ISOLATION AND IDENTIFICATION OF CANDIDA SPECIES FROM ORAL AND VAGINAL AND DETERMINATION OF VIRULENCE FACTOR

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Abstract
Candida species are eukaryotic pathogens that live on the mucosa layer in esophagus, mouth cavity and vagina. Candida pathogenicity is enabled through number of virulence factors, the greatest important of which are those for adherence to host tissues and medical devices, biofilm formation and secretion of hydrolytic enzymes (e.g. proteases, phospholipases and haemolysins). The purpose of this study was to isolation; identify the Candida species and detection of virulence factor.

Isolation of Candida spp. from oral and vaginal and identification by Directs examination, Morphological tests, Germ tube formation, Growth at 45ºC, CHROM agar Candida culture and VITEK 2 Compact system ID-YST Kit. Determine proteinase, phospholipase, haemolysin and biofilm formation.

One hundred –thirty one specimens including high Vaginal swabs from infected women suffered from Vulvo vaginal Candidiasis (VVC) and Oral swabs. Phospholipase (PLA) and Proteinase (PrA) were produced by 48(87.3%) and 46(83.6%) respectively out of 55 isolates, whereas 7(12.7%) and 9(16.4%) have no PLA and PrA respectively from Oral Thrush. In VVC the PLA and PrA produce by 50(89.3%) and 49(87.5%) respectively out of 56 isolates, whereas 6(10.7 %) and 7(12.5%) have no PLA and PrA respectively. Haemolysin was produced by all 111 isolates Candida spp. The ability of Candida spp isolates to produce biofilm were evaluated, 45 (81.8%) out of 55 isolates in Oral Thrush have the ability to biofilm production, while 10(18.2%) have note. In VVC, out of 56 isolates 50(89.3%) have the ability to biofilm production and 6(10.7%) have note.

Different species of Candida isolated and identification from oral and vaginal. The capacity of Candida species to produce proteinases, phospholipases, haemolysins and biofilm formation.

Key words : Candida species, Vulvo vaginitis, Virulence factors.

Introduction
The Candida species are Gram positive, budding yeast cell that harvests pseudo hyphae both in culture and in tissues and excretions they exhibition filamentous mycelial morphology in the saprophytic phase, grow at 37°C. Mono-cell yeasts with small (4-8µm) ovoid cells, polymorphic. Candida species produce well on most culture media and usually do not need special cases to growth (Ogba et al., 2013). Approximately 30 container species cause injury to humans. C. albicans is the most common species, then other pathological species containing C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, C. lusitaniae, C. guilliermondii, C. kefyr, C. rugosa, C. inconspicua, C. dubliensis, C. famata, C. norvegensis, C. lipolytica, C. sake, C. apicola, C. pulcherriima, C. zeylanoides, C. pelliculosa, C. valida, C. intermedia, C. haemulonii, C. stellatoidea, C. humicola, C. utilis, C. ciferrii, C. lambica, C. holmii, C. marina, C. humicola, C.s phaerica and C. colliculosa ect. (Pfaller, 2010). Virulence is the ability of microorganism to multiply and cause diseases (Casadevall, 2007). Virulence in C. albicans and additional pathogens contains host recognition, allows the pathogen to bind to host cells and proteins. Moreover, degradative enzymes production a special part in virulence. Fungal attack is allowed more by the transition among yeast cells and filamentous growth than by yeast growth (Cullen and Sprague, 2012). Excretion of proteinases by pathogen is required in command to degrade the tissue barriers and obtain nutrition at the
infection site. Secreted aspartyl proteinases (SAPs) from Candida have been described that hydrolyze many proteins for example hemoglobin, albumin, collagen, keratin, laminin, fibronectin, salivary lacto Ferin, mucin, interleukin1b, cystatin A, and Immunoglobulin a (Hube et al., 1998). The excretion of extracellular phospholipases by C. albicans was first described in the 1960s by Costa et al., (1967). The “phospholipases” mentions to a heterogeneous collection of enzymes that decomposes one or more ester bonds from glycerophospholipids (Ibrahim, 1995). The production of haemolysin plays a vital part in virulence. Haemolysin is important for existence and is connected to the acquisition of iron (Vaughn and Weinberg, 1978). Biofilms container is distinct as groups of microorganisms, frequently binds to a surface and encased inside an extracellular polysaccharide matrix that is shaped by the microorganisms (Costerton et al., 1995).

