

**RESEARCH ARTICLE**

**Presence of virulence genes of *Candida albicans* isolated from women with remarks to Antifungal susceptibility**

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**ABSTRACT:**

Two hundred vaginal swabs were collected from clinic visiting women. The study revealed that 102 positive samples (51%) for the yeast isolation. The percentage was 51.11% in female below 30 years, while it was 50.76 % in women with age above 30 years. The highest levels of yeast isolation were found in relation with using of antibiotics, pregnant state and non-aborted women. By using disc diffusion method, several antifungal has the highest activity toward *C.albicans* such as fluconazole and clotrimazole while Itrconazole and Amphotericin-B showed lower activity. The result of multiplex PCR showed that the most of the tested strains had *ALS1*, *HWP* and *INT* genes.

**KEYWORDS:** *Candida*, Yeast, Human, Antibiotics, Virulence genes.

**INTRODUCTION:**

The yeast *Candida albicans* colonizes the mucosa of the oral and vaginal cavity, as well as the digestive tract, and, depending on the severity of the underlying host deficiency, can cause a range of infections. As a result, *C. albicans* infections, which called candidiasis, are extremely rare in healthy people. Candidiasis is a serious clinical condition that can be split into two types: superficial and deep-seated. *Candida* infections have increased considerably during the previous two decades for a variety of reasons such as immunosuppressive medications, catheterization, long use of antibiotics, immune-compromised patients<sup>1,2,3</sup>. Infections with *Candida albicans* have risen considerably in the previous two decades<sup>4</sup>. The fungus must express general as well as stage- and tissue-specific virulence or fitness characteristics during different stages of a *C. albicans* infection and within various host tissue settings<sup>5</sup>. Resistance to antifungal also increased during the past several years<sup>6</sup>. So urgent need for new antifungal therapy to control the high incidence of the disease<sup>7,8,9</sup>. Several studies have been done with animal candidiasis in our area<sup>10,11</sup>.

Present study aimed to investigate the presence of yeast in women vaginal samples regarding species, antifungal susceptibility and correlation with pregnancy state and investigating the presence of some virulence genes.

**MATERIALS AND METHODS:**

Two hundred vaginal swabs were taken from women using cotton swab with transport media. Swabs were directly inoculated onto SDA plates supplemented with chloramphenicol. Cultures were incubated at 37°C and were maintained for 2 weeks<sup>12,13</sup>.

**Yeast Identification:**

Yeasts colonies were diagnosed according to<sup>14</sup>. Growth on Sabaroud dextrose agar and observing the shape, color and size of colony. Inoculated plates were incubated under aerobic conditions at 37°C. Yeast were subjected to the following test for identification, Germ tube test<sup>15</sup>, Dalmau plate technique. Growth on CHROMagar™ *candida*: CHROMAgar™ *Candida*, Paris<sup>16</sup> and EPI Aux system also was used for identification<sup>17</sup>.

**DNA Extraction:**

EZ-10 Column Yeast Genomic DNA Yeast Purification Kit was used for DNA extraction from *C. albicans*.

**PCR:**

Virulence genes *ALSI*, *HWP1* and *INT1* were investigated by PCR using the primers indicated in table-1<sup>18</sup>.

**Table 1. The sequences of primers used at the study.**

Genes	Sequence of primer	product (bp)
<i>ALSI</i>	F, GAC TAG TGA ACC AAC AAA TAC CAG A R, CCA GAA GAA ACA GCA GGT GA	318
<i>HWP1</i>	F, ATG ACT CCA GCT GGT TC R, TAG ATC AAG AAT GCA GC	572
<i>INT1-F</i> <i>INT2-R</i>	F, A A G T A T T T G G G A G A A G G G A A A G G G R, AAAATGGGCATTAAGGAAAAGAGC	310

**Antifungal Test:**

In sterile Phosphates buffer saline, a suspension of overnight cultures of *Candida albicans* was prepared.

The inoculum contained 1-5106CFU/ml after turbidity was corrected to 0.5 McFarland standard density. 25µl of suspension inoculated on SDA plates and rolled on the agar medium surface. Antifungal discs were placed on the inoculated agar with forceps after plates were dried at room temperature in a laminar hood. The plates were incubated at 37°C for 24 hours before being manually measured for zone diameters<sup>19</sup>.

**RESULTS:**

The primary isolation of *Candida* species from vaginal swabs indicated that 102 out of 200 (51%) were positive, (Table 2).

**Table 2. Frequency of positive growth in relation with age in human.**

Age (year)	Vaginal swab	Total positive	%
≤ 30	135	69	51.11
> 30	65	33	50.76
<b>Total</b>	200	102	51

According to pregnancy, the present study found an increase of yeasts isolation in both pregnant female with a percentage of 56.45% (Table 3).

**Table 3. Frequency of positive samples in relation with pregnancy in human.**

Status	Total number of sample	No. of positive yeast culture	%
<b>Pregnant</b>	124	70	56.45
<b>Non -pregnant</b>	76	32	42.10
<b>Total</b>	200	102	51

According to the using of antibiotics drugs, the results of this study showed an increase of yeast isolation human that used antibiotics with a percentage of 50 % as indicated in table 4.

**Table 4. Number of samples and percentage of yeast isolation in relation with using antibiotics.**

Status	Total number of sample	No. of positive yeast culture	%
<b>Used antibiotic</b>	28	22	78.57
<b>Non-used antibiotic</b>	172	80	46.51
<b>Total</b>	200	102	51

According to abortion cases, the results of this study revealed that the yeast isolation in human, the results indicated that non –abortion cases were more than abortion ones with a percentage of 51.08% as showed in table 5.

