Characterization, Virulence Factors and Antifungal Susceptibility of Vulvovaginal Candida Isolated from Women at Qena, Egypt

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ALTHOUGH the incidence of vaginitis caused by non-albicans Candida tends to be increased, C. albicans still the main causative agent of vaginitis Candida. Eighty-eight vaginal swab samples were collected from women with acute vaginitis in Qena, Egypt. Of 50 isolates, 39 admitted into C. albicans and 11 non-albicans Candida isolates (78% and 22% prevalence, respectively) were identified. Youths were more susceptible to infection with vulvovaginal Candida, the rate of infection decreased with increase education levels and the risk of infection was greater among douching use women. All isolates belonging to Candida taxa were positive to proteinase activity and 48 (96%) were lipase producers. Non-albicans Candida (C. glabrata, C. tropicalis and C. krusei) were more proteinase producers than C. albicans (P< 0.000). Compared with C. tropicalis, the other isolated Candida exhibited less lipase activity (P< 0.000). The higher lipase capacity of C. tropicalis may reflect their increased prevalence among non-albicans Candida group. Among five essential oils, cinnamon and clove oils showed strong efficacy against isolated Candida strains compared with miconazole antifungal.

Keywords: Vaginitis, Conventional, Candida, Virulence factors, Antifungal susceptibility.

Introduction

Vaginal infection is the common disease affects the wide sector of women worldwide, especially during childbearing age. The second causative agents of this infection after bacteria are Candida (Achkar & Fries, 2010). Candida species are part of normal microbiota of several regions of the human body including vagina (Shao et al., 2007). The type and prevalence of Candida spp. recovered from women with vaginitis depending mainly on the population site and ecological conditions (Galan-Ladero et al., 2009). In African countries, C. albicans is the common species (78.3-96.1%) isolated from vaginitis, followed by C. glabrata (3.9-12%) and C. tropicalis (3.5-5.4) (Konate et al., 2014 and Shaaban et al., 2015). The incidence of vaginitis caused by non-albicans Candida spp. tend to increase (Majumdar et al., 2016). Recently in Burkina Faso, Sangaré et al. (2018) reported the prevalence of non-albicans Candida species, including C. glabrata (32.69%) and C. tropicalis (15.38) and C. krusei (11.54) from the isolated Candida.

Different expression levels of virulence factors were observed among different Candida species (Mane et al., 2012). The virulence factors that contribute to pathogenesis include the production of various exoenzymes. Extracellular proteases and lipases play the fundamental role in the adhesion and invasion of cell membrane enabling penetration of the tissue (Staniszewska et al., 2012 and Mayer et al., 2013). The expression level of different enzymes in vaginitis may affect the disease severity (Haynes, 2001). Recently, in Iran, Fatahinia et al. (2017) conducted that activities of proteinase and phospholipase in non-albicans Candida species (C. glabrata and C. krusei) were found to be lower than the C. albicans species complex including C. albicans and C. dubliniensis.
Several literatures were documented the side effects of synthetic antimicrobials. Moreover, during 2006-2013 it has been observed that the susceptibility of different *Candida* species to azole treatment has decreased (Wang et al., 2016). Therefore, attention has been increasing to alter synthetic preservatives with natural, effective and nontoxic compounds (Smid & Gorris, 1999). The essential oils contain a variety of volatile molecules including terpenes, terpenoids, aldehydes, ketones, lactones and phenolic compounds which have antifungal consequences (Bakkali et al., 2008 and Akthar et al., 2014). Several studies have been emitted to clarify the effect of essential oils and their main compounds on different *Candida* species (Benlafya et al., 2014; Radwan et al., 2014 and Karo et al., 2017).

Therefore, this work aimed to identification of vulvovaginal *Candida* isolated from women at Qena, Egypt, evaluate some virulence factors and assessment the susceptibility of *Candida* spp. to essential oils.

