

Epidemiology of *Candida* infection among pregnant women

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Abstract: vulvovaginal candidiasis(VVC) is a common disease in pregnant women in the last years, the infections may be cause abortion or preterm delivery , also the infections can transfer from the vagina of the infected women to the neonate, giving rise to congenital Candidiasis Therefore, the present study has been focused to isolate *Candida* from (50) pregnant women suffered from vulvovaginal candidiasis(VVC) in AL-Batool maternity Hospital at Waist province (Iraq) and to identify the different species involved in vulvovaginal Candidiasis. The samples were collected between July 2016 and November 2016.. Fifty samples from pregnant women (25 first - pregnancy and 25 multi- pregnancy) with CCV were examined.

A total of 60 isolates were obtained from vaginal swabs samples. *C. albicans* accounted for (43.3%) of the strains including single and mixed infections. The other identified species were *C. krusi* (28.3%), *C. rugosa* (11.6%), *C.tropicalis* (8.3%), *C. parapsilosis* (5%), *C.kefyr* (3.3%) respectively. Mixed infection with two species of *Candida* was recorded in 12 % of vulvovaginal Candidiasis. The most common mixed infection was the combination of *C.albicans* and *C.rugosa*. *C.albicans* was the most common cause of vulvovaginal candidiasis. The second most common species was *C.krusi*.

By using disc diffusion method for seven antifungal drugs toward *Candida* species isolates. They were ketoconazole , nystatin , miconazole, and amphotericin B, gave the highest inhibition to all yeasts isolates. while clotrimazole gave lower inhibition zones towards *C.krusi*, also *C.kefyr* and *C.tropicalis* revealed the resistant to the itraconazole and fluconazole, but *C.krusi* only resistant to itraconazole.

1. Introduction

vulvovaginal candidiasis(VVC) is a common disease in pregnant women in the last years , the infections may be cause abortion or preterm delivery , also the infections can transfer from the vagina of the infected women to the neonate, giving rise to congenital Candidiasis (Samuel *et al.*,2015).

(CCV) caused by overgrowth of yeast (*Candida*), *C.albicans* is the causative agent in most cases compared with other species of *Candida* (Rathod *et al.*, 2015). It is characterized by irritation, purities, burning sensation cord -like patches on vaginal mucosa, thick yellow white discharge and inflammation of the perineum. There are many of Predisposing Factors for developing CCV in women such as use of certain drugs like: antibiotics, Contraceptive pills, or anything with cortisone or steroids , Breast thrush , fatness , diabetes , Immunocompromised , meal with high sugar and yeast content, or tiny in vitamins and minerals (Ellis, 2008). Pregnant women are more susceptible to vaginal candidiasis than healthy women with chronic *Candida* infection (Mitchell, 2004). the CCV can be superficial , deep , acute or chronic, and has broad clinical spectrum. During the pregnancy period the estrogen level increasing and leads to

production of glycogen in the vagina which allows for reproduction of yeast in the vagina cavity . however , an imbalance between estrogen and progesterone can be affect the good bacteria in the vagina would alter The acidity of the vagina reducing(pH more than 6) by enhancing the growth of harmful organisms Such as *Candida* (Akinbiyi, 2008). Vaginal acidity decrease with age , sexual activity , pregnancy, contraception choice and use of antibiotic (Ohmit *et al.*, 2004). Nearly 40% of pregnant women worldwide may have VVC (Prakash and Yadav,2015). Almost 75% of women experience at least one episode of VVC at some point during their lifetime. Nearly 1-3 of patients diagnosed with VVC are infected with different *Candida* species. *Candida* species are among the normal flora in vagina of 25–55% of women without any clinical symptoms. *Candida albicans* is the most widespread species according to the many of studies (Soble,1997). *Candida glabrata* , *Candida krusei* , and *Candida parapsilosis*, however, has more resistance to antifungal drugs, especially to the first line treatments. Some previous studies indicate an increase in the incidence of *Candida glabrata* infection which might be due to the extensive and long term use of antifungal drugs such as azoles(Odds,1988).Thus, the differentiation of diverse species of *Candida* in the laboratory seems imperative. On the other hand, a sample from infected pregnant women may contain mixed species of *Candida* and the isolation and separation process of those different species see culture mediums, API *Candida* kit has high sensitivity and specificity in the differentiation of *Candida* species ,Using this kit, it is possible to identify *Candida* species based on color and other morphologic characteristics , This convenient and yet reliable method has been used predominantly in different studies(Berek,2007). Very little is known regarding the spread of VVC in Iraq. In this study, different species of *Candida* were identified in patients of Iraqi pregnant women.

2. Materials and Methods

2-1 Sample collection and analysis

Fifty samples were taken from pregnant women within the age 20-42 years (in 2nd and 3rd trimester of gestation) who were attending the AL-Batool maternity Hospital by sterile cotton swab from vagina, and transferred to the laboratory for diagnosis after adding few drops of sterile distilled water in laboratory . The samples have been collected during the period from July 2016 up to November 2016.

