*Journal of* Genetic and Environmental Resources Conservation, 2013,1(3):254-261.
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# Detection of *Candida* spp. responsible for vulvovaginitis in women with contraceptives

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## Abstract

From October 2010 to February 2011, 124 high vaginal swabs were collected, 88 swabs from different women with contraceptives and 36 swabs from women with-out all of which were suffering from vulvovaginitis. The specimens were collected from two hospitals in Iraq; Al-Kadhymia Teaching Hospital in Baghdad and Al-Batool Teaching Hospital in Diyala. Fifty-five specimens from 124 with high vaginal swabs were identified as *Candida* spp. the percentage was 44.35%. Forty out of 88 specimens from contraceptive users showed positive results for *Candida* spp., whereas 15 out of 36 specimens from non-contraceptive users showed positive results for *Candida* spp. of which 35(63.6%). The isolates were identified as *Candida albicans*, whereas (36.4%) were identified as non-*albicans* spp., of which 17 (30.9%) were *C. glabrata*, while 3(5.5%) were *C. tropicalis* the statistical analysis showed that there were high significant differences (P<0.01) between *Candida* spp. concerning contraceptives. The isolation rate of *Candida* spp. were higher (P<0.01) among oral contraceptive pills (OCP) which was 47.05% than intrauterine contraceptive device (IUD) which was 43.47%. *Candida albicans* was higher among IUD users (80%) than OCP (50%). Among non- *C. albicans* spp., *C. glabrata* was the most (frequently in all contraceptive users from non-*albicans* spp.), and it's equal to *C. albicans* (50%) among OCP users. *C. tropicalis* was isolated from non contraceptive users (20%). Transmission electron microscope examination showed that *C. albicans, C. glabrata* and *C. tropicalis* have the ability to adhere to vaginal epithelial cells and *C. albicans* was the most adherent more than the other two species. By using dilution method the minimum inhibitory concentration (MIC) of (ketoconazole, clotrimazole, metronidazole and nystatin) for both *C. albicans* and *C. tropicalis* was 12.5 µg/ml and 6. 25 µg/ml in *C. glabrata* except clotrimazole which gave MIC 12.5 µg/ml. Minimum fungicidal concentration (MFC) was 50 µg/ml for all antifungal.

Keywords: *Candida* spp., Vulvovaginitis, Contraceptives, Anticandida drugs.

## Introduction

Human vagina is an elastic muscular canal that extends from the cervix to the vulva. Genital system is consists of Fallopian tubes and uterus, the upper part of uterus duct is empty of microorganisms, but the lower part of uterus and vagina is usually contain microorganisms e.g. *Lactobacillus* (Howkins and Bourn, 1971), in addition other microorganisms e.g. Group B *Streptococci, Diphtheroids, Streptococcus faecailis, Coliform,* an aerobic *cocci, yeasts, Bacteroides, Staphylococcus albus* (Ross, 1979). pH of vagina is acidic due to lactic acid, pH is 5.7 in new infants, in children is equal 6-8, while in pregnancy pH is 4 (Chowdhary, 1986). It was proved that lactic acid in vagina is come from glycogen fermentation in vagina by *Lactobacillus*; this process is controlled by estrogen hormone (Howkins and Bourn, 1971).

Vaginal inflammation is the main reason for vaginal discharges (Quan, 2000), it can be fungal infection due to *Candida* species (candidiasis) and *Tricophyton* spp. in low percentage (Studd, 1998; Bhatla, 2001), or parasitic due to *Trichomonus vaginalis* called Trichomonasis or bacterial infection e.g. *Nisseria gonorrheae* in addition to other kinds of bacteria (Berg *et al.,* 1999; Suzuki *et al*., 2000).

Infections are transferred during sexual transmission or because of normal flora in genital organs when the environmental conditions are

suitable to cause infection (Williams and Willkins, 1995; Baron and Finegold, 1990; Al-Zuhairi, 2001), like immunocompramized diseases (Kauffman and Hedderwicks, 1997).

Birth control is techniques and methods use to prevent fertilization or to interrupt pregnancy at various stages. Contraception includes barrier methods, such as condoms or diaphragm, and oral and injectable contraceptives. Contraceptives, also known as post-coital birth control, include intrauterine devices and what is known as the morning after pill (Hendrick, and Judith, 1997). Physical methods may work in a variety of ways, among them: physically preventing sperm from entering the female reproductive tract; hormonally preventing ovulation from occurring; making the woman's reproductive tract inhospitable to sperm; or surgically altering the male or female reproductive tract to induce sterility. Some methods use more than one mechanism. Physical methods vary in simplicity, convenience and efficacy.

