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Isolation and Identification of *Candida spp.* Isolated from Renal Failure Patients

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Abstract: Background: *Candida* species are perilous fungal pathogens that can cause various human infections. Accurate and timely identification of these fungi is crucial for appropriate treatment selection and effective disease management, Traditional methods for *Candida* species identification and characterization, such as phenotypic assays and culture-based techniques, have limitations in accuracy, time-consuming processes molecular methods have emerged as powerful tools for rapid and accurate identification and characterization of *Candida* species. The aim of this study was to isolate and identification and isolate *Candida spp*. associated with patients with End-Stage Renal Disease. Methods: 120 blood sample were collected from patient at end-stage renal disease Al-Imamin Al-Kadhimin Hospital in Baghdad city, The blood was taken from double-lumen or arteriovenous fistulas in patients who came to the hospital for dialysis in the morning as part of the regular monthly examination Eight ml of blood were taken from each participant (male and female) and divided into two separate tubes , Blood samples were cultivated on Sabroud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Chromogen agar .The plates were incubated at 28 ^{o}C for 24 hours, Then Microscopic and Macroscopic examination and to ensure diagnosis of *Candida spp.* using VITEK2 system and the genomic DNA was extracted from *Candid*a spp. isolated using a commercial Wizard Genomic DNA Purification Kit (Promega ,USA) with some modifications. Results: The number of isolates of *Candida spp.* found in patients who did not receive dialysis was 13 isolates with a percentage of 21.66%. However, 23 isolates with a percentage of 38.33% were found in patients receiving dialysis. The following species were also indicated by *Candida* molecular diagnostics: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*. All *Candida* species were registered with the National Centre for Technology Information. In addition, this study also identified three strains that were discovered for the first time at the molecular level in Iraq due to mutations. The critical NCBI accession numbers are: Accession numbers OR815974 and OR820558 were used to register *C. albicans* and *C. glabrata*, respectively. The identification numbers OR820540 and OR821996 were assigned to the species *C. parapsilosis* and *C. tropicalis*, respectively. Conclusion: The current study concluded that the most common pathogenic fungi in renal failure patient was *C. albicans* , Then *C. tropicalis* , *C. glabrata and C. parapsilosis*. These are reasons that increase the risk of infection with pathogenic fungi. The study has shown that molecular diagnosis is an ideal method for diagnosis at the species level cultured in Iraq, as some mutations in some *Candida*.

Key word: *Candida*, Renal failure, PCR.

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Introduction

The *Candida* genus spreads rapidly due to its ability to cause infections of the respiratory tract, digestive tract, urinary tract and reproductive organs, as well as its numerous virulence factors. It is considered an opportunistic pathogen because it causes infection when the host is immunocompromised and can lead to septicaemia ,The spread of candidiasis is related to the type of

immunity of the host and its ability to change its form the yeast form to the filamentous form and vice versa (1,2,3). The urinary system can sometimes be exposed to urinary tract infections Chronic renal failure (CRF) is characterised by the progressive destruction of renal mass with irreversible sclerosis) and an increase in interleukin 6 , In the case of kidney inflammation, both creatine and interleukin 6 increase , It is considered a disease End-stage renal disease is a recognised global public health problem. Dialysis is a life-saving treatment for patients suffering from this disease (4), and renal failure affects cellular motility and immunoglobulins Inflammation increases interleukin 6 and the level of interleukin 4 and some immunoglobulins decreases in treated and untreated people (5). reported that interleukin 6 levels increase after treatmen (6,7). Autosomal dominant polycystic kidney disease is the most common hereditary kidney disease and leads to end-stage renal failure. It is the reason for dialysis in 7 to 10 per cent of patients (8). Chronic kidney disease (CKD) is described as kidney dysfunction, which is a slow loss of kidney function over time. When the kidneys stop functioning, the patient requires dialysis or a kidney transplant, Fungi make up about 7% of all eukaryotic organisms on earth, and hospitals are full of pathogenic fungi (9). There are numerous approaches and procedures in the field of fungal diagnosis, including PCR (polymerase chain reaction). The idea behind this laboratory technique is the amplification of DNA outside the living organism. With this technique, it is possible to distinguish between genera and species. Due to the accuracy and time-saving nature of this technology, researchers have worked with fungi and strains within the same species (10,11).