2-2 culture media

The patient swabs were inoculated on to plates of sabouraud dextrose agar (oxoide). The plates were incubated at 37 °C for 72 h. (komeman and Roberts,1985).the yeasts on the media were examined under low power magnification for the presence of budding cells, hyphae, blastospores, and chlamyospores. Those which contained chlamyospores were considered species using the *Candida albicans*. Carbohydrate assimilation test was done on non-albicans API 20canda -AUX kit.(BioMerieux), which consists of 20 cupules containing dehydrated 19 assimilation tests. API 20C stripes were substrates which enable the performance of inoculated according to manufacturer's instructions, incubated at 38 °C and scored by two blinded observers at 24, 48, and 72 h. Reactions were read by comparing the strips to growth controls and identification was obtained by referring to the Analytical Profile Index .

2-3 disc diffusion method

Candida inoculate suspension (0.2 ml) was pipetted on the surface of Emmons modified sabouraud dextrose agar (oxoide) plates . the antifungal disc (10µg/disc) were placed on the surface of the medium .after (24-48 h) of incubation . the zone of the inhibition of the growth were expressed as clear zones around the antifungal discs in millimeters. Duplicate plates were used (Al-tamemy , 2009).

2-4 Statistical Analysis

the Statistical Analysis performed with statistical package for social sciences (SPSS) 19.0 and Microsoft Excel 2010.

3. Results and Discussion

3-1 Isolation and Diagnosis Candida species

Out of 50 samples, 60 isolates were identified Among vagina swabs of Pregnant women(20-42 years) whom suffered CCV . 48 (80 %) contained only species of *Candida* and 12 (20%) contained more than one species *Candida*. The prevalence of different species of *Candida* was as follow: *C.albicans* (44%),*C. krusi* (28.3%), *C. rugosa* (11.6%), *C.tropicals* (8.3%), *C. parapsilosis* (5%), *C.kefyr* (3.3%) respectively (Fig.1,2). Among pregnant women with multi –pregnancy patient with CCV , 69.7% were infected with one species of *Candida* while 29.3% were infected with more than one species. Of those with first- pregnancy, 92.6% were infected with one species compared to 7.4% who were infected with more. There was statistically significant difference between these groups

($p < 0.05$). The prevalence of the isolated species among patients with multi – pregnancy and first – pregnancy is shown in (Table 1).

Candida yeast is commonly viewed as an opportunistic yeast pathogen and is the causative agent 85% of the cases of vagina infection. On the normal host, the *Candida* has evolved to become a prosperously commensally. It expresses different traits critical for subsistence on mucosal surfaces. In the abnormal host the same traits become properties characteristic accounting for invasive capabilities as the critical balance of *Candida* with the host shifts in useful yeast. The findings of current study is consistent with the results of (Khushbu and Satyam, 2016). they reported that *C. albicans* was the most common isolated species with prevalence 64.5% in pregnant women suffering from VVC and *C. krusi* the second most common isolated species with a prevalence of 12% then *C. glabarata* 10%. *Candida albicans* is the species most frequently causes superficial and invasive infection at different anatomical sites in patients all over the world. It has a well known pathogenic potential and its main pathogenicity and virulence factors, are capacity to adhere to different mucosa and epithelia, dimorphism, with production of pseudohyphae helping tissue invasion, thermotolerance, and exoenzymes like proteinase and phospholipase and germ tube formation with consequent development of the filamentous form (Moris, *et al.*, 2008). The mannan (glycoprotein present on the cell surface of *C. albicans*), adhesion responsible for the attachment of *C. albicans* to host cells more strong than *C. tropicalis* and *C. prapsilosis* (Tavares *et al.*, 2003). The prevalence of other species had the same pattern in present study compare to earlier findings. The prevalence of non-albicans species seems to increase steadily and that raises the concern regarding the increase of anti-fungal drug resistance (Kontoyiannis, 2002).

3-2 Effect predisposing Factors on CCV infection

There are many of Predisposing Factors for developing CCV in women such as use of certain drugs like: antibiotics, Contraceptive pills, or anything with cortisone or steroids, Breast thrush, fatness, diabetes, Immunocompromised, meal with high sugar and yeast content, or tiny in vitamins and minerals. In this study the patients women with Multi- pregnancy, 24% used broad spectrum antibiotics, 24% diabetic, 20% eating diet with high sugar content, 20% used birth control pills, 8% were suffered Breast thrush infection, 4% fatness. Of patients with first- pregnancy 48% used broad spectrum antibiotics, 28% eating diet with high sugar content, 16% fatness, 8% diabetic. While,

Breast thrush infection and used of birth control pills not mentioned among first – pregnancy in pregnant women (Table 2). The findings of this study is consistent with the results of (Grigoriou *et al.*,2006) who reported that risk factors as overweight, diabetic , vaginal infection, used antibiotic drugs increasing fungal infections in pregnant women .The host defenses against *Candida* infection include T. cell immunity to prevent colonization and superficial invasion and phagocytic immunity to prevent deeper tissue invasion and hematogenous dissemination. These conditions that suppress any of these arms of the immune system include premature neonates severe burns, cancer especially hematologic malignancies, AIDS or immunosuppressive therapy such as steroids or cancer chemotherapy and organ transplantation especially liver transplantation (Anaissie *et al.*,2002). The prolong use of antibiotics are a common cause of candidiasis. Antibiotics destroy both harmful bacteria and good bacteria. When antibiotics destroy friendly bacteria, it gives the *Candida* a chance to begin to multiply (Quinn *et al.*, 2002; Brooks *et al.*, 2007).

