



Epidemiological Study of *Candida* Species among Vaginal and Oral Candidiasis from different clinical states.

Najwan Abbas Mohammed^{1*}; Nemat Jamel Abdulbaqi²; Hamzia Ali Ajah³

^{1,2}Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

³Department of Biology, College of Science, AL-Mustansiriya University, Baghdad, Iraq.

Abstract : *Candida albicans* is an opportunistic fungus that infects the mucosae of oral and vaginal cavities. The aim of this study was to study the epidemiological distribution of *Candida* species in Iraqi patients with oral and vaginal candidiasis. 104 high vaginal swabs were collected from different infected women (pregnant and non- pregnant) suffered from vulvovaginal candidiasis (VVC) aged between 16- 50 years old during the period from March 2015 to the end of June 2015, 90 oral swabs were collected from children (49 male and 41 female) aged between two days to ten years during the period from May 2015 to the end of June 2015. The laboratory examination results showed that; out of 104 high vaginal swabs (HVS); 49/104 (47.12%) were positive for *Candida* Spp., *Candida albicans* was the most predominant species in percentage 22/49 (45%) and 27(55%) were non *albicans*. out of 90 oral specimens (OS) 47(52.2%) were positive for oral candidiasis and 22/47(47%) were *C. albicans*, whereas 25(53%) were non *albicans*. There are high significant differences of VVC among different age groups with ($P<0.01$), it was predominant at age group 26-35 in percentage 48.98%. VVC was higher among pregnant women in percentage 12/22(54.55%) followed by contraceptive users in percentage 21/39 (53.8%) and among contraceptive users; There are high significant differences ($P<0.01$) in the infection among different types of contraceptives. Oral candidiasis (OC) was high frequency ($P<0.01$) in females than males. There are high significant differences ($P<0.01$) in OC according to ages, child feeding and antibiotics usage, babies at age group 1-5 months showed high frequency of OC. Babies with breastfed have less frequency of OC, whereas those with formula fed have the high frequency with OC, in addition to that antibiotics users showed more prevalence of OC.

Key Word : Vaginal Candidiasis, Oral Candidiasis, *Candida*, Contraceptives, Breastfed, formula fed.

Introduction

Candida species are the normal flora microorganisms, they can be isolated from most sites of a human body and also opportunist pathogens, they can cause different clinical appearances of candidiasis^{1, 2} affecting mucosa, skin, nails, and internal organs of the body, it is also a common opportunistic infection in immunocompromised patients^{3, 4}. The infection caused by *Candida* species is termed as candidiasis⁵. It has almost 150 strains from which about 50% of all infections are caused by *Candida albicans*, but there are at least four other pathogenic species of this fungus, namely *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*⁶. Person to person transmission of *Candida* infections is uncommon; it is seen primarily in oral thrush of

newborns whose mothers have vaginal infections, acquiring them during birth. Sexual transmission from patients with vaginitis to their sexual partners has been reported⁷.

An inflammation of the vagina is termed vulvovaginitis, James⁸ reported that moniliasis or candidal vulvovaginitis is an infection of the vaginal mucous membranes by *Candida albicans*. *Candida* spp. is the most causative agents of vulvovaginitis in women⁹. Vulvovaginal candidiasis is common in adults; 70-75% of women are affected by this infection at least once in their lives¹⁰. About 90% of vulvovaginal candidiasis is due to *Candida albicans* and 5% are due to *Candida glabrata*¹⁰. pregnant women or women who used contraceptives usually suffered from Vulvovaginal candidiasis, infection appears to be red ulcers with characteristic edges with thick white or yellow secretions with burning and pain in infection area^{9, 11, 12}. Oral candidiasis or thrush appears to be ulcers develop to become white spots may accompanied to gather to produced membrane, it is appear in new infants due to low PH of saliva, or due to transferred infection from pregnant infected mother to the new infants during delivery¹³.

The mouth and throat tissues are invaded by the organism, the infections are seen inside the cheek, on the roof of the mouth, tongue, gums and lips, these white patches when wiped from the mouth may cause bleeding and a painful patch of ulcer can also be seen. Esophageal thrush that spreads to the esophagus is known as esophagitis, this infection makes it very painful and hard to swallow food and water^{13, 14, 15}. The infection has traditionally been regarded as an acute condition often affecting newborn babies where there is an immature immune system. In older individuals, acute pseudomembranous candidiasis often occurs when there is a nutritional limitation, local immune suppression (e.g. steroid inhaler administration for the treatment of asthma), or an underlying disease most notably HIV-infection and AIDS^{13, 16}. Such infections are predominantly caused by *C. albicans* and can affect the oropharynx and/or the esophagus of persons with dysfunctions of the adaptive immune system. Indeed, HIV is a major risk factor for developing OC. Further risk factors for developing OC include the wearing of dentures and extremes of age^{17, 18, 19}. Prolonged use of systemic drugs like broad-spectrum antibiotics, immune-suppressants and drugs with xerostomic side-effects, alter the local oral flora or disrupt mucosal surface or reduce the salivary flow, creating a favorable environment for *Candida* to grow²⁰. The causes of this type of yeast infection are reduced resistance to *Candida* in women due to the use of birth control pills. Antibiotic used for a long time causes reduction of the bacteria that do not allow yeast to grow^{21, 22}. Though *C. albicans* remains the predominant cause, several less common species are emerging which exhibit resistance to antifungals. Therefore, non-pharmacologic preventive strategies should be emphasized^{23, 24}.

Materials and methods

Samples collection and isolation

High vaginal swabs collection:

High vaginal swabs were collected from 104 patients aged between 16-50 years, presented with vulvovaginitis, during the period from March 2015 to the end of June 2015. Clinical presentations were done by specialized doctors. 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 cultured on SDA medium.

Oral swabs collection:

Oral swabs were collected from 90 patients aged between two days to ten years, during the period from May 2015 to the end of June 2015. Clinical presentations were done by specialized doctors. The sampling approach involves gently rubbing a sterile cotton swab over the moth tissue (the tongue and roof), and were divided in to two smears: one smear was examined immediately under microscope for direct examination; the other usually was cultured on SDA medium.

Identification of Candidal Isolates:

Candida was identified depending on the morphological features on culture medium and germ tube formation with the use of API-*Candida* systems and then conferring the diagnosis by identifying *Candida* Spp

By using Vitek 2 system. Examined under the microscope looking for *Candida* budding cells. The isolates were stained by Gram stain to detect their response to stain, shapes, their arrangement and yeast budding form. All isolates were grown on sabouraud dextrose agar. The plates were incubated at 30°C for 24-48hrs to isolate pure candidal colonies to examine their shape, size, color and consistency. After confirmation that the colonies were belong to *Candida*; the isolates were purified by streaking on sabouraud dextrose agar by using ABC methods then incubated at 37°C for two days to obtained one isolated pure colony. This isolated colony was transferred to SDA by streaking all the plate, and then incubates at 37 °C over night.

