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ORIGINAL ARTICLE

Candidiasis in Immunnocompromised Patients; Comparison between *C. albicans* and Non-*albicans* regarding the Type of Infection, Biofilm Formation and Virulence Genetic Profile

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Key words:
Candida; Albicans, Non-albicans, Genetic profile of Candida spp., Biofilm, Immunnocompromised patients

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ABSTRACT

Background: *Fungal infections have been on the rise during the last few decades. Immunnocompromised patients are at risk for developing deep mycotic infections of which invasive candidiasis (IC) is particularly serious. Although Candida albicans is the most common cause of IC, other species such as C. krusei, C. tropicalis, and C. parapsilosis have also been recovered with variable virulence factors.* ***Objectives:*** *We compared between albicans and non-albicans Candida infections among immunnocompromised patients admitted to the National Liver Institute (NLI) and Menoufia University Hospitals (MUH) as regards the incidence and type of infection, ability of biofilm formation as well as the virulence genetic profile.* ***Methodology:*** *42 Candida isolates were collected from different types of infections and classified as C.albicans and C. non-albicans according to their characteristic reactions on Brilliance™ Candida Chromogenic Agar. The biofilm-forming ability of the isolated species was demonstrated phenotypically by the microtitre plate method (MTP) and multiplex PCR assay verified the existence of the contributing virulence genes.* ***Results:*** *Out of 42 Candida isolates, 9 (21.4%), 26 (61.9%) and 7 isolates (16.6%) were recovered from superficial, invasive and device associated infections respectively. The number of non-albicans spp. exceeded that of C.albicans [25/42(59.52%): 17/42(40.48%)] and the highest frequency was for C. parapsilosis (14/25:56%) followed by C. tropicalis (8/25:32%) and finally C. krusei (3/25:12%).The percentage of biofilm-forming isolates was 94.1% for C. albicans and 72% for non-albicans Candida spp. with no significant statistical difference (P> 0.05). The expression of HWP1 gene was significantly higher in biofilm-forming Candida spp. (P= 0.02).* ***Conclusion:*** *Infections due to non-albicans species are rising especially in immunosuppressed patients. HWP1, ALS1, SAP5 and PLB1 genes were all detected in both C. albicans and non- albicans and the majority of isolated Candida spp. were biofilm producers.*

INTRODUCTION

The proportion of invasive fungal infections in critically-ill patients has increased dramatically in recent decades with poor outcomes and rising mortality [1].

Candida naturally colonizes the female reproductive tract, gastro-intestinal tract and skin. Pathogenic *Candida* is enhanced by diverse virulence features including; adherence to host tissues, biofilm formation as well as production of extracellular hydrolytic enzymes contributing to an array of infectious candidiasis ranging from superficial mucocutaneous infections to life-threatening invasive diseases[2]. The latter is the fourth most frequent health care-associated infection particularly in immunnocompromised patients[3].

Candida is mainly divided into *Candida albicans* and *Candida* non-*albicans* species. Out of 200 known *Candida* species, *C. albicans* is the most common etiological agent of infectious candidiasis[4]. Although *C. albicans* are more characterized and clinically relevant, non-*albicans* species have become more evident in more serious infections especially blood stream infections[5]. In fact, 95% of infections are essentially caused by four species: *C. albicans, C. tropicalis, C. parapsilosis* and *C. glabrata*[6].

Candida infections are frequently associated with therapeutic failure, mainly due to antifungal resistance.

Several mechanisms were identified to provide resistance to commonly used antifungals[7]. Most importantly are biofilm-forming strains which exhibit dramatically reduced susceptibility to antifungals and host immunity[8].

Several genes are documented to mediate the formation of biofilm, including *HWP1*, *ALS1* and *ALS3* genes[9,10]. In addition to biofilm formation, secretion of extracellular hydrolytic enzymes adds more virulence to *Candida*. For instance, secretion of aspartic proteinases (Saps; a family of 10 enzymes) enhances the proteolytic cleavage cell wall proteins which mediates adherence to host tissues and invasion into deeper epithelial layers[11,12]. Moreover; the ability of the fungus to break down the phospholipids (a major component of the cell membrane) by phospholipases plays a potential role in promotion of host tissue invasion. The production of such enzymes is regulated by the expression of *SAP1, SAP2, SAP3, SAP4, SAP5, SAP6, PLB1, PLB2* and *LIP1-10* genes[3].

