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# Cervico-Vaginal Candidiasis in Married Women

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**ABSTRACT**

The present study is aimed to identify the isolated yeasts from vagina and cervix of the pregnant and non-pregnant women. The study included 100 patients (50 pregnant; 50 non-pregnant women), in addition to 50 apparently healthy women. The clinical specimen were collected during the period from December 2013 to May 2014. From each patient high vaginal and endo cervical swabs were collected, in addition to the control group. Three slides were prepared from each swab for direct examination (Slide immersed in normal saline, slide immersed in 20% KOH wet mount and Gram stained slide).The clinical specimens cultured on Sabouraud's agar and Brain heart infusion blood agar. Each culture was identified to yeast species by germ tube test, Chrom agar and API 20 C system. The tested women considered infected with Candida spp when the culture from the clinical specimen for each contain > 10 colonies together with positive direct examination and symptoms and signs. The main isolates were C.albicans from pregnant (84.8% from vagina ; 89.7% from cervix) and non-pregnant (66.7% from vagina ; 64.3% from cervix) women. In addition to the control (50% from vagina ; 0% from cervix) group.

Keywords: Genital candidiasis, Cervico-vaginal yeast infection, Vulvovaginitis.

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# التهاب المهبل وعنق الرحم بداء المبيضات (الفطريات المهبلية)

# عند النساء المتزوجات

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**الملخص**

تهدف الدراسة الحالية الى تمييز الخمائر المعزولة من المهبل وعنق الرحم للنساء المصابات الحوامل وغير الحوامل. شملت الدراسة ١٠٠ مريضة (٥٠ من الحوامل ، ٥٠ من غير الحوامل) بالإضافة الى مجموعة السيطرة التي شملت ٥٠ امرأة من الاصحاء ظاهريا ومن غير الحوامل. تم جمع العينات السريرية للفترة من كانون الأول ٢٠١٣ لغاية أيار ٢٠١٤. اخذت مسحة من المهبل وأخرى من عنق الرحم من كل امرأة مصابة، بالإضافة الى مجموعة السيطرة. تم تحضير ٣ شرائح من كل مسحة للفحص المباشر (شريحة مغمسة بالسيلان، شريحة مغمسة في ٢٠% KOH وثالثة صبغت بصبغة Gram). العينات السريرية زرعت على Sabouraud's agar وBrain heart infusion blood agar. تم تمييز كل مزرعة الى جنس الخميرة بواسطة Germ tube، Chrom agar ونظام API 20 C. النساء اللاتي اجري فحصهن اعتبرن مصابات بالفطريات المهبلية عندما كان عدد المستعمرات في كل زرعة لكل عينة سريرية اكثر من ١٠ مستعمرات بالإضافة الى ايجابية الفحص المباشر والاعراض والعلامات. اهم العزلات كانت المبيضات البيضاء *C.albicans* من النساء الحوامل (٨٤.٨% من المهبل، ٨٩.٧% من عنق الرحم) ومن النساء الغير الحوامل (٦٦.٧% من المهبل ، ٦٤.٣% من عنق الرحم). بالإضافة الى مجموعة السيطرة (٥٠% من المهبل ، ٠% من عنق الرحم).

الكلمات الدالة: التهاب الجهاز التناسلي بالمبيضات، التهاب المهبل وعنق الرحم بالمبيضات (الفطريات المهبلية)، التهاب المهبل.

## 1. INTRODUCTION

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Cervico-vaginal yeast infection is also known as genital candidiasis [1,2]. It is a common gynecological problem in women of childbearing age [3]. The infection occurs when there is overgrowth of the yeasts, mostly *Candida* species [4].

*Candida* species (spp) are usually coexisting with Lactobacillus spp in the vagina. There is a balance between *Candida*, normal bacterial flora, and immune defense mechanisms, when this balance is disturbed; colonization is replaced by infection [5]. Under some conditions, such as reduced immunity, prolonged antibiotic therapy, use of steroids, pregnancy, use of oral contraceptives and diabetes, *Candida* spp may become pathogenic and cause candidiasis [6].