Germ tube formation (GT):

The production of germ tube by the yeast isolates was tested by mixing a small portion of an isolated colony in 0.5 ml of human serum. This suspension was incubated at 37°C for two and half hours. 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^{25,26}.

Identification of *Candida* Spp by Using CHROMagar*Candida* (CaC):

The isolates corresponding to yeasts morphology were inoculated in CHROM agar *Candida* and incubated at 32°C for 72 hrs, for the presumptive differentiation of *Candida* species through the color developed by the colonies, due to development of enzymatic activity.

Statistical Analysis

The Statistical Analysis System- SAS program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentages in this study.

Results and Discussion

Samples Collection

Vaginal Swabs Collection:

The patients enrolled in this study were divided to three basic groups: Pregnant women, Contraceptive devices users' women and non-contraceptive users' women who considered as control group. The high vaginal swabs (HVS) collected in this study were illustrated in figure 1

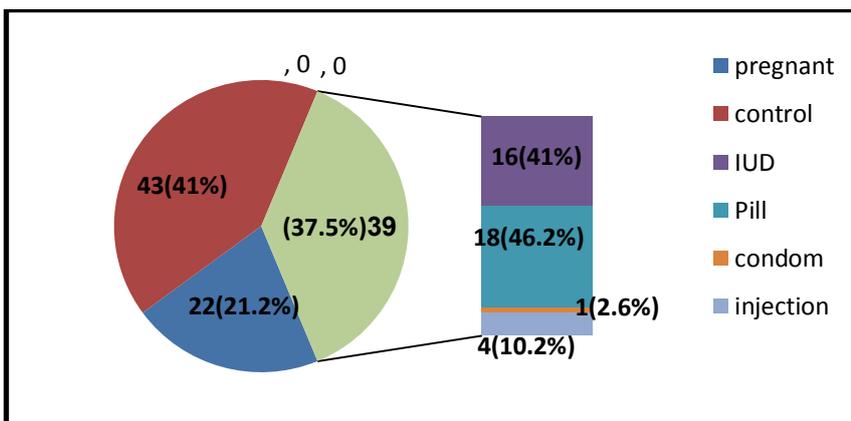


Figure 1: distribution of HVS collected in this study

Oral Swabs Collection

Ninety oral swabs were collected from children (49 male and 41 female) aged between two days to ten years.

The Results of Vaginal and Oral Specimens Examined by Direct Examination and Culture on SDA:

The laboratory examination results showed that; 55(52.88%) out of 104 high vaginal swabs specimens; were negative for candidal growth while 49(47.12%) out of 104 specimens were gave positive results for *Candida* Spp. by direct examination on 10% KOH and cultured on SDA. These results are closely related with Mohammed⁹ showed that 55 specimens from 124 high vaginal swabs of *Candida* spp. were obtained in study in percentage (44.35%), Fuleet *al.*²⁷ results that (37.82%) women showed laboratory evidence of VVC; while Mintz and Martens²⁸ find that: In total, 29.1% (30/103) of the women tested positive for any *Candida* species. out of 90 oral specimens 43 (47.8%) were negative for *Candida* spp., 47(52.2%) were positive for oral candidiasis. There were high significant differences ($P < 0.01$) between positive and negative results of *Candida* spp. from oral swabs as appeared in table 1.

Table 1: Positive and Negative results of *Candida* Spp. from Oral Swabs

Gender	Total No.	Positive No. (%)	Negative No. (%)
Male	49	21(43%)	28(57%)
Female	41	26(63%)	15(37%)
Total	90	47(52.2%)	43(47.8%)
Chi-square χ^2	---	8.422 **	8.422 **
P-value	---	0.00361	0.00361

** ($P < 0.01$).

Identification of *Candida* Species from Oral and Vaginal Swabs:

Candida was identified depending on the morphological features on culture medium and germ tube formation. The identity of non-*albicans* *Candida* spp. was confirmed by API *Candida* and commercial sugar assimilation tests (API- 20 C AUX; Bio Merieux, Marcy l'Etoile, France and Viteck 2 compact system). Different species had been obtained in the current study; but the really focus will be to the most common species: *Candida albicans*, it was identified as follows:

KOH Direct amount:

High vaginal swab examined directly by using 10% KOH, *Candida* appear as oval to spherical budding cells around epithelial cell. On saline and KOH microscopy, numerous budding yeasts are seen but hypha elements are absent.

Cultural Characteristics:

The morphology of *Candida* colonies on Sabouraud dextrose agar were white to cream, round, curved, soft and smooth to wrinkled, with a characteristic yeast odor, it was grew rapidly in 24 h and matured in 3 days. These results are agreed with Bahavan²⁹.

Microscopic characteristic of *Candida albicans*:

In gram stain, *C. albicans* isolates appeared gram positive, spherical to oval with the present of budding and were much larger than bacteria (Figure 2A), it is agree with Emmons *et al.*¹¹ and Webb *et al.*¹⁴

Germ tube formation:

Candida albicans formed short one piece germ tubes (fig.2B) which discriminated them from non *albicans* species; there was no restriction at the point of attachment to the yeast cells. These tubular extensions signify an early stage in the formation of true hyphae. The germ tubes were formed within two hours of incubation and this is a distinctive diagnosis characteristic of *C. albicans* differentiates it from other fungi. Other yeasts usually do not form germ tubes within this 3 hour²⁹, these results agree with this of Sheppard *et al.*³⁰, when they said that "All *C. albicans* strains were germ tube test (GTT) positive when tested directly from the colony, and all non-*albicans* species were GTT negative when tested directly from the colony".

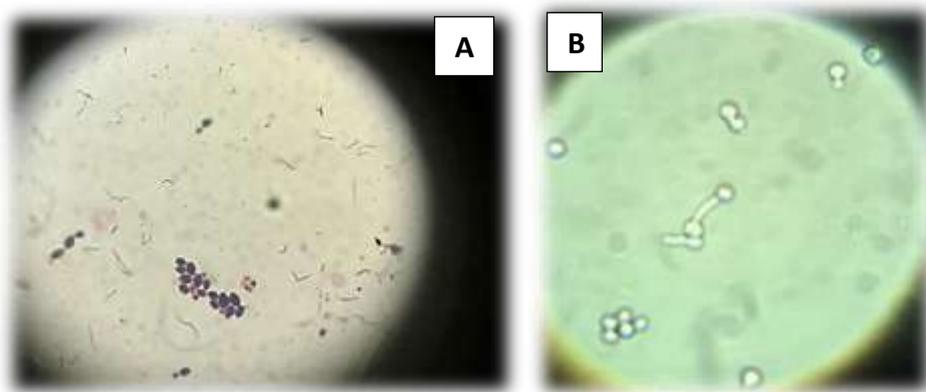


Figure2: A) Gram stain of *Candida albicans* (40X).B) Germ tube of *C. albicans* grown on human serum at 37°C for 2h and half (100 x).