In the present study, we compared between *C.albicans* and non-*albicans* infections among immunosuppressed patients of the National Liver Institute and Menoufia University Hospitals. We investigated the incidence and type of infection and the virulence factors expressed by each type using phenotypic and molecular characterization methods.

METHODOLOGY

Study design

This cross-sectional study was conducted over a period of one year (December 2017 to August 2018) at the Department of Medical Microbiology and Immunology, National Liver Institute in collaboration with Faculty of Medicine, Menoufia University Hospitals (MUH).Written informed consents were taken from all participants before the enrollment and the study protocol was approved by the Ethical Committee of the National Liver Institute and Faculty of Medicine, Menoufia University, Egypt.

Specimen collection:

Specimens were taken from patients admitted to different ICUs, Liver Transplantation Unit as well as Oncology Unit of MUHs. A total of 42 *Candida* isolates were recovered from different clinical specimens including oropharyngeal swabs, urine, blood, sputum, bile and swabs of device-associated infections such as central venous catheter, endotracheal tube and indwelling catheters.

Identification and storage of *Candida* isolates

The collected specimens were inoculated into Sabaroud's dextrose agar (SDA) (Oxoid, England) and incubated at 37ºC for 24-48 hrs. The growing colonies were identified by the standard microbiological features (colony morphology and germ tube test)[13] . Identification of species was performed by culture on *Brilliance™ Candida* Agar (formerly Oxoid Chromogenic *Candida* Agar (OCCA) CODE: CM1002; England) which is a selective differential medium for the rapid isolation and identification of clinically important *Candida* spp. The medium was prepared according to the manufacture s instructions and the inoculated plates were inspected for the growth of *Candida* spp. at 24, 48 and 72 hrs. Colonies were identified according to their expected reactions on *Brilliance™ Candida* Agar as shown in table 1 and figure 1. Further confirmation of the isolated species was done by the Vitek-2 compact system (bioMérieux, France).Confirmed isolates were suspended in nutrient broth supplemented with16% glycerol and stored frozen at -80°C[14].

Table 1: Expected reactions of *Candida* spp. on *Brilliance Candida* Agar

Chromogen: Enzyme:	**X-NAG Hexosaminidase**	**BCIP Alkaline phosphatase**	**Typical colony appearance**
C. albicans	+		Green ‡
C. non- albicans			
C. tropicalis	+		Dark blue
C. krusei		+	Dry, irregular pink-brown
C. glabrata *C. kefyr* *C. parapsilosis* *C. lusitaniae*		Variable	Beige/yellow/ brown †

BCIP, 5-bromo-6-chloro-3-indolyl phosphate p-toluidine saltX-NAG, 5-bromo-6-chloro-3-indolyl Nacetyl B-D-glucosaminide.

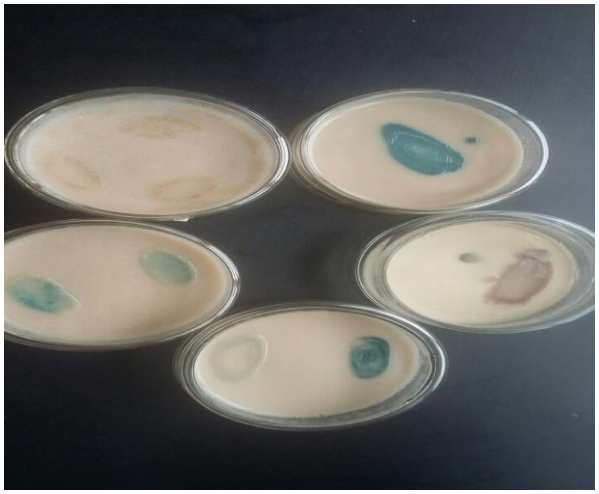

Fig. 1: Characteristic reactions of different *Candida* spp. on chromogenic agar plates

