

Epidemiological and Molecular Study for *Candida* spp in Vagina

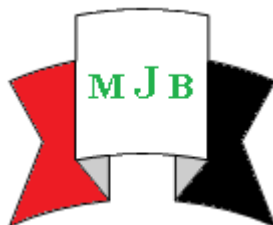
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Abstract

The present study is a case control study where 120 clinically patient women during the period from 1st of March – 28th of December 2012 who were tested to prove *C. albicans* presence in their vagina by culture of vaginal swab on two media, the first was used for primary isolation which was **Sabouraud's dextrose agar** media and the second was to differentiate *Candida* spp. The last identification was achieved by conventional PCR. Results of this study presented that the highest invasion of the vagina of *Candida* spp was accounted for *C. albicans* (38.3%) while other species were as follows: *C. glabrata* (3.3%), *C. tropicalis* (2.5%) and *C. krusei* (1%).

Key words: *C. albicans*, CHROMagar, vaginitis, Non-albicans species, Hay/Ison criteria, PCR.

الخلاصة

هذه الدراسة هي عبارة عن دراسة الحالات و مقارنتها بأشخاص اصحاء حيث تم جمع عينات (١٢٠ عينة) في الفترة من الاول من آذار و لغاية الثامن و العشرين من كانون الاول من نساء تم فحصهن من قبل الاختصاصية النسائية و تشخيصهن على انهن مريضات بالمبيضات البيضاء. بعد تشخيصها يتم اخذ مسحة مهبلية ثم زرع هذه المسحات على وسط ال SDA و الذي استخدم للتشخيص الأولي للفطريات و بعد ظهور العزلات تم زراعتها على الوسط الثاني و هو الوسط المولد للالوان و ذلك للتفريق بين الانواع المختلفة للمبيضات، اما التشخيص الاخير فكان بواسطة تقنية تفاعل البلمرة المتسلسل. و اظهرت النتائج ان اعلى نسبة بين المريضات كانت لنوع المبيضات البيضاء (٣٨,٣%) بينما كانت النسب للانواع الاخرى من جنس المبيضات كالتالي : المبيضات من نوع *glabrata* ٣,٣% و من نوع *tropicalis* ٢,٥% و من نوع *krusei* ١%.

Introduction

The stability of the vaginal microbial ecosystem preclude many other organisms but sometimes the vaginal microbiota is disturbed and there is a change in the normal balance causing symptoms like abnormal or increased vaginal discharge, redness and itching. Irritation of vagina caused by inflammation or infection is called vaginitis or vulvovaginitis if both vagina and vulva are inflamed. Vaginitis is a very common disease for women of reproductive age all over the

world. Children and postmenopausal women can also be affected, but not as commonly [1 , 2].

Bacterial vaginosis and *Candida* vaginitis are the two most common causes of vaginitis [3, 4]. Candidal vulvovaginitis or vaginal thrush, the etiology of which is *C. albicans* which is the most common cause (> 90%) of vaginitis, and it has been found that up to 75% of women have experienced symptomatic vaginal candidiasis at least once. Of these infections, the minority is caused by non-*C. albicans* spp. (< 10 %), including *C. glabrata*,

C. krusei, *C. parapsilosis* and *C. tropicalis* [5, 6, 7].

The correct identification of *Candida* species is of great importance, as it presents prognostic and therapeutical significance, allowing an early and appropriate antifungal therapy [8]. Identification might also be useful for studying their epidemiology, spread and modes of transmission [9]. Nowadays, a large variety of *Candida* spp. identification methods are commercially available, and they differ in principles, discrimination power and cost. Traditional microbiological procedures are based on macroscopic and microscopic analysis of colonies and cells (presumptive tests) and on biochemical characteristics of the yeasts (confirmative tests) [10]. Also, several molecular methods have been developed for the identification of the yeasts [11, 12]. The conventional methods of yeast identification, which mainly consist of assimilation and fermentation characteristics, are reported to be cumbersome and beyond the expertise range available in local laboratories. In non-specialized clinical laboratories [10], especially in resource-limited settings, identification of yeast and yeast-like organisms requires the evaluation of microscopic morphology and biochemical studies. Some unusual yeasts may require unique morphological and biochemical studies for identification, occasionally requiring up to 21 days of incubation [13]. Effective treatment requires both early diagnosis and prompt initiation of therapy against fungal infection [14].

