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Detection The Prevalence of Adhesins and Extracellular hydrolytic enzymes genes in *Candida albicans* Biofilm Formation

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Abstract

Biofilm formation (BF) is one of the most important virulence factors of *Candida* spp. The aim of this study was to detect the prevalence of genes responsible in biofilm formation of *C. albicans* by conventional PCR technique. Among 49 vaginal specimens (VC), *C. albicans* was the most predominant species in percentage 22/49 (45%) and 27(55%) were non *albicans*. Out of 47 oral specimens (OS), 22/47(47%) were *C. albicans*, whereas 25(53%) were non *albicans*. At the present study; all *C. albicans* were biofilm producers with variable strength, out of 44 BF producers, 18 (40.9%) were low biofilm (LBF) with significant differences ($P<0.05$) between HVS and OS, 25 (56.8%) moderate or high biofilm (HBF) and just one isolate from oral was strong (very high) biofilm in percentage about 2.3% with no significant differences between HVS and OS.

Molecular study of virulence genes correlated with biofilm *C. albicans* show that *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* genes were detected in 100% of *C. albicans* from vaginal and oral infections.

Keywords: *Candida albicans*, Biofilm, *ALS1*, *ALS3*, *SAP5*, *LIP8*, *PLB1*, *HWP1*.

التحري عن سيطرة جينات الالتصاق وجينات الانزيمات خارج خلوية في عزلات المبيضة البيضاء المكونة للغشاء الحياتي

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الخلاصة

الغشاء الحياتي هو واحد من اهم العوامل الامراضية في انواع المبيضات. الهدف من هذه الدراسة هو التحري عن انتشار الجينات المسؤولة عن تكوين الغشاء الحياتي في المبيضات البيضاء باستخدام تقنية التفاعل التضاعفي المتسلسل. من بين 49 عينة مهبلية؛ كانت المبيضات البيضاء هي النوع الاكثر سيادة بنسبة 49/22 (45%) و 27(55%) كانت نسبة الانواع الاخرى. من بين 47 عينة فموية؛ 47/22(47%) كانت نسبة المبيضات البيضاء بينما 25(53%) كانت نسبة الانواع الاخرى. في الدراسة الحالية كل عزلات المبيضات البيضاء كانت منتجة للغشاء الحياتي بدرجات قوة مختلفة، من بين 44 عزلة منتجة للغشاء الحياتي ؛ 18 (40.9%) كانت منخفضة الغشاء الحياتي مع وجود فروق معنوية بين مسحات الفم و المهبل،

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25(56.8%) متوسطة او عالية الانتاجيه للغشاء الحياتي او عزلة واحدة فقط كانت ذات غشاء حياتي قوي بنسبة 2.3% ولم يكن هناك فروق معنوية بين العزلات الفموية و المهبلية.

بينت الدراسة الجزيئية لجينات الضراوة ذات العلاقة بالغشاء الحياتي في المبيضاء البيضاء؛ ان *HWP1, ALS1, ALS3, SAP5, PLB1, LIP8* قد تحددت بنسبة 100% في عزلات المبيضات البيضاء المعزولة من اصابات الفم والمهبل.

Introduction

As the most common yeasts in humans, *Candida* spp. are responsible for most fungal diseases. Therefore, understanding the mechanisms by which these microorganisms colonize and cause disease in humans is a great challenge for planning and establishing treatments [1]. In the recent years, the prevalence of serious fungal infections, invasive *Candida* infections particularly, has been increasing due to an increased number of patients receiving immunosuppressive therapy, increased major surgeries and broad-spectrum antibiotherapy, hyperalimentation, prolonged intensive care unit stay for patients with poor health status [2, 3]. *Candida albicans* is responsible for more than 50% of human candidiasis, including two major types of infections, superficial infections (nonlethal), such as oral or vaginal candidiasis; and systemic infections [4- 6]. *Candida* spp. is the most causative agents of vulvovaginitis in women and *C. albicans* was the most predominant candidal spp. [7].

Like other microorganisms, free-living *Candida albicans* is mainly present in a three-dimensional multicellular structure, which is called a biofilm [8]. Biofilms are defined as structured microbial communities that are attached to a surface and surrounded by a self-produced extracellular matrix [9, 10]. Biofilms form on tissue surfaces, such as infections of the oral and vaginal mucosa. In such infection models, *C. albicans* produces dense three-dimensional biofilms embedded inextracellular matrix material [11].

C. albicans biofilm formation characterized as a series of sequential steps: (1) biofilm formation begins with adherence step which cell-wall protein-mediated adherence of yeast cells to a surface (2) Initiation step, the yeast cells proliferate across the surface and produce elongated projections that grow into filamentous forms, including hyphae or/ and pseudohyphae (3) Maturation step extracellular matrix accumulates as the biofilm matures, and high-level drug resistance is also acquired Finally, (4) non-adherent yeast cells are released from the biofilm into the surrounding medium (dispersal step). While these steps may occur concurrently rather than sequentially during natural biofilm formation [12, 13].