**Materials and Methods**

**Isolation of Candida isolates**

From November 2016 to December 2016 one hundred specimens including high Vaginal from patients presented with Vulvovaginitis and Oral swabs from children suffering from Oral thrush sample were inoculated on the fit culture media including (Sabouraud dextrose agar with chloramphenicol). All plates were raised aerobically at 37°C for 24,48 hrs (Vandepttie et al., 1991; Colle et al., 1996).

**Identification of Candida isolates**

Single colonies were isolated after primary positive cultures and identified giving to the standards of (Murray et al., 1999; Milan and Zaror., 2004) that were comprised the following test:

**Directs examination**

Specimens of high vaginal and oral swabs were placed on clean slide mounted with drop of KOH 10 %, cover-slip and then the slide wormed mildly and then examined under the microscope looking for Candida budding cells, Moreover the isolates were stained with Gram stain to detect their reply to stain.

**Morphological tests**

All isolates were grown Sabouraud dextrose agar plates and incubated at 37°C for 24-48 hrs. To isolate the Candida colonies are pure to study their shape, size, and color and texture.

**Germ tube formation**

The production of germ tube from yeast isolates done by inoculation a small portion of an isolated colony in 0.5ml of human serum, The suspension was inoculated at 37°C for 3hrs. Then a drop of this suspension was put on clean glass slide covered by cover-slip and examined under light microscope. The germ tube seemed as short lateral hyphen filament. The serum used in this test was prepared by aspirating blood from healthy human being into test tube without anticoagulant agent and then incubated in an upright position at room temperature for 30 minutes, then the samples were centrifuged at 3000 rpm for 15 minutes, then the serum was aspirated carefully by sterilized micropipette and placed in sterilized tube and stored at deep freeze at -8°C until use (Yan et al., 2013).

**Growth at 45°C**

Growth at 45 °C was considered a useful test of differentiation C. albicans from C. dubliniensis. The medium (SDA) was inoculated with Candida colonies and incubated at 45°C for 24 hrs(Abood, 2014).

**CHROM agar Candida culture**

All isolates were cultured on a dam at 30 ° C for 48 hours. After that, she was seeded on Chromagar Candida and incubated at 30 ° C for 48 hours. Chromag allows selective yeast isolation, and colonies identified of C. dubliniensis, C. albicans, C. krusei and C. tropicalis by morphology and color reaction due to development enzymatic activity (Hospenthal et al., 2006). The strains were identified according to the manufacturer’s instructions known as C. albicans or C. dubliniensis as green colonies and C. tropicalis as blue steel colonies and C. krusei colonies. The color of roses and the wooden side, and other species as white colonies.

**VITEK 2 Compact system ID-YST Kit**

The VITEK 2 Compact system is devoted to the check identification of the significant Candida species clinically next protocols described by the manufacturer (BioMerieux Inc., Durham, NC 27712, USA), counting the VITEK® Compact instrument, 21 CFR parts 11 compliance (for electronic records and signatures) and a colorimetric regent card (YST). Reagent cards have 64 wells that can each contain an individual test substrate. The substrates measure different metabolic activities such as acidification, alkalinity, enzymatic degradation, and growth in the presence of inhibitory substances. Visually clear film is located on both sides of the card allowing the proper level of oxygen transmission while maintaining a sealed vessel that prevents contact with the animal substrate mixtures. Cards contain bar codes containing information about the product type, piece number, expiry date, and unique identifier that can be linked to the sample either before or after the card is loaded.
A. Suspension preparation

A sterile swab was used to transfer sufficient colonies of pure culture of Candida spp that growth on SDA and to hang the microorganism in 3 ml of sterile saline in a 12×75 mm clear plastic (polystyrene) test tube. The turbidity is attuned (1.80-2.20) and measured using a turbidity meter called the DensiChekTM.