Materials and Methods

This case control study was conducted at outpatient consultation clinics for Gynecology in Baghdad Teaching Hospital (Medical City) and outpatient consultation clinics for Gynecology and Obstetrics in Al-

Zahra'a Teaching Hospital in (An Najaf governorate) for molecular characterization study for clinically diagnosed women infected with recurrent vulvo vaginal candidiasis. During the period from 1st of March – 28th of December 2012, a total of 155 specimens were collected from 120 apparently infected with recurrent vulvovaginal candidiasis by vaginal swab, and also the study included 35 apparently healthy women considered control group. The patients and control groups were aged from 15-50 years.

After physical examination with a moistened speculum by a gynecologist, high vaginal swabs from anterior fornix have been taken from married women while low vaginal swabs from the labia minora have been taken from unmarried and pregnant women. Cotton sterile disposable swabs have been used for vaginal collection. Then the swabs have been transported as soon as possible to the laboratory for incubation at 37°C for 24 - 96 hours onto Sabouraud's dextrose agar. Gram stain and microscopical examination have been also done to detect if having other causing agents for vulvovaginitis. Subsequently the positive cultures were plated on CHROMagar *Candida* at 37 °C for 24 hours to ensure detection of mixed infections.

Identification of *Candida albicans*

Morphology on Sabouraud's dextrose agar (SDA) plates

The growth of colonies on Sabouraud's dextrose agar was noticed. In Some cases, the growth of which was scanty while most of them was heavy growth. Scanty growth has been excluded supposing *Candida albicans* as a normal flora in this case.

Microscopical examination

Direct examination was done to determine the shape and size of cells of the yeast by picking a colony from the culture and emulsifying it within a drop of normal saline then covered by

a cover slide. Examination will be done by light microscope on the power 40 X.

Gram stain was done from vaginal swabs to detect other causing agent for vulvovaginitis, mostly bacterial vaginosis. Identification and diagnosis of bacterial vaginosis and other causing agents depending upon Hay/Ison criteria that is defined as follows:

Grade 1 (Normal): Lactobacillus morphotypes predominate

Grade 2 (Intermediate): Mixed flora with some Lactobacilli present, but Gardnerella or Mobiluncus morphotypes also present

Grade 3 (BV): Predominantly Gardnerella and/or Mobiluncus morphotypes, Few or absent Lactobacilli [15].

The Bacterial Special Interest group of BASHH (British association of sexual health and HIV) recommend using the Hay/Ison criteria in Genitourinary medicine clinics.

Morphology on CHROMagar-Candida medium

CHROMagar Candida is a novel, differential culture medium that is claimed to facilitate the isolation and presumptive identification of some clinically important yeast species and the differentiation of these species from other yeasts on the basis of strongly contrasted colony colors produced by reactions of species-specific enzymes with a proprietary chromogenic substrate. The medium

greatly facilitates the detection of specimens containing mixtures of yeast species. All isolates were inoculated on CHROMagar-Candida medium and incubated at 37°C for 24 hrs looking for light green colonies (a typical color of *C. albicans*).

Polymerase chain reaction (PCR) assay

DNA Extraction and Purification

A pure culture was made on SDA and incubated overnight. A colony was isolated and the *Candida albicans* cells were grown overnight at 37°C in 5ml of SDB in a plastic tube. Genomic DNA was extracted using the DNA-Pure Yeast Genomic Kit according manufacturer's instructions (BioBasic Company).

***Candida albicans* Identification by PCR**

After DNA isolation process was completed, process of PCR has been established as in Genekam Biotechnology instructions.

Results

Identification

Appearance of yeast colonies

On Sabouraud's dextrose agar, colonies of *Candida albicans* were white to cream colored, smooth, glabrous and yeast-like in appearance after 72 hrs of incubation. Microscopic morphology showed spherical to subspherical budding yeast-like cells or blastoconidia as obviously was showed in figure (1).