Over the past years, the genetic network controlling biofilm formation has been investigated and partially elucidated, both *in vitro* and *in vivo* [14-17]. The genes that govern *C. albicans* biofilm formation fit into several broad functional categories. Many of these genes are required for production of hyphae (filamentation). Some of the first *C. albicans* biofilm genetic studies indicated that hyphae are required for stable biofilm formation [18, 19]. Many biofilm genes encode known or predicted cell wall proteins. These proteins are of special interest because they may play a direct role in cell-substrate or cell-cell adherence, heterologous expression studies indicate that *HWP1, ALS1, and ALS3* have such roles [20]. Previous works demonstrated that the expression of *HWP1* and of genes belonging to the *ALS, SAP, LIP* and *PLB* gene families is associated with biofilm growth on mucosal surfaces [21], as mentioned previously, the main function of *SAPs* is to degrade proteins, but they also play a role in cell-cell adhesion [22].

Materials and Methods:

Samples collection and isolation

High vaginal swabs were collected from 49 patients aged between16-50 years, presented with vulvovaginal candidiasis. Oral swabs were collected from 47 patients with oral candidiasis aged between two days to ten years, during the period from March 2015 to the end of June 2015. Clinical presentations were done by specialized doctors. and were divided in to two smears: one smear was examined immediately under microscope for direct examination; the other usually was cultured on SDA medium.

Identification of Candidal Isolates*:*

C. albicans was identified depending on the morphological features on culture medium with the use of API-*Candida* systems and then conferring the diagnosis by identifying *C. albicans* by using Vitek 2 system. Examined under the microscope looking for *Candida* budding cells. The isolates were stained

by Gram stain to detect their response to stain, shapes, their arrangement and yeast budding form. All isolates were grown on sabouraud dextrose agar. The plates were incubated at 37ºC for 24-48h to isolate pure candidal colonies to examine their shape, size, color and consistency. After confirmation that the colonies were belong to *C. albicans*; the isolates were purified by streaking on sabouraud dextrose agar by using ABC methods then incubated at 37ºC for two days to obtained one isolated pure colony. This isolated colony was transferred to SDA by streaking all the plate, and then incubates at 37 ºC over night.

Biofilm Formation Assay

In the present study, biofilm formation was determined using pre-sterilized polystyrene 96-well microplates using method described in [23] with modifications. Yeast was inoculated using a loop into tube containing 2 ml of YPD broth incubated at 37°C for 24 h, all tubes were diluted at a ratio of 1:20 by using freshly prepared YPD with 1% glucose, each well of the microplate was filled with 200 µL of this final solution. Microplates were covered with lids and incubated at 37°C for 24 h.

The medium in wells was removed and washed two times with sterile phosphated buffer solution (PBS) and then inverted to blot and let to dry. Microplates were stained by adding 200 µL of 0.1gm/100ml crystal violate to each well incubated for 20 minutes which then washed two times with PBS then inverted to blot and let to dry, finally 200 µL of acetone: ethanol mixture (20:80 v/v) was added to each well, waiting for about 10 min then the results were read at 450nm by an Elisa reader.

All tests were done in triplicates and means were calculated. Finally, the optical density (OD) of each strain was compared with the mean absorbance of negative controls (ODnc), (negative control is medium without inoculum). The following classification was used for the determination of biofilm formation: no biofilm production (ODs = ODnc), weak biofilm production (ODnc < ODsP ≤2ODnc), moderate biofilm production (2ODnc < ODsPc ≤4ODnc) and strong biofilm production (4ODnc < ODs) [24].

Statistical Analysis:

The Statistical Analysis System- SAS program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentages in this study.

Extraction of *Candida albicans* DNA:

Samples tubes were mixed thoroughly, and then DNA was extracted from each sample by using Wizard Genomic DNA purification kit (Promega, USA)

Measurement of concentration and purity of extracted DNA

Using NanoDrop spectrophotometer, DNA concentration and purity was determined. One drop for each extracted DNA was loaded then the software was calculated the purity and concentration depends on the following equilibrium:

$1OD_{260}$ = 50ng

Purity = 260 /280

DNA rehydration solution was used as blank.

Primers selection and preparation

All primers Table-1 were supplied in lyophilized forms. Dissolved in nuclease-free water to give a final concentration of 100 picomol⁄µl as recommended by provider and stored in deep freezer as stock solution until used in PCR amplification. Work solution was prepared by added 90 µl of nuclease free water to 10 µl of stock solution of primer to get 10picomol/ µl concentration.