Material and methods

Sample collection

Blood samples were collected from 120 Iraqi patients were diagnosed with Renal failure and admitted to Al-Imamin Al-Kadhimin Teaching Hospital in Baghdad city. They were classified as patients with end-stage renal disease and divided into two groups: sixty patients who received dialysis (twenty-three males and thirty-seven females) and sixty patients who did not receive dialysis (twenty-five males).

Blood was done after disinfecting the area with a medical syringe sprayed with a 70% ethanol and 15% iodine solution and before the start of dialysis. Eight millilitres of blood were taken from each participant (male and female) and divided into two separate tubes. 4 ml were placed in two gel tubes, which were then centrifuged at 3000 rpm for 20 minutes. Centrally, the samples were then refrigerated.

Identification of *Candida spp.*

Blood samples were cultivated on Sabroud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Chromogen agar. The plates were incubated at 28 ^{o}C for 24 hours. Then Microscopic and Macroscopic examination according to (12,13) and to ensure diagnosis of *Candida spp*. using Vitec2 system (14).

DNA extraction from *Candida* yeast isolates

Briefly, Loopful of grown hyphal of *Candida spp.* Growth for 24 hours at 28 ^{o}C in Sabroud Dextrose Broth (SDB) (sigma, USA) was transfored to 1.5 ml. microcenter fuge tube. The genomic DNA was extracted from *Candida spp*. isolated using a commercial Wizard Genomic DNA

Purification Kit (Promega, USA) according to Hussain *et al.*, (15) and Abdullah *et al.*, (16). Manufactur's instruction with some modifications.

Primer selection

To select PCR primer that can give specific amplification DNA for detection ITS region of rDNA gene the specific – species primers, ITS Forward primer and Reverse for *Candida spp.* were used according to (17) and then general properties for these primers were checked by using oligonucleotide properties program, the name, sequence and the expected product size of these primers are listed below in (Table 1).

Table(1): The name , sequence and the expected product size of *Candida spp.* primers .

Name of Primer	Sequence of primers	Expected Product size(bp)
Forward ITS1	5′- TCCGTAGGTGAACCTGCGG -3′	550 base pair
Reverse ITS4	3′ TCCTCCGCTT ATTGATATGC -5′	

PCR Master Mix

Optimization of PCR master mix for amplification of ITS of rDNA gene were accomplishedafter several traik, thus, the following mixture was adopted for *Candida* spp. as (Table 2).

Table (2): PCR master mix to detect the ITS region of rDNA gene for *Candida spp.* isolates.

Components	Concentration	Amount (ML)
Go Taq master mix 2x	10 m µ each	12
ITS primer	1 µl (10 picomols/µl) each primer	2
Nuclease free water	2.5Mm	3
DNA sample	10 µl	8
Total volum	-	25

PCR Program

Optimazation of PCR program amplication of ITS gene was accomplished after several trails ,thus , the following programs were adopted for *Candida spp.* as (Table 3)

Table (3): PCR program amplication of ITS gene for *Candida spp.*

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	1 cycle
2-	Denaturation	95°C	45sec	35 cycle
3-	Annealing	52°C	1 min	
4-	Extension	72°C	1 min	
5-	Final Extension	72°C	7 min.	1 cycle
6-	Storage	4° C	Hold	

PCR product analysis

The PCR product of ITS gene for *Candida spp.* isolates were analyzed as (17).

Results and discussion

The results of isolation, morphological and microscopic *Candida* from the blood of patients with end-stage renal disease and those not undergoing dialysis showed that the number of *Candida* isolates reached 13, with a rate of 21.66%. The *C. albicans* species ranked first with 6 isolates and a frequency of 46.15 %. *C. glabrata*

species ranked second with 4 isolates and a frequency of 30.76%, while the number of *C. parapsilosis* isolates was 3 and the frequency was 23.07%, (Table 4).

Table (4): The species of *Candida* isolated from the blood of end-stage renal patients not undergoing dialysis, the number of isolates and the percentage of fungal frequencies.