B. Inoculation

Identification the cards are vaccinated with suspension of microorganisms using an integrated vacuum device. A test tube containing candida suspension was placed in a special rack (cassette) a select wire was placed in the adjacent slot while inserting the transfer tube into the corresponding suspension tube. Audiobooks can accommodate up to 10 tests, filled cassette manually placed in the vacuum room station.

C. Card sealing and Incubation

The vaccination cards are passed by a mechanism, which cuts the transfer tube and card seals before loading into a circular incubator.

D. Optical system

The optical permeability system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure the turbidity or color products of substrate metabolism. In addition, a special algorithm is used to eliminate false reading because small bubbles may exist.

E. Test Reactions

The calculations were performed on the raw data and compared with the thresholds to determine the reactions for each test. On the VITEK 2 antibody, the result of the test interaction appears as “+”, “-”, “+” or “.”. The interactions shown in parentheses are indicative of weak reactions that are very close to the test threshold”

Virulence factor test

Preparation of yeast inoculums

Candida species isolates was activated on Sabouraud dextrose agar after overnight incubation at 37°C on SDB, a small amount of activated pure colonies transfer by using a sterile loop into 5 ml of PBS at turbidity equal to 1 McFarland. The final comment has been modified to contain 3×108 yeast cells/ml (Deepa et al., 2015).

Detection of proteinase production

Ten microliter of freshly prepared yeast inoculum equal to one McFarland (3×108 CFU/ml) was inoculated into our face of the proteinase agar medium. Plates incubated at 37°C for 48-72 hrs. The diameters of together colony and proteinase zone were measured. Record the protease activity as the grade (-) when not clear zone around colony was present, grade (+) for mild activity when visible proteolysis was limited to 1-2 mm around the colony and double positive (++) for strong activity when the area is analyzed from 3-5 mm around the colony (Akâdarâlar et al., 2011)

Detection of phospholipase production

Ten microliter yeast suspension equal to one McFarland (3×108 CFU/ml) was incubated on the surface of the egg yolk agar, after the medium was incubated at 37°C for 24-48 hours. A positive result was the pilot when a clear area appeared around the colony (Tsang et al., 2007). The result of phospholipase activity was calculated by using this formula: Pz value= Colony diameter/ (Colony diameter + Zone of precipitation. When the value of Pz 1 means that the test strain is negative for phospholipase activity, while the value of Pz <0.64-0.99 = moderate Activity of phospholipase (+++) and <0.63 = large Activity of phospholipase (+++) (Deepa et al., 2015).

Detection of haemolysis activity

Ten microliter yeast suspension equal to one McFarland (3×108 CFU/ml) was spotted on human blood SDA with 3% glucose. Plates were incubated at 37°C for 48hrs. The colony diameter ratio was used for a translucent area of hemolysis (mm) as the degradation index (Hz value) (Tsang et al., 2007). The results of hemolytic activity (Hz) where calculated by using this formula: Hz value = Colony diameter / (Colony diameter + Zone of precipitation) According to this system, activity ranges were established according to the Hz index: Hz<0.69=very strong (++++), Hz = 0.70- 0.79= strong (+++); Hz = 0.80- 0.89=mild (++);Hz = 0.90- 0.99=weak (+); and Hz =1 mean Negative.