Table 1-The primers and their sequences used in conventional PCR technique

Primers	Primers sequences (3`-5`) Forward reverse	Annealing temperature (℃)	Product size (bp)	Reference
ALS1	GAC TAG TGA ACC AAC AAA TAC CAG A CCA GAA GAA ACA GCA GGT GA	52	318	[25]
ALS3	CCA CTT CAC AAT CCC CAT C CAG CAG TAG TAG TAA CAG TAG TAG TTT CAT C	58	342	[26]
HWP1	ATG ACT CCA GCT GGT TC TAG ATC AAG AAT GCA GC	52	572	[25]
LIP8	AGA GTG ATA CAG ACA AAA AAT CAG AAG ACC ATT CAG CAT CAT GGT G	59	521	[27]
SAP5	AGA ATT TCC CGT CGA TGA GAC TGGT CAA ATT TTG GGA AGT GCG GGA AGA	60	277	[28]
PLB1	CCT ATT GCC AAA CAA GCA TTG TC CCA AGC TAC TGA TTT CAC CTG CTC C	58	179	[28]

Multiplex PCR working solution:

Optimization of Multiplex PCR was accomplished according to [25]; thus, 12.5 µl of go tag master mix, 1 µl for each forward and revers part of *ALS1*and *HWP1*and 4 µl of DNA sample, then nuclease-free water was added to obtain 25 µl as final volume.

Multiplex PCR program:

To detect both of *ALS1* and *HWp1* genes of *C. albicans*, the Multiplex PCR program was mentioned in Table- 2.

Table 2- The Multiplex PCR program for both *ALS1* and *HWP1* gene amplification.

Step		Temperature	Time
1	Initial denaturation	95	4 min
2	denaturation	95	30 sec
3	annealing	52	1 min
4	extension	72	1 min
5	Repeat steps 2-4 for 35 cycles		
6	Final extention	72	7 min
7	hold	4	-

Identification of *PLB1, SAP5, LIP8* and *ALS3* genes of *Candida albicans* by singleplex PCR

PCR Amplification:

The PCR reaction was carried out in a 25 µl reaction containing 12.5 µl of Green Master Mix, 1 µl of 10pmol/ µl from each primer, 2 µl of DNA template and the volume was completed to 25 µl using nuclease-free water. Thermocycling conditions were as follows:

Table 3- The Singleplex PCR Condition for *PLB1, SAP5, LIP8* and *ALS3* gene amplification

Step		Temperature	Time
1	Initial denaturation	95	4 min
2	denaturation	95	30 sec
3	annealing	Depending on Primer (Table- 2(8))	30 sec
4	extention	72	1 min
5	Repeat steps 2-4 for 35 cycles		
6	Final extention	72	7 min
7	hold	4	-

Gel Electrophoresis

PCR products were resolved on 1% agarose gel.The gel was prepared by dissolving 1g of agarose in 100ml of 1x TBE buffer using a microwave oven. The mixture was left to cool to about 55-60 C. one microliter of 10mg/ml of ethidium bromide was added. It was then poured into the electrophoresis tray, secure the combs in place, and left to cool and solidify for about 30min. After the gel was set; the combs were removed carefully and the tank was placed in the electrophoresis system containing running buffer consisting of 1x TBE. The buffer was poured until it covered the gel for about 2mm. Five microliter of each PCR product along with the negative control and a 100 bp DNA ladder were loaded into the wells, the system cover was then put into place and the system was turned on. The gel is left to run for 90min with a 100volt/50 mAmp current. Following electrophoresis, visualization was conducted with a UV transilluminator and the image was captured by digital camera (Canon, US). This camera has the appropriate filter and a suitable program for illumination of EtBr-stained gels.

Results and Discussion:

From 49 *Candida* isolates from vaginal swabs; 22(45%) were *Candida albicans* and 27(55%) were non *albicans*. Whereas 22(47%) out of 47 *Candida* isolates from oral swabs were *C. albicans* and 25(53%) were non *albicans*.

In the present study, non *albicans Candida* isolates collectively contributed to more than half (55%) and (53%) of the candidial infections in both of vaginal and oral infection respectively this result agree with Jose *et al*. [29] in a previous study, also observed that the non *albicans Candida* was predominant (70%) as compared to *C. albicans* (30%), which indicate that the non a*lbicans Candida* infections are on the rise.

Similar finding have been reported in the literature by different authors [30], Whereas *C. albicans* was the most dominant species in both of vaginal and oral infections with percentages 45% and 47% respectively. These results are agree with [31] that *C. albicans* and *C. glabrata* were the most common yeast species isolated from patients. Mohammed [7] indicated that *C. albicans* was the predominant species (63.6%) out of 124 HVS, followed by *C. glabrata* (30.9%) and *C. trupicalis* (5.5%), [32] find 63.8% isolates were *C. parapsilosis*20.34% were *C. albicans*.

Biofilm Formation of *C. albicans*

Biofilm formation is one of the most important virulence factors of *Candida* spp. *Candida* biofilms occur on tissue surfaces as well as the biomaterial of medical devices [33]. Biofilm formation by *C. albicans* has shown to be highly variable and is directly associated with pathogenicity and poor clinical outcomes in patients at risk [34]. Biofilm formation was variable among *C. albicans* strains that were isolated from different anatomical sites [35].

At the present study; all *C. albicans* were biofilm producers with variable strength value depended on the OD value with using crystal violate (Figure-1). In general out of 44 BF producers, 18 (40.9%) were weak BF (low biofilm) with significant differences ($P<0.05$) between oral and vaginal specimens, 25 (56.8%) moderate BF (high) and just one isolate from oral was strong (very high)

biofilm in percentage about 2.3%. Among vaginal isolates; 10/22 (45.5%) were low BF whereas moderate or high BF were 12/22 (54.5%). In comparable with those; the biofilm among oral isolates was higher than those of vagina, that weak BF were produced by 8/22 (36.4%), moderate BF 13/22 (59.1%) in addition to the appearance of one oral isolates produced strong BF in percentage about 4.5% Table- 4. As any other research these results are agree with some and different with others in some sides. These results show that BF is higher among oral infection than vaginal infection.