Barrier methods place a physical impediment to the movement of sperm into the female reproductive tract. The most popular barrier method is the male and female condom (Grimes, 2004).

There are various delivery methods for hormonal contraception. The combined oral contraceptive pill (COCP), often referred to as the birth-control pill or simply "the Pill", is a birth control method that includes a combination of an estrogen (oestrogen) and a progestin (progestogen) (Mosher *et al*., 2004).

Intrauterine contraceptive devices that are placed inside the uterus. They are usually shaped like a "T" the arms of the T help hold the device in place. There are two main types of intrauterine contraceptives: those that contain copper (which has a spermicidal effect), and those that release a progestin (a synthetic progestogen). Both types are very effective forms of contraception and can stay in place for at least five years (Trussell, 2007).

## Materials and Methods

High vaginal swabs were collected from 124 patients aged between16-55 years, presented with vulvovaginitis, during the period from October 2010 to February 2011. All specimens were collected from two hospitals; Al-Kadhymia Teaching Hospital in Baghdad and Al-Batool Teaching Hospital in Diyala. Clinical presentations were done by specialized doctor. Informational data were recorded from all patients using a private data. The specimens were taken by sterilized cotton swabs, and were divided in to two smears: one smear was examined immediately under microscope for direct examination; the other usually was transferred to the laboratory for culturing. Forty six from 124 women (37.09%) were used Intra uterine device (IUD).

Direct examination: Specimens of vaginal swabs subjected for examination were usually done by placing the specimens on a clean slide mounted with a drop of KOH 10%, covered with cover-slip, then the slide wormed gently (but not boiling). Examined under the microscope looking for *Candida* cells. The isolates were stained by Gram stain to detect their response to stain, shapes, their arrangement and yeast budding form.

Lactophenol cotton blue examination: After obtain the growth; a touch of colony was taken and placed on a clean slide mixed with drop of lactophenol cotton blue, covered with cover-slip then examined under light microscope to examined blue budding cells of *Candida.*

Germ tube formation test: The inoculums of yeast cells obtained from an isolated colony was suspended in a 0.5 ml of human serum in a small tube (blood centrifugation at 1500 rpm for 15mins and the serum was obtained), the tubes was incubated at 37 ºC for 2-3 hrs. The incubation period must not exceed 3hrs as other yeast species can begin to form germ tubes. A drop of the incubated serum was placed on a slide, covered by cover-slip and examined by the microscope for the presence of germ tubes (Forbes *et al*., 2007).

Chlamydospore Formation Assay: It was prepared according to Wikerham and Pettger (1939); Rose and Harisson (1969), that petri dish was prepared contained slide, filter paper and V shape glass rod (were sterilized by oven at 180 ºC for 2 hrs), drops of corn meal-tween-80 were added on the slide and left until become dry and solid, a part of four days old colony has been grown up on SDA; was streaked on the slide, then drops of sterile distilled water were added on the filter paper; to keep it moist and humid. The dishes were incubated at 25-35 ºC for 4-6 days. After incubation period; a drop of lactophenol cotton blue stain was added to the slide, covered with cover-slip, then examined under microscope 40x to observed chlamydospores.

Identification of *Candida* spp. by using API *Candida* and API 20 C AUX system: API 20 C AUX is a system for the precise identification of the most frequently encountered yeasts, API 20 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation testes and 12 tests for API *Candida*, the cupules are inoculated with a semi-solid minimal medium and the yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source. The reactions are read by comparing them to growth controls and identification is obtained by referring to the analytical profile index.

Attachment of *Candida* to the vaginal epithelial cells: According to Segal *et al*. (1984); Sobel *et al.* (1984) vaginal epithelial cells (VEC) were prepared from healthy young age adult women by taking vaginal swabs. Vaginal swabs were placed into 20 ml of phosphate buffer saline (PBS) then centrifugated at 250 rpm for 5 minutes, and washed three times by using 20 ml of PBS. VEC were hanged in 4 ml PBS. VEC concentration were calculated by haemocytometer, the concentration was adjusted to be 2 x $10^5$ cell/ml. 0.5 ml, yeast suspension was incubated with 0.5 ml VEC for 90 minutes at 37ºC in a shaker water bath, and examined under transmission electron microscope to asses *Candidal* cells adherence to VEC.

Antifungal sensitivity test: Sensitivity of *Candida* spp. to (Metronidazole, Clotrimazole, Ketoconazole and Nystatine ) was studied to determine minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC), by using sabouraud's dextrose broth.

