar medium (Figure 1) .CHROMagar which was demonstrated to be the presumptive test but less accurate evidence. These results were coincidence with Odds & Bernaerts (1994). Also all *C. albicans* produced germ-tube ,this results agree with Ibrahim et al. (1995).

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Table 2:Evaluated biofilm formation by *C.albicans*

Number of *C.albicans* and percentage	Biofilm formation scale		
	Strong +++	Moderate ++	Low +
	34(56.6%)	23(38.3%)	3(5%)

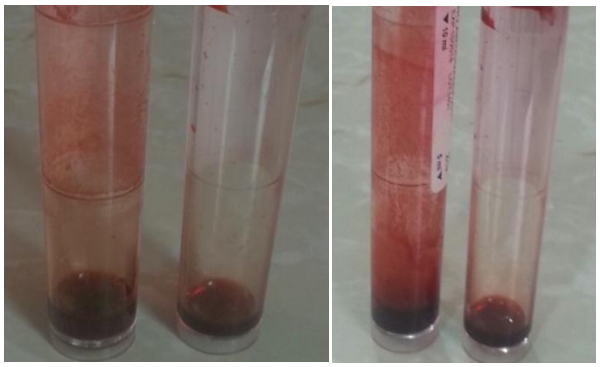

Figure 3. Biofilm formation as virulence factors produced by *C.albicans* isolates in vitro.

Many articles detected the virulence factors for clinical isolates of *C. albicans*, which is considered the most pathogenic member of the genus Sardi et al.,2013 .However, quite a few research articles refer to virulence factor production soil *C.albicans*. In the present study, biofilm formation was noted in 100% of *C. albicans* isolates. Seneviratne, et al., 2008 Singhai et al. ,2012

Phospholipases facilitate the invasion of the host mucosal epithelia by hydrolyzing one or more ester linkages in glycerophospholipids Singhai et al. ,2012 . In our study phospholipase was the major virulent factor expressed by *C. albicans* isolates(Deorukhkar, and Saini ,2013). The present study also demonstrated high phospholipase activity in biofilm forming isolates of clinical and environmental isolates . Screening of phospholipase production in biofilm forming isolates can be used as an important parameter to

Figure 2:Phospholipase precipitation sing around *C.albicans* colonies.

The P_Z values(Table 1) showed that the mean phospholipase activity of isolates from the clinical group was higher than that of the soil isolates. PZ scale of 33 clinical isolates and 12 soil isolates) *Candida albicans* isolates, All isolates of *C. albicans* tested, 60 (100 %) were Phospholipase positive according to the plate assay. No significant difference in phospholipase activity could be detected between the isolates from the clinical and environmental isolates (Table 1).Our results showed that phospholipase enzyme was the major virulent factor produced by *C.albicans* isolates. *C.albicans* isolated from vaginal swabs and soil cultures showed maximum phospholipase activity.. A similar result (79 %) was reported for 41 oral *C. albicans* isolates by Samaranayake et al. (1984) using an identical plate assay.

Table 1:Hydrolytic enzyme activity for 60(33 clinical isolates and 12 soil isolates) *Candida albicans* isolates

Activity zone of phospholipase enzyme(pz)				
Scale	**High 0.69-0.70**	**Moderate 0.71-0.79**	**Low 0.8-.89**	**Negative 0.99-1**
percentage	**66.6%(55+11.6)**	**(31.66%)**	**(1.66%)**	**(0%)**
Total isolates	**33*+7**=40**	**14*+5**=19**	**1***	**0**

*clinical isolates,**soil isolates

3.2.Biofilm formation

Biofilm formation as virulence factors produced by *C.albicans* isolates are shown in Table 2 and Figure 3. Biofilm formation was high scale seen in 34 (56.6%) , moderate in 23 (38.3%) and low scale in 3(5%) isolates. *C.albicans* isolated from buckle and vaginal samples demonstrated high biofilm production capacity.

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4.Conclusion

The identification and demonstrated high phospholipase activity in biofilm forming isolates of clinical and environmental isolates . Screening of phospholipase production in biofilm forming isolates can be used as an important parameter to determinate the risk factor of between clinical and environmental isolates will aid in the understanding of the pathogenesis of infection.

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