Determination of biofilm-forming ability of the isolated *Candida* spp.:

The ability of the isolated species to form biofilms was determined by the 96-well microtitre plate method (MTP)[15]. The collected isolates were inoculated into tube containing 2 ml of YPD broth and incubated at 37°C for 24 h. After incubation, all tubes were diluted at a ratio of 1:20 by using freshly prepared YPD with 1% glucose and each well of the plate was filled with 200 µL of this final solution. The plate was covered and incubated at 37°C for 24h. The content of the wells was removed and washed twice with phosphate- buffer solution (PBS) and then inverted and left to get dry. The plate was stained with 200 µL of 0.1gm/100ml crystal violate added to each well and incubated for 20 minutes. The plate was washed two times with PBS then inverted to blot and left to dry. Finally 200 µL of acetone: ethanol mixture (20:80 v/v) was added to each well, left for about 10min then the results were read at 450nm by an ELISA reader and classified into no, weak, moderate and strong biofilm formation[16] as shown in figure 2.

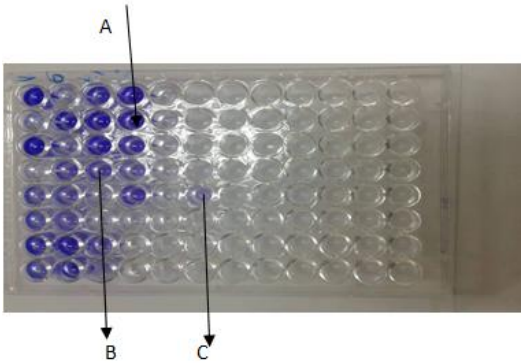

Fig. 2: Biofilm formation among the isolated *Candida* spp. using microtitre plate method: A, B and C represent strong, moderate and weak biofilm formation respectively.

Genotypic characterization of the suspected virulence genes in the isolated *Candida* spp.:

– The isolated *Candida* spp. were subjected to multiplex PCR assay to verify the presence of some of the suspected virulence genes that contributes to biofilm formation and production of extracellular hydrolytic enzymes. The target genes and their specific sequences are illustrated in table 2.

Table 2: Primers used in the study

Target genes	Primers sequences (3`-5`)	Annealing temperature	Product size (bp)	Reference
HWP1	FW CCATGTGATGATTACCCACA RV GCTGGAACAGAAGATTCAGG	52	572	(17)
ALS1	FW CCATCACTGAAGATATCACCACA RV TGGAGCTTCTGTAGGACTGGTT	52	318	(17)
SAP5	FW AGAATTTCCCGTCGATGAGACTGG RV CAAATTTTGGGAAGTGCGGGAAGA	60	277	(18)
PLB1	FW GGTGGAGAAAGATGGCCAAAA RV AGCACTTACGTTACGATGCAACA	58	179	(18)

– The PCR technique involved the following steps:

- ***DNA extraction:*** Samples tubes were mixed thoroughly, and then DNA was extracted from each sample by using DNeasy plant Mini Kit (50) (Cat.No.69104, Germany) according to the manufacture s instructions.
- ***Multiplex PCR program*:** For *HWP1 and ALS1* genes involved the following steps:

Step	Temperature	Time
Initial denaturation	95	4 min
Denaturation	95	30 sec
Annealing	52	1min
Extension	72	1min
Repeat steps 2-4 for 35 cycles		
Final extension	72	7 min
Hold	4	-

– The PCR reaction mixture was prepared by adding 12.5 µl of Taq green PCR Master Mix, 1µl forward primer, 1µl reverse primer (Qiagen, Germany), 4 µl of DNA sample, then nuclease- free water was added to obtain 25 µl as final volume17.