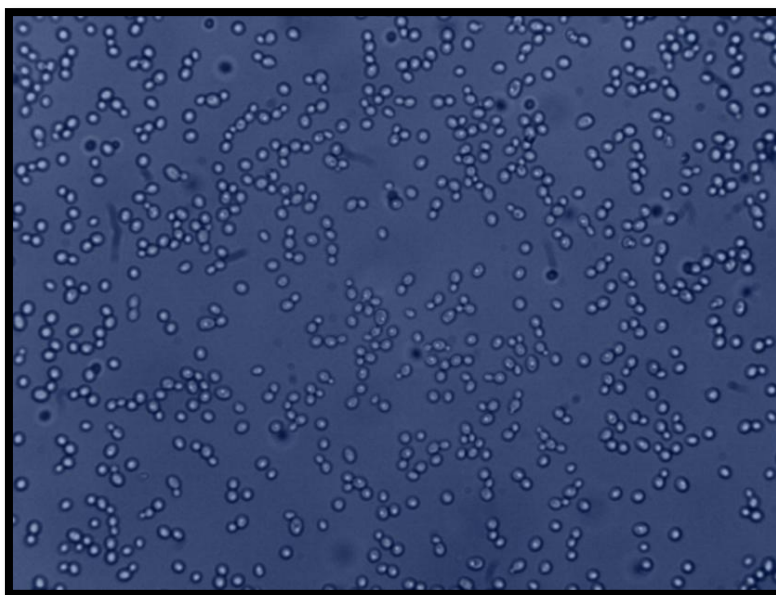


Figure 1 *Candida albicans* blastospores grew on SDA media under light microscopy 400X magnification.

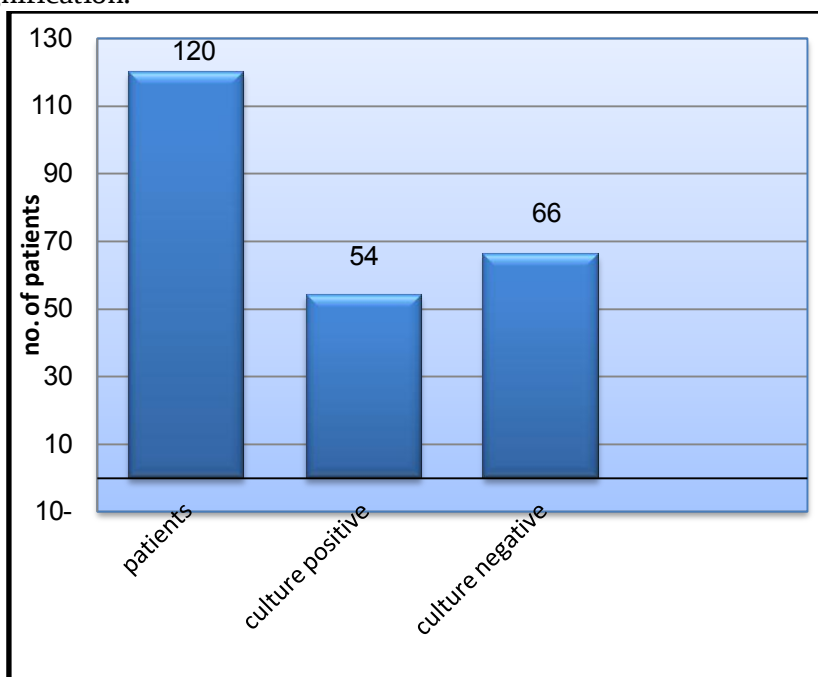


Figure 2 Histogram showed *Candida* detection by SDA culture in patients group.

While growth on HiCrome Candida Differential medium showed good luxuriant light green colonies after 24 hrs of incubation at 37°C. The color

was consistent after 24 – 48 hrs and then the color began to be lighter than the first time (figure 3).