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3-3 Antifungal susceptibility test

The results obtained revealed that the ketoconazole , nystatin, miconazole, and amphotericin B inhibited all *Candida* species isolates, while Clotrimazole gave lower inhibition zone against *C.krusi*. Itraconazole and fluconazole resist by *C.kefyr* and *C.tropicals* , while *C.krusi* isolates were able to resist fluconazole only (Table 3). Our result agreement with (Abduallah *et al.*, 2001) , who used five antifungal against *Candida* species isolated from vaginal swab of women in Basrah (Iraq) by using disc diffusion showed ketoconazole , Clotrimazole , miconazole, and amphotericin B are more active except nystatin gave smaller inhibition zone. Muhasin, (2007) used five antifungal against 19 isolates of *Candida* species from mucocutaneous candidiasis in Iraqi babies by using minimum inhibition concentration , he found ketoconazole was more active than other antifungal drugs which inhibition 89% from isolates in 6.25 µg/ml and 100% at 12.5 µg /ml , followed by Clotrimazole which inhibition 35.5% at 6.25 µg/ml . size of inhibition zones might be influenced by volatilization of antimicrobial substances ,disc size, amount of compound added to disc adsorption by the disc , agar type,

agar strength ,pH, volume of agar and microbial strains used (Scorzoni *et al.*,2007).

4. Conclusions

The current study concluded that VVC is a common disease among Iraqi pregnant women especially in the 2nd and 3rd trimester of gestation. *C.albicans* was the most common cause of vulvovaginal candidiasis; The second most common species was *C.krusi*. *C. albicans* accounted for (43.3%) of the strains including single and mixed infections , The most common mixed infection was the combination of *C.albicans* and *C.rugosa*. Antibiotic utilization is most common risk factors for developing VVC in first pregnancy , while , in the multi pregnancy diabetes and antibiotic utilization were the most common Factors . by susceptibility test amphotricine B, nystatin, clotramazole, itraconazole, ketoconazole, fluconazole, miconazole, all effective against *Candida albicans*, while amphotricine B, nystatin, ketoconazole, miconazole, were most effective antifungal to all yeasts species, study has also shown an increasing resistance of *C.kefyr* and *C.tropicalis* to Itraconazole and fluconazole. Therefore, appropriate drugs without any side effect should be given to culture positive women in order to prevent subsequent infection of the neonate and secondary infection to the mother.



Fig.1 *C.albicans* by API 20canda -AUX kit



<i>Candida species</i>	*Multi -- pregnancy	*First -- pregnancy	Total
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Fig.2 *Candida* species on SDA

<i>C.albicans</i>	6	14	20
The Risk Factors	Multi– pregnancy	First - pregnancy	Total
<i>C.rugosa</i>	2	1	3
<i>C.tropicals</i>	4	1	5
<i>C.parapsilosis</i>	2	1	3
<i>C.kefyr</i>	1	1	2
<i>C.albicans+C.krusi</i>	4 (2 for each species)	0	4
<i>C.albicans+C.rugosa</i>	6 (3 for each species)	2 (1 for each species)	8
<i>Total</i>	(55 %)33	(45 %)27	60
$(P<0.05)$			

Table.1 prevalence of *Candida species among pregnant women*

*The age between (20-42) years

Antibiotic utilization	6 (24%)	12(48%)	18(36%)
Diabetes	6(24%)	2(8%)	8(16%)
<i>Candida</i> species	Inhibition zone in millimeters		
Diet with high sugar content	5(20%)	7(28%)	12(24%)
Breast thrush infection	2(8%)	0(0%)	2(4%)
Contraceptive pills	5(20%)	0(0%)	5(10%)
Total	25(100%)	25(100%)	50(100%)
$(P < 0.05)$			

Table.2 predisposing Factors developed of VVC Infections in pregnant women

Table. 3 Antifungal susceptibility test for *Candida* species isolates

	Amphotericin - B	Nystatine	Clotrimazole	Itraconazole	Ketocanazole	Fluconazole	Miconazole
<i>C.albicans</i>	10	20	13.5	18	32	33	21
<i>C.krusi</i>	11	12	6	0	15	16	10.5
<i>C.rugosa</i>	10	14	17	16	32	27	15
<i>C.tropicals</i>	12	18	13	0	32.5	0	23
<i>C.parapsilosis</i>	12	14	15	20	34	24	18
<i>C.kefyr</i>	10	14	19	0	29	0	19
$\chi^2=6.97 (P<0.05)$							

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