Not infected with *Candida*	Species of *Candida*	Number of isolates	Frequency percentage	Chi (x2)	P value
	No	/	/	/	/
Infected with *Candida*	*C. albicans.*	6	46.15%	38.16	**<0.0001**
	C.glabrata	4	30.76%		
	C. parapsilosis	3	23.07%		
Total number of isolates		13	21.66 %		

The results of isolation, morphological and microscopic diagnosis also showed the diagnosis of *Candida* isolated from blood samples of patients with end-stage renal disease and those undergoing dialysis, which amounted to 60 patient samples. The number of *Candida* isolates reached 23 isolates with a frequency of 38.33%, in which *C. albicans* species ranked first with 10 isolates and a frequency of 43.47%, while the number of *C. parapsilosis* isolates was 2 isolates with a frequency of 8.69%, (Table 5).

Table (5): The species of *Candida* isolated from the blood of end-stage renal patients undergoing dialysis, the number of isolates and the percentage of fungal frequencies.

Not infected with *Candida*	Species of *Candida*	Number of isolates	Frequency percentage	Chi (x2)	P value
	No	/	/	/	/
Infected with *Candida*	*C. albicans.*	10	43.47%	**43.40**	**<0.0001**
	C.glabrata	8	34.78%		
	C.tropicallis	3	13.04%		
	C. parapsilosis	2	8.69%		
Total number of isolates		23	100%		

As for the results of the molecular diagnosis, the results of the duplication of the ITS region by polymerase chain reaction (PCR) using specific primers and electrophoresis of the duplication products showed the appearance of bands at approximately bp570 compared to the DNA ladder (1000 plus) (Figure 1).

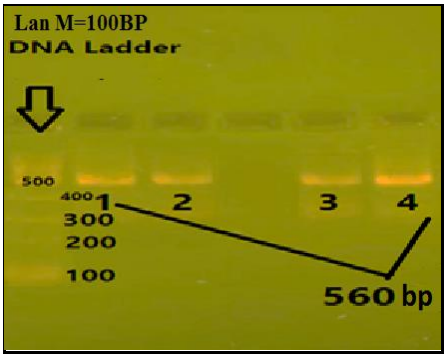

Figure (1): Gel Electrophoresis of PCR products of *Candida* spp. isolates using (1.5 % agarose at 7 V\cm for 1 h) , Lanes (1-4) PCR product of ITS gene showed positive result with positive bands of 560 bp of *Candida spp.*

Phylogenetic tree of *Candida spp.*

After sequence ,High identify to *Candida* spp. was found with all isolates when a systematic comparsion of theye sequence to these available in Gen bank database NCBI was conducted , The accession numbers of these isolated were OR815974 , OR821996, OR820558 and OR820540. Phylogentic analysis showed that isolates *Candida albicans* strain OR815974 from Iraq is very close to *C. albicans* strain KU987839.1 from United States of America then *C. tropicalis* strain OR821996 from Iraq is very close to *C. tropicalis* strain ON142635.1 from India , also *C. glabrata* strain OR820558 from Iraq is very close to *C. glabrata* strain OQ024920.1 from Iran and *C. parapsilosis* strain OR820540 from Iraq is very close to *C. parapsilosis* strain MT640028.1 from Iran (Table 6).

Table (6): International isolates, their accession numbers and the name of the country in NCBI compared with them by the BLAST programme, showing the type of variation in the rules, as well as the percentage of identity with the isolates studied.

No	Type of substitution	Nucleotide	Sequence ID with compare	Country	Source	Identities
1	Transvertion	T\A	ID: KU987839.1	USA	*Candida albicans*	99.55 %
	Transition	T\C				
2	Transvertion	G\C	ID: ON142635.1	India	*Candida tropicalis*	99.36%
	Transition	A\G				
3	-	-	ID: OQ024920.1	Iran	*Candida glabrata*	100%
4	Transvertion	T\G	ID: MT640028.1	Iran	*Candida parapsilosis*	98.56%

(Figure 2) shows the genetic relationship tree between four Candida species, and with the help of the Mica programme it becomes clear that the genetic tree has branched into two groups, one of which is *C. tropicalis* and the second branch comprises the other three species. The reason for the uniqueness of this species, *C. tropicalis*, with its own branch is that it has genetic mutations.