- ***Multiplex PCR program*:** For *SAP5* and *PLB1* genes involved the following steps:

Step	Temperature	Time
Initial denaturation	95	4 min
Denaturation	95	30 sec
Annealing	Depending on Primer (Table- 2(8))	30 sec
Extension	72	1min
Repeat steps 2-4 for 35 cycles		
Final extension	72	7 min
Hold	4	-

– The PCR reaction was carried out in a 25 µl reaction containing 12.5 µl of Green Master Mix, 1 µl of 10 pmol/µl from each primer, 2 µl of DNA template and the volume was completed to 25 µl using nuclease-free water[18]. The PCR programs were performed in a thermal cycler (Biometra, Germany).The amplified DNA products were detected on 1.5% agarose gels by ethidium bromide staining (Sigma, USA). A DNA ladder (100-1000bp) (Fermentas, Germany) was used to estimate allele sizes in base pairs (bp) for the gel. Following electrophoresis, visualization was conducted with a UV transilluminator and the image was captured by digital camera (Canon, US) as shown in figures 3,4.

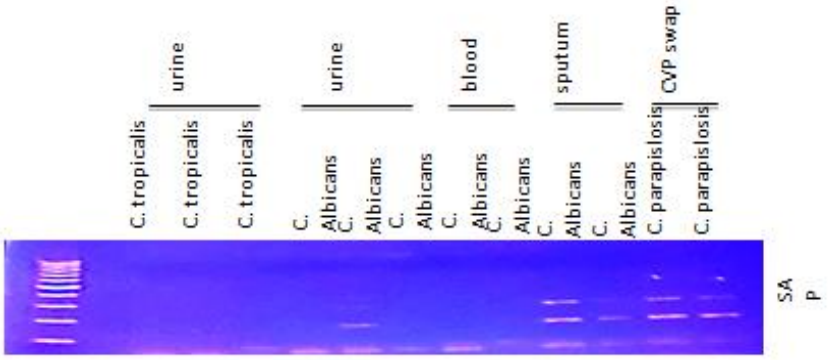

Fig. 3: Agarose gel electrophoresis of *Candida* spp. showing positive samples for SAP5 (277bp) and BLP1 (179bp) genes

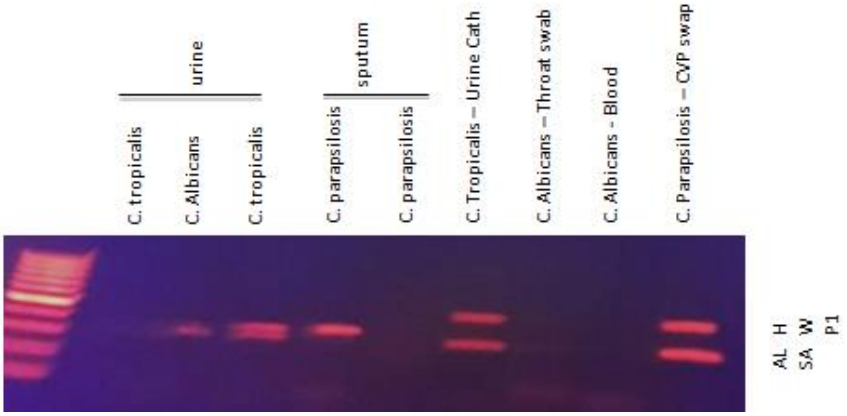

Fig. 4: Agarose gel electrophoresis of *Candida* spp. showing positive samples for HWP1 (572bp) and ALS1 (318bp) genes.

Statistical methods:

Data collected and analyzed using SPSS (Statistical Package for Social Science) program version 22, SPSS Inc., Chicago, Illinois, USA. Analytical statistics as Chi-square test and Fisher exact test were used to measure association between two sets of qualitative variables. P (probability) value considered to be of statistical significance if it is less than 0.05.

RESULTS

During the period from December 2017 to August 2018 a total of 42 *Candida* isolates collected from immunosuppressed patients of both NLI and MUHs suffering from different types of infection. Of these, 42 isolates, nine (21.4%), twenty six (61.9%) and seven isolates (16.6%) were recovered from superficial, invasive and device associated infection respectively. The majority of *Candida* spp. were isolated from urine samples (10/42:23.8%). For device-associated infections, the most commonly affected devices were the central venous catheters (CVC) (4/42; 9.5%) as shown in table 3.

