## Prevalence of Vaginal Candidiasis among women and Diagnosis of *Candida* species from vaginal infection in Kirkuk city

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#### **Abstract**

Vaginal candidiasis is a common complaint among women of different age groups in any society whether or not they are sexually active. Although it is both treatable and mild, when left untreated, The study was set to detect Candida organisms in women vaginal. A prospective study of women vaginal swabs collected from Gynecological Clinic in Azadi teaching Hospital and some privet clinical in Kirkuk city and a total of two hundred (200) women swab were analysed for microscopy and culture, from May 2014 to April 2015. Data on epidemiologic indices were collected from the patients, using structured interviewer-administered questionnaires. fifty- seven positive cultures were obtained, constituting 28.5% (n = 57) in a total of 200 women vaginal samples. A total of 28.5% (n= 57) isolates were detected which comprises four different Candida species, namely Candida albicans, C. glabrata, C. krusei and C. kefyre respectively as well as isolated Cryptococcus lorynti with frequency of occurrence of 23%, 2%, 1.5%, 0.5% and 1.5% respectively. The isolates were related to age, the age range of 26-35 years had the highest incidence (35%), followed by age range of 36-45 years (29.5%) and 56-88 years had the least (2%) percentage of occurrence. The isolates were also related to used contraceptives, copper-T contraceptives and combined oral contraceptives 41.5% was the most common methods used by recurrent vaginitis patients while the male condom contraceptives users had lower cure (26.5%). The distribution of vaginal candidiasis according antibiotic using was highest in women take antibiotic with 64.4% of the total 200 cases, while the women don't take antibiotic was recorded lowest occurrence (35.5%). The result shows that *Candida* species has assumed the role of the most common cause of vaginitis, with Candida albicans as the most prevalent species. vaginal candidiasis was common in the young adults o age range of 26-35 years, contraceptive, broad spectrum antibiotic users, other diseases and operation. We therefore recommend prevention, early diagnosis and prompt treatment of vaginal candidiasis especially among the risk groups in order to avert its complications.

#### Introduction

Vaginal candidiasis is a kind of vaginal mucosis infection which is caused by Candida species . During fertile period, it is one of the most common vaginal infections in women, and most frequent as well as most important fungal disease of vaginal content (1). Candida spp. are genus of yeasts and are the most common cause of fungal infections worldwide. (2) Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. (3) Candida albicans is an opportunistic fungal pathogen, this dimorphic yeast is a commensal that colonizes in the human skin, gastrointestinal tract and the female lower genital tract<sup>(4)</sup>. It is a unique parasite capable of colonizing, infecting, and persisting on mucosal surfaces, and also of stimulating mucosal responses. As a pathogen C. albicans is associated with a wide spectrum of diseases in humans, ranging from allergy, severe intractable mucocutaneous diseases, to threatening bloodstream infections<sup>(4)</sup> Non-C. albicans species are emerging pathogens and can also colonize human mucocutaneous surfaces<sup>(5)</sup>. Consequently, they are also isolated in the setting of candidiasis, albeit at a lower frequency. Candidal infections are significant clinical problem for a variety of immunocompetent and immunocompromised patients<sup>(4,6)</sup>. The pathogenesis and prognosis of

candidial infections are affected by the host immune status and also differ greatly according to disease presentations. Therefore, diagnosis, management, and treatment choices vary and need to be considered in the overall setting of the affected human host <sup>(7)</sup>.

#### Aim of Study:

- 1. The aim of this study was to determine the frequency of *Candida species* isolated from women with vaginitis in azadi hospital and some privet clinical in Kirkuk city.
- 2. Determine related infection according to age, contraceptives, antibiotics, diseases clinic and impotent.

#### Materials and Methods Patients

The study was conducted on 200 female patients attending the Obstetrics and Gynecological Clinic of Azadi Hospital and some privet clinical, In Kirkuk city, from May 2014 to April 2015. All women were complaining of symptoms of vaginitis (Discharge that looks like cottage cheese, Itching, pruritus, redness and burning (especially during urination). After careful history taking. Information about age, Women with severe medical disorders, taking oral contraceptive pills, had taken a course of antibiotics or corticosteroids within the preceding 7 days or had vaginal douching during the previous 48 hours were excluded. Written informed consent was obtained from each participant.

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#### **Sampling**

The patient was placed in lithotomy position and vaginal exposure was done through introduction of a non-lubricated sterile Cusco speculum. Double high vaginal swabs (HVS) were collected using sterile cotton tipped swabs and they were sent to the laboratory without delay.