**Table 5. Number of samples and percentage of yeast isolation in relation with abortion state.**

Status	Total number of sample	No. of positive yeast culture	%
<b>Abortion</b>	34	16	47.05
<b>Non -abortion</b>	166	86	51.80
<b>Total</b>	200	102	51

**Antifungal sensitivity test:**

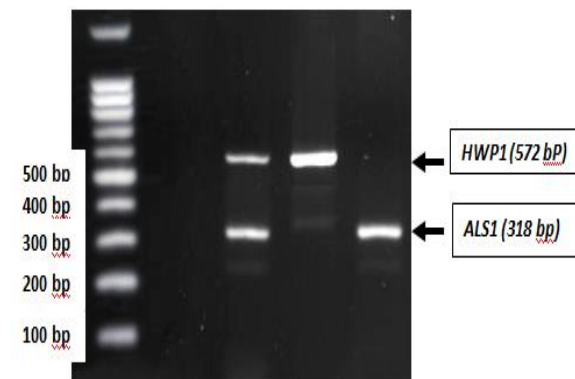
Twenty isolates from *C. albicans* isolated from women were tested for their antifungal susceptibility toward antifungal drugs using disc diffusion method. Results showed high sensitivity toward fluconazole and clotrimazole and high resistance toward amphotericin B and itrconazole.

**Detection of virulence gene:**

The *HWP1* gene was found in 6 out of 10 isolates (60 %), whereas the *ALSI* gene was found in 7 out of 10 isolates (70%). *INT* gene was found in 5 out of 10 isolates (50%) as shown in table (6) and in Fig 1 and 2.

**Table 6. Number and percentage of positive INT, ALSI and HWP gen in yeast isolates.**

genes	Samples Number	positive samples	% positive
<b>INT gene</b>	10	5	50 %
<b>ALSI gene</b>	10	7	70 %
<b>HWP gene</b>	10	6	60 %



**Fig 1. PCR results of HWP1 and ALSI genes of C. albicans ( 3,4,8 HWP1 gen positive ),(3,5, 6,8 ALSI gen positive)**

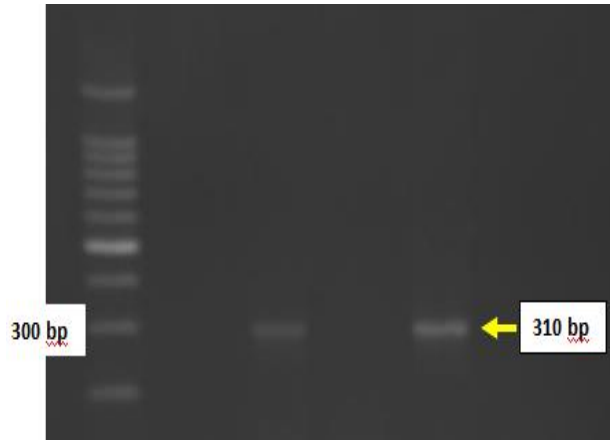


Fig 2. Multiplex PCR amplification with 100 bp ladder showing the presence of *INT* gene of *C. albicans*.

### DISCUSSION:

In relation with age of women, the results indicated that the highest percentage of *Candida* were in age less than or equal to 30 years with a percentage of (51.11%). That is agree with previous report<sup>20</sup>, they reported that (46.8 %) of *Candida* were growth on SDA agar with the age between 25 to 31 years. Studies found the most frequent infection of the female genital tract is vulvovaginal candidiasis. During a woman's lifespan, nearly 75% will have a candidal vulvovaginitis<sup>21</sup>.

In relation with pregnancy, results showed that the percentage of *Candida* were 56.45% and 42.10% in pregnant and non-pregnant respectively. This result was in agreement with other study<sup>22</sup>, they found that 46.8% and 55.4% of *Candida* were growth on SDA agar from pregnant case and non-pregnant, respectively.

In relation with antibiotic used in human, the result revealed that (78.57%) of *Candida* were growth on SDA agar. Any types of antibiotics can increase the risk of developing thrush, but in order to develop the condition, the *Candida* fungus must already be present. Prevalence of vulvovaginal candidiasis (VVC) as a result of antibiotic use is also indicated previously<sup>23</sup>, who reported 28% and 33% frequently of VVC with antibiotic use respectively.

The *ALS1* gene was positive in 13. This result was in agreement with Pakdel *et al*<sup>24</sup>, they found 57% of strains were positive. The result of *HWP* gene was in agreement with Nas *et al*<sup>25</sup>, they showed that 62% of strains were positive. On the other hand, this result was in disagreement with others who reported that 5.3% and 28% of strains were positive<sup>26,27</sup>. The result of *INT* gene was in disagreement with another work that found 100 % of strains were positive<sup>28</sup>.

The *ALS* gene family and the *HWP1* gene family encode cell-surface-related glycosylphosphatidyl inositol, which binds to glycoprotein and mediates *C. albicans* strain adherence to mucosal surfaces<sup>29</sup>. Both in vivo and in vitro, the *HWP1* gene and the *ALS1* gene of the *ALS* family have been shown to play a role in *C. albicans* biofilms formation<sup>30</sup>. *Candida*'s capacity to attach to epithelial cells may be aided by the *INT1* gene<sup>31</sup>.

### CONCLUSION:

It can be concluded that the highest levels of yeast isolation were found in relation with using of antibiotics and pregnant state, and the most of the tested *candida* strains had *ALS1*, *HWP* and *INT* genes.

### CONFLICT OF INTEREST:

Authors declare that there is no conflicts of interest regarding this investigation.

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