*Candida albicans* is responsible for the largest number of symptomatic episodes of vaginal candidiasis [7]. Non-albicans spp are most commonly represented by *C. tropicalis*, *C. glabrata*, and *C. krusei*. Accurate species identification is important for the treatment of the *Candida* infections, as the non-albicans species of *Candida* continue to be increasingly documented [8].

Nowadays, large varieties of *Candida* spp identification procedures are available. Chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentations [9]. These enzymes are species specific, allowing organisms to be identified to the species level by their color and colony characteristics. Chrom agar *Candida* has been shown to allow differentiation of *Candida*l yeasts by color and morphology [10,11]

## 2. MATERIALS AND METHODS

### 2.1 Patients and Control

One hundred married female patients with genital infection were included in this study. For each patient a special Questionnaire form of relevant clinical data was completed with detailed history and special reference to predisposing factors. The studied patients were 50 (50%) pregnant and 50 (50%) non-pregnant women. The age of the patients ranged from 16 – 50 years.

Fifty apparently healthy married female attending the Clinic for other causes were enrolled in the current study as a control group. All of them non-pregnant women. Their age ranged between 16 – 50 years.

**2.2 Sample collection and processing**

A total of 200 samples were collected from symptomatic women attending the Outpatient Clinic of Al-Batool and Al-Khansaa Teaching Hospitals for various gynecological and obstetrical problems in Mosul. The samples consisted from 100 high vaginal swabs and 100 endocervical swabs collected aseptically under full illuminated condition using sterile cotton swabs. From the 50 control married females, both vaginal and endocervical swabs were also obtained and processed in same manner as for the patients.

All the swabs from patients and control group were collected in sterile containers, then brought to the laboratory within two hours.

**2.3 Isolation of the Yeasts**

Each swab (vaginal and cervical) was inoculated onto both Sabouraud dextrose agar with chloramphenicol and Brain – Heart Infusion (BHI) blood agar with chloramphenicol. The specimens were streaked on all the surface of the media to obtain separated colonies. The plates were then incubated aerobically at 37Cº for 2 – 3 days, then checked for growth of yeasts depending on colony morphology and microscopy in lactophenol mount and considered negative and discarded after a third day of incubation [12]. Pure cultures of the yeasts were obtained by subculture of each isolate on Sabouraud dextrose agar at 37Cº for 2 days then preserved as stock culture at 4Cº for further study [13].

**2.4 Direct examination**

Three slides were prepared from each clinical specimen. The first slide immersed in normal saline, the second slide immersed in 20% KOH solution with parker ink, and the third heat fixed smear was stained by Gram's stain.

**2.5 Tests for identification of the yeasts**

1- Lactophenol mount of small portion of the isolated colonies, to determine the morphological features of different yeasts [14].
2- Germ tube test for the production of short initial hyphae [15].
3- Chrom agar medium was inoculated with a small portion of each yeast colony, then incubated for 2 – 3 days at 37C°, producing colonies of different colors [16].
4- API – 20 C system was used for the identification of yeasts according to the analytical profile index.

**2.6 Statistical analysis**

Data will be recorded on a specially designed questionnaire, collected and entered Statistical Package for Social Sciences (SPSS) version 22, and then analyzed statistically by using tables, pie and bar charts according to Dunn and Clark 2009 [17].Chi square and T-test were used to find out the relationship (association) between different variables. Statistical results were considered significant at P level of $< 0.05$

## 3. RESULTS

The 100 pregnant (50%) and non-pregnant (50%) women with genital infection that were included in the present study were of age ranging between 16 – 50 years. They were categorized into 4 age groups (Table 1). The age group between 21 – 30 showed the highest number of patients (52 ; 52%) with a significant difference between pregnant (31 ; 62%) and non-pregnant (21 ; 42%) women at p-value = 0.04. The control group included 50 apparently healthy non-pregnant women , and their age match the patients (Table 1).

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**Table (1):** The age of the pregnant, non-pregnant women and control group

| Age Groups (years) | Total | | Pregnant women | | Non pregnant women | | Control group | |
|---|---|---|---|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| **≤ 20** | 15 | 15 | 10 | 20 | 5 | 10 | 4 | 8 |
| **21 – 30** | 52 | 52 | 31 | 62 | 21 | 42 | 12 | 24 |
| **31 – 40** | 22 | 22 | 9 | 18 | 13 | 26 | 21 | 42 |
| **41 – 50** | 11 | 11 | 0 | 0 | 11 | 22 | 13 | 26 |
| **Total** | 100 | 100 | 50 | 100 | 50 | 100 | 50 | 100 |

A significant difference between pregnant and non-pregnant women in the age group of 21 – 30 years, P = 0.04 according to proportions and Fisher's exact test.