Determination of biofilm formation

The Candida was evaluated for the composition of the biofilm using the method described Melek et al. (2012).96-well sterile microplates were used to evaluate the formation of biofilm. The yeast culture was grafted using a loop in a tube containing 2 ml of brain heart infusion broth medium with glucose (0.25%) and incubated at 37°C for 24hrs. Then all tubes were diluted by 1:20 using fresh BHIB. From this final solution, 200µl are placed in 96-well sterile polystyrene plates. The microscopic plate was covered with lids and incubated at 37 °C for 24hrs after incubation and rinsed the microplate with PBS three times and then inverted the
splash. Then add 200 µl of 1% violet crystal to each well, followed by incubation for 15 minutes. After incubation, rinse again a microplate with PBS three times. Then 200 µl of ethanol: the acetone mixture (80:20 w/v) was added to each well. They were read at 450nm using an enzyme-linked immunosorbent assay (ELISA) reader and I recorded for each well. Three wells were used to form biofilm and the mean was used for three readings in the analysis. Negative BHIB was used without a dynamic object as a negative control, and samples with a value greater than the value of the pieces were considered positive, while those with a lower value were considered negative. Mean values of OD values were calculated in the sterile medium and subtracted from all test values. The optical density (OD) of each isolate was compared with the mean absorbance of negative controls (ODnc) sample with a ODs =ODnc Means any biofilm production, Weak production of biofilm (ODnc < ODsP < 2ODnc), moderate biofilm production (2ODnc < ODsP < 4ODnc) and strong production of biofilm (4ODnc < ODs) (Rodrigues et al., 2010).

Results and Discussion
Distribution of Candida vulvo vaginitis between women in diverse age groups

One hundred and eleven cases (84.73%) were gave positive results for Candida spp., fifty six (42.74%) were isolated from VVC. The result indicated that the highest infection with VVC occurred in the age collection of 20-30 years with the percentage of 59%, followed by age group less than 20 years with percentage of 25%, then the age group 31-40 years with percentage of 12.5%, finally the age group of 41-50 years 3.5% (Table 1).

Table 1: Age distribution of VVC infection.

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>No. of infected woman</th>
<th>Age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>14</td>
<td>Years&gt; 20</td>
</tr>
<tr>
<td>59%</td>
<td>33</td>
<td>20-30 years</td>
</tr>
<tr>
<td>12.5%</td>
<td>7</td>
<td>31-40 years</td>
</tr>
<tr>
<td>3.5%</td>
<td>2</td>
<td>41-50 years</td>
</tr>
<tr>
<td>100%</td>
<td>56</td>
<td>Total</td>
</tr>
</tbody>
</table>

Vulvovaginal Candidiasis is the second most recurrent infection of the female reproductive system. Through a woman’s lifetime, nearly 75% will have a Candida vulvovaginitis (Grigoriou et al., 2006). Increased incidence in this age group is due to appropriate conditions such as pregnancy, antibiotics and contraception. Estrogen level will increase in vaginal epithelial cell with the increase of glycogen content which enhance lactic acid bacteria that consumed glycogen which cause decrease in vaginal pH, and that considered as an encouraging condition to distribution and Candida growth and that is essentially agree with Sobel (1993), Mahdi and AL–Hamadani (1998).

The distribution of oral candidiasis in patients according to age groups

Fifty five cases (41.98%) out of (111) cases, showed positive results for Candida spp, were isolated from Oral Candidiasis. The highly positive results of oral thrush occurred in the age group that less or equal one month with the percentage of 84% followed by age group of 2-6 months with percentage of 16%, as in table 2.

Table 2: Oral Candidiasis in children according to age.

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>No. of infected children</th>
<th>Age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>84%</td>
<td>46</td>
<td>month ≤ 1</td>
</tr>
<tr>
<td>16%</td>
<td>9</td>
<td>2-6 months</td>
</tr>
<tr>
<td>100%</td>
<td>55</td>
<td>Total</td>
</tr>
</tbody>
</table>

The result of our study partially compatible with the result of Sachdeva et al., (2016) who evaluated the rate of neonatal oropharyngeal Candidiasis has increased 4.7 times after few days from childbirth. That also compatible with Martins et al., (2014) who reported after some day’s childbirth and that species colonize the mucosa of the gastrointestinal tract (40-50%) and the upper respiratory passages, as well as the mouth, pharynx and larynx.

