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The Prevalence of *Candida* spp. Associated with Primary and Secondary Infertility

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Zaidan Khlaif Imran* Faris Naji Abbod Ruqaya Yahya Abd

Babylon University, All women college of Science, Biology Department, Iraq.

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ABSTRACT

The present study aims to survey and diagnosis of *Candida* species associated with women infertility based on their colors on CHROMagar and determine their virulence factors based on detection of their abilities to produce Phospholipase, urease and biofilm formation. 127 vaginal swab samples collected from the vagina and cervix. 78 samples collected from primary Infertility and 49 samples from women with secondary infertility, from patients, attenuated to women Rajan unit infertility, a maternity hospital and children, and private clinics in Babylon province. The results showed that *C.albicans* associated 61.53% with primary infertility while 38.46% with secondary infertility associated with Non-*C.albicans*. *C.albicans* and *C.krusei* have the ability to produce phospholipase .The results showed that the incidence of candidiasis in women with primary infertility was more than the unemployed in secondary infertility.

INTRODUCTION:

Infertility of couples affects 19% of the general population. The problem of infertility has become a public health problem. Most women suffer from severe valve vaginal candidiasis (VVC) caused by *Candida* spp. Vaginal infections represent the second type of common disease (Imran and Al.Shukry 2014). High percentage of women are exposed to vaginal candidiasis caused by a group of *Candida* spp. once or twice a year or at least once in their lifetime (Corsello *et al.*, 2003). *Candida albicans* is responsible for 70-90% of all vaginal candidiasis in Austria while many other studies showed that *Candida glabrata has* been the most frequent pathogen that causes vaginal Candidiasis (Novikova *et al.*, 2002). Ozcan *et al.* (2006) reported that *C. glabrata* is responsible for 14% of infections in immune competent women. There are many predisposing factors that can lead to these infections such as pregnancy, long-term use of antibiotics, diabetes mellitus, using of corticosteroids, HIV infection, and being immunocompromised (Fidel and Sobel, 1996).

In America, it is estimated that about six million (10% of the reproductive age population) couples are infertile. In rural Nigerian community, an overall prevalence of 30% was reported, of which 9.2% and 21.1% represent primary and secondary infertility respectively (Adetoro and Ebomoyi 1991). Unfortunately no data available about women infertility in Iraq. Imran and Al.Shukry (2014) mentioned to high occurrence of vaginal candidiasis in Iraq.

This study aims to determine the prevalence of *Candida* species associated with women infertility consulting at fertility clinics in Babylon, Iraq.

MATERIALS AND METHODS:

1. Samples collection

One hundred and twenty seven (127) vaginal swabs were collected from primary and secondary infertile women attending fertility clinic of Babylon hospital (age ranged from 13 to more than 40 years) women were seen by gynaecologists at Babylon hospital in Babylon province, Iraq between January 2013 and February 2014 were recruited for enrolment in the study. Vaginal swabs streaked on Sabouraud's dextrose agar and incubated at 37°C, overnight, in aerobic conditions, and identified by the CHROMagar candida medium.

2. CHROMagar test

Purified single colonies from Sabouraud's dextrose agar were streaked on CHROMagar and incubated for 24-48 h at 30°C. *Candida* isolates were classified according to their colors on the CHROMagar medium based on the color key described by Nadeem *et al*., (2010). Our diagnosis based on Williams and Lewis 2000; Hospenthal *et al*., 2006). One colony for each *Candida* species was selected randomly representative for each vaginal swab sample revealed positive growth on CHROMagar.

3. Phospholipase production

Phospholipase production was assayed by using an egg yolk agar plate method. SDA plates containing 1 M NaCl, 0.005 M $CaCl_2$, and 8% sterile egg yolk emulsion were used. Standard inoculum of the test and control *Candida* isolates (5 µL; 10^8 yeast cells/mL) was spot-inoculated on the plates. Then, 5 µL of sterile saline was also spot-inoculated on the plates. The plates were incubated at 37°C for 5 days, following which the diameter of the zone of precipitation around the colony (in mm) was determined (Hankin and Anagnostakis 1975; Kumar et al., 2006).