The risk factor that leads to the genital yeast infection of the studied women was pregnancy (50%). For the non-pregnant women, the risk factors were contraception (19%) , diabetes mellites (15%) , antibiotics (9%) and corticosteroids (7%) use.

The clinical presentation for the studied patients showing the highest number of pregnant and non-pregnant women presented with vaginal discharge (50/ 50 , 50/ 50), followed by itching (41/ 50 , 36/ 50), odor (20/ 50 , 31/ 50) and oedema (13/ 50 , 11/ 50) respectively.

Slides in normal saline prepared from the high vaginal swab (HVS) and endo cervical swab (ECS) from pregnant and non-pregnant women revealed yeasts, bacteria, pus and epithelial cells as presented in Table(2).

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**Table (2):** Wet mount examination in normal saline of the clinical specimens for pregnant and non-pregnant women

| Clinical specimens | Pregnant women | | | | | | Non-pregnant women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of samples | No. (%) | | | | | No. of samples | No. (%) | | | | |
| | | **Yeast** | **Bacteria** | **Pus** | **Epithelial** | **Negative** | | **Yeast** | **Bacteria** | **Pus** | **Epithelial** | Negative |
| **HVS *** | 50 | 32 (64) | 28 (56) | 29 (58) | 20 (40) | 9 (18) | 50 | 35 (70) | 25 (50) | 24 (48) | 34 (68) | **4 (8)** |
| **ECS **** | 50 | 22 (44) | 9 (18) | 26 (52) | 6 (12) | 18 (36) | 50 | 22 (44) | 7 (14) | 25 (50) | 11 (22) | **15 (30)** |

* High Vaginal Swab
** Endo Cervical Swab

Stained slides with Gram's method and others mounted slides with 20% KOH solution with parker ink for all the clinical specimens showed budding yeast cells with or without pseudohyphae as shown in Table 3. In pregnant women, 64% of the HVS showed the presence of budding yeast cells and in 44% of the ECS with no significant difference between them at P-value of 0.16 . In non-pregnant women, 70% of the vaginal swabs revealed budding yeast cells, while in 40% of the ECS with a significant difference between them at p-value of 0.001. On the other hand, the control group showed the presence of budding yeast cells in 24% of the HVS only with a significant difference between the tested women and control group at P-value of 0.01.

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**Table (3):** Direct examination of the clinical specimens with Gram stain & KOH mount for pregnant, non-pregnant women and control group

| Clinical specimens | No. of samples | Pregnant women No. (%) | | No. of samples | Non-pregnant women No. (%) | | No. of samples | Control group No. (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Budding yeast cells with or without pseudohyphae** | **Negative** | | **Budding yeast cells with or without pseudohyphae** | **Negative** | | **Budding yeast cells** | **Negative** |
| **HVS** | 50 | 32 (64) | 18 (36) | 50 | 35 (70) | 15 (30) | 50 | 12 (24) | 38 (76) |
| **ECS** | 50 | 22 (44) | 28 (56) | 50 | 20 (40) | 30 (60) | 50 | 0 (0) | 50 (100) |

No significant difference between HVS and ECS of pregnant women at P-value of 0.16. Chi square test was used. A significant difference between HVS and ECS of non-pregnant women at P-value of 0.001 using proportions and Chi square test. A significant difference between the tested women and the control group at P-value of 0.01 using Chi square test.

Out of the 50 HVS from pregnant women, 33 (66%) of them showed positive culture for yeasts on Sabouraud's agar and Brain heart infusion blood agar, while from the 50 ECS of the same group of the patients 29 (58%) showed yeast colonies. On the other hand, from the 50 HVS of non-pregnant women, 30 (60%) showed positive culture, while from the 50 ECS, 28 (56%) of them were positive yeast culture with no significant difference between HVS and ECS of both pregnant and non-pregnant women at P-values of 0.84 and 1.00. From the 50 HVS of control group, 10 (20%) revealed positive culture, while all of the 50 ECS were negative yeast culture (Table 4).