Identification of Candida species from Vaginal and Oral swab

Candida spp identified contingent on the morphological features on culture medium, germ tube formation and Vitek 2 compact system.

KOH direct amount

High Vaginal and Oral Thrush swab observed directly by using KOH10%. Candida spp seem as oval to spherical budding cells around epithelial cell. Analysis of C. glabrata vaginitis is harder than that of typical Candida vaginitis. This is for the disappointment of the C. glabrata organisms to form pseudo hyphae and hyphae in vivo. Accordingly, on the saline and KOH microscope many of the yeasts are seen in its infancy but the Hypha elements are absent.

Cultural Characteristics

Morphology of colonies Candida species on Sabouraud dextrose agar (SDA) were, curved, white to cream, round, soft and smooth to wrinkled (Figs 1-1), by characteristic yeast odor, It has grown rapidly and matured in 1 days. These results are agreed with Larone (1995) and Bhavan et al., (2010). Candida glabrata
Shiny, smooth, and creamy colored colonies are relatively indistinguishable from those other Candida species except their relative size, which is very small. *C. glabrata* is the only type of candida that does not form pseudo hypha at temperatures above 37 °C. *C. Tropical* was similar to *C. albicans* in the profile properties, this results agree with Martin and White (1981).

**Microscopic characteristic**

Microscopy after g-staining of *Candida* isolates spawned a positive gram, spherical to oval with present from the bud and was significantly larger than bacteria, it is agree with Emmons *et al.*, (1974) and Webb *et al.*, (1998).

**Germ Tube Formation Test**

*Candida albicans* isolates were showed positive result the formation of germ tubes was understood as a long tube such as the extended projections of yeast cells (Fig. 2), there was no contraction at the point of attachment to the yeast cells. These tubular extensions represent an early stage in the formation of the real thread. Germ tubes are forming within two hours of incubation and this is a unique diagnostic feature of *C. albicans* distinguishes them from other fungi. Other yeasts generally do not form germ tubes within this 2-hour time frame. About 95% of *C. albicans* isolates harvest germ tubes, possessions also common by *C. stellatoidea* and *C. dubliniensis* (Byadarahally and Rajappa, 2011).

Growth at 45°C was firstly careful to be a valuable test for difference between *C. dubliniensis* (not grow) from *C. albicans* (grow) in the unique report of this species (Ibraheem *et al.*, 2015).

Fig. 1: Colonies of *Candida albicans* cultured on Sabouraud dextrose agar at 37 °C for 24hrs.

Fig. 2: Germ tube of *C. albicans* grown on human serum at 37°C for 2hrs and half (40 X).

**Identification of Candida species by CHROMagar media and Vitek 2 compact system**

*Candida* spp. grew well in chromo agar media and developed distinctive colored colonies after incubation *C. albicans* produced green smooth-type colonies after incubation for 24hrs. *C. glabrata* isolates formed Pink with a darker mauve center colored colonies on CHROM agar *Candida*. After 48 hrs, *C. krusei* colonies they can be easily distinguished from those other yeasts that formed smooth, brownish brown to brown purple colonies on CHROM agar. *C. Tropicalis* isolates all advanced a distinctive dark blue gray central color afterward 48 hrs of incubation (Manikandan and Amsath, 2013). Final identification for the isolates has been done with Vitek 2 compact system, an indefinite bio pattern is associated to database of responses for every taxon, and a numerical probability calculation is done Identifications isolated contingent on 64 biochemical test (BioMerieux, USA).