4. Urease test

The urease test was performed by streaking the surface of a urea agar slant with a portion of well-isolated colony. The cap of the slant tube was loosened and incubated at 35°C in ambient air for 48 h to 7 days. Positive urease production was detected by change in the color of the slant to light reddish color. Negative urease production was noted when the agar slant and butt remained light orange in color (Taira *et al*., 2011)

5. Biofilm test

Biofilm test included visual inspection of a thin layer or scattered cells adhesive on a glass surface as per the method of Aboul-Nasr, *et al.*, 2013. The isolate to be tested for the production of biofilm was inoculated in conical polystyrene test tube containing Sabouraud dextrose broth supplemented with glucose (final concentration of 8%) and incubated at 35°C for 48 h. After incubation, the broth from the tubes was gently decanted. The tubes were twice washed with distilled water to remove any non-adherent cells, followed by staining with 2% safranin for 10 min. The isolate was considered positive for biofilm formation when

a visible film was seen on the wall and bottom of the tube and absence of the same indicated that the isolate could not produce biofilm.

RESULTS AND DISCUSSION:

1. Morphological Identification

A total of 127 *Candida* species were isolated as representative for positive VVC cultures, of which 78,49 were isolated from women undergo primary and secondary infertility respectively, 48 / 78 and 29 / 49 isolates of *Candida* from women severing of (VVC), 43 and 29 isolates from primary and secondary infertility respectively were classified as *C.albicans* showed green color on CHROMagar medium these results coincidence with the results of Imran and Al.Shukry (2014). Whereas the remaining isolates were non-albicans showed pink to white pink or white color on CHROMagar medium. The non-albicans were classified into *C. glabrata*, *C. kruzi*, *C. tropicals*, *C. prapsilosis* and *C. dubliniensis*. *C. albicans* showed high incidence percentage with the vaginal swabs of infertility and secondary infertility 61.53% and 59.18%, respectively, followed by *C.glabrata* 17.9% and 18.36% in women with primary and in secondary infertility respectively (Table 1 and Figure 3).

2. Biofilm and enzymes production

In our study, biofilm formation was tested for both *C. albicans* and non-albicans spp. The *C. albicans* formed thicker and denser biofilms or scattered aggregates of cells compared to non-albicans (Figure 1A). The *Candida* spp. showed variation in their abilities of enzymes production; *C.albicans* produced produce lipase more activity than others species, and not produced urease enzyme, most *Candida* species under interest produced phospholipase enzyme (Figures 1B, 2). Our results coincidence with Singhai *et al*.(2012).

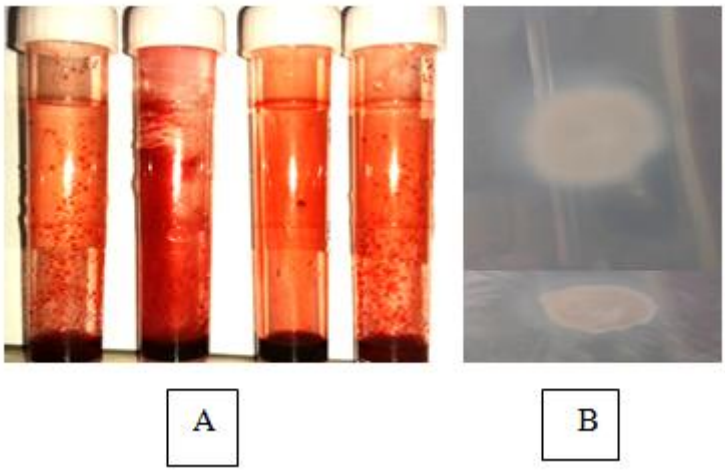

Figure 1: A- biofilm formation of *C.albicans* on tube surface. B-Phospholipase production on egg yolk agar plate

This study concluded that all *C.albicans* isolates had ability to biofilm formation (Figure 1A), the result came, coincidence with Ramage *et al.* (2001) and Silva *et al*. (2011) showed that the majority of disease produced by *C.albicans* is associated with biofilm growth . This finding is consistent with the results of studies conducted by Calderone and Fonzi (2001) **.** Traditionally, urease test is used for the presumptive evidence of the presence of *Cryptococcus* sp. in tissue biopsy material. Our study showed inabilities of isolated *Candida* species for urease production (Figure 2).