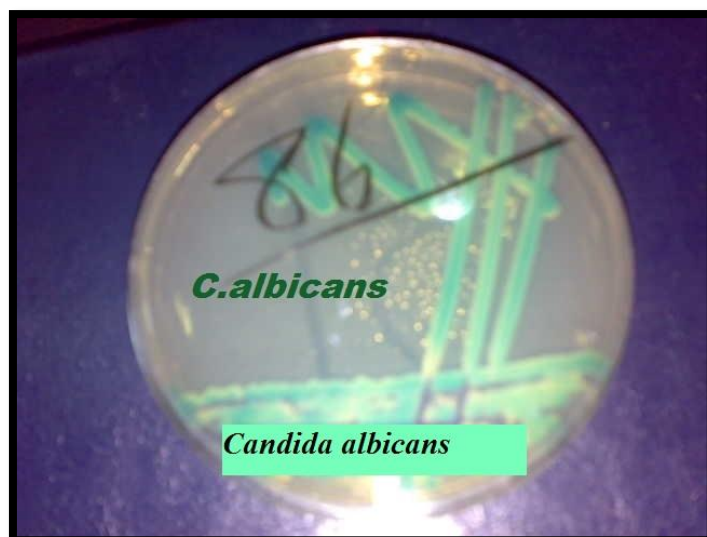


Figure 3 *C.albicans* green colonies on HiCrome Candida Differential medium.

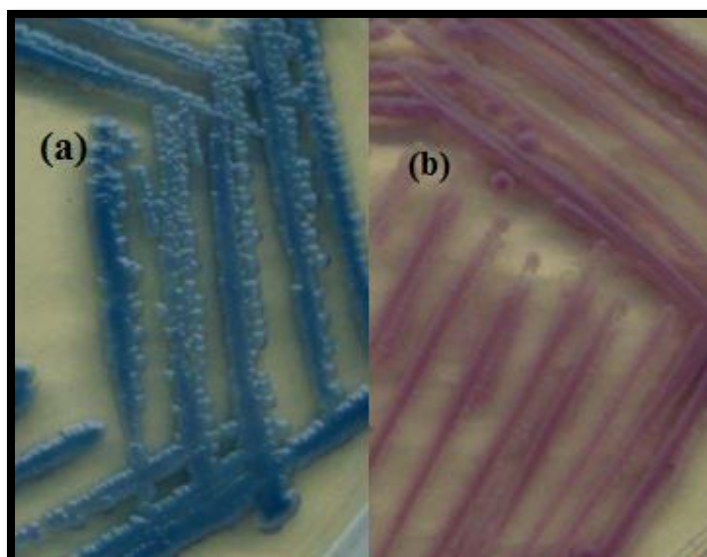


Figure 4 Colonies of *Candida* spp. On HiCrome Candida Differential medium
(a): *Candida tropicalis*, (b): *Candida krusei* .

Table 1 *Candida* spp. percentage isolated from patients on chromogenic medium.

<i>Candida</i> spp.	No. of isolates of total 120 pts.	Percentage %
<i>C. albicans</i>	46	38.3
<i>C. glabrata</i>	4	3.3
<i>C. tropicalis</i>	3	2.5
<i>C. krusei</i>	1	0.9
Negative cultures	66	55
Total no.	120	100