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**Table (4):** The positive cultures of yeasts obtained from the clinical specimens taken from pregnant, non-pregnant women and control group

| Clinical specimens | Pregnant women | | Non-pregnant women | | Control group | |
|---|---|---|---|---|---|---|
| | **No. of samples** | **Positive cultures No. %** | **No. of samples** | **Positive cultures No. %** | **No. of samples** | **Positive cultures No. %** |
| **HVS** | 50 | 33 66 | 50 | 30 60 | 50 | 10 20 |
| **ECS** | 50 | 29 58 | 50 | 28 56 | 50 | 0 0 |

No significant difference between HVS and ECS of both pregnant and non-pregnant women at P-values of 0.84 and 1.00, Chi square test was used.

Table (5) showed the number of the yeast colonies < and > 10 colonies for each positive culture isolated from pregnant, non-pregnant women and control group from all the clinical specimens. From pregnant women, 20 out of 33 positive culture showed more than 10 colonies for each culture isolated from HVS that means infection, while 15 out of 29 culture from ECS showed more than 10 colonies for each.

From non-pregnant women, 20 0ut of 30 positive culture showed more than 10 colonies for each culture isolated from HVS, while 15 out of 28 culture obtained from ECS showed more than 10 colonies. On the other hand, the number of colonies of all positive cultures (10) obtained from clinical specimens of control group were < 10 colonies for each.

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**Table (5):** The positive yeast cultures isolated from the clinical specimens of pregnant, non-pregnant women and control group with < and > 10 colonies for each

| Clinical specimens | Pregnant women | | | Non-pregnant women | | | Control group | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of positive cultures | Positive cultures < 10 colonies | Positive cultures > 10 colonies | No. of positive cultures | Positive cultures < 10 colonies | Positive cultures > 10 colonies | No. of positive cultures | Positive cultures < 10 colonies | Positive cultures > 10 colonies |
| HVS | 33 | 13 | 20 | 30 | 10 | 20 | 10 | 10 | 0 |
| ECS | 29 | 14 | 15 | 28 | 13 | 15 | 0 | 0 | 0 |

The positive germ tube test identified all the isolates of *C.albicans* from the test group and control group (Figure 1-A), then confirmed by the growth on the selective medium (Chrom agar)-Figure 2. The NAC and other unidentified yeasts were tested to species level by API-C-20 (Figure 1-B).

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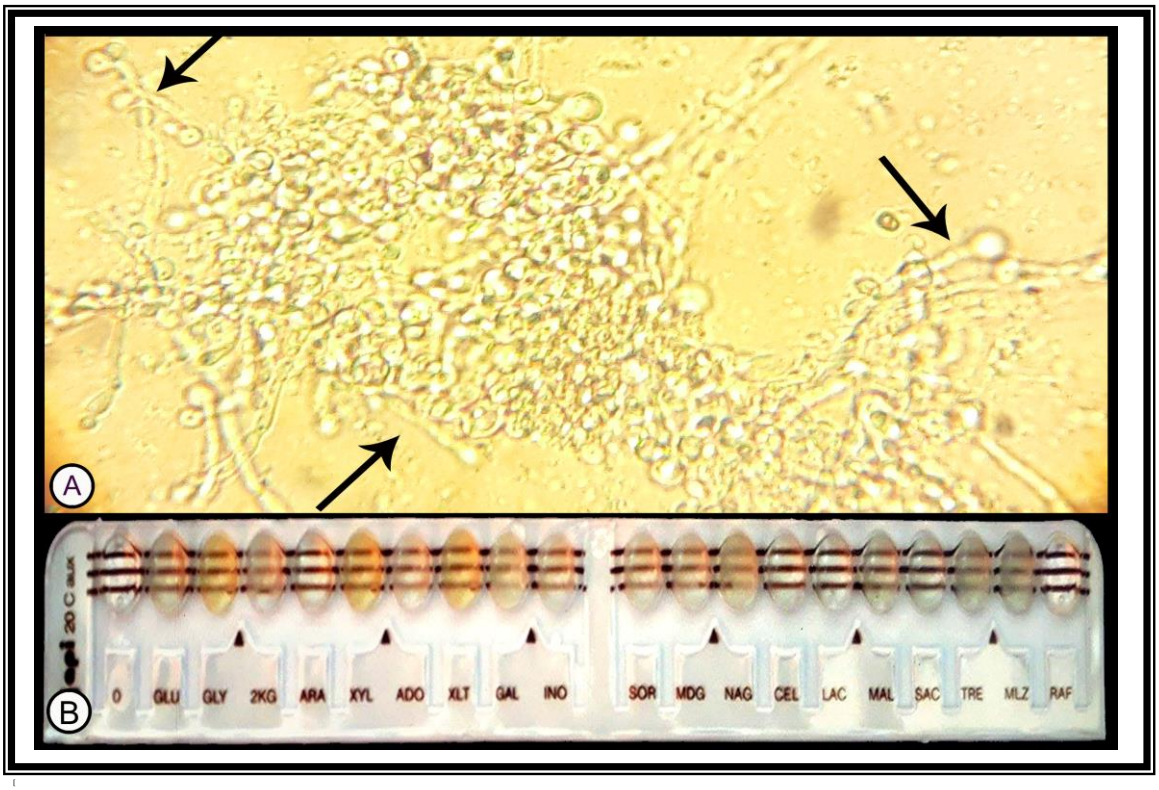