#### **Microscopic examination**

One swab sample was examined microscopically (40x) after 10% KOH preparation to detect the presence of budding yeast cells and pseudohyphae of *Candida species*. For yeast isolation, the other swab sample was inoculated on Sabouraud's dextrose agar (SDA) supplemented with 0.05g/ L Chloramphenicol and CHROMagar Candida to detection of mixed infections with more than one species of *Candida*. After 48 hours incubation at 37°C, cultures were examined for pasty, creamy and smooth white colonies of yeasts which were further identified.

Using the formula = O/P \*100

where: O = The number of individuals with the disease.

P = Total number of individuals in the population involved in the study at the study period.

#### Yeast identification

Yeast identification was done to each positive growth on SDA as follows:

## a) Cultivation on the selective medium (CHRO Magar Candida)

CHRO Magar Candida is a selective fungal medium that includes chromogenic substances allowing for quick identification of several different *Candida spp*. It can be used for identification of individual nonalbicans species, as well as *C. albicans*, if germ tube test was not characteristic. After incubation for (48 hours at 37°C) identification of yeast was preformed based on a colony colour. Using this method, we were able to identify the following individual nonalbicans species: *C. glabrata* (dark pink colonies,

wet), *C. tropicalis* (blue colonies, wet), *C. krusei* (light pink colonies, dry), and *C. albicans* (green colonies, wet)., which also facilitates the detection of mixed infections with more than one species of *Candida* <sup>(8, 9)</sup>. The method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species following differential exoenzyme activity <sup>(10)</sup>.

### **Germ Tube Test** (10)

#### Chlamydospores Formation Test (10) RapID Yeast Plus System

The Rap ID Yeast Plus System" Remel Co. USA" consists of 18 wells containing the following tests: utilization of glucose, maltose, sucrose, trehalose, and raffinose; hydrolysis of fatty acid ester; pnitrophenyl-*N*-acetyl-b, D-galactosaminide; nitrophenyla, D-glucoside; p-nitrophenyl-b, Dglucoside; o-nitrophenyl-b, D-galactoside, nitrophenyl-a, D-galactoside; p-nitrophenyl-b, Dfucoside; p-nitrophenyl phosphate; p-nitrophenyl phosphorylcholine; urea; proline b-naphthylamide; histidine b-naphthylamide; and leucylglycyl bnaphthylamide. Procedure for Panels were inoculated according to the manufacturer's instructions RapID Yeast Plus System is a qualitative micromethod that uses conventional and chromogenic substrates for identification of medically important yeasts, yeastlike fungi, and similar organisms isolated from human clinical specimens (11)

#### **Result and Discussion**

Results of microscopic examination from total number samples of test 25.5% (51/200) showed positive using the microscopic examination, while 74.5% (149/20) of microscopic findings were determined as negative, negative microscopic findings (Figure 1) were more than positive microscopic examination.

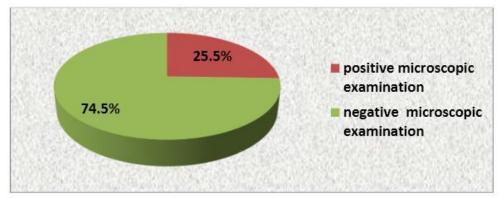


Figure 1: The results of microscopic examination from total number samples

#### **Results of culture isolation**

Two hundred (200) of women with symptoms of vaginal candidiasis visiting the antenatal Gynecological Clinic of Azadi Hospital, participated in this study. The results growth of *Candida* species on Sabourauds agar and CHRO Magar Candida

showed 28.5% (57/200) positive cultures and 71.5 (143/200) negative cultures tested for vaginal candidiasis infection in the laboratory as shown in Figure 2. This finding is agree with studies recorded in Baghdad AL-Hachami ,(2001)<sup>(12)</sup>. The high

prevalence of vaginal candidiasis among women may be due to inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits, shortage of effective treatment, increased levels of estrogens and corticoids, wearing of tight-fitting synthetic underclothing, prolonged use of antibiotics which kill the good and beneficial bacteria (13, 14)