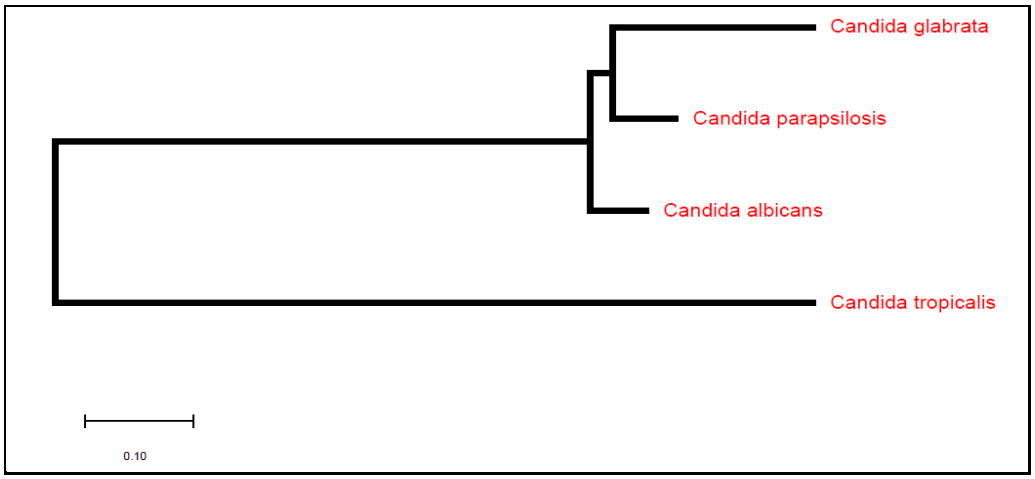

Figure (2): Neighbor-Joning Phylogenetic bootsrap consensus tree of PCR- ITS DNA sequences of clinical *Candida spp.* Strain.

Once the analysis of the nitrogenous base sequences of the samples analysed was completed, these sequences were registered in the American GenBank and the data were assigned accession numbers for identification. As shown in (Table 7), each isolate has an identification accession number.

Table (7) : NCBI Accession Numbers for clinical isolates.

No.	Type of *Candida*	Accession number
1	***C. albicans***	**OR815974**
2	***C. tropicalis***	**OR821996**
3	***C. glabrata***	**OR820558**
4	***C. parapsilosis***	**OR820540**

The cause of a fungal infection is an opportunistic infection, i.e. an infection caused by fungi that live peacefully and harmlessly in the host's body and turn into pathogenic fungi that cause disease when the host's immune system is weakened for some reason, e.g. acquired immunodeficiency, cancer or the intake of medication. Immunosuppressive effects associated with organ transfers and transplants, contaminated surgery or the use of contaminated medical devices and equipment (19). These results do not agree with those of Shuaib et al. (20), who isolated 277 fungal isolates from 115 clinical blood samples from dialysis patients with kidney failure and healthy people. He found that the main fungal genera isolated from the blood were: *Alternaria, Penicillium, A. flauvs*, *Candida* and *Cladosporium*, but they agree with the same researcher regarding the Candida species, as the researcher distinguished the species: *C. albicans* and *C. glabrata*. It has been shown that many complications can occur during dialysis sessions and/or with long-term use of dialysis. In a study showed that fungal infections associated with catheters are common in patients on double lumen dialysis as out of (129) patients, the percentage of catheter-related infections was 19 or 14.7% (21). Non-culture-based methods such as polymerase chain reaction (PCR) allow rapid detection and identification of yeast infections and offer the possibility of prescribing pathogen-specific and effective species-targeted therapy and identifying genetic markers associated with antifungal resistance , By directly detecting and analyzing minute amounts of fungal DNA in a clinical sample without the need for previous culture, molecular amplification techniques also allow for quick and sensitive detection and identification, which makes these tests appealing for early disease diagnosis (22,23). Molecular evaluation of the fungal ribosomal DNA region in the ITS region has been shown to be necessary and important for species identification and has implications for the selection of the appropriate treatment, which is preferable as it is a direct, rapid and reproducible tool in the laboratory (24, 25, 26).

Conclusion

The current study concluded that the most common pathogenic fungi in renal failure patient was *C. albicans* , Then *C. tropicalis* , *C. glabrata* and *C. parapsilosis.* These are reasons that increase the risk of infection with pathogenic fungi. The study has shown that molecular diagnosis is an ideal method for diagnosis at the species

level cultured in Iraq, as some mutations in some *Candida.*

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