## Results and Discussion

patients according to the type of contraceptives:

1. Thirty four from 124 women in percentage (27.42%) were used birth control pills (the pills).
2. Four women from 124 (3.23%) were used injection.
3. Four women from 124 (3.23%) were used suppositories.
4. Thirty six women from 124 (29.03 %) were considered as a control group, with no contraceptives (Figure 1).

Results of specimens examined by direct examination and culture on SDA: Laboratory examination results showed that 55 specimens from 124 (44.35%) were positive for *Candida* spp., 34 specimens (27.41%) were identified as bacterial infections, 11 specimens (8.87%) were identified as Trichomonasis, only 24 specimens (19.35%) were negative. there was high significant differences ($P<0.01$) between these results (Figure 2).

These results are close to others in some cases and far from others. Balaka *et al.* (2003) mentioned that *Candida* infection ratio was 33.3% from 306, Bacterial infection was 35.7%, while *trichomoniasis* was 10.6%.

While Al-Zuhairi (2001) mentioned that bacterial infections were 37.33% in pregnant women, 20% in non pregnant, *Candida* infection was 28.57% in pregnant and 6.66% in non pregnant. While the percentage of *T. vaginalis* was 5.55% in pregnant and 3.33% in non pregnant.

Bourgeosis *et al.* (1998) mentioned that bacterial vaginosis was the most frequent cases, fungal vaginitis comes in the second level. We improved that *Candida* spp. was the most predominant one, more than 75% of vaginal infection was related to *Candida* spp. Especially *C. albicans, C. glabrata, C. tropicalis and C. krusei.* Also there was another types of yeasts *Torulopsis glabrata* were faced in such cases. Less than 10% of fungal vaginitis was related to *Tricophyton* spp. (Studd, 1998; Campbell and Monga, 2000; Bhatla, 2001).

Distribution of *Candida* vulvovaginitis among contraceptive and non contraceptive users:

Table (1) shows that the number of positive specimens for *Candida* spp. in contraceptive users' was 40 specimens out of 88, while the control groups (non contraceptives users) were only 15 out of 36 specimens. There was no significant differences between positive and negative results of patient groups, while there were a significant differences between positive and negative results of control groups, and there were no significant differences between positive results of both patients and control group, so for negative ones.

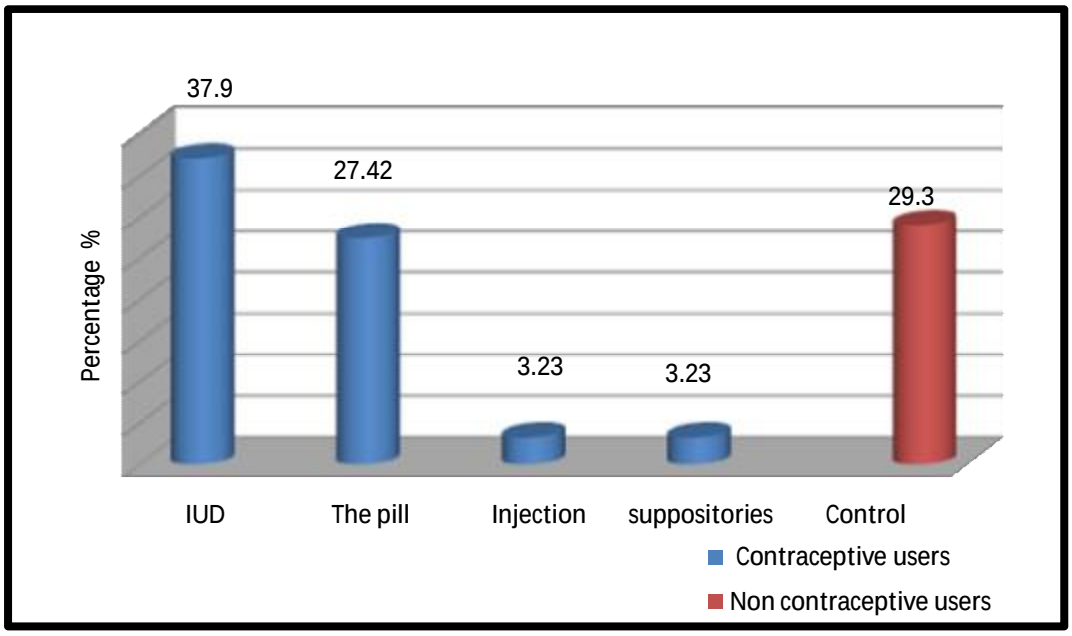


Figure 1: The percentage value of contraceptive users in comparison with control group.