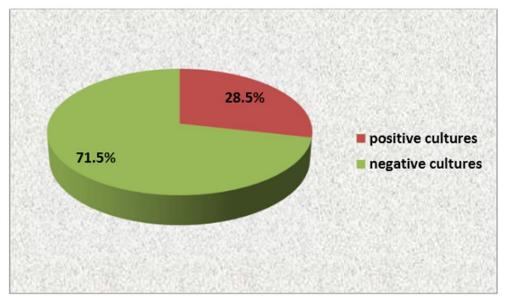


Figure 2: The results of Candida species growth on Sabourauds agar and CHROMagar

## Etiological agent that isolated in this study: *C. albicans*:

The colore colonies of *C. albicans* cultures on Sabourauds (SDA) agar Colonies was white to cream smooth, glabrous, on CHROMagar colonies was green, wet Figure 3. Microscopic examination

Spherical to sub spherical budding blastoconidia Figure 4. Germ tube positive Figure 5. Dalmau Plate Culture on Cornmeal and Tween 80 Agar: branched pseudo hyphae with dense blastoconidia. Spherical chlamydospores, mostly terminal Figure 6,7.

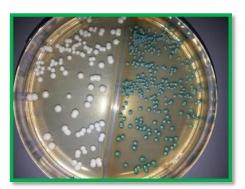




Figure 3: C.albicans on Sabourauds (SDA) agar and CHROMagar

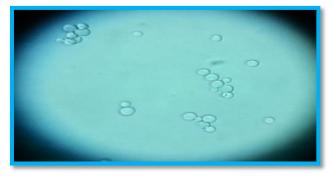


Figure 4: Microscopy of *C.albicans* showing Budding and yeast cells (400x).



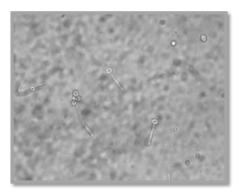


Figure 5: Germ tube of Candida albicans after three hours incubation.

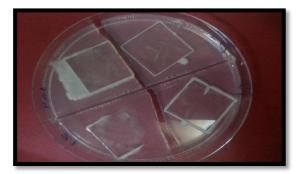


Figure 6 : Dalmau Plate Culture on Cornmeal and Tween 80 Agar

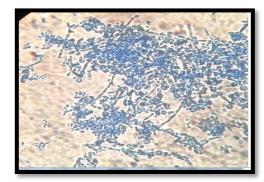


Figure 7: Candida albicans on Cornmeal agar showing branched pseudo hyphae and chlamydospores

#### C. glabrata:

The colonies of *C. glabrata* cultures on Sabourauds (SDA) agar colonies was small, white, shiny and smooth, on CHROMagar colonies was dark pink colonies, wet Figure 8, microscopic examination

small, raised, smooth with an entire periphery Figure 9; Dalmau Plate Culture on Cornmeal and Tween 80 Agar: negative. Spherical chlamydospores, mostly terminal. Germ tube negative.



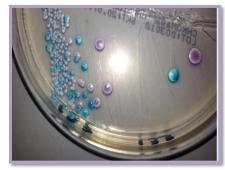


Figure 8 : C. glabrata on Sabourauds (SDA) agar and CHROMagar and shwing mixed infections with more than one species of Candida

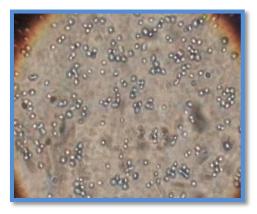


Figure 9: Microscopy of C. glabrata showing yeast cells (400x).

#### C. krusei:

The colonies of *C. krusei* cultures on Sabourauds (SDA) agar colonies was flat, dry, spreading ,ground-glass appearance; variants dull and no separated, glabrous, on CHROMagar colonies was light pink

colonies, dry Figure 10. microscopic examination Large, flat, dry with a delicate feathery periphery Figure 11. Dalmau Plate Culture on Cornmeal and Tween 80 Agar: negative . Germ tube negative.





Figure 10: C. krusei on Sabourauds (SDA) agar and CHROMagar

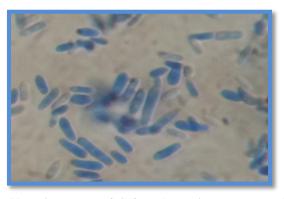


Figure 11: Microscopy of C. krusei showing yeast cells (400x).

#### C. kefyre:

The colonies of *C. kefyre* cultures on Sabourauds (SDA) agar colonies was white to cream colored smooth, glabrous, on CHROMagar colonies was

white, wet Figure 12. microscopic examination spherical to sub spherical budding blastoconidia Figure 13. Dalmau Plate culture on Cornmeal and Tween 80 Agar: negative. Germ tube negative.