Identification of *Candida* spp. by HiChrom Agar Medium, API Systems and Vitek 2 Compact System

Candida colonies that grew on SDA were sub cultured on HiChrom agar media. The isolates grew well and developed distinctive colored colonies after overnight incubation. Presumptive identification was made by noting the color of the colonies as per the manufacturer’s Instructions (*C. albicans*-green, *C. tropicalis*- blue, *C. krusei*- pink colonies with matt surface, *C. norvegenensis*- cream to pale pink, *C. dubliniensis*- green, *C. glabrata* – Pale cream and *C. famata* – light green (fig. 3)

The chromogenic medium, CHROM agar *Candida*, is a simple and rapid method for identification of common *Candida* species with a sensitivity and specificity of 98 and 86–97%³¹. The current study found that Hichrom agar was not specific to identifying *C. albicans*, *C. dubliniensis* and *C. famata*. API AUX and API 20 *Candida* were good choice to identifying yeast in general and *Candida* spp. respectively (fig.4 and 5). Laura and Yolanda³² indicated that HiChrom agar and API were too sensitive to identifying *Candida* spp. in about 98.60% and 97% respectively. Then the identification results were conforming by using recent identification method; Vitek system; it is the most specific method for *Candida* spp. Identification.

***Candida* Identification Results:**

In this study from 49 *Candida* isolates from vaginal swabs; 22(45%) were *Candida albicans* and 27(55%) were non *albicans*. Whereas 22(47%) out of 47 *Candida* isolates from oral swabs were *C. albicans* and 25(53%) were non *albicans* (table2).

Table 2: *Candida* spp. isolated in this study

<i>Candida</i> spp.	Vaginal swab no.(%)	Oral swab no.(%)
<i>C. albicans</i>	22/49 (45%)	22/47 (47%)
<i>Non-albicans</i>	27/49 (55%)	25/47 (53%)
<i>C. trropicalis</i>	2/27 (7.4%)	7/25 (28%)
<i>C. dubliniensis</i>	4/27 (14.8%)	8/25 (32%)
<i>C glabrata</i>	12/27 (44.5%)	3/25 (12%)
<i>C. krusei</i>	0	3/25 (12)
<i>C. famata</i>	4/27 (14.8%)	0
<i>C. kefyfyr</i>	2/27 (7.4%)	0
<i>C. norvegenensis</i>	3/27 (11.1%)	0
<i>C. lusitaniae</i>	0	4/25 (16%)

In the present study, non *albicans Candida* isolates collectively contributed to more than half (55%) and (53%) of the candidial infections in both of vaginal and oral infection respectively this result agree with Jose *et al.*³³, In a previous study, also observed that the non *albicans Candida* was predominant (70%) as

compared to *C. albicans* (30%), which indicate that the non *albicans Candida* infections are on the rise. Similar finding have been reported in the literature by different authors ³⁴, Whereas *C. albicans* was the most dominant species in both of vaginal and oral infections with percentages 45% and 47% respectively. These results are agree with Javadet *al.* ³⁵ that *Candida albicans* and *Candida glabrata* were the most common yeast species isolated from patients. Mohammed ⁹ indicated that *C. albicans* was the predominant species (63.6%) out of 124 HVS, followed by *C. glabrata* (30.9%) and *C. trupicalis* (5.5%). Imran and Alshammry³⁶ find 71 out of 136 oral swabs were culture positive. Out of which 63.8% isolates were *C. parapsilosis*20.34% were *C. albicans*. Among the non *albicans Candida* isolates in vaginal candidiasis, *C. glabrata* (44.5%) was found to be the predominant species followed by *C. dubliniensis* and *C. famata* in percentage (14.8%) for both, whereas *C. trupicalis* and *C. kefy*r were less dominant (7.4%) for both. Jose *et al.*³³ reported that: Among the non *albicans Candida* species, *C. tropicalis*(37%) was the most common isolate, followed by *C. krusei* (18%) and *C. parapsilosis* (2%)that disagree with present results.

In oral candidiasis; *C. albicans* was the predominant species (47%), Among non *albicansC. dubliniensis* was the most frequent (32%) followed by *C. trupicalis* (28%), *C. lusitaniae*(16%) then *C. glabrata* and *C. krusei* (12%) for both. The results agree with Da Silva-Rocha *et al.* ³⁷ and Tinoco-Araujo *et al.*³⁸ that*C. albicans* was the most prevalent species in premature neonates. Mohamadi *et al.*³⁹ indicated that: The most common infective agent in oral infection in children was *Candida albicans* (64.4%). Fatahinia*et al.*⁴⁰ reported that *C. albicans* was (66.7%) in oral cavity followed by *C.krusie*(14.3%), *C. dubliniensis* (12.7%) and (6.4%) for *C. glabrata*. The findings of the present study about *C. albicans* is lower than Fatahinia *et al.*⁴⁰ these results may be due to lower average age of this subjects, living conditions and some host dietary habits.

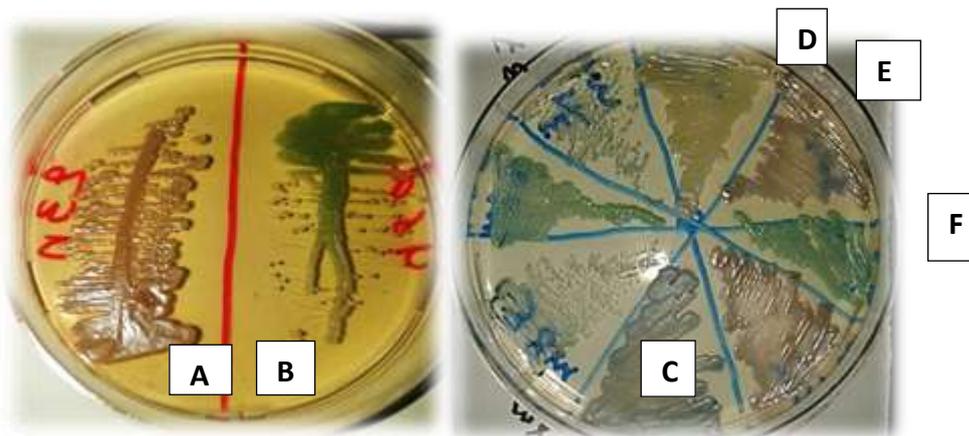


Figure 3:*Candida* species on HiChrom agar media (24h./ 37°C). A) *C. norvegeninsis*, B) *C. dublianinsis* C) *C. trupicalis*, D) *C. glabrata*, E) *C. krusei*, F) *C. albicans*.