**Figure (1):** Identification of the isolated yeasts:
**A-** Germ tube production by *C.albicans* (arrowed).
**B-** API 20 C system for identification of Non-*Candida albicans* and other yeasts

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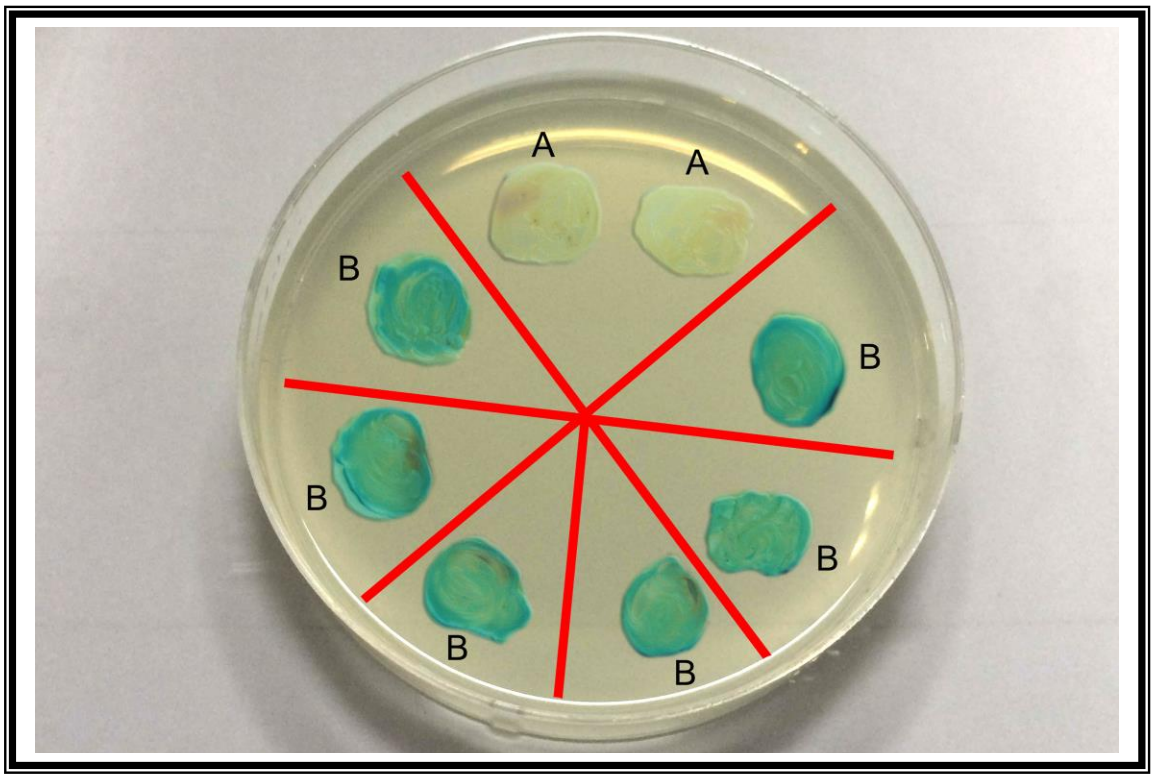


**Figure (2):** The growth of *Candida* species on Chrom agar:
**A-** *C.glabrata* (creamy colored colonies).
**B-** *C.albicans* (light green colored colonies).