Table 3: Number and percent of the isolated *Candida* spp. among the collected specimens

Types of infection		**Specimens**	***Candida* isolates (n=42)**		
			C.albicans	***C.non albicans***	**Total**
Superficial candidiasis	No.=9 % (21.4)	Oropharyngeal swabs	4	0	4 (9.5%)
		Skin wounds	1	4	5 (11.9%)
Invasive candidiasis	No.=26 % (61.9)	Urine	3	7	10 (23.8 %)
		Blood	3	4	7 (16.7 %)
		Sputum	4	1	5 (11.9 %)
		Bile	1	3	4 (9.5%)
Device-associated candida infection	No.=7 % (16.6)	Indwelling catheter	0	2	2 (4.7%)
		Endotracheal tube	0	1	1 (2.3%)
		Central venous catheter	1	3	4 (9.5%)
Total			**17 (40.48%)**	**25 (59.52%)**	**42**

The number of non-*albicans* spp. 25/42 (59.52%) exceeded that of *C.albicans* isolates, 17/42 (40.48%). Among non-*albicans* spp. the highest frequency was for *C. parapsilosis* 14/25 (56%) followed by *C. tropicalis* 8/25 (32%) and finally *C. krusei* 3/25 (12%).The majority of *Candida* isolates recovered from invasive infections belonged to non-*albicans* spp. (57.7%) compared to *C. albicans* (42.3%) as shown in table 4.

Table 4: Distribution of the isolated *Candida* spp. (*albicans* and non- *albicans*) regarding the type of infection

***Candida* spp.**		**Types of infection**						**Chi x^2test**	**P - value**
		Superficial candidiasis (9)		**Invasive candidiasis (26)**		**Device- associated candida infection (7)**			
***C.albicans* (n=17)**		5		11		1			
***C.non albicans* (n=25)**	*C. parapsilosis* (14)	4		8		2		**11.22**	**0.82**
	C. tropicalis (8)	0		4		4			
	C. krusei (3)	0		3		0			
Total number (42)		***C.albicans*** **No (%)**	***C.*non *albicans*** **No (%)**	***C.albicans*** **No (%)**	***C.*non *albicans*** **No (%)**	***C.albicans*** **No (%)**	***C.*non *albicans*** **No (%)**		
		5 (55.5)	4 (44.5)	11 (42.3)	15 (57.7)	1 (14.3)	6 (85.7)		

The percentage of biofilm-forming isolates was 94.1% for *C. albicans* (16/17;of which 31.25%, 25% and 43.75% were strong, moderate and weak biofilm producers respectively) and 72% for non-*albicans* *Candida* spp. (18/25;of which 27.8%, 16.7% and 55.5% were strong, moderate and weak biofilm producers respectively) with no significant statistical difference (P>0.05) as shown in table 5.

Table 5: Number and percent of biofilm producers among the collected *Candida* spp.

	Non- biofilm producers	**Biofilm producers**			**Chi x^2 test**	**`P- value**
		Strong biofilm producers (n= 10)	Moderate biofilm producers (n= 7)	Week biofilm producers (n= 17)		
***C.albicans* (n=17)**	1 (5.9 %)	5 (31.25 %)	4 (25%)	7 (43.75%)	**3.78**	**0.2**
***C.non- albicans* (n=25)**	7 (28 %)	5 (27.8%)	3 (16.7%)	10 (55.5 %)		
Total number of biofilm producers: *C.albicans* = 16 (94.1%) C. *non- albicans* =18 (72%)						

The highest percentage of biofilm-forming isolates of *C.albicans* was from invasive infections (11/16; 68.75%) followed by device-associated infection (4/16; 25%) and finally superficial infection (1/16; 6.25%). For non-*albicans Candida*, 55.6% (10/18) of biofilm-forming isolates were from invasive infections, 33.34% (6/18) from device-related infection and finally superficial infection (2/18; 11.12%) as shown in table 6.