Figure4: API 20 *Candida* for *Candida albicans*



Figure 5: API AUX for *Candida albicans*

Distribution of Candidal Vulvovaginitis between Women in Different Age Groups

One hundred four infected women with vulvovaginitis between ages 16-50 years old were evaluated in this study. The range of ages was classified in groups 16-25, 26-35, 36-45; 46 and above years. From 49 women with candidal vulvovaginitis; 14(28.57%) patients show positive results for candidal vulvovaginitis were found in age group 16-25, 24(48.98%) at age group 26-35, at age 36-45; 8 (16.33%), while 3(6.12%) at age 46 and above(Fig.6). Vulvovaginal candidiasis (VVC) is caused by the overgrowth of *Candida* species, most commonly *Candida albicans*, in the vagina and is characterized by curd-like vaginal discharge, itching and erythema⁴¹.

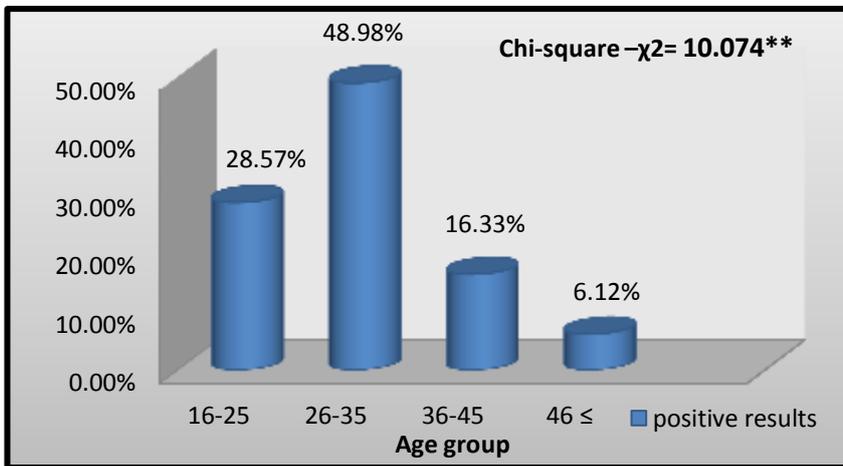


Figure 6: Distribution of Vulvovaginal Candidiasis among Different Age Groups, Chi-square -χ²= 10.074**, (P<0.01).

At the present results showed that VVC was predominant in women enrolled in this study with age group 26-35 in percentage 48.98% followed by ages 16-25 (28.57%), then ages 36-45; whereas only 6.12% of women who have VVC were in age 46 or above years. Statistical analysis show that, there are high significant differences of VVC among different age groups with (P<0.01). The obtained results are agree with the recent results of Sopianet *al.*⁴² who find that the infection rate is highest among women aged 27 -32 years, followed by ages 33 -38 and 21 -26 years old. Only 2 out of 42 patients (4.8%) aged 39 to 45 years old have yeast infection. Mohammed⁹ showed that (50.9%)of patients at the age group 26-35 year had the highest frequency (highly significant) of *Candida* spp., followed by the age groups 36-45 (25.45%) and 16-25 year (18.18%).Whereas (5.45%) specimens at age group 46 and above year had the lowest frequency of *Candida* spp. Nurat *et al.*⁴³ reported that candidiasis was predominantly detected in women in the age group of 20-29 years (33.8%), followed by 30-39 years (24.3%) and no woman in the age group of 40 to 49 years had candidiasis. Jombo *et al.*⁴⁴ and Nelson *et al.*⁴⁵ reported similar results. However, the prevalence of yeast infection was higher in the younger age groups, Younger and more sexually active women may be more prone to develop vaginal yeast infection. They may be more likely to use contraceptives to prevent pregnancy or to space pregnancies and misuse antibiotics. However, Kent⁴⁶ reported a contradictory result; he found that women in the 26–35 year age group were sexually active have low vaginal defense mechanisms against *Candida* species. The lowest frequency of VVC in age 46 and above years which was (6.12%) in the present study is due to women in this age group are nearing their menopause age and are becoming less sexually active, they rarely use contraceptives to prevent pregnancy and vaginal immunity may increase as the levels of estrogen and corticoids decrease⁴⁷.

Grigoriouet *al.*⁴⁸ mentioned that reproductive age, pregnancy, diabetes, contraception and antibiotic use correlated positively with both *C. albicans* and non albicans spp. Isolates. Okungbowa *et al.*⁴⁹ reported a prevalence rate of 10% and 2% within the 36–45 and over 46 years age groups, respectively, and they suggested that this low incidence was probably due to the increased of vaginal immunity with age. The high concentration of estrogen hormone during pregnancy provides favorable environment for the growth of *Candida*. However, the reduction in the effect of estrogen hormone in women as they advance in age could lead to lower infection rates in pregnant women above 40⁵⁰.

Distribution of Vulvovaginal Candidiasis among Study Groups (pregnant & non-pregnant)

The table (3) shows the number of specimens which show positive results for *Candida* spp., in pregnant women were 12 out of 22 in percentage about (54.5%); whereas 10(45.5%) out of 22 show no vulvovaginal candidiasis. While from 39 patients with different contraceptive devices; 21(53.8%) gives positive results for VVC, 18(46.2%) were negative. In contrast with control group 16(37.2%) were positive; whereas 27(62.8%) were negative for VVC.

Table 3: Distribution of VVC among patients groups:

Patients groups	Total No.	Positive results No. (%)	Negative results No. (%)	Chi-square – χ^2
Pregnant	22	12 (54.55)	10 (45.45)	4.248 *
Contraceptives users	39	21 (53.8)	18 (46.2)	4.309 *
Control group	43	16 (37.2)	27 (62.8)	9.512 **
Total no.	104	49 (47.12)	55 (52.88)	1.369 NS
Chi-square $-\chi^2$	---	7.249 **	7.249 **	---
P-value	---	0.00269	0.00269	---

* (P<0.05), ** (P<0.01), NS= Non-significant.

There are a significant differences between positive and negative results for both of pregnant and contraceptives users women (P<0.05) and high significant differences between positive and negative results among control group at (P<0.01). Whether; there are high significant differences in positive results among the groups of study.

VVC is an important cause of morbidity in pregnant women. In pregnant women, vaginal candidiasis has been related to emotional stress and suppression of immune system which steps up the risk of *Candida* species overgrowth and become pathogenic⁴⁷.