The types of yeasts and their number that were isolated from vagina and cervix of pregnant and non-pregnant women present in (Table 6). Out of the 33 isolates from vagina of pregnant women, *C.albicans* represents 84.8% and from the 29 isolates from cervix , *C.albicans* represents 89.7%, while 2 isolates of *C.glabrata* obtained from vagina (6.1%) and cervix (6.9%). The other yeasts that were isolated from vagina and cervix of pregnant women were one isolate of *Cr.laurentii* (3% ; 3.4%) respectively, while 2 isolates of *S. cerevisiae* (6.1%) identified from their vagina only.

In the non-pregnant women, the main isolates were *C.albicans* from vagina (66.7%) and cervix (64.3%) . The other *Candida* species isolated from vagina and cervix of non-pregnant women were *C.glabrata* (13.3% ; 14.3%), and *C.tropicalis* (6.7% ; 7.1%) respectively. *Cryptococcus laurentii* was identified from vagina

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(13.3%) and cervix (14.3%) of 4 women. The difference between *C.albicans* and other yeasts is statistically significant (P = 0.001 and 0.01).

On the other hand, from the control group , *C.albicans* was isolated from the vagina (50%), in addition to *C.glabrata* (30%) , and *S.cerevisiae* (20%) .

**Table (6):** Number and percentage of yeasts isolated from pregnant, non-pregnant women and control group

| Isolated Yeasts | Isolated yeasts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pregnant women** | | | | **Non-pregnant women** | | | | **Control group** | | | |
| | **V*** | | **C**** | | **V** | | **C** | | **V** | | **C** | |
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| ***C.albicans*** | 28 | 84.8 | 26 | 89.7 | 20 | 66.7 | 18 | 64.3 | 5 | 50 | - | - |
| ***C.glabrata*** | 2 | 6.1 | 2 | 6.9 | 4 | 13.3 | 4 | 14.3 | 3 | 30 | - | - |
| ***C.tropicalis*** | - | - | - | - | 2 | 6.7 | 2 | 7.1 | - | - | - | - |
| ***S.cerevisiae*** | 2 | 6.1 | - | - | - | - | - | - | 2 | 20 | - | - |
| ***Cr.laurentii*** | 1 | 3 | 1 | 3.4 | 4 | 13.3 | 4 | 14.3 | - | - | - | - |
| **Total** | 33 | 100 | 29 | 100 | 30 | 100 | 28 | 100 | 10 | 100 | - | - |

*C = Candida ; Cr = Cryptococcus ; S = Saccharomyces*
* Vagina ** Cervix
A significant difference between *C.albicans* and other yeasts was found in both vaginal and cervical specimens among pregnant and non-pregnant women. P = 0.001 and 0.01.

In table 7, the percentage of vaginal infection in the studied women was 40% including 20% of the pregnant and the same percentage for the non-pregnant women. On the other hand, the percentage of cervical infection in the same group of the studied women was 30% involving 15% of pregnant and 15% of non-pregnant women. The higher incidence of infection caused by *C.albicans* for both vagina and cervix (85% ; 86.6%) in pregnant and in non- pregnant (70% ; 66.7%) women respectively.

The other NAC was *C.glabrata* (5% ; 6.7%) from vagina and cervix of pregnant women and (10% ; 13.3%) from non-pregnant women respectively, in

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addition to one isolate (5%) of *C.tropicalis* from vagina of non-pregnant women. Furthermore, the other yeasts isolated were *S.cerevisiae* (5%) from vagina of pregnant women and *Cr.laurentii* (5% ; 6.7%) from vagina and cervix of pregnant women and (15% ; 20%) from non-pregnant women respectively with no significant difference between vaginal and cervical positive culture of both pregnant and non-pregnant women at P-values of 0.29 and 0.29 .