Figure 12 : C. kefyre on Sabourauds (SDA) agar and CHROMagar

## Distribution frequency of isolates in vaginal infection

Candida species were isolated from the Four women, namely, C. albicans, C. glabrata, C. krusei and C. kefyre with percentage occurrence of 23%, 2%, 1.5%, and 0.5% respectively as well as isolated Cryptococcus lorynti 1.5%. C. albicans had the highest occurrence C. glabrata found next after C. albicans with 1.5%, C. krusei and C. kefyre in this study which is comparable with the reports of (15, 16). (Fig. 14). This is similar to the reports of Shivan and Saldanha 2011 (17) in India and Rad *et al.* 2012 ( in Iran.The high occurrence rate (23%) of C. albicans observed in this study is an indication that it is a leading causative agent of the reproductive tract yeast infections in women of child bearing age as also observed<sup>(19)</sup>. These results are comparable to

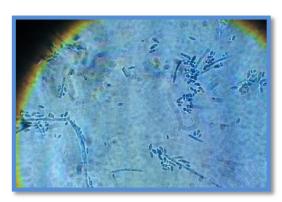


Figure 13: Microscopy of *C. kefyre* showing yeast cells (400x).

Kalkanci et al. 2012<sup>(20)</sup>. who reported similar results. This may be due to its virulent factors which include dimorphism and phenotypic switching. Candida albicans produces protease and phosphatase which enhance its attachment to human epithelium. It can also be deduced that the high incidence rate of C. albicans could be due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favor its growth during pregnancy<sup>(21)</sup>. However, Wise et al 2007<sup>(21)</sup>. and Trofa et al. 2008  $^{(22)}$ , reported a low occurrence of C. albicans in New York. The low occurrence of C. albicans reported by Wise et al. 2007 (21), and Trofa et al. 2008<sup>(22)</sup> may be as a result of good personal hygiene, appropriate nutrition, adequate diagnostic facilities and treatment..

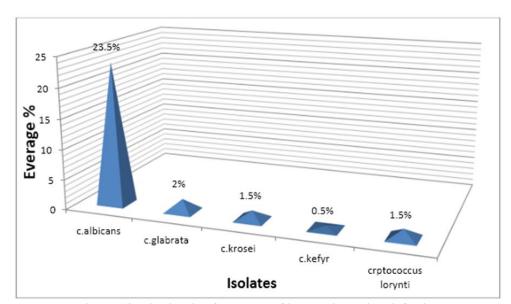


Figure 14: Distribution frequency of isolates in vaginal infection

#### **Relation of Infection Cases with Age Groups**

In this study, 200 patients with recurrent vaginal candidiasis were arranged according to the age groups of the patients ranging from 15-80 years. In the age range 15-25 years, only 49 (24.5%) *Candida species* were detected. The highest occurrence of

vaginal candidiasis 70 (35%) was recorded between ages 26-35 followed by ages 36-45 with percentage occurrence of 29.5% while the lowest occurrence (2%) was recorded between age group 56-80 (Fig. 15). Predominance of candidiasis in the study was in the age group 26-35 The age decade of 26-35 is the

most sexually active age group with highest risk of pregnancies, indulgence in family planning pills and immunosuppression due to HIV/AIDS. The inflammation of the vagina, as in any inflammatory STI (sexually transmitted infections,( increases the risk of acquisition of HIV<sup>(23)</sup>. The highest prevalence of vaginal infections in this study was noted among

the age groups 26-35 years followed by 36-45 age group .This report agreed with<sup>(24)</sup> which reported a peak vaginal infections between age group 20 and 40. This may be due to high sexual activity, poor personal hygiene, the use of contraceptives and drug abuse among this age group.

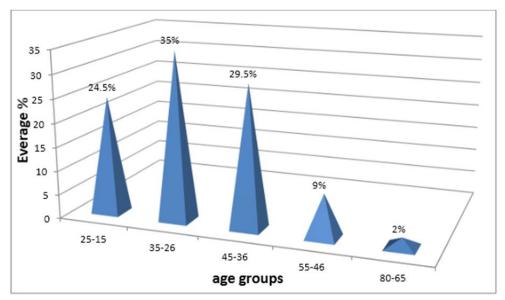


Figure 15: Relation of Infection Cases with Age Groups

#### **Relation of Infection Cases with contraceptive**

The results of study showed that the women used contraceptives and combined copper-T contraceptives pill users the highest occurrence of vaginal candidiasis was calculated to give the percentage prevalence (41.5%) was recorded, followed by no contraceptives prevalence (32%). While the male condom contraceptives users had lower cure (26.5%), as shown in Figure 16. In this study, more number of women with copper-T contraceptives users and combined contraceptives pill users had vaginitis followed by no contraceptive users. This finding suggests that barrier contraception such as copper-T contraceptives and combined oral contraceptives pill has role in recurrent vaginitis. The findings from the present study partly agrees with findings from similar studies in: Indian<sup>(25)</sup> contraceptives (34%) were the most common methods used by recurrent vaginitis cases. Male