Nuratet *al.*⁴³ concluded a high incidence of asymptomatic vulvovaginal candidiasis among pregnant women. The present results are in line with the result of Dias *et al.*⁵¹ whom reported that vulvovaginal candidiasis percent was 34.5% in non-pregnant women and 44.8% in pregnant women, but higher than the finding of Babic and Hulic⁵² that Pregnant showed 46.8% positive cultures for *Candida* spp. and 53.2% negative cultures, and agree with same study in the control group which showed 25.4% positive cultures and 74.6% correlated to negative cultures in contrast with 37.2%. Positive cultures and 62.8% negative in the present study. Fule *et al.*²⁷ concluded that Pregnancy was found to be major risk factor for development of VVC. *C. albicans* was prevalent species but non *albicans* species were also frequently isolated.

Cetin *et al.*⁵³ reported that *Candida* spp. were found in 44.2% of contraceptive users, non-contraceptive users had an isolation rate of 37.9% ($p>0.05$), which agree with present results, Enweani *et al.*⁵⁴ find that contraceptive users showed 51.5% *Candida* spp. in of whereas a somewhat lower isolation rate (40.6%) was found in non-contraceptive users, with referring to the difference in percentages is due to differences in numbers of specimens which be taken in each research. The altered pH and sugar content in vaginal secretions during pregnancy lead to vaginal candidiasis. The acidity level of the vagina is maintained at pH 4.0- 4.5.

However, any physiological changes that affect the beneficial bacteria in the vagina would alter the acidity of the vagina decreasing its pH to 5.0-6.5 therefore improving the establishment of pathogenic organisms such as *Candida*⁵⁵. A higher level of estrogen during pregnancy causes the vagina to produce more glycogen, making it easier for the yeast to grow. Babic and Hukic⁵² concluded that estrogen may cause yeast to

grow faster and stick to the walls of the vagina. Many health practitioners believe that nylon underwear and tight insulating clothing in temperate environment predispose to vaginal candidiasis by increasing the temperature and moisture of the perineum⁵⁶.

Table 4: Distribution of VVC among contraceptives users:

Type of Contraceptive	Total No.	Positive results No. (%)	Negative results No. (%)	Chi-square $-\chi^2$
IUD	16	9 (56.3%)	7 (43.7)	5.024 *
The pill	18	10 (55.6%)	8 (44.4)	4.284 *
Injection	4	1 (25%)	3 (75)	12.50 **
Condom	1	1 (100)	0	15.00 **
Total no.	39	21 (53.8)	18 (46.2)	2.638 NS
Chi-square $-\chi^2$	---	11.648 **	11.648 **	---
P-value	---	0.001	0.001	---

* (P<0.05), ** (P<0.01), NS= Non-significant.

Among contraceptives users; in present study 9(56.3%) from 16 IUD users were diagnosed as VVC, 10(55.6%) from 18 used pills were positive for VVC, 1(25%) from four patients were used birth control injection were positive too, in addition to one (100%) patient used condom was positive for VVC (table 4).

There are high significant differences (P<0.01) in the infection among different types of contraceptives in contrast, there are no significant differences between positive and negative candidiasis among contraceptives types. These results are closely to the results of other researchers in the same field and far with others, Cetin *et al.*⁵³ found that the isolation rate of *C. albicans* was higher among OCP users (57.5%) than IUCD users (38.5%), coitus interrupts (48.5%) and condom users (42.8%), but these differences were not significant. Some studies have shown that there were no significant differences for acquiring *Candida* infection between contraceptive users and non-users⁵⁷. Mohammed⁹ reported that the isolation rate of *Candida* spp. was higher ($\rho<0.01$) among oral contraceptive pills (OCP) users (47.05%) than IUD users (43.47%). The prolonged usage of Cu-IUCD may predispose the cervico-vaginal flora for *Candida* especially for the infectious "hyphae" form although Demirezen *et al.*⁵⁸ were said statistically the correlation between IUCD usage and candidiasis was not significant ($p>0.05$).

Distribution of Oral Candidiasis in Patients According to Gender and Age Groups:

From 49 male, 21(43%) were positive for oral candidiasis (Table 1), while 26(63%) from 41 female were positive for oral candidiasis which is higher than males (P<0.01). These results disagree with Mohamadi *et al.*³⁹ that find the males were having oral candidiasis more than females. However, the differences in the results of these studies may be due to differences in race, age, and disease severity of the study population or related to the different in samples numbers among two studies. In the present study; the oral specimens which collected from patients were organized on groups according to age. The age groups are less than one month premature babies, (1–5 month), (6-11 month), (1year-5) and six and children in above years.

The results show the infection with oral candidiasis in premature babies less than one month were 8(17%) out of 47 positive oral swabs, 15 (32%) at age (1-5month), 9 (19%) at age (6-11 month), 11(23%) at age (1-5 years) and 4 (9%) at age six and above years (fig.7). Oral candidiasis is frequent in the extremes of age⁵⁹. Approximately 5–7% of infants develop oral candidiasis. Its prevalence in AIDS patients is estimated to be 9–31% and close to 20% in the cancer patients⁶⁰. The present results show that oral candidiasis is predominant in children at ages (1-5 months) followed by ages (1-5 year) and (6-11 month) then ages less than one month with high significant differences (P<0.01), these results agree with the results of Mohamadi *et al.*³⁹ whom reported that oral candidiasis is higher in babies at ages (1-3) then (4-7) months in percentages 68% and 25% respectively, whereas the babies less than one month have low percent of infection, which agree with the current study. The major predisposing factors were low birth weight, prolonged hospital stay and associated increased risk of exposure to environmental factors. The colonization of oral mucosa by *Candida* organism plays a decisive role in development of invasive candidiasis; hence, as a prophylactic measure, maintenance of oral hygiene is believed to be an important preventive measure³⁸.

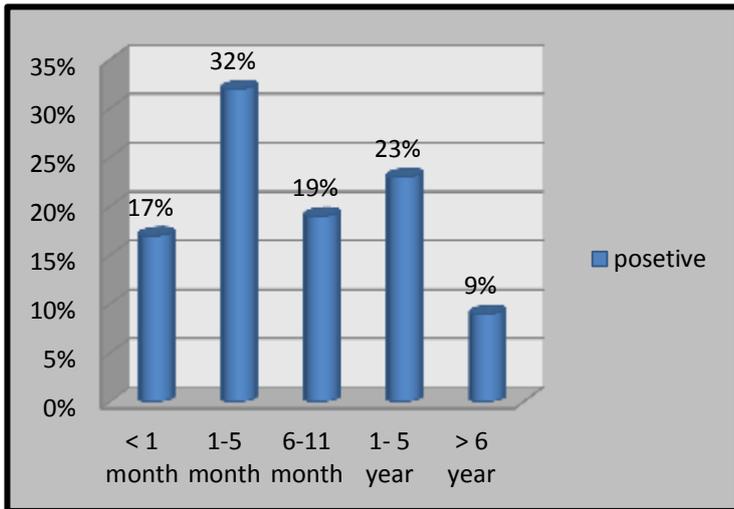


Figure 7: Oral Candidiasis in Children According to Age Groups, Chi-square $\chi^2 = 10.415$, (P<0.01).