Mahmoudabadi *et al*. [36] indicated that 100% of *C. albicans* isolated from different sources had the ability to produce biofilm *in vitro*. It also agreed with [37] found all tested isolates of *C. albicans* produced biofilm on polystyrene. [38] Found that total of 198 of 327 (60.6%) *Candida* species isolates were biofilm-positive. Of these, 72 (36.4%) and 126 (63.6%) isolates were low and high biofilm producers, respectively.

Among 40 *C. albicans*, 22(55%) were strong- moderate biofilm producers [39], which agree with current results. In [29] found that Among the 100 isolates of *Candida*, 69% were found to be biofilm producers. Among them, 42% were weakly adherent, 23% moderately adherent, and 4% were strongly adherent.

Table 4- Biofilm formation by *C. albicans*

***Candida albicans* source**	**Biofilm Strength OD_c= 0.060 No. (%)**		
	Weak BF	**Moderate**	**Strong**
VVC (n= 22)	10 (45.5%)	12 (54.5%)	0
OC (n= 22)	8 (36.4%)	13 (59.1%)	1 (4.5%)
Total (n= 44)	18 (40.9%)	25 (56.8%)	1 (2.3%)
Chi-square $-\chi^2$	4.367 *	2.159 NS	1.077 NS
P-value	0.0488	0.148	0.375

* (P<0.05), NS= Non-significant.

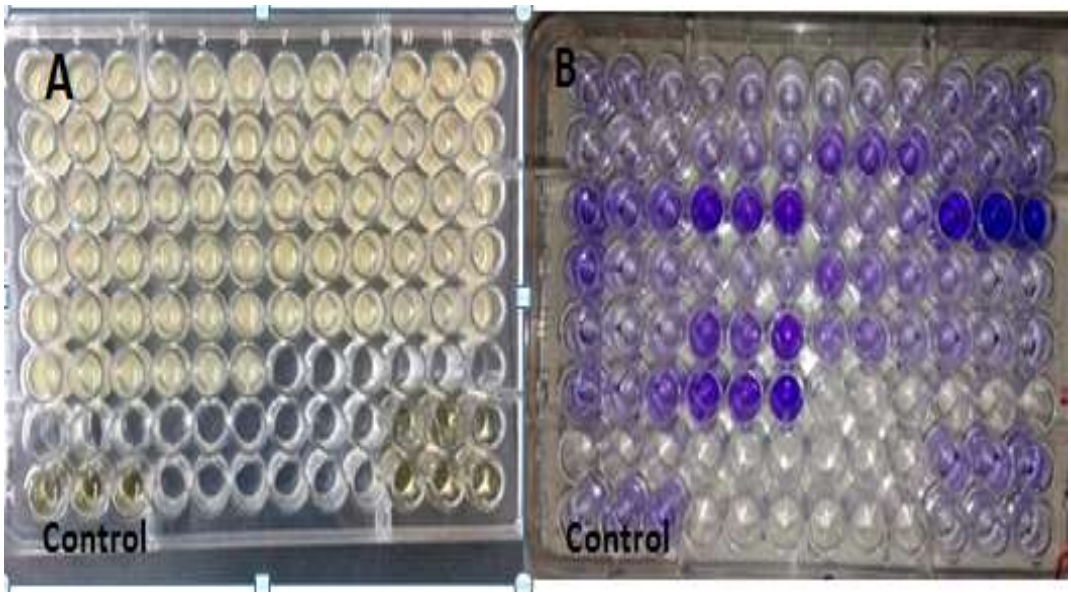

Figure 1- Microtiter plate for biofilm formation, *Candida albicans* isolates biofilms after 24h at 37℃ cultured on YPD with 2% glucose. A) before staining B) after staining with 0.5 mg/100ml crystal violate.

Molecular Study of Virulence Genes Correlated with Biofilm *Candida albicans*:

DNA Extraction:

Forty four extracted and purified DNA were obtained from 22 candidal isolates from vagina and 22 from oral. DNA was extracted and purified by using Wizard Genomic Kit.

Detection of *HWP1* and *ALS1* from Isolated *Candida albicans* by Using Multiplex PCR Technique:

Forty four (100%, 22 from oral, same from vaginal infection) of *C. albicans* were positive for *HWP1* and *ALS1* when detected by multiplex PCR technique. PCR products of these genes were 318 bp and 572 bp for *ALS1* and *HWP1*, respectively (Figure- 2).