condom users were found in (22%) cases and others (copper-T users and combined OC pill users) in (5.2%). Diddle et al. 1969<sup>(26)</sup> showed had study the incidence of yeast infection was more common in women given the oral contraceptive pills for year or more than among either women not given the pills or those taking the pills for less than 1 year. Group using male condom as method of contraception also showed recurrent vaginitis in this study. Unhygienic factors may be possible explanation for this category. Counselling about good hygiene and proper menstrual care may help in prevention of recurrent vaginitis in this group. In India, National AIDS Control Programme was launched in 1987, and started promoting condom for prevention of AIDS and STDs . Hence use of male condom should be promoted in recurrent vaginitis cases even if they are sterilized (27).

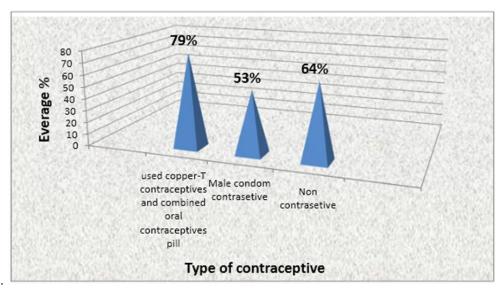


Figure 16:Relation of Infection Cases with contraceptive

#### **Relation of Infection Cases with Antibiotic**

The results of Figure 17 showed that the women used antibiotic have the highest occurrence of vaginal candidiasis was calculated to give the percentage prevalence 64.4% was recorded ,while the women don't take antibiotic was recorded lowest occurrence (35.5%). The findings from the present study partly agrees with findings from similar study Pirotta et al, his reported increased Candida colonization from 21% at baseline to 37% 2 weeks after antibiotics, and 23% of women developed symptomatic VVC after antibiotics, similar to our findings. However, none of these studies included a nonantibiotic group for comparison. Broad spectrum antibiotic users posed a risk to vaginal candidiasis in the study. Antibiotics and vaginal douching suppress normal bacterial flora and allow Candida organisms to proliferate. Of interest is that sulfonamide decrease neutrophil intracellular killing of Candida organisms, and

tetracyclines and amino glycosides have been shown to decrease neutrophil phagocytosis (28). Antibiotics alter the bacterial microflora of the vaginal and gastrointestinal tracts and thus allow for overgrowth of Candida spp. After antibiotic use, the increase in vaginal colonization with Candida spp,. mostly C. albicans, is estimated to range from 10 to 30%, and VVC occurs in 28 to 33% of cases (29) It is commonly hypothesized that the reduction of lactobacilli in the vaginal tract predisposes women to Lactobacilli play a key role in the vaginal flora through the production of hydrogen peroxide, bacteriocins, and lactic acid, which protect against invasion or overgrowth of pathogenic species (30). However, studies have failed to provide evidence that an altered or abnormal vaginal bacterial flora predisposes women to recurrent episodes of VVC in the absence of antibiotic intake  $^{(1,31)}$ .

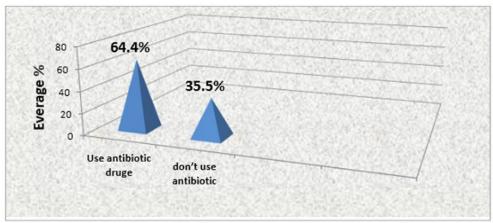


Figure 17: Relation of Infection Cases with Antibiotic

#### Relation of Infection Cases with other diseases

The highest rate (76 %) of vaginal candidiasis was found in women have other diseases such as diabetes millets ,thyroid gland diseases, allergy and ulcer

,while the lowest occurrence (24%) was recorded in women do not suffer from any diseases as shown in Figure 18. vaginal candidiasis VC is both treatable and mild, when left untreated, is a possible risk for

acquisition of HIV/AIDS as well as other complications include pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, menstrual disorders, spontaneous abortion and premature birth. It is now well established that the presence of infective vaginal discharge greatly facilitates transmission and acquisition of HIV between sexual partners (32,33). Candida species e.g. C. albicans outgrows other friendly organisms in the genital tract, it disrupts the balance in the host as a

result of response to the changes in the environment and becomes disease-causing pathogen  $^{(34)}$ . This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids, birth control pills and hormonal drugs and nutrient deficiency  $^{(35,36)}$ . Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defence mechanisms  $^{(35)}$ .