**Table (7):** Yeasts isolated from pregnant and non-pregnant women with positive culture > 10 colonies for each

| Type of yeasts | Isolated yeasts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Pregnant women** | | | | **Non-pregnant women** | | | |
| | **Positive culture >10 colonies** | | | | **Positive culture >10 colonies** | | | |
| | **V** | | **C** | | **V** | | **C** | |
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| ***C.albicans*** | 17 | 85 | 13 | 86.6 | 14 | 70 | 10 | 66.7 |
| ***C.glabrata*** | 1 | 5 | 1 | 6.7 | 2 | 10 | 2 | 13.3 |
| ***C.tropicalis*** | - | - | - | - | 1 | 5 | - | - |
| ***S.cerevisiae*** | 1 | 5 | - | - | - | - | - | - |
| ***Cr.laurentii*** | 1 | 5 | 1 | 6.7 | 3 | 15 | 3 | 20 |
| **Total** | 20 | 100 | 15 | 100 | 20 | 100 | 15 | 100 |

No significant difference between vaginal and cervical positive culture of both pregnant and non-pregnant women at P-values of 0.29 and 0.29, Chi square test was used.

## 4. DISCUSSION

Vaginal candidiasis is considered the second most common cause of genital infection in women of reproductive age, although it represents a problem of global importance in public- health, its exact incidence is unknown [18,19]. The main reservoir for *Candida* is thought to be the rectum, but vaginal colonization is also common [20].

The present study showed that women aged from 21-30 years had highest number of cervico-vaginal infections in both pregnant (62%), and non-pregnant (42%) women. These results were not consistent with the results of Ahmed *et al*., 2016 who reported that the cervico-vaginal infection by *Candida* was observed in the women aged 41 to 50 years [21], while Brandolt *et al*., 2017 [22] reported that more than 65% of the women with cervico-vaginal candidiasis under 31 years of age, which in agreement with our results.

The clinical presentations in the studied pregnant and non-pregnant women were vaginal discharge followed by itching, odor and oedema. Vaginal discharge is one of most frequent gynecological problems encountered in females especially during their reproductive stage [23]. Other authors mentioned that the signs and symptoms of vaginitis include thick cottage cheese-like vaginal discharge associated with vulvar pruritus, pain, burning, erythema and /or oedema [24].

The pregnancy represents a risk factor in the occurrence of vaginal candidiasis. Odds, 1988 [25] mentioned that the Documented risk factors of vaginal candidiasis are pregnancy (30-40%), use of high estrogen content oral contraceptives, antibiotics, steroids and chemotherapeutics. The risk factors in the current study were pregnancy (50%), contraception (19%), diabetes mellites (15%), antibiotics (9%) and corticosteroids (7%). The increased secretion of reproductive hormones during pregnancy favors the formation of infection [26]. Other investigators reported that the incidence of infection, as well as the increase in colonization of the mucosa by the yeasts, is also higher in women with diabetes due to their higher glycogen levels and in those with HIV due to immune suppression [27].

The accurate diagnosis of vaginal candidiasis (VC) is important so that patients do not have to rely on empirical treatment, which may be inappropriate. It

was reported by Schwiertz *et al*., 2006 [28] a rate of misjudgment of VC by physicians of 77% on the basis of clinical evidence alone. During this study, the wet mount preparation in normal saline showed budding yeast cells in 64% of the HVS and 44% of ECS in pregnant women and in 70% of HVS and 44% in non-pregnant women. Moreover, bacteria was also detected in HVS and ECS of the tested women (Table 2). Narasimha *et al*., 2014 [29] reported that the microorganisms observed in their study were bacteria, *Candida* and trichomonas. The present study was in agreement with a previous study done by Maria *et al*., 2014 who reported that most cervical-vaginal infections were attributable to *Candida* [30].

The current study clearly demonstrate significantly increased the number of the positive microscopical finding from vagina and cervix (by Gram stain and KOH mount) in pregnant (64% , 44%) and non-pregnant (70% , 40%) women compared to 24% , 0% respectively from healthy control group. Newmann *at al*., 1975 [31] investigated two groups of respondents, pregnant and normal women. Their results indicated a greater representation of the positive microscopic findings in the test group (36.7%), compared to the control group (19.9%), and their values corresponded to the present findings in this study.

