



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 isolate; 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 45°C, 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. dubliniensis*, *C. famata*,

C. norvegensis, *C. lipolytica*, *C. sake*, *C. apicola*, *C. pulcherrima*, *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

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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 Ferrin, 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 one 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. Chromgar 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 reagent 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 DensiChek™.

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 matured. 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 (Ak«aðlar *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 ($ODnc < ODsP \leq 2ODnc$), moderate biofilm production ($2ODnc < ODsP \leq 4ODnc$) 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.

| Percentage % | No. of infected woman | Age group |
|--------------|-----------------------|-------------|
| 25 % | 14 | Years > 20 |
| 59 % | 33 | 20-30 years |
| 12.5 % | 7 | 31-40 years |
| 3.5 % | 2 | 41-50 years |
| 100 | 56 | Total |

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.

| Percentage % | No. of infected children | Age group |
|--------------|--------------------------|----------------|
| 84 % | 46 | month \leq 1 |
| 16 % | 9 | 2-6 months |
| 100 % | 55 | Total |

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. Tropicalis* was similar to *C. albicans* in the profile properties, this results agree with Martin and White (1981).



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

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).

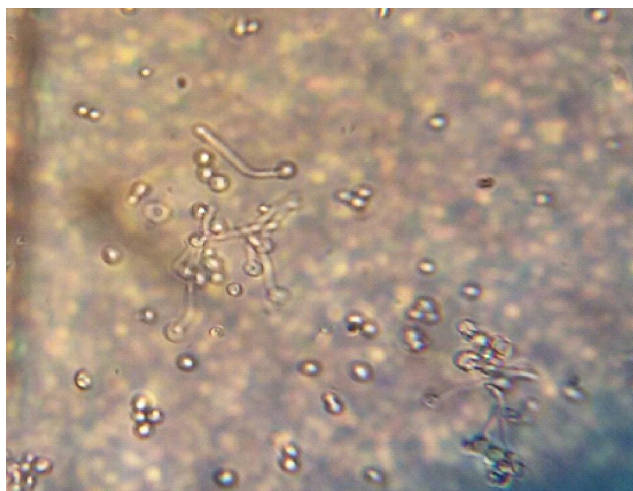


Fig. 2: Germ tube of *C. albicans* grown on human serum at 37°C for 2hrs and half (40 X).

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

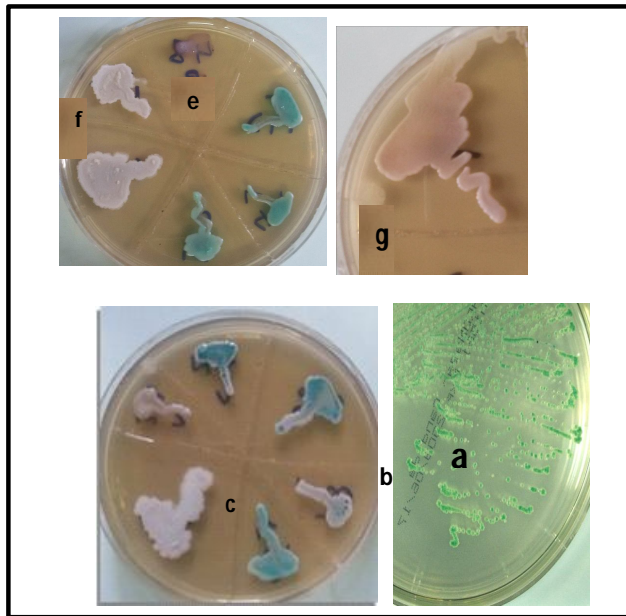


Fig. 3: Colonies of *Candida* spp on CHROMagar *Candida* medium (a-*Candida albicans*, b *C.tropicalis*, c-*C.sphaerica*, d-*C.famata*, e-*C.glabrata*, f-*C.lambica*, g-*C.krusei*).