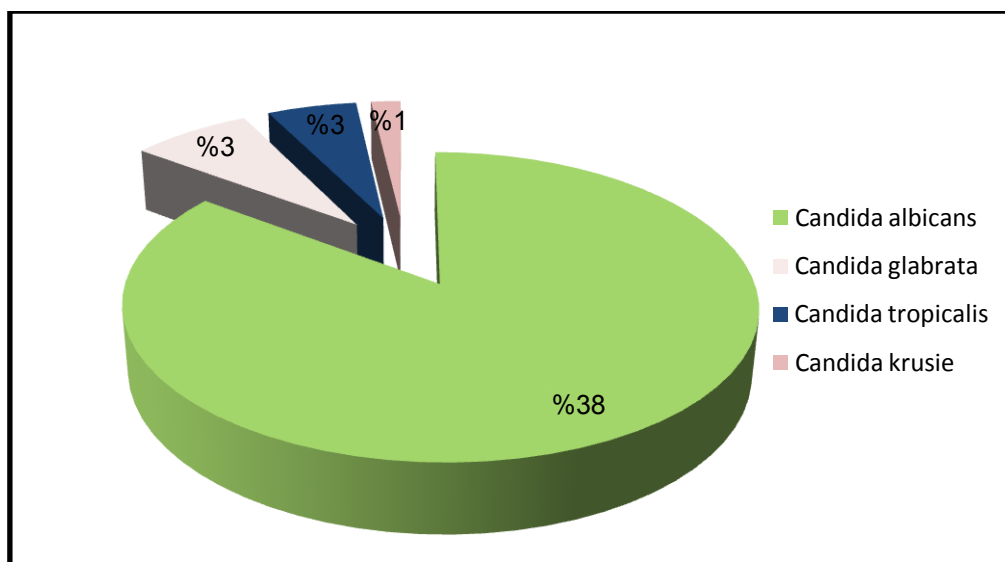


Figure 5 Percentage of *Candida* spp according to its appearance on CHROMagar.

Molecular analysis of samples by PCR: PCR results showed that only 35

patients were having *Candida albicans* from the 46 patients (fig 6).

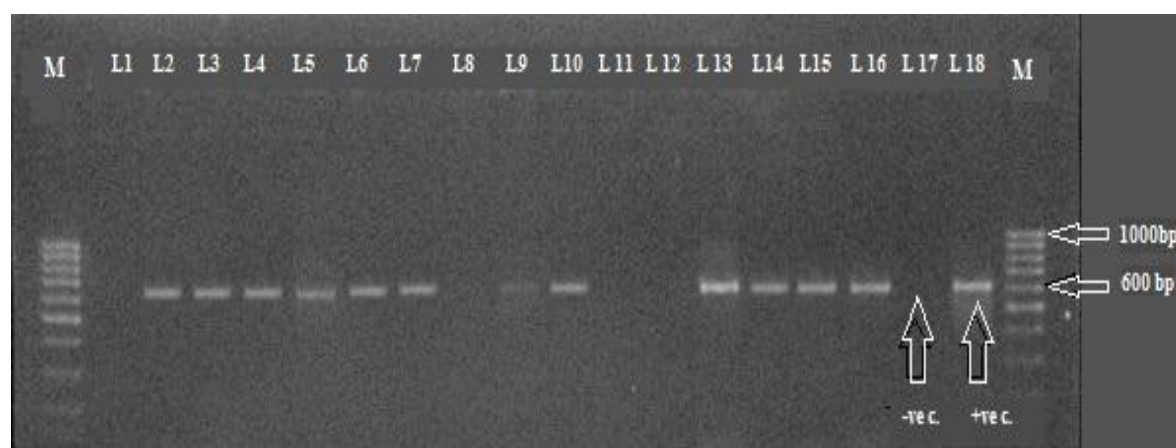


Figure 6 PCR amplified products of *Candida albicans* on agarose gel stained by Ethedim bromide from vaginal swab. Lane(M) DNA molecular size marker (100 bp ladder). Lanes 17 & 18, show negative control & +ve control sequentially. Lanes 2,3,4,5,6,7,9,10,13,14,15&16, show positive *Candida albicans* with (600 bp) PCR product size, while Lanes 1,8,11&12 show negative *Candida albicans* . (1.0 % agarose gel, 100V-1hour).

Discussion

Appearance of yeast colonies

The results of this study clarified that all the isolates of *Candida albicans* were grown well on HiCrome Candida Differential medium and this agrees with the fact that this medium having good performance, less time wasting and having sensitivity for the isolation and detection of *Candida albicans* [16]. Almost most of the

isolates after 24 hrs of incubation on HiCrome Candida Differential medium revealed good luxuriant light green colonies (*C. albicans* colonies). In figure (3), Albicans species is the predominant as was showed in Table (1) and this agrees with the previous studies that almost all colonies form this color which was the light green on chromogenic media [17,18, 19].

