



## DETECTION OF CANDIDA PHOSPHOLIPASE ACTIVITY FROM CLINICAL ISOLATES

### Microbiology

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### ABSTRACT

The virulence of *Candida* species is contributed by the secretion of enzymes like phospholipase and proteinases as they play a role in invasion of host mucosal epithelia. The samples collected were urine, sputum, pus, and blood. A total of 100 samples that yielded *Candida* species on culture were studied for Extracellular phospholipase production on in-house prepared Sabouraud's dextrose egg yolk agar. Out of 100 isolates, 38 showed extracellular phospholipase activity and 62 showed absence or weak phospholipase activity.. This study will help clinicians correlate the virulence of the organism with the severity of the infection in the patient.

### KEYWORDS

*Candida*, Phospholipase secretion, Sabouraud's dextrose egg yolk agar

### INTRODUCTION

The virulence of *Candida* species is contributed by the secretion of enzymes like phospholipase and proteinase<sup>1</sup>. Extracellular phospholipase plays a major role in the invasion of host mucosal epithelia<sup>2</sup>. They hydrolyse one or more ester linkages of glycerophospholipids<sup>3</sup>. Secretion of extracellular phospholipase from *Candida* is shown by growing the yeast on solid media containing egg yolk<sup>4</sup>.

### AIMS AND OBJECTIVES

- To isolate *Candida* from various clinical samples
- To determine the secretion of phospholipase enzyme by the isolated *Candida* species.

### MATERIALS AND METHODS<sup>5,6,7,8,9,10</sup>

The samples collected were urine, sputum, and pus from out-patient and in-patient departments of Government General Hospital from September 2018 to November 2018 at random for the present study. History of the cases like age, sex, complaints, clinical diagnosis, history of chronic steroid usage, history of antibiotic treatment and the immune status of the patient were taken into consideration. All the samples were stained by Hucker's modification of Gram's stain and examined microscopically for Gram positive, budding, oval shaped yeast cells with pseudohyphae along with pus cells. The samples were inoculated onto Sabouraud's dextrose agar supplemented with 0.05% Chloramphenicol and incubated at 37°C for 48 hours. Cultures were examined for smooth, pasty, and creamy white colonies for yeasts which were again stained to confirm for Gram positive oval budding yeast cells.

Following identification of yeast-like growths on SDA, germ tube test was performed to distinguish *Candida albicans* and *non-albicans Candida*. A single colony of yeast from a pure culture was inoculated in 0.5mL human serum and incubated at 37°C for 1-2 hours. A drop of the incubated serum was placed on a glass slide and covered with a cover slip. The wet mounts were examined under the 40X objective for Germ tube formation.

All the *Candida* species isolated were screened for production of phospholipase secretion by growing them on in-house prepared egg yolk agar. The egg yolk agar was prepared by dissolving 65g SDA, 58.4g NaCl, 5.5g CaCl<sub>2</sub> in 980 ml distilled water and sterilized at 121°C for 15 minutes. Egg yolk was centrifuged at 5000 x g for 30 minutes and 2 ml of the supernatant was added to the medium cooled at 45-50°C. A 10µL suspension of 24hr old yeast culture was spot inoculated on egg yolk agar medium and incubated at 37°C for 4 days.



**Figure 1: Spot inoculation of *Candida* species on SDA-Egg yolk agar**

Phospholipase secretion is indicated by a zone of precipitation around the colony. The diameter of the precipitation zone is measured.



**Figure 2: Hazy zone of precipitation around the colony**

Phospholipase secretion, i.e. Pz value was calculated by applying the following equation.

$$Pz = \frac{\text{Colony diameter(mm)}}{\text{Colony diameter(mm)} + \text{Zone of precipitation(mm)}}$$

Based on the equation above, phospholipase secretion is classified into 5 types as follows:

Pz value	Phospholipase secretion grade
1	No phospholipase secretion
0.90-0.99	+ (Weak phospholipase secretion)
0.80-0.89	++ (Poor phospholipase secretion)
0.70-0.79	+++ (Moderate phospholipase secretion)
<0.70	++++ (Intense phospholipase secretion)

**RESULTS:**

This study was conducted to determine the secretion of phospholipase by *Candida* species. A total of 100 isolates were tested for phospholipase secretion. The specimens collected were urine, sputum, and pus. Out of 100 isolates, 72 urine samples and 28 sputum samples yielded *Candida* species on culture. None of the pus samples were positive for *Candida* species.