The study of [40] that *ALS1* gene was detected in 39/39 (100%) of *C. albicans* isolates and *HWP1* was detected in 35/39 (89.7%) and all strains were positive for *HWP1* 35/35 (100%) expressed this gene during infection. The current detection results were much higher than other Iraqi thesis by [41] who found that gene detection of each of *ALS1* and *HWP1*was done by PCR methods. Out of 25 *C. albicans* twelve isolates were positive for *ALS1* gene *and* only nine samples positive for *HWP1* gene, eight isolates were positive for both genes by multiplex PCR method. The study of [25] found that the presence of the *ALS1* gene was detected in 53.9% of all strains, while the *HWP1* gene was present in 5.3% which are much lower than present findings. The high frequency of detected *ALS1* and *HWP1* in this study may be related to the high pathogenicity of *C. albicans* that isolated from patients in ruled in this study. Transcript profiling and functional analysis pointed to the same conclusion: *Bcr1* is required for expression of genes for cell surface adherence proteins (called adhesins), such as *ALS1*, *ALS3*, and *HWP1*. Many of these genes, including *ALS3* and *HWP1*, are induced strongly during hyphal growth. It is important to note that overexpression of adhesin genes *ALS1*, *ALS3*, or *HWP1* in bcr1Δ/Δ mutant background restored biofilm formation ability, both *in vitro* and in a catheter infection model [42]. *C. albicans* Bcr1, a C_2H_2 zinc finger protein, has a significant role in biofilm formation [43].

Bcr1 is required for full expression of adhesins *ALS3*, *ALS1*, and *HWP1*. Gene mutation and overexpression analyses together prove that *ALS3* is necessary and sufficient among Bcr1 targets for biofilm formation *in vitro*. Overexpression analysis indicates that *ALS1*, *HWP1*, can also restore biofilm formation in the absence of Bcr1 *in vitro*. The fact that overexpression suppressors*ALS1*, *ALS3* and *HWP1* are all known adhesins indicates that adherence is the property through which Bcr1 governs biofilm formation. Bcr1 is required for biofilm formation *in vivo*, and overexpression of *ALS3* permits biofilm formation in the absence of Bcr1 *in vivo*. Thus, Bcr1-dependent adherence is critical for biofilm formation *in vivo* and *in vitro* [42].

The production of hyphae is a hallmark of initiation, and many initiation-defective mutants grow solely as yeast cells under biofilm conditions. Expression of a surface-directed *ALS3* fusion protein permits biofilm formation *in vitro*; therefore, the major way that hyphae promote biofilm formation is through expression of their surface protein complement [44].

The idea that *ALS1* might function in the initial adherence step is consistent with the fact that expression of gene is detectable in cells grown as either yeast or hyphal cell types this is not the case for *ALS3*, which is expressed primarily or exclusively in hyphae [45]. It is possible that the initial adherence step leading to biofilm formation *in vivo* can be carried out by either yeast-form cells, which express *ALS1*, or by hypha, which express *ALS3* [46].

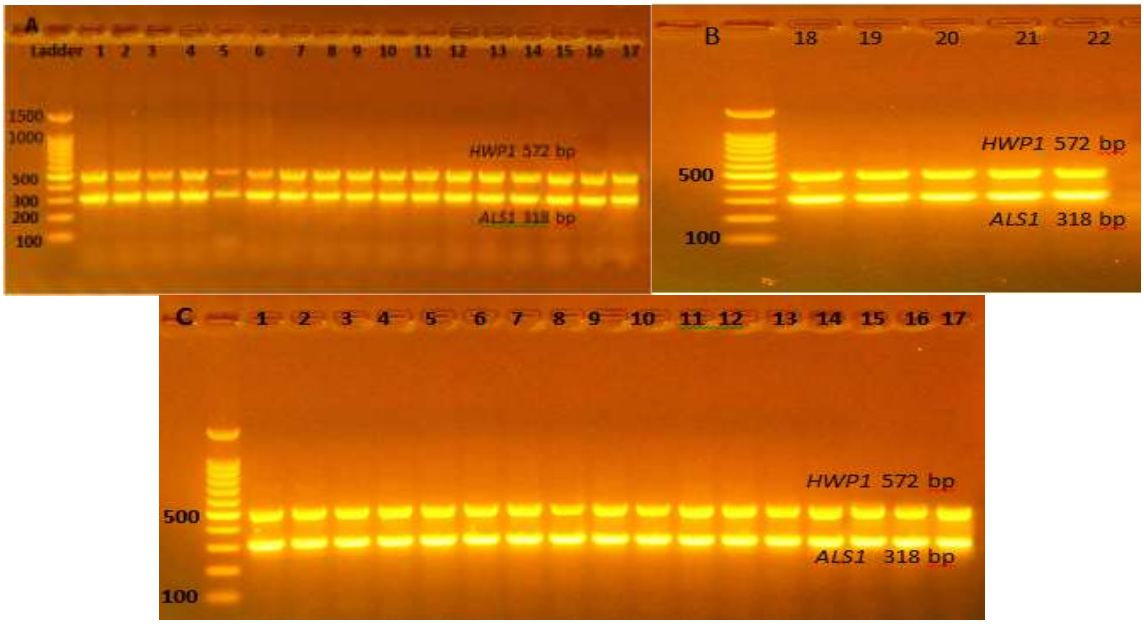

Figure 2-Gel electrophoresis of multiplex PCR products of both *ALS1* and *HWP1* genes of *Candida albicans* on 1% agarose gel at 7volt /cm for 1 hour with 100bp DNA ladder. A, B) isolated No. 1-17, 18-22 from oral candidiasis C) 1-17 for *C. albicans* from vaginal candidiasis.