Table 3: Distribution of *Candida* spp isolation in this study.

| <i>Candida</i> spp | Positive No/ Total No (%) | |
|---------------------|---------------------------|------------|
| | Vaginal swab | Oral swab |
| <i>C. albicans</i> | 41/56(73%) | 36/55(65%) |
| <i>C glabrata</i> | 5/56(9%) | 7/55(13%) |
| <i>C.famata</i> | 4/56(7%) | 7/55(13%) |
| <i>C. krusei</i> | 2/56(3.5%) | 2/55(3.6%) |
| <i>C.sphaerica</i> | 1/56(2%) | 2/55(3.6%) |
| <i>C.tropicalis</i> | 1/56(2%) | 1/55(1.8%) |
| <i>C.lambica</i> | 2/56(3.5%) | 0 |

among non-*albicans* spp in the present study. These results are agree with Mohammed (2017) that *C. glabrata*, *C. albicans*, and *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-*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.

Detection of Virulence Factor for *Candida* species Production of phospholipase Activity

Table 4 shows the phospholipase activity(PLA) of *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, *C. albicans* 35(97.2%), *C. glabrata* 3(42.8%), *C. famata* 5(71.4%), while for each *C. krusei* and *C. sphaerica* 2(100%) and *C. tropicalis* 1(100%) (Fig. 4).

Table 4: Phospholipase activity of *Candida* spp isolated from Oral Thrush.

| <i>Candida</i> spp | Number of Isolate (%) | | Total |
|---------------------|-----------------------|----------|-------|
| | Large PLA | Negative | |
| <i>C.albicans</i> | 35(97.2%) | 1(2.8%) | 36 |
| <i>C.glabrata</i> | 3(42.8%) | 4(57.2%) | 7 |
| <i>C.famata</i> | 5(71.5%) | 2(28.5%) | 7 |
| <i>C.krusei</i> | 2(100%) | 0 | 2 |
| <i>C.sphaerica</i> | 2(100%) | 0 | 2 |
| <i>C.tropicalis</i> | 1(100%) | 0 | 1 |
| Total | 48(87.3%) | 7(12.7%) | 55 |

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 *C. albicans* 40(97.5%), *C. glabrata* 4(80%), while for each *C. krusei*, and *C. lambica* 2(100%), *C. tropicalis* and *C. sphaerica* 1(100%), moreover all isolates of *C. famata* have no phospholipase production 4(100%).

Table 5: Phospholipase activity of *Candida* spp isolated from Vulvovaginal Candidiasis.

| <i>Candida</i> spp | Number of Isolate (%) | | Total |
|---------------------|-----------------------|----------|-------|
| | Large PLA | Negative | |
| <i>C.albicans</i> | 40(97.5%) | 1(2.5%) | 41 |
| <i>C.glabrata</i> | 4(80%) | 1(20%) | 5 |
| <i>C.famata</i> | 0 | 4(100%) | 4 |
| <i>C.krusei</i> | 2(100%) | 0 | 2 |
| <i>C.sphaerica</i> | 1(100%) | 0 | 1 |
| <i>C.tropicalis</i> | 1(100%) | 0 | 1 |
| <i>C.lambica</i> | 2(100%) | 0 | 2 |
| Total | 50(89.3%) | 6(10.7%) | 56 |

The result was similar to that of Jasim et al., (2016) who initiate Out of 50 *Candida* species studied; action of phospholipase in 40(80%) isolates. Kantarcioğlu and Yücel (2002) as stated the rate of almost positive action of phospholipase, in samples of patients with aggressive *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.