Following Germ tube test, 56 out of 100 showed terminal chlamydoconidia and 44 were negative for Germ tube. Those that were positive for Germ tube test were considered as *Candida albicans* and those that were negative were considered as *non-albicans Candida*.

All the isolates were screened for phospholipase secretion on Sabouraud's dextrose egg yolk agar. Intense phospholipase secretion was shown by 38 isolates of *Candida albicans*. Absence or weak phospholipase secretion was shown by 18 isolates of *Candida albicans*. None of the *non-albicans Candida* showed any phospholipase secretion.

**Table 1: Phospholipase secretion of the isolates.**

Phospholipase secretion-Pz value	Number of strains showing phospholipase secretion (no.of strains tested n=100)
1	62
0.90-0.99	0
0.80-0.89	0
0.70-0.79	0
<0.70	38

**Table 2: Phospholipase activity of *Candida albicans* and non-*albicans Candida*.**

Intense phospholipase secretion by <i>Candida albicans</i>	38/56
Absence or weak phospholipase secretion by <i>Candida albicans</i>	18/56
Phospholipase secretion by non- <i>albicans Candida</i>	0/44

**DISCUSSION:**

The immune status of the host plays a vital role in determining *Candida* pathogenicity<sup>11</sup>. Minor impairments of the host immune system can enable *Candida albicans* to cause infections<sup>12</sup>. Pathogenic enzymes secreted by *Candida* contribute to its virulence<sup>13</sup>. Mucosal *Candida* infections are more common compared to deep candidiasis<sup>14</sup>.

In the present study, it is seen that the *Candida* species were isolated highest from urine samples (72%) followed by sputum samples (28%) which correlates with Nisha Vincu Jose et al (44% from urine and 14% from respiratory samples)<sup>15</sup>. *Candida* species are a part of the normal microbial flora in healthy humans. They colonize on the external side of the urethral openings in females. Any predisposing factors such as pregnancy, diabetes, immunosuppression may lead to urinary tract infections by *Candida* species in the host<sup>16</sup>. Isolation of *Candida* species from respiratory tract is common because the organism is one of the colonizers of the respiratory region<sup>17</sup>. It causes opportunistic *Candidal* infections in patients with predisposing factors like pulmonary tuberculosis and Acquired Immune Deficiency Syndrome<sup>18</sup>.

*Candida albicans* was the most commonly isolated species (56%) and 44% of the isolates were *non-albicans Candida*, which correlates with Ravinder Kaur et al (82.51% were *Candida albicans* and 17.41% were non-*albicans Candida*)<sup>18</sup>. However, few studies show that the prevalence of *Candida albicans* is declining and that of *non-albicans Candida* is increasing<sup>19</sup>.

In the present study, 38% of the isolates had exhibited phospholipase secretion. In a study conducted by Yukthi Sharma et al, phospholipase production was seen in 81% of the total isolates<sup>20</sup>. Amongst the *Candida albicans* isolated in this study, 64% of the isolates had secreted phospholipase enzyme. This correlates with Basu et al in which it is reported that 66.6% of the isolates had shown the enzymatic secretion<sup>21</sup>. Amongst the *non-albicans Candida* tested for phospholipase secretion, none of the isolates produced the enzyme. This correlates with Samaranyake et al where no strains of *C.tropicalis*, *C.glabrata*, and *C.parapsilosis* produced the enzyme<sup>22</sup>. But, some studies show that *non-albicans Candida* species also produced extracellular phospholipase<sup>23,24</sup>.

In this study the highest phospholipase producers were isolated from urine samples. Yucel et al had shown in their study that the highest phospholipase producing *Candida* species were isolated from the respiratory tract<sup>25</sup>.

**CONCLUSION:**

*Candida* species is a general constituent of the normal microbial flora of the genitourinary tract, gastrointestinal tract and respiratory tract. In the presence of predisposing factors, the colonizers cause opportunistic *Candidal* infections<sup>26</sup>.

The absence of extracellular phospholipase enzyme may indicate less virulent nature of *Candida*. The degree of pathogenicity and virulence can be correlated to the extent of phospholipase production<sup>27</sup>.

SDA egg yolk agar is a reliable assay to detect *Candidal* phospholipases<sup>1</sup>. Detection of this enzyme might play an important role in informing the clinicians about the possible virulence of the strain<sup>28</sup>.

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