Detection of *ALS3, SAP5, PLB1* and *LIP8* Genes in *Candida albicans*

ALS3, *SAP5*, *PLB1* and *LIP8* were detected in all 44(100%) tested *C. albicans* isolated from both of vaginal and oral infections. Gel electrophoresis show the PCR products for *ALS3* was 342 bp, *SAP5* 277bp, *PLB1* 179 bp and 521 bp for *LIP8* Figures- 3, 4, 5 and 6 respectively. These results agree with some previous studies and disagree with others the reason of disagreement could be related to differences in *Candida* strains, *Candida* pathogenicity, sampling numbers, sites and degree of infection.

The study of [40] detected *SAP5*, *PLB1* in 39/39 (100%) of *C. albicans*, and their expression were also 100%, whereas detection frequency of *LIP8* was nearly agree with the current results it was detected in 38/39 (97.4%) with 100% expression fold, in contrast with 100% in this study. In the same previous study; *ALS3* was detected in 14/39 (35.8%) which is much lower than 100% detection in this study in spite of 100% expression fold of *ALS3* in [40] study. by using PCR technique; [47] results showed that *LIP8* was detected in only 1(100%) *C. albicans*, and *PLB1* gene was detected in 9(90%) *C. albicans*, in other previous study of [48] found the genotyping frequencies of the *SAP5* gene was 100% with 90% expression, suggesting that the Sap proteins play an important role in the pathogenesis of infection. The main function of *SAPs* is to degrade proteins, but they also play a role in cell-cell adhesion [22]. [49] showed that *C. albicans* biofilms secrete more *SAPs* than do planktonic cells, while [50] showed that an *in vitro C. albicans* biofilm induced *SAP* activity, and that *SAP8* expression within the biofilm correlated with *in vivo* denture stomatitis severity.

Of the 10 *SAP* genes, *SAP4 – 6* are predominantly expressed in hyphae [22] and hyphae are the predominant forms in biofilm growth in the *in vivo* model [51], on the other hand, *SAP9* - and *SAP10* - encoded proteins maintain cell surface integrity by processing cell wall proteins, which mediate biofilm formation [52, 53]. The study of [53] proposed that *Sap9* and *Sap10* influence distinct cell wall functions by proteolytic cleavage of covalently linked cell wall proteins which mediate biofilm formation and promote adherence to host cells and invasion into epithelial cell layers.

In addition to *SAPs*, *C. albicans* also has two other gene families, namely the lipases (*LIP*) and phospholipases (*PL*) that produce extracellular hydrolytic enzymes that could play roles in candidal adhesion, nutrient acquisition and invasion of epithelial surfaces [27]. Constitutive expression of the *LIP* genes and *PLB* has been demonstrated in *C. albicans* biofilms [54], However, it is known that the expression of *ALS, SAP, LIP and PLB* genes can be influenced by other factors such as the growth medium, temperature and other environmental conditions [22, 55].