Table 6: Biofilm production among the collected *Candida* isolates (*albicans* and non -*albicans*) regarding types of infection

	Candida spp.	Types of infection		
		Superficial candidiasis (6)	Invasive candidiasis (21)	Device- associated infection (7)
Biofilm producers	*C. albicans* (n=16)	1 (6.25%)	11(68.75%)	4 (25%)
	C.non- albicans (n=18)	2 (11.12%)	10 (55.6%)	6 (33.34 %)

Regarding virulence genetic profile, all of the tested genes; *HWP1*, *ALS1*, *SAP5* and *PLB1* genes were detected in both *C.albicans* and *C.*non-*albicans* with no significant statistical difference (P>0.05) as shown in table 7. There was also a statistically significant association between the existence of *HWP1* gene and biofilm formation among the isolated *Candida* spp. (P= 0.02) as shown in table 8.

Table 7: Comparison between *C.albicans* and *C.*non- *albicans* as regards virulence genetic profile

Target genes	*C.albicans*		*C.*non- *albicans*		Statistical test	P – value
	No.	%	No.	%		
HWP1					Chi-square	
Positive	7	38.9%	7	29.2%	0.438	0.50
Negative	11	61.1%	17	70.8%		
ALS1						
Positive	11	61.1%	10	41.7%	1.556	0.21
Negative	7	38.9%	14	58.3%		
SAP5			**Fisher Exact**			
Positive	6	33.3%	2	8.3%	4.169	0.05
Negative	12	66.7%	22	91.7%		
PLB1						
Positive	6	33.3%	2	8.3%	4.169	0.05
Negative	12	66.7%	22	91.7%		
HWP1+ALS1			Chi-square			
Positive	7	38.9%	6	25%	0.928	0.33
Negative	11	61.1%	18	75%		
All genes						
Positive	7	38.9%	6	25%	0.928	0.33
Not all genes positive	11	61.1%	18	75%		

Table 8: Association between HWP1 and ALS1 genes expression and biofilm among the isolated *Candida* spp

Target genes	Negative		Low biofilm producers		Moderate biofilm producers		High biofilm producers		Statistical test	P – value
	No.	%	No.	%	No.	%	No.	%	Fisher Exact	
HWP1										
Positive	0	0%	3	30%	5	71.4%	6	35.3%	8.446	**0.02***
Negative	8	100%	7	70%	2	28.6%	11	64.7%		
ALS1										
Positive	2	25%	3	30%	4	57.1%	12	70.6%	6.434	0.08
Negative	6	75%	7	70.0%	3	42.9%	5	29.4%		
HWP1+ ALS1										
Positive	0	0%	3	30%	4	57.1%	6	35.3%	6.085	0.10
Negative	8	100%	7	70%	3	42.9%	11	64.7%		

DISCUSSION

Candida species are considered important pathogens due to their versatility and their ability to survive in different anatomical sites as well[19]. Previously, it was believed that yeasts passively participated in the pathogenesis of fungal infections in immunnocompromised hosts. Nowadays, this concept has been changed. The current consensus is that, such pathogens actively participate in the disease process depending on variable mechanisms of aggression known as virulence factors [20].

Considering the importance of *Candida* infection among immunosuppressed patients, the present study aimed to compare between *C.albicans* and non-*albicans* infections among immunnocompromised patients of NLI and MUH as regards the incidence and types of infection and the presence of different virulence factors by both phenotypic and genotypic methods.

In the present study, the majority of *Candida* spp. were recovered from invasive infections (61.9%) represented by urine samples (23.8%) followed by blood (16.7%), sputum (11.9%) and bile (9.5%). The number of non-*albicans* spp. exceeded that of *C.albicans* isolates (59.52% vs. 40.48%). Our findings were parallel to that of *Sachin et al*, *Alvarez-Lerma et al* and *Kauffmann*, where the majority of *Candida* spp. were isolated from urine samples and that more than 50% of the urinary *Candida* isolates belonged to non-*albicans* spp.[21,22,23,24]. They explained this observation by the fact that, non-*albicans* spp. are not only well adapted to the urinary tract but also, the presence of indwelling urinary catheters, advanced age, diabetes mellitus, and immunosuppression are major risk factors associated with candiduria. The abuses of antibiotics as a "pill for all ills," and starting broad spectrum antibiotics as the first line treatment have led to suppression of the commensal flora and increased colonization by *Candida* spp.