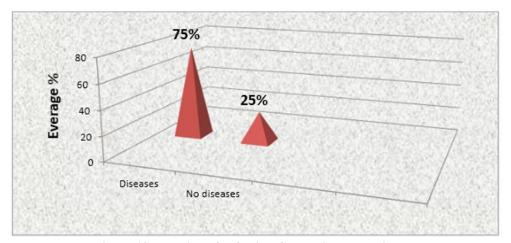


Figure 18: Relation of Infection Cases with other diseases

#### conclusion

1- The result shows that *Candida* species has assumed the role of the most common cause of vaginitis, with *C. albicans* as the most prevalent species. Vaginal candidiasis was common in young adults of age range 26 to 35 years, contraceptive and broad spectrum antibiotic users.

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- 2- Our findings indicate that barrier method of contraception offers protection from recurrent vaginitis. Male condom use should be combined with hygienic practices to reduce relapse.. Usage of male condom should be promoted and combined with other contraceptive methods for prevention of recurrent vaginitis.
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# أنتشار داء المبيضات بين النساء وتشخيص انواع المبيضات في الاصابات المهبلية في مدينة كركوك

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#### الملخص

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يعتبر داء المبيضات المهبلي من اكثر المشاكل شيوعا بين النساء في مختلف الفئات العمرية سواء كانت نشطة او غير نشطة جنسيا . وقد تكون الاصابة شديدة او معتدلة عندما تترك بدون علاج .اعدت الدراسة الحالية للكشف عن انتشار المبيضات في مهبل النساء. وتم جمع المسحات المهبلية من النساء اللواتي يراجعنا الاستشارية النسائية في مستشفى الازادي التعليمي وبعض العيادات الخارجية في مدينة كركوك. وقد جمعت 200 مسحة من النساء في الفترة من شباط 2014 ولغاية نيسان 2015 واجريت لها الاختبار المجهري المباشر والزرع على الاوساط الخاصة, وتم جمع المعلومات من كل مريضة في قائمة استبيان خاصة معدة للدراسة. واظهرت نتائج الزرع على الاوساط الزرعية بنسبة 28.5 % (57) من المجموع الكلى 200 مسحة. وتم عزل اربعة انواع من المبيضات منها C. kefyre و C. krusei , C. glabrata , C. albicans بالاضافة الى عزلة Cryptococcus lorynti وينسية 23% , 2% , 1.5 , % و 1.5 % على التوالي . اما بالنسبة لعلاقة الاصابة بالعمر سجلت اعلى نسبة في الفئة العمرية الواقعة بين 26-35 سنة (35%) وبتبعه الفئة العمرية 36-45 سنة وبنسبة (29.5%) وسجلت اقل نسبة اصابة في الفئة العمرية الواقعة بين 56-88 سنة وينسبة (2%) . واظهرت علاقة الاصابة بموانع الحمل بان النساء اللواتي يستعملنا اللولب وحبوب لمنع الحمل هم اكثر عرضة للمبيضات والتي كانت بنسة (41.5%) وسجلت اقل نسبة في النساء اللواتي تعتمدنا اى موانع الحمل الذكرية والتي كانت بسبة (26.5%). وبالنسبة لتوزيع اصابة داء المبيضات اعتمادا على تناول المضادات الحياتية للنساء حيث سجلت اعلى نسبة اصابة وهي (64.5%) في النساء اللواتي يتناولنا المضادات الحياتية وسجت اقل نسبة اصابة وهي (35.5%) في النساء اللواتي لم يتناولنا المضادات الحياتية. اظهرت نتائج الراسة بان انواع المبيضات تكون اكثر شيوعا في التهابات المهبلية ويعتبر خميرة C.albicans اكثر انتشارا بين الانواع الاخر. وداء المبيضات المهبلية تكون اكثر شيوعا بين النساء اللواتي تقع اعمارهم بين (26–35) سنة وبين اللواتي يسخدمنا موانع الحمل والمضادات الحيوية او يعانين من امراض اخرى واللواتي اجريت لهن عمليات جراحية. ونوصى بالوقاية والتشخيص المبكر والعلاج الفوري من داء المبيضات وخاصة بين الفئات المعرضة للخطر من أجل تجنب مضاعفاته .