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. guilliermonidii 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 mucocutaneous 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 proteinase (Ghanoum, 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 Sabouraud's agar and CHROM agar plates and incubated at 37°C 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 proteinase production

Detection of extracellular proteinase was tested using Casien media, after inoculation of the medium with yeast isolates, and incubation for 24–48 hrs at 37°C. 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 37°C 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 proteinase 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 proteinase 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 proteinase 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 proteinase production, 0/8 (0%) isolates were found to be positive for phospholipase production, 1/5 (20%) isolates of C. krusei were found to be positive for proteinase production, 1/5 (20%) isolates were found to be positive for phospholipase production and one isolate of Candida guilliermondii has ability to produce proteinase and phospholipase 100%. Finally, C. dubliniensis has the ability to produce proteinase 1/1 (100%) and has no ability to produce phospholipase (0%). Fig (1), Fig (2).

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Proteinase (no%)</th>
<th>Phospholipase (no%)</th>
</tr>
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<tbody>
<tr>
<td>C. albicans</td>
<td>60 (48.7%)</td>
<td>52 (42.2%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>15 (39.4%)</td>
<td>12 (31.5%)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>1 (4.1%)</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>1 (12.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>79/200 (39.5%)</td>
<td>68/200 (34%)</td>
</tr>
</tbody>
</table>

Results shown in table (2) indicate that the Candida spp. have the ability to produce phospholipase and proteinase. It appears that the two species of Candida: Candida albicans and Candida tropicalis were more efficiency in producing phospholipase and proteinase 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. parapsilosis and C. guilliermondii, C. glabrata, C. dubliniensis and C. parapsilosis appeared positive reactions for proteinase with diameter (3, 3, 10) respectively, whereas no positive reactions for hydrolysis (proteinase) for isolates Candida krusei, and C. guilliermondii.

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

<table>
<thead>
<tr>
<th>Candida spp. (no)</th>
<th>Phospholipase (mm)</th>
<th>Proteinase (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>C. krusei</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

LSD (0.05) for proteinase = 1.214, (significant)
LSD (0.05) for phospholipase = 1.086, (significant)

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

DOI: 10.9790/3008-10454447 www.iosrjournals.org 45 | Page
IV. Discussion

In the present study, different percentage of secretion for proteinase 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 proteinase 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- Hedaitly, 2005; Oksuz et al., 2007).

Variations in different hydrolytic zones of proteinase and different phospholipase were statistically significant: LSD (0.05) for proteinase = 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 proteinase 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 groups of patients, also Samaranake et al. (2006) showed that phospholipase gene expression has been affected by growth conditions.

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

Lower virulence of C. dublindiensis 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.
References