**Materials and Methods**

**Samples collection**

Eighty-eight vaginal samples collected from women aged from 16-60 years complaining from vaginal infection at Center of Obstetrics and Gynecology (Dr. Safaa Adly) Qena, Egypt. Samples were taken from patients using sterile spectrum and sterile cotton swabs with long handle. The swabs were immediately covered by its sterile cover and transported to the laboratory for quick examination and culturing (Shaaban et al., 2015).

**Direct microscopic examination and samples culturing**

Distinctive features of yeasts can be determined by observing the morphology. Microscopes can be used to rapidly identify and detect possible yeasts in the clinical sample slide that was prepared from each vaginal swab and was examined under the light microscope on power 40x and 100x. Culturing of samples was obtained by shaking well the sterile swab of each vaginal sample in sterilized distilled water and poured in a sterile plate followed by addition 20ml of Sabouraud Dextrose Agar (SDA). Plates were incubated at 37°C for 96hr to appear colonies.

**Conventional identification**

**Germ tube test**

Germ tube is a quick check of *Candida albicans* and *Candida dubliniensis*. Germ tube appeared as extending outgrowth from the yeast cells (Ellis et al., 2007) by inoculation one or two colonies of culture suspected *Candida* with 0.5ml of human serum, which contains 0.5% of glucose in the Eppendorf tube and incubated at 37°C for 2-3hr. After required incubation time, a complete loop of the culture on a glass slide and overlaid with a sliding lid and examined microscopically for the presence or absence of the formation of the germ tube.

**Growth on HiChrome Candida differential agar**

HiChrome agar is a differential and selective medium, for rapid differentiation of *Candida* species namely *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* according to coloration and colony morphology (Mahajan et al., 2014).

**Molecular characterization of selected strains**

Based on HiChrome differential agar medium results, 4 strains selected randomly (one from each *Candida* species) to further identification. Yeast strains were cultured on Sabouraud Dextrose Broth for 2 days at 30°C. DNA was extracted as previously described (Robert et al., 1995). Internal transcribed spacer ITS 1– 5.8S rDNA–ITS 2 fragment of yeast species were amplified using universal primer pair ITS1 and ITS4 (ITS1: 3'-TCCGTAGGTGAACCTGCGG-5', ITS4: 3'-TCCTCCGCTTATTGATATGC-5') (White et al., 1990), with the following amplification conditions: 95°C for 15sec. followed by 30 cycles of 95°C for 20sec, 50°C for 40sec and 72°C for 1min, with a final extension step at 72°C for 5min. PCR fragments were detected by 1.2 agarose gel electrophoresis and visualized on a UV transilluminator. Purification of PCR product was done using SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) and sequenced. Sequences were edited with Chromas Lite (Technelysium Pty. Ltd.). The resulted sequences were blasted against GenBank to identify the selected isolates to species level. Phylogenetic analysis was done with the help of software mega 4.0 (Tamura et al., 2007) using *Saccharomyces cerevisiae* DQ674252 as outgroup.

**Some virulence factors (Enzyme activity)**

This study focused on identifying the activity of extracellular enzymes of different isolates of
vulvovaginal Candida. Different isolated taxa were subjected to qualitative analysis of proteinase and lipase enzymes. The activities of proteinase were tested on Casein hydrolysis medium (Paterson & Bridge, 1994). The medium was distributed into 15 ml test tubes (10ml/tube), autoclaved at 121°C for 20min. Tubes were inoculated with 50µl from spore suspensions (1x10⁵/ml) and incubated at 37°C for 7 days. Positive results were taken by degradation of casein protein as clear depth in the tube. Proteinase activity expressed as; weak (1-9mm), strong (10-19mm) and very strong producers (≥20mm).

The production of lipase was tested on Ullman & Blasins (1974) medium. As described before, tubes with medium autoclaved, inoculated by different isolates and incubated for 7 days at 37°C. The lipolytic activity was detected as a visible white precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured. Lipase activity expressed as; weak (1-4mm), strong (5-14mm) and very strong producers (≥15mm). The enzymes activities were performed in triplicate.