Detection of *C. albicans* by PCR

Since Polymerase Chain Reaction (PCR) has proven to be a powerful tool in the early diagnosis of several infectious diseases, it might also be a more sensitive alternative assay in the diagnosis of vaginal candidiasis. Several PCR methods are used for the detection of *Candida* spp. [20, 21, 22]. The main aim of this study to compare between culture based method (Hicrome Candida Differential Agar) and non-culture based method (PCR) for the detection and identification of *Candida* spp. isolated from women with signs & symptoms with VVC.

A large proportion of women suspected on the basis of history and clinical examination prove negative for *Candida* on culture of vaginal anterior fornix samples. By utilizing the very sensitive PCR, *C. albicans* was identified in only 29.1% of symptomatic women with recurrent vulvovaginitis as in figure (6). Therefore, the vaginal symptoms in the majority of these patients were not due to the presence of *Candida* at this anatomical site. These results are in agreement with Stephanie and his colleagues (2000) [23] who clarified that not all the patients who having signs and symptoms of VVC, necessarily have infection with *C. albicans*.

The present study came to the conclusion that PCR seems to be more sensitive detection method in comparison to the cultural methods. Giraldo *et al.* (2000) [24] also came to this conclusion, but CHROM agar has the advantage of rapid identification of *Candida* species, technically simple, rapid and cost effective compared to PCR which is time consuming and expensive conventional method. This is in agreement with Vijaya *et al.* (2011) [18].

Prevalence of *Candida* spp. in VVC

Najwan (2008) [25] has found a high proportion of infection with VVC especially infection with *C. albicans* with a proportion a round 63.6%. In the present study, in Table (1), most *Candida* spp. isolated from the vagina were as follows: *C. albicans* followed by *C. glabrata* to a lower extent *C. tropicalis* & *C. krusei* which can be prevalent in vulvovaginal region. The result of this study agreed with Schamidt *et al.* (1997) [26] and Najwan (2008) [25]. However, other investigators observed an increased infection with non- albicans species in VV region [27, 28]. Parazzini *et al.* (2000) [29] demonstrated that an isolation rate was 57.3% for non-albicans spp. from patients with VV symptoms, with 36% and 8.6% isolation rates for *C. glabrata* and *C. krusei*, respectively. Abu-Elteen *et al.* (2001) [30] isolated non-albicans spp. at a proportion of 56.9%, of which 32.5% were *C. glabrata*.

In the present study, in figures (5 & 6), *C. albicans* was the predominant yeasts species isolated from female vagina. This could be due to the fact that *C. albicans* can adhere easily to the vaginal epithelial cells through their surface mannoprotein. A study by Saporiti & Gomez (2001) [31] who reported that mannoprotein in the surface of *C. albicans* as well as germ tube formation and mycelium formation facilitates vaginal mucosal invasion. Also, this may be due to its virulent factors which include dimorphism and phenotypic switching. *C. albicans* produces protease and phosphatase which enhance its attachment to human epithelium. It can also be deduced that the high incidence rate of *C. albicans* could be due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa there by providing an adequate supply of utilisable sugar

that favor its growth [32]. It seems to be the reason why is the *C. albicans* considered one of the major components of normal vaginal flora. So under certain conditions such as use of broad spectrum antibiotics or corticosteroids and other risk factors that increase the incidence of VVC, *C. albicans* will proliferate and the number of which will increase, transforming it to pathogenic *C. albicans*.

Non- albicans species, in figures (4) & (5), were observed but at lesser extent than *C. albicans*. These species play an important role in VVC, *C. glabrata* & *C. tropicalis* are not producing mycelia but they produce proteolytic enzymes that help fungi to adhere to VECs. This study is in agreements with Lubna (2006) [33].

It can be concluded that CHROM agar has the advantage of rapid identification of *Candida* species, technically simple and cost effective compared to technically demanding time consuming and expensive conventional method and 2. PCR is the more sensitive method for the identification of *C. albicans* but cost and time consuming.

Recommendations

1. Gynecologists should not only depend on signs & symptoms of the disease in patients suspected having VVC. Vaginal swab for culture & sensitivity is the first choice for diagnosis.
2. Using CHROMagar *Candida* medium for primary identification and isolation of *Candida* spp.
3. Identification of *C. albicans* by molecular techniques must be used only in researches and not for rapid diagnosis.

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