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Genotype Comparisons of *Candida albicans* From Patients With Vulvovaginal Candidiasis.

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ABSTRACT

This study was intended to isolate and identification of *Candida albicans* which causing vaginitis in women. A total 204 samples of vaginal tract swabs were collected from patients women from April to November, 2015 in Dyala/Iraq. Standard microbiological methods were used to isolate and identify *candida* from vaginal swabs obtained from study object, Sabouraud dextrose agar used for isolation of *candida*, CHROMagar of *candida* used to identification of *C. albicans*, germ tube test done. The final identification done by using VITEK Yeast Biochemical Card. From 204 infected women 75 have vaginal candidiasis about (36.76%), *C. albicans* was causative agent of 54 isolates about (72%).

The genetic diversity of recent clinical isolates of *Candida albicans* was studied basis on amplified DNA bands fragment that spans the side of the transposable intron in the 25s rDNA by using CA-INT-L and CA-INT-R primers. Our analysis of 54 *C. albicans* isolates showed that genotype-A with (450bp) band presented in 27 isolates about (50%) was a predominant *C. albicans* genotype, genotype-B with (840bp) band was found in 16 isolates with (29.62) ratio, and finally genotype-C with both bands (450-840bp), was found in 11 isolates about (20.37%).

INTRODUCTION

Candida species is a commensal microflora in human vagina, but it can cause a variety of infections in superficial mucosal infections, it has to cross physical barriers that presented by the epithelial cells layers and could active penetration and induced endocytosis (Zakikhany *et al.*, 2007). We could defined vulvovaginal candidiasis (VVC) as a disorder characterized by signs and symptoms of vaginal inflammation when the *candida species* found, (Workowski *et al.*, 2010) and stated that it is the second common causes of vaginitis symptoms after bacterial infections and accounts for one-third of vaginitis cases. *Candida spp.* Infections may be occurred in mouth, throat, skin, scalp, vagina, nails, lung, bronchi, in the gastrointestinal tract and may be become systemic as in septicemia, meningitis and endocarditis (Rippon, 1988) and he stated that the clinical manifestations may be acute, sub-acute, or chronic to episodic.

Life threatening infection is acted by invasive candidiasis it occurs in immunocompromised hosts like organ transplant and bone marrow recipients, AIDs patients and patients receiving intensive chemotherapy (Perlroth *et al.*, 2007). Patients with intensive antibiotic therapy, pregnant women, extensive surgery or burns, indwelling, catheters, local warmth and moisture, skin irritancy, elderly patients and patient that have cancer or leukemia have more probability to infected by systemic *Candida spp.* (Richardson and Lass0Florl,2008), vaginal culture positive was found in (10-55)% of healthy women who are completely asymptotic (Sobel J., 1993) and he stated that founding of *Candida spp.* during the symptoms free period would refer to resistance of the organism to complete eradication by drugs, in adequate treatment, recolonization and insufficient use of antifungal drugs.

The main causes of VVC is *C. albicans* which is causes about(70-90)% vulvovaginitis cases (Fan SR., *et al.*, 2008). There is very important to know the differences among *C.albicans* strains to given appropriate treatment, and pathogenicity vary among strains (Costa JM., *et al.*, 2010).

There were some new techniques have been used to found *C. albicans* genotypes, such as high resolution DNA melting analysis (HRM), polymerase chain reaction melting profile (PCR-MP), Multilocus sequence typing (MLST) (Garcia HD., *et al.*, 2007), in this study the method that used to characterize the *C. albicas* genotypes is the PCR based with specific primers CA-INT-L and CA-INT-R to amplify the region of the transposable group I intron of 25 rDNA gene which is easy and quick method that developed by Mu Cullough *et al.*, (1999) to determine *C. albicans* genotypes affecting women with VVC in Dyala, Iraq.

MATERIALS AND METHODS

Isolation and identification of *Candida albicand*:

Clinical isolates obtained as vaginal swaps from patient with VVC, the identification was based on physiological and morphological characteristics. The vaginal swaps were streaked out on sabouraud dextrose agar plates amended with chloramphenicol 0.05 mg/L, incubation the plates for (24-48) hours at 37°C (Kown and Bennett, 1992), (Fig. 1). *Candida* species were identified by inoculation on CHROMagar candida (Paris-France) the appearance of light green colonies indicates the presence of *Candida albicans* (Fig. 2).

To identification of *Candida albicans* among other species germ tube test was done, taken a small amount from the colony by sterile loop, inoculated in human serum, incubated at 37°C to about (2.5-3) hours.

The final identification done by using VITEK Yeast Biochemical Card , VITEK 2system instrument:

According to the manufacturer's instructions, a sterile loop is used to transfer a sufficient number of colonies from pure overnight culture and suspend the yeast in 3.0 ml of sterile saline in test tube. The turbidity adjusted to (1.8-2.2) measured by using (Densichek) a turbidity meter, identification cards are inoculated with yeast suspension using an integrated vacuum apparatus. Putted in VITEK 2 system to about 18 hours. The result printed.

Genotyping of *C. albicans*:

DNA extraction

A single colony from yeast were grown on sabouraud dextrose agar was culture overnight on yeast peptone dextrose (YPD), (1% yeast extract, 2% peptone, and 2% dextrose at 37°C.

Genomic DNA of *C.albicans* were obtained following manufactures instructions. Extracted DNA was precipitated by isopropanol and washed with ethanol 70%, derided and used in PCR, (Fig. 3).