Antifungal susceptibility

Five essential oils (Black seed, Cinnamon oil, Clove oil, Coconut oil, and Lemon oil) were tested against different Candida isolates. One ml of the above spore suspension was transferred into petri dish followed by addition of about 20ml liquefied SDA medium. After solidifying, 6mm wells were made in each plate and inoculated with 25µl of each oil. Plates were incubated at 37°C for 48hr. Miconazole (0.01g/ml) was used as positive control and the average inhibition zone was measured.

Statistical analysis

all obtained data were statistically analyzed by one-way ANOVA and Post Hoc Test (LSD). P< 0.05 was considered statistically significant.

Results

Conventional and molecular identification of Candida spp.

Eighty-eight cases of women complaining of funga vaginitis were studied. All vaginal swabs from women were exposed directly to direct microscope examination (DME), which revealed that, 35 samples (39.8%) of patients were positive showing budding cells and pseudo hyphae. 56.8% (50 out of 88) of vaginal swabs were positive on SDA medium producing white to cream colonies. The positivity of culturing on SDA medium is greater than DME (Fig. 1).

Germ tube is the most important criteria to differentiate albicans group (Candida albicans and Candida dubliniensis) from other Candida species. The formation of germ tube took 2hr after inoculation in human serum at 37°C. Thirty-nine (78%) of isolates were positive for germ tube test (Candida albicans or Candida dubliniensis) and eleven (22%) isolates were negative for germ tube (non-albicans group). In relation to the colony colors of the various yeast taxa grown on HiChrome agar, 39 (78%) of taxa identified as C. albicans (appeared as light green colored smooth colonies), 8 (16%) as C. tropicalis (blue colonies), two (4%) taxa as C. glabrata (Creamy white), and the final one as C. krusei (pink fuzzy) (Fig. 2).
The resulted sequences from four selected isolates were edited and deposited in GenBank with accession numbers MG707638, MG707639, MG707640 and MG707641. Comparison of ITS sequences with sequences available in the GenBank nucleotide database indicated that the DNA sequences from selected yeast isolates had 99-100% sequence identity with yeast species sequences from GenBank. Phylogenetic tree was generated from tested four sequences with closely related yeasts species from GenBank indicated that the selected strains formed distinct four clades; MG707638 strain clustered with Candida albicans strains recovered from different places and MG707639 grouped with Candida tropicalis with while MG707640 and MG707641 forming clades with Candida glabrata and Pichia kudriavzevii (teleomorph C. krusei) with 92-100% bootstrap values (Fig. 3).

**Prevalence of Candida taxa in women patients**

The age range of women patients was 16-60 years, with mean 28.1±9.43 years. Most of C. albicans (69.2%), C. tropicalis (75%) and C. krusei (100%) infections were diagnosed in women aged from 21-34 years. In general, the rate of vaginal infection with Candida decrease with increasing the academic qualifications; most of the infected women were illiterate and low education levels by 36% and 38%, respectively, but were for secondary (12%) and university (14%). Of the 50 patients, 38 (76%) were admitted into douching use women and 12 (24%) into douching non-using women. Approximately 37 (74%) of the women patients were not using any method of contraception. The frequency of vaginal infection performed 10% by the first time, 28% by one of the year and 62% by more than one in the year (Table 1).

**Virulence factors**

The abilities of different isolates to produce virulence factors including proteinase and lipase were studied. The obtained results clarified that, all of Candida isolates are positive to proteinase activity and 48(96%) were lipase producers. The mean enzyme activities for proteinase and lipase ranged from 7.5±0.35 - 62±0.58 and 10±0.58 - 16.5±0.19, respectively.

About 23% (9 out of 39 isolates) of C. albicans showed strong activity for protease production, while the remaining (77%) exhibited weak activity. Exactly half of C. tropicalis isolates showed very strong proteinase production and the other one was strong in its production. The remaining isolates of C. glabrata and C. krusei were very strong producers (Fig. 4). In relation to lipase, 41% of the C. albicans isolates were very strong in lipase production, 53.8% were strong, and the remaining two isolates were non-producers. All of C. tropicalis isolates were very strong producers. The isolates belonging to C. glabrata and C. krusei exhibited strong activity (Fig. 5).