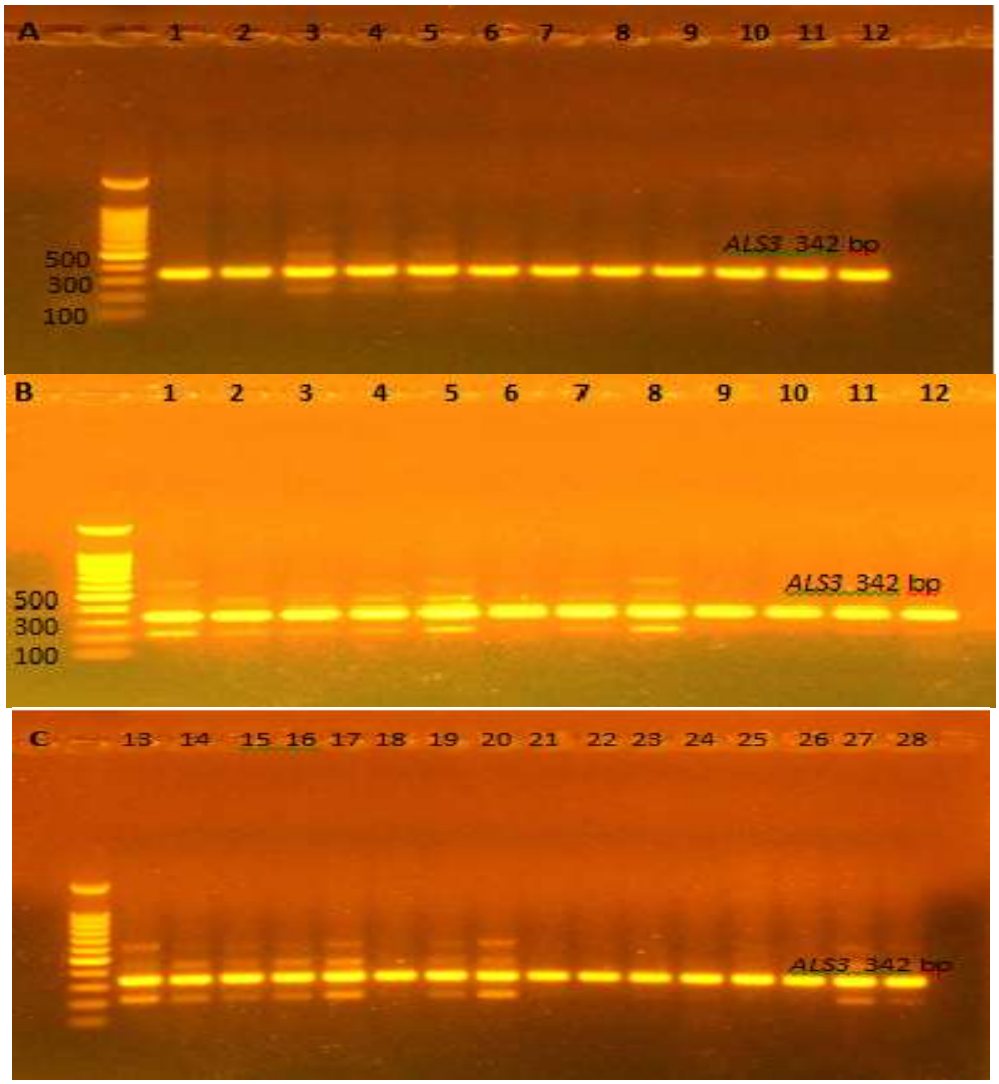

Figure 3- Gel Electrophoresis for PCR Product for *ALS3* Gene of *Candida albicans* on 1% agarose gel at 7volt /cm for 1 hour with 100bp DNA ladder. A) Isolates No. 1-12 from Oral Candidiasis, B) Isolates No. 1-12 for Vaginal Candidiasis. C) 13-22 from vagina, 23- 28 from oral.

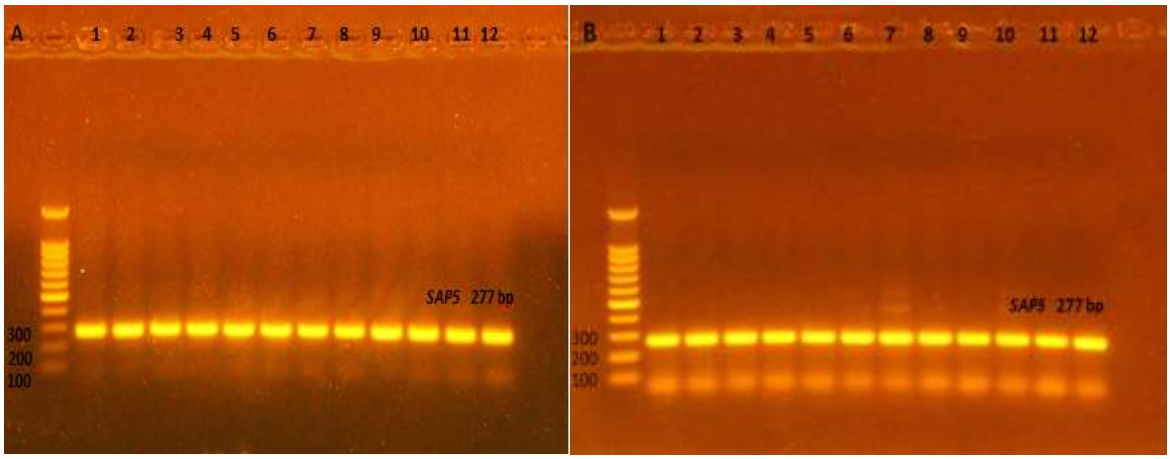

Figure 4- Gel Electrophoresis for *SAP5* Gene of *Candida albicans*, on 1% agarose gel at 7volt /cm for 1 hour with 100bp DNA ladder. A) Isolated No. 1-12 from oral. B) 1-12 from vaginal infection.