Distribution of Oral Candidiasis According to Child Feeding and Antibiotics.

Thirty seven babies depend on their mother's milk (breast feeding) were enrolled in this study, 12(32%) out of those were showed positive results for oral candidiasis, 25(68%) were negative with high significant differences (P<0.01). In contrast with 27(73%) out of 37 child were depend on follow up formula (formula feeding) were positive, whereas 10(27%) with no oral candidiasis with high significant differences (P<0.01). In addition to that out of 16 big children with normal different food there is an equal percent (50%) for both positive and negative results with no significant differences. Figure (8) shows that formula fed babies have the highest (P<0.01). frequency of oral candidiasis.

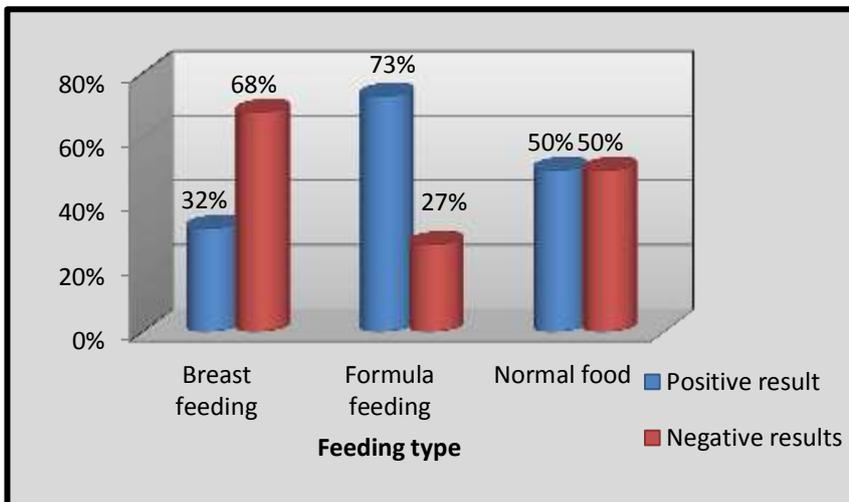


Figure 8: Distribution of Oral Candidiasis According to Child Feeding, **(P<0.01).

Mohamadi *et al.*³⁹ were not found a significant relationship between type of feeding and *Candida* infection. In contrast with another study showed that infants who are breastfed are at low-risk of suffering from moderate to severe candidiasis⁶¹ which agreed with present results. Another important finding was the high incidence of oral and diaper candidiasis among infants of mothers with nipple candidiasis. This concurrence of *Candida* infection in mother and baby has been documented previously. The generation of statistically significant data, has important implications for treating nipple and oral candidiasis⁶². It emphasizes the need to treat both mother and infant with topical antifungal agents even if only one is exhibiting symptoms. The probable explanations include changes in the practice of medicine like introduction of broad-spectrum antibiotics, immunosuppressive agents, transplantations, indwelling catheters, etc., and morbid conditions such as diabetes, severe malnutrition in children and AIDS⁶⁰.

In the present study 29 out of 90 patient were treated with antibiotics. 24(83%) from 29 patient were give oral candidiasis in contrast with 5(17%) were negative with high significant differences ($P < 0.01$). Mohamadi *et al.*³⁹ reported that fifty and thirteen percent of the infants had a history of taking antibiotic and antifungal, respectively. The researchers found that antibiotics ($P = 0.015$) and corticosteroid ($P = 0.002$) usage had a significant relationship with candidiasis⁶³. Growth of *Candida* in saliva is enhanced by the presence of glucose and its adherence to oral epithelial cells is enhanced by a high carbohydrate diet⁶⁴, Systemic factors Extremes of life predispose to infection because of reduced immunity, Drugs such as broad spectrum antibiotics alter the local oral flora creating a suitable environment for *Candida* to proliferate⁶⁵.

Conclusions:

The present study has reported that *Candida albicans* was the predominant species in both of oral and vaginal candidiasis. Vulvovaginal candidiasis was higher among pregnant women fallowed by contraceptive users with high significant differences. Vulvovaginal candidiasis was predominant at age group 26-35. Oral candidiasis (OC) was high frequency in babies at age group 1-5 months. Babies with breast feeding have less frequency of OC, whereas those with formula feeding have the high frequency with OC.

Acknowledgement:

All thanks for patients who accepted to study their clinical states and helped in sampling, with gratitude to biology department, collage of science, Baghdad University and for every one supported this work.

References:

1. Abaci, O. and Haliki-Uztan, A. (2011). Investigation of the susceptibility of *Candida species* isolated from denture wearers to different antifungal antibiotics. African Journal of Microbiology; 5(12): 1398-1403.
2. Nada's, G. C., Taulescu, M. A., Ciobanu, L., Fit, N. I., Flore, C., *et al.* (2013). The interplay between NSAIDs and *Candida albicans* on the gastrointestinal tract of guinea pigs. Mycopathology 175: 221–230.
3. Makwana, G.E., Gadhavi, H. and Sinha, M. (2012). Comparison of Germ Tube Production by *Candida albicans* in various media. National Journal of Integrated Research in Medicine; 3(2):6-8.
4. Mayer, F. L., Wilson, D., and Hube, B. (2013). *Candida albicans* pathogenicity mechanisms, Virulence, 4(2): 119-128.
5. Maganti, H. B. (2011). Species and genotype diversities of yeasts in the clinical and natural environments in Hamilton. M.Sc. Thesis. University of McMaster.
6. Singh, A., Tripathi, P. and Singh, S. (2017). Evaluation of Anti-*Candida* potential of Indigenous Plants and Herbs. International Journal of ChemTech Research, 10(1): 335-341(Abstract).
7. Narkwa, P. W. (2010). Antifungal Susceptibility of *Candida Species* and *Cryptococcus neoformans* Isolated from Patients at the Komfo Anokye Teaching Hospital in Kumasi. M.Sc. Thesis. University of Science and Technology.
8. James, William, D., Berger and Timothy, G. (2006). Andrews' Diseases of the Skin: clinical Dermatology. Saunders Elsevier.
9. Mohammed, N. A. (2012). Detection of *Candida* spp. and other pathogens responsible for vulvovaginitis in women with contraceptive methods. M.S. thesis. Baghdad University. College of Science.
10. Sobel, J. D. (2007). "Vulvovaginal candidosis." Lancet 369(9577): 1961-1971.
11. Emmons, C. W., Binford, C. H. and UtZ, J. P. (1974). Candiasis. In Medical Mycology. Lea and Febiger ed. 2nd ed. Philadephia. Ch. 14: 167-182.
12. Boyd, R. F. (1988). General Microbiology. 2nd ed. Mosby College Publishing St.Louis. Toronto.
13. Thomas, M. S., Parolia, A., Kundabala, M. and Vikram, M. (2010). Asthma and oral health: a review. Aust Dent J. 55:128–33.