For device-associated infections, the most commonly affected devices were the central venous catheters (CVC) (9.5%). Such observation agreed with *Johnson et al* [25]who stated that, the medical device frequently infected by *Candida* is the CVC, which is used to administer fluids and nutrients and/or cytotoxic drugs. The infusion fluid itself, or the catheter core, can be contaminated but, more frequently, *Candida* can have origin on the patient's skin or on the hands of nursing staff (the distal tip of the catheter can be contaminated at the time of insertion or, instead, organisms can migrate down the catheter wound) [26].

Interestingly, infections due to non-*albicans* *Candida* were much higher (59.52%) than *C. albicans* infections (40.48%). *C. parapsilosis* (56%) followed by *C. tropicalis* (32%) and finally *C. krusei* (12%) were the frequently isolated non-*albicans* spp. These results were in consistence with *Bansal et al* and *Golia et al* who reported higher percentage for non-*albicans* spp. as well [27,28]. Similar results were also detected by *Sachin et al* who observed that, the percentage of *C. albicans* vs. non-*albicans* spp. was 36.7% vs. 63.3% and that the incidence of candidiasis due to non-*albicans Candida* spp. was increasing[21]. Several factors like severe immunosuppression, prematurity, use of broad spectrum antibiotics, and empirical use of antimycotic drugs are associated with shift towards non-*albicans* species. Such findings provide further evidence for the growing significance of non-*albicans Candida* in clinical practice. Isolation of such microbes from clinical specimens can no longer be ignored as nonpathogenic isolate nor can it be dismissed as a contaminant[21].

The most striking observation in the current study was that, the majority of *Candida* isolates recovered from invasive infections belonged to non-*albicans* spp. as compared to *C.albicans* (57.7% vs. 42.3%) and that *C. parapsilosis* was the most frequent non-*albicans* isolate (56%). Similarly, *Caggiano et al* found that *C. albicans* and non-*albicans* spp. caused 44.2% and 55.8% of IC respectively[29]. *Matsumoto et al* [30]also addressed that *C. parapsilosis* was the most prevalent one of the non-*albicans* isolates and accounted for 62.3% of non-*albicans* invasive episodes. The dominance of *C. parapsilosis* is by far related to factors common in hospital settings, such as gastrointestinal colonization, or the affinity for intravascular devices and prosthetic materials. Additionally, *C. parapsilosis* has been known to colonize the hands of healthcare workers and is often responsible for nosocomial clusters, so its presence in hospital settings is possibly caused by insufficient implementation of infection control measures[31].

Since biofilms have been considered as significant virulence factors contributing to the establishment of *Candida* infection and that most of biofilm-associated infections exhibit remarkable ability to get more invasiveness into host tissues, we assessed the biofilm-forming ability of the isolated *Candida* spp. by the microtitre plate method. Our study revealed that 94.1% of *C. albicans* were biofilm-forming isolates classified as strong, moderate and weak biofilm producers at 31.25%, 25% and 43.75% respectively and that 72% of non-*albicans Candida* spp. produced biofilm with 27.8%, 16.7% and 55.5%, strong, moderate and weak biofilm producers respectively with no significant statistical difference between *albicans* and non-*albicans* spp. ($P>0.05$). According to *Sachin et al*, the biofilm formation capacity was 72.9% for *C. albicans* as compared to 74.1% for non-*albicans Candida* mainly *C.tropicalis* with no significant statistical difference as well[21].

Importantly, the highest percentage of biofilm-forming isolates of *C.albicans* was from invasive infections (68.75%) followed by device-associated infection (25%) and finally superficial infection (6.25%). In regard to non-*albicans Candida*, 55.6% of biofilm-forming isolates were from invasive infections, 33.34% from device-related infection and finally superficial infection (11.12%). These results were in accordance with *Silva et al* who documented that, biofilms are thought to provide ecologic advantages such as protection from the environment, nutrient availability, metabolic cooperation, and acquisition of new defense mechanisms that enable the organism to evade the host immune system and consequently gain access to deeper layers of the host tissues[32].