**Candida species identification result**

In this study of 56 *Candida* isolated from vaginal swabs; 41(73 %) were *C. albicans*, *C. glabrata* 5(9%), *C. famata* 4(7%), *C. krusei* 2(3.5%), *C. sphaerica* 1(2%), *C. tropicalis* 1(2%) and *C. lambica* 2(3.5%). Whereas 36(65%) out of 55 *Candida* isolation from oral swabs were *C. albicans*, *C. famata* 7(13%),*C. glabrata* 7(13%), *C. sphaerica* 2(3.6%), *C. krusei* 2(3.6%) and *C. tropicalis* 1(1.8%) (Table 3).

In the current study, *C. albicans* was the greatest recurrent etiological agent which accounted for (73%) and (65%) of the *Candida* infections in both of Vaginal and Oral infection respectively. Similar results have been reported by Ogba, (2013) that presented the rate of *C. albicans* isolates were 80% and 74%, respectively. Whereas *C. glabrata* was the greatest recurrent species.
among non-\textit{albicans} spp in the present study. These results are agree with Mohammed (2017) that \textit{C. glabrata}, \textit{C. albicans}, and \textit{C. famata} were the most common yeast species isolated from Vaginal infection with percentages 45%, 44.5% and 14.8 \% respectively. The aim for the low occurrence isolation of non-\textit{albicans} spp of this study may due to preceding antifungal usage may affect the species distribution, presently do not have sufficient data to regulate this option since only a small number of patients had conventional an earlier antifungal treatment in this study.

\section*{Detection of Virulence Factor for Candida species}

\subsection*{Production of phospholipase Activity}

Table 4 shows the phospholipase activity( PLA) of \textit{Candida} spp isolated from Oral Candidiasis cultured on egg yolk agar, it appeared as precipitated zone around colony, 48 (87.3\%) out of 55 isolates were shown phospholipase activity whereas 7(12.7\%) have no PLA. Among the 48, \textit{C. albicans} 35(97.2\%), \textit{C. glabrata} 3(42.8\%), \textit{C. famata} 5(71.4\%), while for each \textit{C. krusei} and \textit{C. sphaerica} 2(100\%) and \textit{C. tropicalis} 1(100\%) (Fig. 4).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Candida spp} & \textbf{Number of Isolate (%)} & \textbf{Total} \\
\hline
\textit{C. albicans} & 35(97.2\%) & 36 \\
\textit{C. glabrata} & 3(42.8\%) & 7 \\
\textit{C. famata} & 5(71.5\%) & 7 \\
\textit{C. krusei} & 2(100\%) & 2 \\
\textit{C. sphaerica} & 2(100\%) & 2 \\
\textit{C. tropicalis} & 1(100\%) & 1 \\
\hline
\textbf{Total} & 48(87.3\%) & 55 \\
\hline
\end{tabular}
\caption{Phospholipase activity of \textit{Candida} spp isolated from Oral Thrush.}
\end{table}

Table 5, In VVC infected woman’s the phospholipase produce by 50 (89.3\%) out of 56 isolates were show phospholipase activity whereas 6(10.7\%) have no PLA. Among the 50 Large PLA \textit{C. albicans} 40(97.5\%), \textit{C. glabrata} 4(80\%), while for each \textit{C. krusei}, and \textit{C. lambica} 2(100\%), \textit{C. tropicalis} and \textit{C. sphaerica} 1(100\%), moreover all isolates of \textit{C. famata} have no phospholipase production 4(100\%).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Candida spp} & \textbf{Number of Isolate (%)} & \textbf{Total} \\
\hline
\textit{C. albicans} & 40(97.5\%) & 41 \\
\textit{C. glabrata} & 4(80\%) & 5 \\
\textit{C. famata} & 0 & 4 \\
\textit{C. krusei} & 2(100\%) & 2 \\
\textit{C. sphaerica} & 1(100\%) & 1 \\
\textit{C. tropicalis} & 1(100\%) & 1 \\
\textit{C. lambica} & 2(100\%) & 2 \\
\hline
\textbf{Total} & 50(89.3\%) & 56 \\
\hline
\end{tabular}
\caption{Phospholipase activity of \textit{Candida} spp isolated from Vulvovaginal Candidiasis.}
\end{table}