Primers:

For genotype determination of *C. albicans* on the basis of 25s rDNA, primers CA-INT-LATAAGGGAAGTCGGCAAATAGAT CCGTAA,CA-INT-CCTTGGCTGTGGTTTCG

CTAGATAGTAGAT were used to amplify a transposable intron in the 25s rDNA. DNA fragment that spans the side of the

Table 1: list of genotypes of *C. albicans* and expected size of PCR-Product.

Genotype	Expected band size (bp) and 25s rDNA type
A	450
B	840
C	450,840
D	1040
E	1080

Pcr conditions:

Genomic DNA were amplified in a reaction mixture (25 µl) containing 12.5 µl master mix (promega, USA), 5 µl DNA template, 1.25 µl each of the primers and complete the volume by PCR grade water.

PCR cycle parameters were as following: 95°C for 4 min, 95°C for 1 min, 56°C for 1 min, 72°C for 1 min and 72°C for 9 min, the band of DNA fragments were characterized by electrophoresis by (Thermocycler PCR, TECHNE,UK). The band running in 1.2% agarose ethidium bromide gel putted in 1x TBE buffer at 120 V for 1 hours (Fig. 1).

RESULTS AND DISCUSSION

A total of 75 *C. albicans* isolates were obtained from 204 women screened within the study period from (April–November) in 2015 in Dyala/Iraq about 36.76%, after screening of these candida isolates resulted that 54 isolates confirmed to be *C. albicans* which represented about 72% of the *candida spp.* isolates.

All 54 *C. albicans* isolates growth on CHROMagar with green color, formed germ tube when incubated in human serum at 37°C for 2.5-3 hours. So this could be a good identification to the *C. albicans*.

Presence or absence of a 25s rDNA transposable introns was used to determination of the genetic diversity among *C. albicans*, to differentiate between the various genotypes was based on the PCR bands products as showed in table.1, polymerase chain reaction amplification bands resulted that genotype-A with (450bp) presented in 27 species about (50%), this result prevalent that genotype-A is predominant *C. albicans* genotype followed by genotype-B which presented 16 isolates with (840bp) about (29.62%), and finally genotype-C found in 11 isolates with two bands (450bp, 840bp) as showed in (Fig.4).

The result of this study agrees with previous studies (Bii, C. *et al.*, 2009; Emmanuel, N. N., *et al.*, 2012; Zhux, *et al.*, 2011), which identified *C. albicans* genotype A as a predominant in VVC in women.

In Al-Baker (2012) studies the results showed that among 104 *C. albicans* isolates from pregnant women, 65 isolates blond to genotype A about (62.5%), genotype-B about 27 isolates (26%) and 12 isolates blond to genotype-C with (11.5%) which agrees with this studies that referred to the predominant *C. albicans* genotype was genotype-A with (450bp).



Fig. 1: *C. albicans* growth on sabouraud dextrose agar.

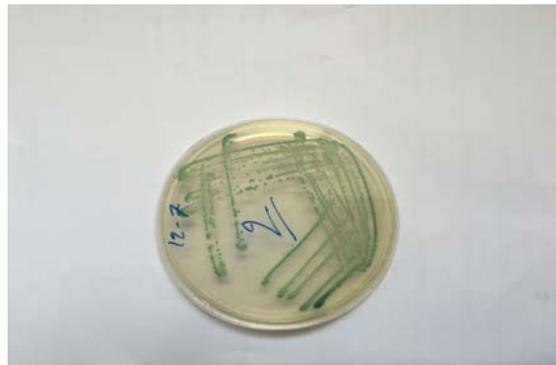


Fig. 2: *C. albicans* growth on CHROMagar (light green).

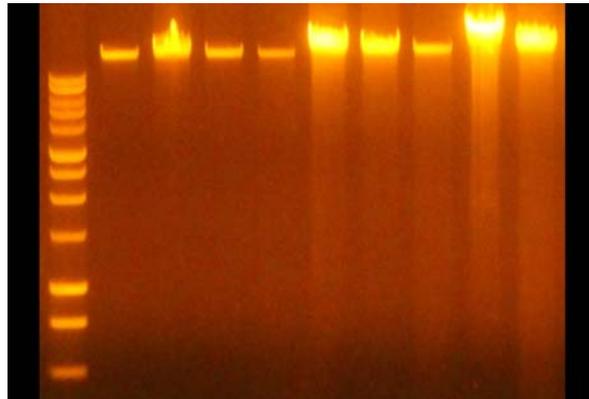


Fig. 3: DNA extracted from *C. albicans*, isolated from patient with VVC in women

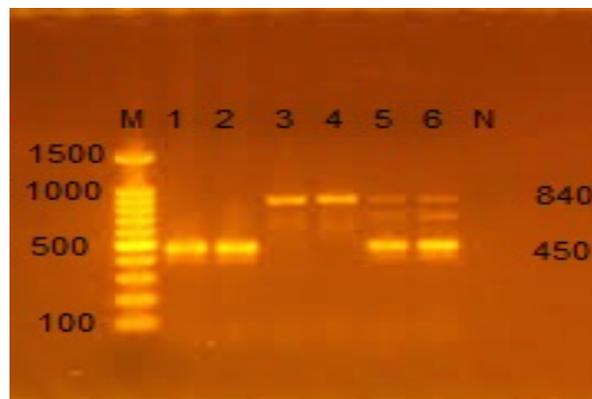


Fig. 4: PCR product bands lanes 1,2 with band 450bp for genotype- A, lanes 3, 4 with 840bp for genotype-B, lanes 5,6 with both (450-840) bp for genotype-C. M indicates the lane containing 100bp DNA ladder, N refers to negative control.

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