For device-related biofilms, the central venous catheter was the most commonly affected device which indicated that *Candida* commonly adheres to biomedical devices, growing as a resilient biofilm capable of withstanding extraordinarily high antifungal concentrations as seen in other studies[33]. *Fox et al* [26] also reported that, each year, in the US, more than five million CVCs are placed and, even with new improved clinical security procedures, biofilm infection still occurs in over 50% of these catheters and that *Candida* biofilms represent a reservoir for continuing infections.

Regarding virulence genetic profile, we also explored virulence genes related to biofilm formation such as *HWP1* and *ALS1*[9]. These genes ultimately function as cell surface adherence proteins (adhesins) enhancing the attachment of *Candida* to mucosal surface of the host. On the other hand, *SAP5* and *PLB1* genes are induced for the production of phospholipase and proteinase enzymes that promote tissue invasion[34].

HWP1 and *ALS1* genes were detected in 38.9% and 61.1% respectively of *C. albicans*, and seven isolates (38.9%) proved co-existence of both genes together. In agreement with our study, Iraqi thesis by *Ali* [35] also found that, out of 25 *C. albicans*, 12 isolates (48%) were positive for *ALS1* gene, 9 isolates (36%) were positive for *HWP1* gene and 8 isolates (32%) were positive for both genes by multiplex PCR. *Melek et al* [17]also found that *ALS1* gene was detected in 53.9% of all *C. albicans* isolates. For *non-albicans Candida*, 29.2% and 41.7% of the isolates were positive for *HWP1* and *ALS1* genes respectively and six isolates (25%) were positive for both genes with no significant statistical difference between *albicans* and non-*albicans* spp. (P=0.5, P=0.2, P=0.3 respectively). Notably, such findings proved that non-*albicans* spp. exhibit variable virulence factors like *C.albicans* that contributed to their pathogenic potential. Non-*albicans Candida* like *C. parapsilosis* and *C. tropicalis* are possibly sharing the identical sequence of *HWP1* with *C.albicans*[34]. This is in contrast to another study which previously reported that *HWP1* was expressed only in *C. albicans*[36].

Candida albicans is not the only *Candida* spp. known to produce extracellular proteinases. Many of the pathogenic *Candida* spp. have been shown to posses *SAP* genes, including *C. tropicalis* and *C. parapsilosis*[18]. According to our results, both *SAP5* and *PLB1* genes were detected in 33.3% of *C. albicans* and 8.3% non-*albicans* isolates respectively with no statistically significant difference. However other studies reported higher percentages such as *Mohammed et al*[11] who detected *SAP5* and *PLB1* genes in 100% of *C. albicans* and *Fattah*[37] reported that, *PLB1* gene was detected in 90% of *C. albicans* isolates, suggesting that both *PLB* and *SAP* proteins play an important role in the pathogenesis of *Candida* infection.

Remarkably, we identified a statistical significant association between the presence of *HWP1* gene and biofilm formation among the isolated *albicans* and non-*albicans Candida* spp. A similar conclusion was reported by *Mohammed et al* who mentioned that, during hyphal growth many adhesin genes including *HWP1* and *ALS1* were strongly induced to restore the biofilm-forming ability among *Candida* spp.[11]

CONCLUSION

Genetic determinants of biofilms and extracellular hydrolytic enzymes play an important role in the pathogenesis of candidiasis particularly in invasive and device associated infections. Although *C. albicans* is the most dominant fungus involved in invasive candidiasis, this study proved that the incidence of infections due to non-*albicans* species is rising especially in immunosuppressed patients. Most of isolated *Candida* spp. were biofilm producers with variable strength. *HWP1, ALS1, SAP5 and PLB1* genes were detected in both *C. albicans* and non *albicans* spp. In addition, the existence of *HWP1* gene was significantly associated with biofilm formation.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
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