During this study, the results of cultivation and isolation of yeasts on Sabouraud dextrose agar and B.H.I blood agar from control group showed fewer positive culture (20%) from vaginal swabs only compared to the tested pregnant (66% from vagina ; 58% from cervix) and the tested non- pregnant (60% from vagina ; 56% from cervix) women with no significant difference between HVS and ECS of both pregnant and non-pregnant women . The results of Enweani *at al*., 2001 [32] showed a greater percentage of the vaginal yeast detected in pregnant women (51%), compared to non-pregnant control group (40%) which was in agreement with our study. Other investigators examined the vaginal swabs in two groups. Their control group contained women who were on a regular gynecological control with positive cultures of 25% , while the test group contained pregnant women with positive cultures elevated to 52% [33].

Mycological diagnosis of vaginal candidiasis is complex due to the fact that *Candida* species is an integral part of normal vaginal flora [5]. Microscopic evidence of *Candida* in the vaginal swab and positive cultures are not necessarily an indicator of the infection. Therefore, attention should be paid to the number of colonies of *Candida* in culture [34]. In this study, the women were considered to have *Candida* infection when swabs were positive for yeasts by both microscopy and culture with number of colonies for each culture more than 10 as presented in Table 5. On the other hand, when the microscopy was negative or showed few budding yeast cells and few numbers of colonies (< 10 colonies) were appeared on culture, this was taken to indicate colonization with *Candida* rather than infection. Hopwood *et al*., 1985 [35] reported that when the number of yeast colonies isolated from clinical specimens > 10 considered the causative agent of infection.

In this study, *Candida albicans* was identified by the production of germ tube and confirmed by growth on Chrom agar with the production of green colored colonies. Babić and Hukić, 2010 [5] mentioned that the germ tube test proves yeast germination, and it is characteristic for the detection *Candida albicans*. Furthermore, Chrom agar is a selective medium. It can be used for identification of non-albicans species, as well as *C.albicans*, if germ tube test was not characteristic. During this study, the unidentified yeast species by germ tube formation and color production on Chrom agar mainly these non-albicans *Candida*, they were identified by API 20 C test. The need for rapid identification of *Candida* species has led to the development of several media that differentiate yeast species based on colony color [36]. These media contain chromogenic substrates that react with species-specific enzymes secreted by various *Candida* species producing colonies with various pigmentations [37]. Bhesania and Narayankhedkar *et al*., 2017 [24] reported that assimilation tests is of importance for identification of yeast isolates, and the method consists of essentially growing yeast on a basal carbohyhdrate-free medium supplemented with test sugar.

In this study, *C.albicans* was predominant, accounting the higher incidence of isolates in both colonized patients and those with VC. It represents 84.8% of the

isolate from vagina and 89.7% from cervix of pregnant women, while represent 66.7% from vagina and 64.3% from cervix of non-pregnant women with a significant difference in comparison to the other *Candida* species. Moreover, *C.albicans* represented 50% of the isolates from the control group (Table 6 , 7). In fact, this species is the most pathogenic of the gender, being related to most cases of vaginal candidiasis (VC) described. Ying et al., 2016 [38] reported that *C.albicans* is considered as the most common causative agent and isolated from 85%-90% of cases of VC. *Candida glabrata* was the second common pathogen detected in our study followed by *C.tropicalis*, which was in agreement with previous studies reported by several investigators [39]. In many parts of the world, non-albicans isolates notably *C.glabrata* affect 10-20% of women [24]. Vaginitis induced by non-albicans species is clinically indistinguishable from that caused by *C.albicans* but such species are more resistant to treatment [40].

## 5. CONCLUSION

The studied cases of pregnant and non-pregnant women have cervico-vaginal candidiasis when both direct examination and culture were positive for the presence of yeasts, in addition to the sign and symptoms. Furthermore, the number of colonies > 10 on culture media for each patient. The main isolates were *C.albicans* in addition to non-albicans *Candida* including *C.glabrata* and *C.tropicalis* .

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