The result was similar to that of Jasim et al., (2016) who initiate Out of 50 \textit{Candida} species studied; action of phospholipase in 40(80\%) isolates. Kantarcioçlu and Yücel (2002) as stated the rate of almost positive action of phospholipase, in samples of patients with aggressive \textit{Candida} infection. The result obtained is partly in agreement with the results Sachin et al., (2012) activity of phospholipase in 67(60.9\%) isolates. The phospholipase enzyme breaks down the phospholipid cell of the host cell membrane, causing cell degradation and changes in surface features that enhance adherence and resulting infections. Thus, phospholipase production can be used as a parameter to differentiate invasive strains of non-
Fig. 4: Phospholipase activity from *C. albicans* on egg yolk agar medium at 37°C for (24-48) hrs.

invasive colonists (Deepa et al., 2015).

**Production of proteinase activity**

Table 6 shows the proteinase activity (PrA) of *Candida* spp. isolated from Oral candidiasis cultured on proteinase media, it appeared as clear zone around colony, 46 (83.6%) out of 55 isolates were shown proteinase activity whereas 9(16.4%) have no PrA. Among the 46, *C. albicans* 30 (83.3%), *C. glabrata* 7(100%), *C. famata* 7(100%) and *C. tropicalis* 1(100%) also *C. krusei* 1(50%), In addition to that 2(100%) isolates of *C. sphaerica* have no PrA (Fig. 5).

**Table 6: Proteinase activity of *Candida* spp isolated from Oral Thrush.**

<table>
<thead>
<tr>
<th><em>Candida</em> spp</th>
<th>Number of Isolate (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large PLA</td>
<td>Negative</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>30(83.3%)</td>
<td>6(16.7%)</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>7(100%)</td>
<td>0</td>
</tr>
<tr>
<td><em>C. famata</em></td>
<td>7(100%)</td>
<td>0</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>1(50%)</td>
<td>1(50%)</td>
</tr>
<tr>
<td><em>C. sphaerica</em></td>
<td>0</td>
<td>2(100%)</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46(83.6%)</td>
<td>9(16.4%)</td>
</tr>
</tbody>
</table>

Table 7, In VVC infected woman’s the proteinase produce by 49(87.5%) out of 56 isolates were show proteinase activity whereas 7(12.5%) have no PrA. Among the 49 Large PrA, *C. albicans* 36(87.8%), *C. famata* 3(75%), while for each *C. sphaerica* and *C. tropicalis* 1(100%), and *C. lambica* 2(100%), also *C. krusei* 1(50%).

These results have been decided with Dan et al., (2002) who showed that the proteinase activity noticed in *Candida* isolates was 34(85.0%) while stretched to 100 % in non-*albicans* *Candida* species. In additional study, the positivity for proteinase production was originate in 65(59.1%) of *Candida* isolates (Sachin et al., 2012). Proteinases are capable of debasing host epithelial and mucosal barrier proteinase such as collagen, keratin, and mucin. They also aid *Candida* to battle cellular and humoral immunity by humiliating antibodies, complement, and cytokines Deorukhkar et al., (2014).

**Table 7: Proteinase activity of *Candida* spp isolated from Vulvovaginal Candidiasis.**

<table>
<thead>
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<th>Number of Isolate (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>36(87.8%)</td>
<td>5(12.2%)</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>5(100%)</td>
<td>0</td>
</tr>
<tr>
<td><em>C. famata</em></td>
<td>3(75%)</td>
<td>1(25%)</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>1(50%)</td>
<td>1(50%)</td>
</tr>
<tr>
<td><em>C. sphaerica</em></td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td><em>C. lambica</em></td>
<td>2(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49(87.5%)</td>
<td>7(12.5%)</td>
</tr>
</tbody>
</table>

Fig. 5: Proteinase activity from *C. albicans* on proteinase media at 37°C for (48-72) hrs.