| <i>Candida</i> spp | Number of Isolate (%) | | Total |
|---------------------|-----------------------|----------|-------|
| | Large PLA | Negative | |
| <i>C.albicans</i> | 30(83.3%) | 6(16.7%) | 36 |
| <i>C.glabrata</i> | 7(100%) | 0 | 7 |
| <i>C.famata</i> | 7(100%) | 0 | 7 |
| <i>C.krusei</i> | 1(50%) | 1(50%) | 2 |
| <i>C.sphaerica</i> | 0 | 2(100%) | 2 |
| <i>C.tropicalis</i> | 1(100%) | 0 | 1 |
| Total | 46(83.6%) | 9(16.4%) | 55 |

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

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

| <i>Candida</i> spp | Number of Isolate (%) | | Total |
|---------------------|-----------------------|----------|-------|
| | Large PLA | Negative | |
| <i>C.albicans</i> | 36(87.8%) | 5(12.2%) | 41 |
| <i>C.glabrata</i> | 5(100%) | 0 | 5 |
| <i>C.famata</i> | 3(75%) | 1(25%) | 4 |
| <i>C.krusei</i> | 1(50%) | 1(50%) | 2 |
| <i>C.sphaerica</i> | 1(100%) | 0 | 1 |
| <i>C.tropicalis</i> | 1(100%) | 0 | 1 |
| <i>C.lambica</i> | 2(100%) | 0 | 2 |
| Total | 49(87.5%) | 7(12.5%) | 56 |

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).



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).



Fig. 6: Haemolysin activity from *C. albicans* on Sabouraud dextrose blood agar with 3% glucose at 37°C for 48hrs.

Table 8: Biofilm formation of *Candida* spp. isolated from Oral thrush.

| Total | Number of Isolates (%) | | | | <i>Candida</i> spp |
|-------|------------------------|----------|-----------|-----------|---------------------|
| | Negative | Moderate | Weak | Strong | |
| 36 | 1(2.8%) | 0 | 9(25%) | 26(72.2%) | <i>C.albicans</i> |
| 7 | 5(71.4%) | 1(14.3%) | 0 | 1(14.3%) | <i>C.glabrata</i> |
| 7 | 2(28.6%) | 0 | 2(28.6%) | 3(42.8%) | <i>C.famata</i> |
| 2 | 0 | 0 | 2(100%) | 0 | <i>C.krusei</i> |
| 2 | 2(100%) | 0 | 0 | 0 | <i>C.sphaerica</i> |
| 1 | 0 | 0 | 0 | 1(100%) | <i>C.tropicalis</i> |
| 55 | 10(18.2%) | 1(1.8%) | 13(23.6%) | 31(56.4%) | Total |

Table 9: Biofilm formation of *Candida* spp isolated from Vulvovaginal Candidiasis.

| Total | Number of Isolates (%) | | | | <i>Candida</i> spp |
|-------|------------------------|----------|-----------|-----------|---------------------|
| | Negative | Moderate | Weak | Strong | |
| 41 | 1(2.4%) | 0 | 26(63.4%) | 14(34.2%) | <i>C.albicans</i> |
| 5 | 1(20%) | 1(20%) | 0 | 3(60%) | <i>C.glabrata</i> |
| 4 | 4(100%) | 0 | 0 | 0 | <i>C.famata</i> |
| 2 | 0 | 0 | 2(100%) | 0 | <i>C.krusei</i> |
| 1 | 0 | 1(100%) | 0 | 0 | <i>C.sphaerica</i> |
| 1 | 0 | 1(100%) | 0 | 0 | <i>C.tropicalis</i> |
| 2 | 0 | 0 | 2(100%) | 0 | <i>C.lambica</i> |
| 56 | 6(10.7%) | 3(5.4%) | 30(53.5%) | 17(30.4%) | Total |

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 *C.albicans* 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* prevailed (54.1%) over *C. albicans* (45.9%).

Conclusions

The data suggests that isolates of *Candida albicans*, *C. glabrata*, *C. famata*, *C. krusei*, *C. sphaerica*, *C. tropiclis* and *C.*

lambica. The capacity of *Candida* spp. to produce proteinase, phospholipase, haemolysin and biofilm formation.

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