The resulted analysis for proteinase and lipase activities revealed a significant difference between different species of Candida (P< 0.000). Compared with C. albicans, non albicans Candida (C. glabrata, tropicalis and krusei)
produced significant more protease (P< 0.000). Non-significant was observed in proteinase production between C. glabrata and C. krusei (P= 0.689). On the other side, C. tropicalis and C. krusei produced significant more and fewer lipase activities (P< 0.000) respectively than C. albicans. There was no significant difference in lipase activity produced by C. albicans and C. glabrata (P= 0.244) (Table 2).

Antifungal susceptibility

Among five tested essential oils, all tested Candida species showed strong antifungal susceptibility to cinnamon and clove oils, but in case lemon oil, C. albicans and C. krusei were affected. The other remaining oils (black seed and coconut oil) did not show any detectable effect against the tested isolates. Compared with miconazole as control, cinnamon and clove oils showed significantly higher efficacy against tested isolates, except in case of cinnamon with C. glabrata was less efficiency. The susceptibility of C. albicans to essential oils was greater than non-albicans Candida (Table 3).

Discussion

Vaginal candidiasis is a common vaginal infection attributed to different species of Candida of 88 symptomatic women, 50 (56.8%) were diagnosed as vaginal candidiasis infection with a higher prevalence rate than that reported by several researchers (Paulitsch et al., 2006; Yusuf et al., 2007 and Ogouyèmi-Hounto et al., 2014). In this work, C. albicans was the predominant cause of vaginal candidiasis, followed by C. tropicalis, C. glabrata and C. krusei. Although many literatures have illustrated a shift towards an increase in non-albicans Candida species (Dan et al., 2002; Fatahinia et al., 2017 and Sangaré et al., 2018). This result collaborating with those obtained by Jasim et al. (2016), they reported the prevalence of C. albicans, C. tropicalis and C. glabrata were 78%, 14% and 2% from different clinical specimens, respectively. In Egypt, Shaaban et al. (2015) indicated that the most prevalent vaginal Candida species was C. albicans (78.3%) followed by C. glabrata (12%) then C. tropicalis (5.4%). In most regions of the world, C. glabrata was the common taxa among non-albicans vulvovaginal Candida (Paulitsch et al., 2006; Konate et al., 2014 and Ameen et al., 2017).

Recently, several researches were done to clarify the prevalence of different vulvovaginal Candida to age, educational level, work, personal hygiene and contraceptive method of the women (Yusuf et al., 2007; Rezaei-Mateholaei et al., 2016 and Swaminathan et al. 2017). The current study showed that the maximum incidence of vaginal candidiasis was found between youths (20-34 age), the rate of vaginitis was decreased by increasing educational level and the douching using women were more susceptible to the risk of infection with vaginitis Candida. This finding is in full agreement with data obtained by Hassan et al. (2017) they concluded that 71% of vaginal infection in Egypt were admitted into women aged 19-30 years. The reason for the high incidence rate in this age group includes increased sexual activity and a new effect of reproductive hormones (Sobel et al., 1998). The vaginal douching increases the infection rate due to change in normal flora, but not affect the type of Candida species (La Ruche et al., 1999 and Shaaban et al., 2015).

In our work, protease activity was seen in 100% of different taxa with the more active producer belonging to non-albicans group (C. glabrata, C. tropicalis and C. krusei). The correlation between the taxa and proteinase was significant, reflecting that non-albicans produced the highest levels of proteinase activity. These results are partially agreed with Moharram et al. (2013) they showed that protease was produced by 83 (89.2%) out of 93 isolates tested with active isolates belonging to C. albicans and C. krusei. The opposite finding was reported by de Melo Riceto et al. (2015) who detected the proteinase activity in C. albicans, C. tropicalis and C. parapsilosis isolates but not in C. glabrata and C. krusei.