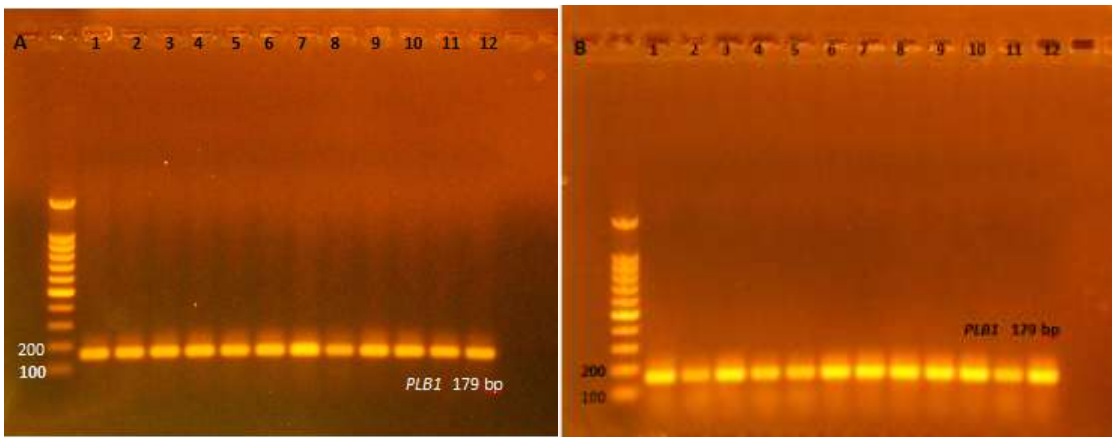

Figure 5- Gel Electrophoresis for *PLB1* Gene of *Candida albicans*, on 1% agarose gel at 7volt /cm for 1 hour with 100 bp DNA ladder. A) Isolated No. 1-12 from oral. B) 1-12 from vaginal infection.

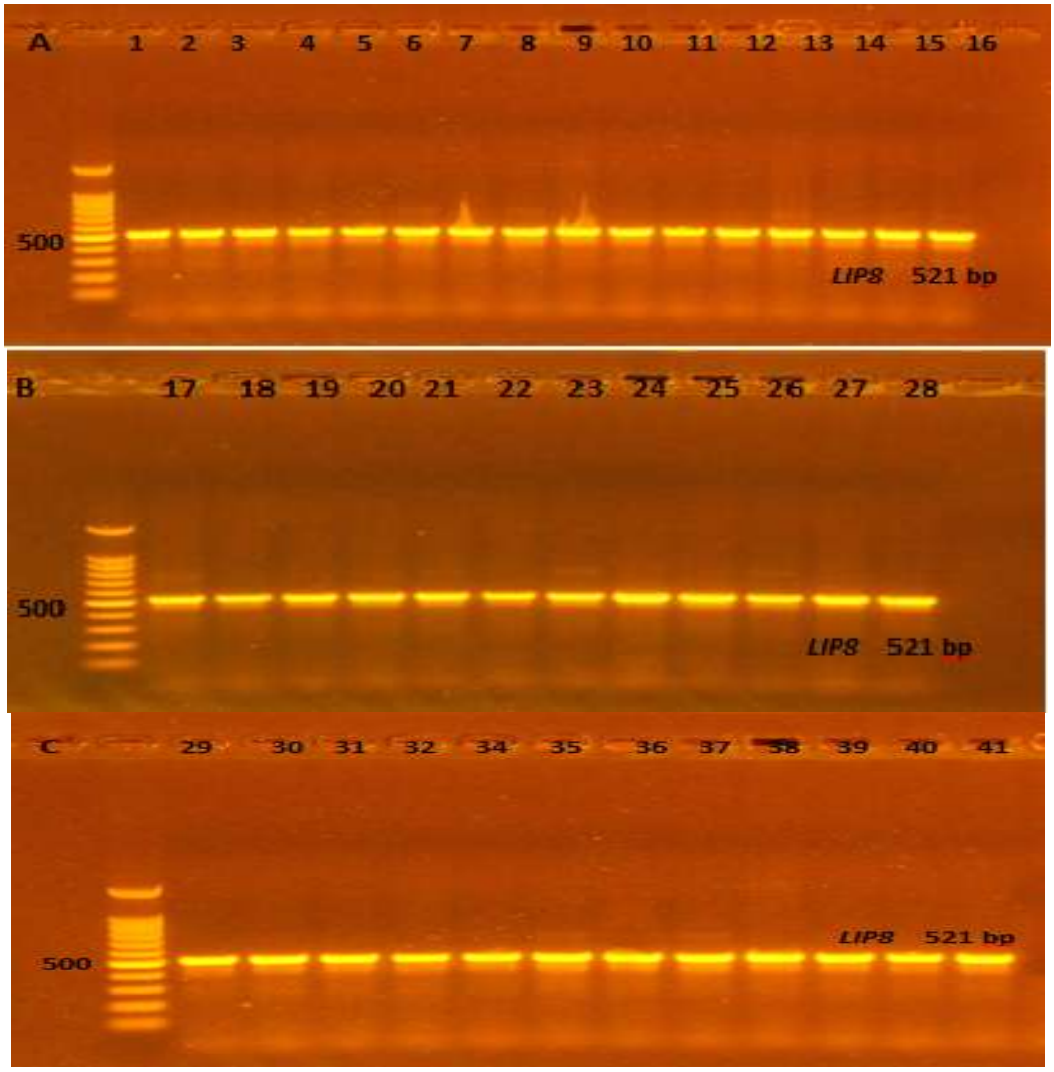

Figure 6- Gel Electrophoresis for *LIP8* Gene of *Candida albicans*, on 1% agarose gel at 7volt /cm for 1 hour with 100 bp DNA ladder. A) Isolated No. 1-16 from vaginal candidiasis B) 17-22 from vaginal candidiasis, 23-28 from oral candidiasis C) isolates from oral.

Conclusions**:**

This study concluded that all *C. albicans* were biofilm producers with variable strength. *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* genes were detected in 100% of *C. albicans* from vaginal and oral infections.

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