Virulence Compartment between Clinical and Environmental Candida Albicans Isolates

Zaidan Khlaif Imran¹, Zahra Abd Al-Karrem²

All women Science college, Babylon University, Hilla, Iraq

*Correspond author: Zaidan Khlaif Imran

Email- zaidan.omran@yahoo.com

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).
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.

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

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 = negative 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 1x10⁷ cell/ml loop full of suspension was added to 500 µL of human serum and incubated at 37°C 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 °C. The yeasts were maintained in epipindroff tubes and subcultured monthly on SDA and maintained at 4 °C 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 °C, 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 10⁶ 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,
Colonies of Candida 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).
Figure 2: Phospholipase precipitation sing around Candida albicans colonies.

The PZ 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

<table>
<thead>
<tr>
<th>Activity zone of phospholipase enzyme (PZ)</th>
<th>Scale</th>
<th>High 0.69-0.70</th>
<th>Moderate 0.71-0.79</th>
<th>Low 0.8-0.89</th>
<th>Negative 0.99-1</th>
</tr>
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<tbody>
<tr>
<td>percent age</td>
<td>66.6 % (55 +11.6)</td>
<td>(31.66 %)</td>
<td>(1.66 %)</td>
<td>(0 %)</td>
<td></td>
</tr>
<tr>
<td>Total isolates</td>
<td>33*+7**=40</td>
<td>14*+5**=19</td>
<td>1*</td>
<td>0</td>
<td></td>
</tr>
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</table>

*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.

Table 2: Evaluated biofilm formation by C. albicans

<table>
<thead>
<tr>
<th>Number of C. albicans and percentage</th>
<th>Biofilm formation scale</th>
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<tbody>
<tr>
<td></td>
<td>Strong +++</td>
</tr>
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<td>34(56.6%)</td>
<td>23(38.3%)</td>
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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 determine the risk factor of between clinical and environmental isolates will aid in the understanding of the pathogenesis of infection.

References