Candida albicans revealed more phospholipase activity than non-albicans Candida species (Chin et al., 2013; de Melo Riceto et al., 2015 and Fatahinia et al., 2017), while in the present work C. tropicalis strains showed the highest level of lipase among albicans and non-albicans Candida which corroborate with results obtained by Thangam et al. (1989) and Moharram et al. (2013) they reported high lipase activity in C. tropicalis. Generally, the microorganism that produces lipolytic enzymes might have abilities to lysing competing microflora and have a protective role against host by suppressing the cellular and humoral responses which reflecting the increase in their incidence (Stehr et al., 2003 and Toth et al., 2017).

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Fig. 3. Maximum likelihood tree based on the rDNA-ITS sequences data for 4 isolates of yeast. The tree was rooted with *Saccharomyces cerevisiae* DQ674252 as the outgroup.
### TABLE 1. Demographic data of the women affected by vulvovaginal Candida.

<table>
<thead>
<tr>
<th>Patients criteria</th>
<th>C. albicans n= 39</th>
<th>C. tropicalis n= 8</th>
<th>C. glabrata n=2</th>
<th>C. krusei n= 1</th>
<th>Total n= 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>5 (12.8)</td>
<td>1 (12.5)</td>
<td>1 (50)</td>
<td>-</td>
<td>7 (14)</td>
</tr>
<tr>
<td>21-34</td>
<td>27 (69.2)</td>
<td>6 (75)</td>
<td>-</td>
<td>1 (100)</td>
<td>34 (68)</td>
</tr>
<tr>
<td>≥35</td>
<td>7 (18)</td>
<td>1 (12.5)</td>
<td>1 (50)</td>
<td>-</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Academic Qualifications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>12 (30.8)</td>
<td>5 (62.5)</td>
<td>1 (50)</td>
<td>-</td>
<td>18 (36)</td>
</tr>
<tr>
<td>Primary</td>
<td>16 (41)</td>
<td>2 (25)</td>
<td>-</td>
<td>1 (100)</td>
<td>19 (38)</td>
</tr>
<tr>
<td>Secondary</td>
<td>5 (12.8)</td>
<td>-</td>
<td>1 (50)</td>
<td>-</td>
<td>6 (12)</td>
</tr>
<tr>
<td>University</td>
<td>6 (15.4)</td>
<td>1 (12.5)</td>
<td>-</td>
<td>-</td>
<td>7 (14)</td>
</tr>
<tr>
<td>Personal hygiene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Douching use</td>
<td>30 (76.9)</td>
<td>6 (75)</td>
<td>1 (50)</td>
<td>1 (100)</td>
<td>38 (76)</td>
</tr>
<tr>
<td>Non-use</td>
<td>9 (23.1)</td>
<td>2 (25)</td>
<td>1 (50)</td>
<td>-</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Contraceptive method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-use</td>
<td>29 (74.4)</td>
<td>6 (75)</td>
<td>1 (50)</td>
<td>1 (100)</td>
<td>37 (74)</td>
</tr>
<tr>
<td>IUD</td>
<td>3 (7.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>4 (10.2)</td>
<td>1 (12.5)</td>
<td>1 (50)</td>
<td>-</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Injectables</td>
<td>3 (7.7)</td>
<td>1 (12.5)</td>
<td>-</td>
<td>-</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Frequency of vaginal infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First time</td>
<td>4 (10.2)</td>
<td>1 (12.5)</td>
<td>-</td>
<td>-</td>
<td>5 (10)</td>
</tr>
<tr>
<td>One in year</td>
<td>9 (23.1)</td>
<td>3</td>
<td>1 (50)</td>
<td>1 (100)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>More than one in year</td>
<td>26 (66.7)</td>
<td>4 (50)</td>
<td>1 (50)</td>
<td>-</td>
<td>31 (62)</td>
</tr>
</tbody>
</table>

IUD: Intra Uterine Device

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**Fig. 4.** Proteinase production by *Candida* species.