**Detection Haemolytic Activity**

In this study all *Candida* spp isolated from both of Vaginal and Oral Candidiasis have haemolytic activity when grew on Sabouraud dextrose blood agar with 3% glucose, the isolates give Beta -haemolysis. Haemolysin action is identified to be the supposed virulence factor that donates to the spread of *Candida*. By facilitating the acquisition of iron from the host erythrocytes. It was first described that cysteine-induced hemolysis is caused by *C. albicans* (Manns et al., 1994). However, there have been no reports of hemolytic activity of non-*albicans* *Candida* species. In the meantime, Luo et al., (2001)
established the adjustable expression of haemolysin by dissimilar Candida species. Sathiya et al., (2015) stated that 100% of C. albicans and non-albicans Candida species, isolates presented β-haemolytic activity. There is about reasons principal to numerous responses of Candida spp. to the antifungal drugs with: genetic difference, in all tested isolates, diseases, treatment history of the patients who stretch the sample and antifungal structure difference (Pfaller et al., 2005).

Biofilm formations

All the 45 (81.8%) positive Candida spp isolates, isolated from Oral Thrush had the ability to harvest biofilm at varying range (Table 8). Out, of 36 Calbicans isolates 26(72.2%) were the Strong producer of biofilm, 9 isolates (25%) formed weak biofilm, whereas 1 isolates (2.8%) were non-produce biofilm. Among the 19 non- albicans, C. glabrata isolates 1(14.3%) were strong, 1(14.3%) moderate producer of biofilm whereas 5(71.4%) non-produce biofilm, C. famata 3 isolates (42.8%) formed strong biofilm, 2 isolates (28.6%) were weak biofilm formers, and the same percentage (28.6%) non-produce biofilm, C. krusei isolate 2(100%) were strong whereas 2(100%) of C.sphaerica non-produce biofilm, and 1(100%) of C. tropicalis were the Strong producer of biofilm.

Table 9 shows Biofilm formation of Candida spp isolated from Vulvovaginal Candidiasis, 50(89.3%) positive Candida spp isolates had the ability to harvest biofilm at variable range. Out of, 41 C.albicans isolates 14(34.2%) were Strong producer of biofilm. Though, 26 isolates (63.4%), formed weak biofilm, whereas 1 isolates (2.4%), were non-produce biofilm. Among the 15 non- albicans, C. glabrata isolates, 3(60%) were strong, whereas 1(20%) moderate, non-produce biofilm, C. famata 4 isolates (100%) non-produce biofilm, 2 (100%) of C. krusei and C. lambica were weak biofilm formers, and the same percentage of C. sphaerica and C. tropicalis 1(100%) were moderate biofilm.

As any other research these results are agree with some and different with others in some sides. Khater and Al-Nory (2014) originate that 41isolates (54.7%) out of 75 Candida species isolates produced biofilm, (Jasim et al., 2016) found out of 39 C. albicans isolates 20 (51.3%) shaped biofilm, although out of, 36 non-albicans Candida species isolates 21 (58.3%), produced biofilm. Deepa et al., (2015) stated that 78.9% of Candida isolates presented biofilm formation. While both C. albicans and non-albicans Candida were accomplished producing, biofilm formation like results were obtained in a study conducted by Vijaya et al., (2011) who stated that non-albicans Candida species isolates 21 (58.3%), produced biofilm.

Conclusions

The data suggests that isolates of Candida albicans, C. glabrata, C. famata, C. krusei, C. sphaerica, C. tropicalis and C.
Isolation and identification of *Candida* Species from oral and vaginal and determination of Virulence Factor

*Candida* spp. to produce proteinase, phospholipase, haemolysin and biofilm formation.

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