14. Webb, B.C., Thomas, C.J., Willcox, M.D.P., Harty, D.W.S. and Knox, K.W. (1998). *Candida* associated denture stomatitis aetiology and Management; A review. Part I factors in influencing distribution of *Candida* species in oral cavity. *Austrian Dental J*.43(1): 45-50.
15. Monod, M. and Borg-von Zepelin, M. (2002). Secreted proteinases and other virulence mechanisms of *Candida albicans*. *ChemImmunol*, 81:114–28.
16. Thompson, G. R., Patel, P. K., Kirkpatrick, W. R., Westbrook, S. D., Berg, D., Erlandsen, J., *et al.* (2010). Oropharyngeal candidiasis in the era of antiretroviral therapy. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod.* 109:488–95.
17. Pappas, P. G., Kauffman, C. A., Andes, D., Benjamin, D. K. J, r., Calandra, T. F and Edwards, J. E. (2009). Infectious Diseases Society of America. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 48:503- 35.
18. Zomorodian, K., Haghighi, N. N., Rajaei, N., Pakshir, K., Tarazooie, B., Vojdani, M, *et al.* (2011). Assessment of *Candida* species colonization and denture-related stomatitis in complete denture wearers. *Med Mycol.* 49:208–11
19. Pereira, C. A., Toledo, B. C., Santos, C. T., Pereira Costa, A. C., Back-Brito, G. N., *et al.* (2013). Opportunistic microorganisms in individuals with lesions of denture stomatitis. *DiagnMicrobiol Infect Dis* 76: 419–424.
20. Martins, N., Ferreira, I.C., Barros, L., Silva, S., and Henriques, M. (2014). Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*177, 223–240.
21. Koneman, E.w., Allen, S.D., Janda, W.M.J., Schreckenberger P.C. and Winn, W.C. (1992). *Diagnostic Microbiology*. 4th ed. J.B. Lippincott company, Philadelphia.
22. Fidel, P. L. (2004). History and new insights into host defense against vaginal candidiasis. *Trends Microbiol*, 12:220-7.
23. Al-Terehi, M., Al-Saadi, A.H., Zaidan, H.K, Alkaim, Z.H., Habeeb, R.A. and Majed N.(2015). Some herbal medicinal plants activity against *Candida* spp which resistance to antifungal drugs, *International Journal of PharmTech Research*, 8(10),146-150.
24. Tharkar, P.R., Tatiya, A.U., Shinde, P.R., Surana, S.J. and Patil, U.K. (2010). Antifungal Activity of *Glycyrrhizaglabra* Linn. And *Emblica officinalis* Gaertn. by Direct Bioautography Method, *International Journal of PharmTech Research*, 2(2),1547-1549.
25. Forbes, B. E., Sahm, D. F. and Weissfeld, A. S. (2007). *Bailey and Scott's Diagnostic Microbiology*. 12th ed. Mosby Elsevier. Texas, USA.
26. Kumar, R. (2010). Speciation of *Candida* isolates in significant count from urine samples. Ph.D. Thesis. University of Rajiv Gandhi. Kim J, Sudbery P (2011) *Candida albicans*, a major human fungal pathogen. *J Microbiol* 49:171–177.
27. Fule, S. R., Das, D. and Fule, R. P. (2015). Detection of phospholipase activity of *Candida albicans* and non *albicans* isolated from women of reproductive age with vulvovaginal candidiasis in rural area,” *Indian Journal of Medical Microbiology*. 33 (1):92–95.
28. Mintz, J. D., Martens, M. G. (2013). Prevalence of non-*albicans* *Candida* infections in women with recurrent vulvovaginal symptomatology. *Adv Infect Dis.* 3(4):238–242.
29. Bhavan, P. S., Rajkumar, R., Radhakrishnan, S., Seenivasan, C. and Kannan, S. (2010). Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. *Inter. J. Bio.* 2 (1): 84-93.
30. Sheppard, D. C.; Locas, M. C.; Restieri, C. and Laverdiere, M. (2008). Utility of the Germ Tube Test for Direct Identification of *Candida albicans* from Positive Blood Culture Bottles. *Journal of clinical microbiology*, 46(10):3508–3509.
31. Agarwal, S., Manchanda, V., Verma, N. and Bhalla, P. (2011). Yeast identification in routine clinical microbiology laboratory and its clinical relevance. *Indian J Med Microbiol.* 29:172-7.
32. Laura, C. and Yolanda, S. (2013). Clinical and microbiological diagnosis of oral candidiasis. *J ClinExp Dent.* 5(5):e279-86.
33. Jose, N. V., Mudhigeti, N., Asir, J. and Chandrakesan, S. D. (2015). Detection of virulence factors and phenotypic characterization of *Candida* isolates from clinical specimens. *J Curr Res Sci Med.* 1:27-31.
34. Dharmeswari, T., Chandrakesan, S. D., Mudhigeti, N., Patricia, A. and Kanungo, R. (2014). Use of chromogenic medium for speciation of *Candida* isolated from clinical specimens. *Int J Curr Res Rev.* 6:1-5.