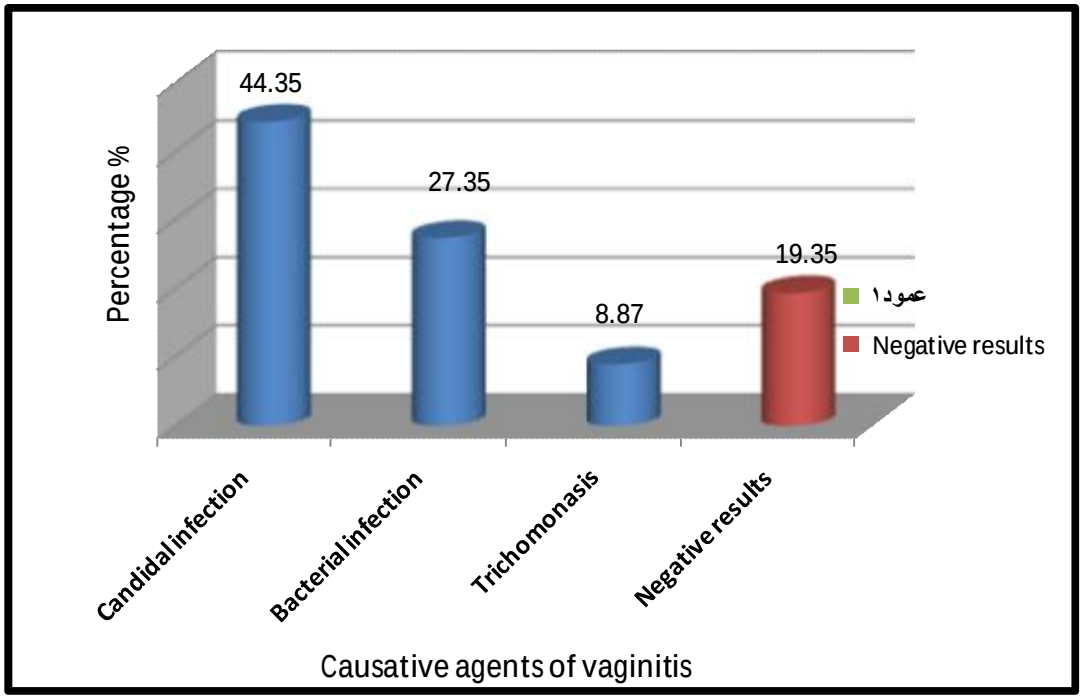


Figure (2): Direct examination results

Table (1): Distribution of patients according to vulvovaginitis by *Candida* spp.

| Specimens groups | Positive results | | Negative results | | Chi-square-$X^2$ value |
|---|---|---|---|---|---|
| | No. of specimens | Percentage % | No. of specimens | Percentage % | |
| Test group, n= 88 | 40 | 45.45 | 48 | 54.54 | 2.774 ns |
| Control group, n= 36 | 15 | 41.67 | 21 | 58.33 | 3.916 * |
| Chi-square-$X^2$ value | -- | 0.683 ns | -- | 0.279 ns | -- |

*(P<0.05), ns= non significant

These findings are agreed with those of Abu-Elteen *et al*. (2001), the isolation rate was 44.9% in women with vaginal discharge, But it was higher than this of Mendoza *et al*. (1999) who mentioned that the isolation rate was 24%, isolation rate of Bauters *et al.* (2002) was 21.7%, and Consolaro *et al.* (2004) mentioned that the isolation rate in women with vaginal discharge was 20.1%. Other investigators have reported isolation rates up to 79.3% of *Candida* spp. from patients with VV (Parazzini *et al.,* 2000; Ribeiro *et al.*, 2001). This results were in agreement with this of Cetin *et al.* (2007) that *Candida* spp. were isolated from 44.2% of contraceptive users, while this isolated from non-contraceptive users had an isolation rate of 37.9% (P<0.05). It has been reported that reproductive age, pregnancy, diabetes, contraception and antibiotic use correlated positively with both *C. albicans* and non *albicans* spp. isolates (Grigoriou *et al.,* 2006). Some studies have shown that there were no significant differences between contraceptive and non-contraceptive users concerning *Candida* infection (Odds *et al.,* 1988; Erdem *et al.,* 2003), and this agree with our results. Enweani *et al*. (2001) detected *Candida* spp. in 51.5% of contraceptive users, but a somewhat lower isolation rate (40.6%) was found in non-contraceptive users.

vulvovaginitis according to age groups: In this study, 124 women between the age group of 16 and 55 years were evaluated. The range of ages was classified into different groups from 16-25, 26-35, 36-45 and 46 years and above. Twenty five patients suffered from vulvovaginitis within the age group 16-25 (25%), patients of the age group 26-35 with a percentage of 41(41%), those of the age group 36-45 with a percentage of 29(29%), patients of the age 46 years and above revealed a percentage of 5%, there were a high significant differences in distribution of vulvovaginitis between age groups as showed in Figure (3).