Figure 2: Urease test of Candida species on urease substrate show negative test

Calderone and Fonzi 2001 has expressed virulence factor that contributes to pathogenesis as well as the production of lytic enzymes like: phospholipases by *Candida* spp. are most often responsible for causing diseases (Hankin and Anagnostakis ,1975; Taira *et al*., 2011).

The results of our study reinforce the need for further care to control the spread of contagious fungi. We evaluated the causative *Candida* spp. in eye infections showing high clinical signs. These results about the recorded *Candida* spp. causing eye infections are in agreement with earlier reports (Moore *et al*.,1988).

Table 1: Relationship between *Candida albicans* and non-albicans with primary and secondary infertility of women

Total	secondary infertility of women	primary infertility of women	Candida
77 (60.63%)	29 (59.18%)	48 (61.53%)	*C.albicans*
50 (39.37%)	20 (40.81%)	30 (38.46%)	Non-*C.albicans*
127 %100	49 (38.58%)	78 (61.42%)	Total

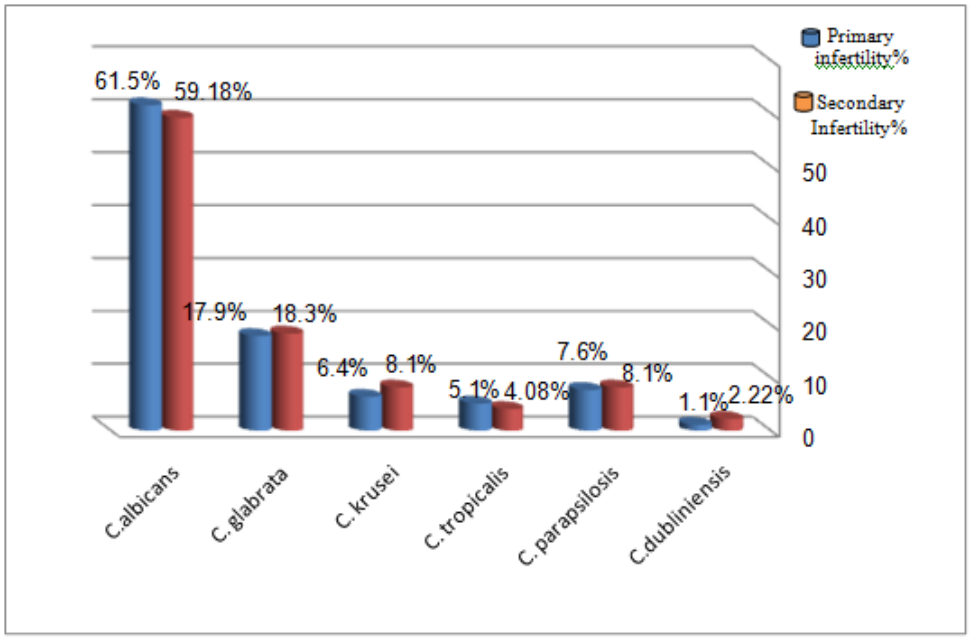

Figure 3: Incidence percentage of *Candida* spp. with primary and secondary infertility

Unfortunately, no previous studies performed in Iraq and neighbor countries. The prevalent rate varies from one region to the other (Fayig *et al*., 2005). In America, it is estimated that about six million (10% of the reproductive age population) couples are infertile. In rural Nigerian community, an overall prevalence of 30% was reported, of which 9.2% and 21.1% represent primary and secondary infertility respectively (Adetoro and Ebomoyi 1991). In a similar study in southeastern Nigeria, 65% and 35% prevalent rate were reported for primary and secondary infertility respectively (Ikechebelu *et al*., 2003). Our results coincidence with Nerurkar *et al*. (2012).

ETHICAL APPROVAL

All authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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