35. Javad, G., TaheriSarvtin, M., Hedayati, M. T., Hajheydari, Z., Yazdani, J., and Shokohi, T. (2015). Evaluation of *Candida* Colonization and Specific Humoral Responses against *Candida albicans* in Patients with Atopic Dermatitis. *BioMed Research International*, 2015, 849206.
36. Imran, Z. K. and Alshammry, Z. W. (2016). Molecular diagnosis of Candidemia of intensive care unit patients based on sequencing analysis of ITS regions. *International Journal of PharmTech Research*, 9(12): 658-668.
37. Da Silva-Rocha, W. P., Lemos, V. L. de B., Svidiziski, T. I. E., Milan, E. P., and Chaves, G. M. (2014). *Candida* species distribution, genotyping and virulence factors of *Candida albicans* isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. *BMC Oral Health*, 14, 20
38. Tinoco-Araujo, J. E., Araujo, D. F. G., Barbosa, P.G., da Silva Santos, P. S., and de Medeiros, A. M. C. (2013). Invasive candidiasis and oral manifestations in premature newborns. *Einstein (Sao Paulo)* 11(1):71-75.
39. Mohamadi, J., Motaghi, M., panahi, J., Havasian, M. R., Delpisheh, A., Azizian, M. and Pakzad, I. (2014): Anti-fungal resistance in *Candida* isolated from oral and diaper rash candidiasis in neonates. *Bio information* 10(11): 667-670.
40. Fatahinia, M., Poormohamadi, F., and Zarei Mahmoudabadi, A. (2015). Comparative Study of Esterase and Hemolytic Activities in Clinically Important *Candida* Species, Isolated From Oral Cavity of Diabetic and Non-diabetic Individuals. *Jundishapur Journal of Microbiology*, 8(3), e20893. <http://doi.org/10.5812/jjm.20893>.
41. Rathod, S. D., Klausner, J. D., Krupp, K., Reingold, A. L. and Madhivanan, P. (2012). Epidemiologic Features of Vulvovaginal Candidiasis among Reproductive Age Women in India. *Hindawi Publishing Corporation, Infect Dis ObstetGynecol* 2012; 8.
42. Sopian, I. L., Shahabudin, S., Ahmed, M. A., Lung, L. T. T., and Sandai, D. (2016). Yeast Infection and Diabetes Mellitus among Pregnant Mother in Malaysia. *The Malaysian Journal of Medical Sciences : MJMS*, 23(1), 27–34.
43. Nurat, A. A., Ola, B. G., Olsula, S. M., Mikhail, T.A. and Ayodeji, A.S. (2015). Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women Attending a Tertiary Hospital in Ogbomoso, Nigeria. *International Journal of Biomedical Research*. 6(07): 518-523
44. Jombo, G. T. A., Opajobi, S. O., Egah, D. Z., Banwat, E. B. and Denen, P. (2010). Symptomatic vulvovaginal candidiasis and genital colonization by *Candida* species in Nigeria. *J Public Health Epi*. 2(6):147–151
45. Nelson, M., Manjiru, W., Margaret, M. W. (2013 a). Prevalence of vaginal candidiasis and determination of the occurrence of *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. *Op J Med Microbiol*. 3:264–272.
46. Kent, H. (1991). Epidemiology of vaginitis. *Am J Obstet Gynecol*. 165(4):1168–1176.
47. Nelson, M., Manjiru, W. and Margaret, M.W. (2013 b). Identification and susceptibility profile of vaginal *Candida* species to antifungal agents among pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. *Op J Med Microbiol*. 3:239–247.
48. Grigoriou, O., Baka, S., Makrakis, E., Hassiakos, D., Kapparos, G. and Kouskouni, E. (2006). Prevalence of clinical vaginal candidiasis in a university hospital and possible risk factors. *Eur J ObstetGynecolReprodBiol*; 126:121-5.
49. Okungbowa, F., Isuehemen, O. and Dede, A. (2003). The distribution, frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigeria cities. *Rev IberoamMicrobiol*. 20 (2): 60–63.
50. Garcia, H. M., García, S. D., Copolillo, E. F., Cora, E. M., Barata, A. D., Vay, C. A., *et al*. (2006). Prevalence of vaginal candidiasis in pregnant women. Identification of yeasts and susceptibility to antifungal agents. *RevistaArgentinadeMicrobiología*. 38(1):9-12.
51. Dias, L. B., de Souza CarvalhoMelhem, M., Szeszs, M. W., Filho, J. M., and Hahn, R. C. (2011): Vulvovaginal candidiasis in MatoGrosso, Brazil: pregnancy status, causative species and drugs tests. *Brazilian Journal of Microbiology*, 42(4): 1300–1307.
52. Babic, M. and Hukic, M. (2010). *Candida albicans* and non *albicans* species as etiological agents of vaginitis in pregnant and non-pregnant women. *Bosnian journal of basic medical science*; 10 (1): 89-97.
53. Cetin, M., Ocak, S., Gungoren, A. and Ulvhlakverdi, A. (2007). Distribution of *Candida* spp in women with vulvovaginal symptoms and their association with different ages and different contraceptive methods. *Scandina formed via J. of Infec. Dis.*; 39: 584–588.

54. Enweani, I. B., Gugnani, H. C., Okobia, R. and Ojo, S. B. (2001). Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria. *Rev IberoamMicol.* 18:171-3.
55. Nviriesy, P. (2008). Vulvovaginal Candidiasis and bacterial vaginosis infections. *North Am J Clin Diseases.* 22:637-652.
56. Alli, J. A. O., Okonko, I. O., Odu, N. N., Kolade. A.F. and Nwanze, J. C. (2011). Detection and prevalence of *Candida* isolates among patients in Ibadan, Southwestern Nigeria. *J MicrobBiotechnol Res;* 1:176-184.
57. Erdem, H., Cetin, M., Timuroglu, T., Cetin, A., Yanar, O. and Pahsa, A. (2003). Identification of yeasts in public hospital primary care patients with or without clinical vaginitis. *Aust N Z J of ObstetGynaecol.* 43: 312_6.
58. Demirezen, S., Dirlik, O. O. and Beksa, M. S. (2005).The association of *Candida* infection with intrauterine contraceptive device .Dep. of Bio. Ankara, Turkey; 13(1):32– 4 (Abstract)
59. Akpan, A. and Morgan, R. (2002). Oral candidiasis. *Postgrad. Med. J.* 78; 455–459. doi: 10.1136/pmj.78.922.455.
60. Lalla, R. V., Patton, L. L., and Dongari-Bagtzoglou, A. (2013). Oral candidiasis: pathogenesis, clinical presentation, diagnosis and treatment strategies. *J. Calif. Dent. Assoc.* 4: 263–268.
61. Singalavanija, S. and Frieden, I. J. (1995). Diaper dermatitis. *Pediatr Rev.*16(4):142-7.
62. Amir, L. H. (1991). *Candida* and the lactating breast: predisposing factors. *7 Hum Lact.* 7: 177-81.
63. Choi, J.H., Lee, C.G., Lim, Y.J., Kang, H.W., Lim, C.Y. and Choi, J.S. (2013). Prevalence and risk factors of esophageal candidiasis in healthy individuals: A single center experience in korea. *Yonsei Med J.*;54: 160–165. doi: 10.3349/ymj.2013.54.1.160. pmid:23225813
64. Ohman, S. C., Jontell, M. (1988). Treatment of angular cheilitis: the significance of microbial analysis, antimicrobial treatment, and interfering factors. *ActaOdontol Scand.* 46:267–72.
65. Epstein, J. B., Truelove, E. L. and Izutzu, K. L. (1984). Oral candidiasis: pathogenesis and host defense. *Rev Infect Dis.* 6:96–106.