Vulvovaginal candidiasis is the second most frequent infection of the female genital tract. During a woman's lifespan, nearly 75% will have a candidal vulvovaginitis (Erdem *et al.*, 2003; Grigoriou *et al.,* 2006).

In the current study, the highest frequency (highly significant) of *Candida* spp. was occurred in the age group 26-35 which was 50.9%., followed by this of the age groups 36-45 which was 25.45% and 18.18% of the age group 16-25. Whereas the lowest one which was 5.45% at age 46 year and above which had the lowest frequency of *Candida* spp. (Figure 3). Parazzini *et al.* (2000) reported that there was no clear association between age and frequency of *Candida* infection. On the other hand, Ako-Nai *et al*. (1993) reported that the highest frequency of *Candida* spp. was found in the age group 20-25 year. Okungbowa *et al*. (2003) reported that the age group 26-30 year had the highest frequency of *Candida* spp., followed by the age group 21-25 year, and age 41years and above had the lowest frequency in women with symptomatic VV infection. Al-Rijabo (2004) reported that the most frequent fungal vaginitis was in the age group (31–50) years old, followed by this of (16–30) years old and the lowest frequent infection was in the age 50years old and above. Predisposing factors for vulvovaginitis are pregnancy, antibiotics and contraceptives. 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 for *Candida* growth our results agreed with Richard and Sweet (1985); Sobel (1993); Mahdi and Al–Hamadani (1998).

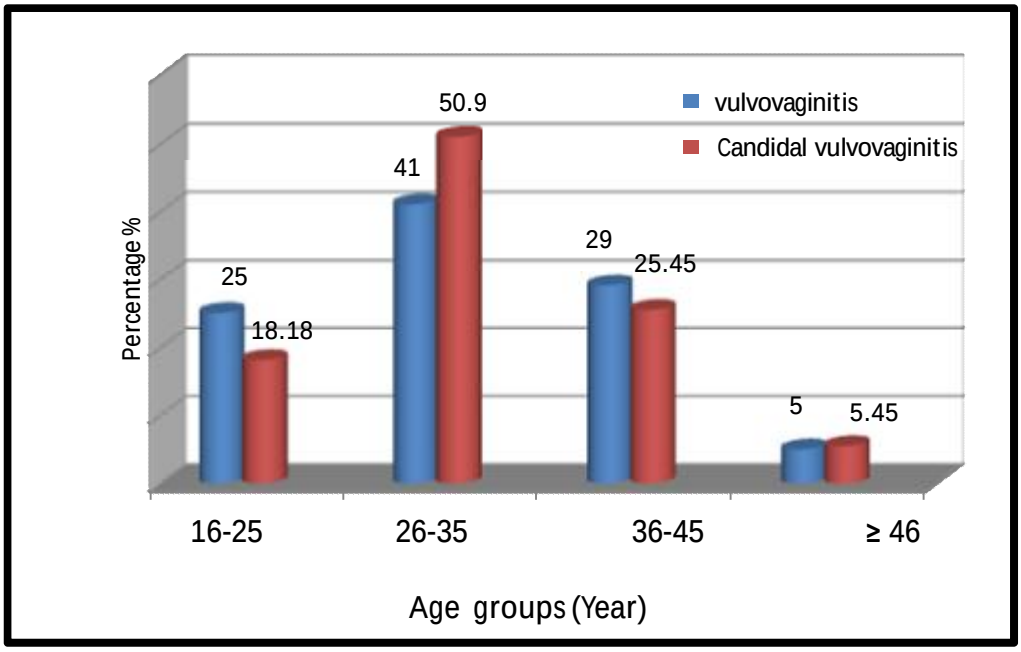


Figure (3): Distribution of vulvovaginitis in women according to age groups
*Chi-square-$X^2$ value: 7.262 ** 6.271 ** (P<0.01)

Contraceptives that contain estrogen hormone increasing candidal adhering to vaginal epithelial cell (VEC), which encouraged transformation from yeast form to filamentous form (Vazques Sobel, 2002), in addition; contraceptive helps in glycogen presentation in VEC which decrease vaginal pH that make suitable environment to yeast growth (Khider, 1985; Spinillo *et al.*,1993). Antibiotics usage corporate to kill normal flora in body which increase *Candida* growth in vagina (Willmott, 1975; De-leon *et al*., 2002). Diabetes is enhance *Candida* infection; due to sugar increasing in vagina that make it suitable environment to yeast growth (De-leon *et. al.,* 2002).

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