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Table 2. Enzymatic activities indicated by different Candida taxa (in mm).

<table>
<thead>
<tr>
<th>Virulence attribute</th>
<th>Candida species</th>
<th>EI (%)</th>
<th>Range</th>
<th>EA Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. albicans (n= 39)</td>
<td>100</td>
<td>4-12</td>
<td>7.5±0.35</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis (n= 8)</td>
<td>100</td>
<td>17-21</td>
<td>19.1±0.48*</td>
</tr>
<tr>
<td></td>
<td>C. glabrata (n= 2)</td>
<td>100</td>
<td>59.5-61</td>
<td>60.2±0.44*</td>
</tr>
<tr>
<td></td>
<td>C. krusei (n= 1)</td>
<td>100</td>
<td>61-63</td>
<td>62±0.58*</td>
</tr>
<tr>
<td>Proteinase</td>
<td>C. albicans</td>
<td>94.8</td>
<td>0-16</td>
<td>13±0.53**</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis</td>
<td>100</td>
<td>16-17</td>
<td>16.5±0.19</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
<td>100</td>
<td>12-13</td>
<td>12.5±0.29**</td>
</tr>
<tr>
<td></td>
<td>C. krusei</td>
<td>100</td>
<td>9-11</td>
<td>10±0.58**</td>
</tr>
</tbody>
</table>

EI = Enzyme index, EA = Enzyme activity.

*: Significant difference between C. albicans and other non-albicans Candida taxa.

**: Significant difference between C. tropicalis and other Candida taxa.

Table 3. Antifungal susceptibility of Candida isolates against different essential oils.

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. glabrata</th>
<th>C. krusei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole</td>
<td>21±0.15</td>
<td>19±0.35</td>
<td>24±0.10</td>
<td>18±0.21</td>
</tr>
<tr>
<td>Black seed oil</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cinnamon oil</td>
<td>34±0.50*</td>
<td>24±0.43*</td>
<td>22±0.25*</td>
<td>25±0.38*</td>
</tr>
<tr>
<td>Clove oil</td>
<td>42±0.22*</td>
<td>27±0.30*</td>
<td>28±0.25*</td>
<td>28±0.22*</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>ND</td>
<td>N.d</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lemon oil</td>
<td>18±0.27*</td>
<td>N.d</td>
<td>ND</td>
<td>15±0.34*</td>
</tr>
</tbody>
</table>

*: Significant difference between essential oils and miconazole (positive control).

N.d = Not detected.

The resistance Candida albicans and non-Candida albicans species isolated from patients, against antifungal agents has increased. Several studies have shown that clove and cinnamon oils had a strong and inhibitory activity against different Candida species (Aneja & Joshi, 2010; Fani & Kohanteb, 2011 and Radwan et al., 2014). In the same situation, the combination
cinnamon with clove oils leading to enhancement of the antifungal activity in all cases (Horváth et al., 2016). Omran & Esmailzadeh (2009) indicated that lemon essential oil has a low inhibited effect on different Candida species. The chemical composition, structure and functional groups of the oils play a fundamental role in determining their antifungal activity (Omidbeygi et al., 2007 and Yeşil Celiktas et al., 2007).

Conclusions

Candida albicans is the predominant cause of vaginitis Candida in Upper Egypt. Compared with Candida albicans, non albicans Candida was more producer to virulence factor. The increase in the prevalence of C. tropicalis among non-albicans species may result from the highest activities of hydrolytic enzymes especially lipase which secreted by this taxon. Cinnamon and clove oils could be considered as the excellent source for new antifungal production.

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توصيف وعوامل الإصابة وقابلية الكانديدا المهبلية المعزولة من النساء في قنا بمصر

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المهبل المعبأ من النساء في قنا بمصر

الكنديدا

توصيف وعوامل الإصابة وقابلية الكانديدا المهبلية

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