AL_KUFA UNIVERSITY JOURNAL FOR BIOLOGY-2023.VOL.15 NO.2 P:49-54
ISSN (Print): 2073-8854 ISSN **(online):** 2311-6544

Original Research Paper *Journal homepage: https://journal.uokufa.edu.iq/index.php/ajb/index*

Detection of *Candida* Species Isolated From Thalassemia Patients in Al-Najaf Province

Majjed T. R.
Ismail M. A.
Al-Amer A. Y.

Al-Furat Al-Awsat Technical University, Iraq

Article history
Received: 4 / 6 /2023
Revised: 2 / 8 /2023
Accepted: 10 / 8 /2023
DOI: 10.36320/ajb/v15.i2.11882

**Corresponding Author:*
Email:
mona.almontafaki@atu.edu.iq

Abstract: *The study was carried out to identify fungal species isolated from patients suffering from Thalassemia in Al-Najaf Province and the detect antifungal Agent (Nystatin and Amphotericin B) towards these isolates. Fifty samples, including oral swabs, were taken from the AL-Zahraa Teaching Hospital in the A.L. Najaf province between October to November 2021, only 14 showed Positive growth. All samples were cultured in the Microbiology Department of the Faculty of Medical Technologies at the Islamic College of Najaf; they plated them first on Sabouraud dextrose agar, then on chromagar medium to identify the species of Candida based on colony color. On chromagar medium. The results on chromagar medium were, ten isolates of Candida albicans, three of Candida parapsilosis and one of Candida tropicalis. Candida albicans' virulence factor which included the formation of germ tubes, the formation of biofilms and the production of phospholipase enzymes, were examined in this study. All virulence factors, such as germ tubes, biofilms, and the phospholipase enzyme, are produced by C. albicans, as evidenced by the culture findings. This study used a disk diffusion technique to describe the effects of the antifungal drugs nystatin and amphotericin B on the yeast species Candida. These results appeared that Amphotercin B was, Amphotercin B is preferable to Nystatin for treating Candida spp.*

Keywords*: *Candida species, Thalassemia, Nystatin, Amphotericin B.

1.Introduction

Thalassemia is a genetic blood disease in which the body fails to produce enough of the protein hemoglobin required for the proper functioning of red blood cells. When hemoglobin levels are low, red blood cells cannot carry out their normal functions for as long, leading to a decrease in the number of healthy red blood cells in circulation [1] Fungus-related illnesses are serious problems that can have severe consequences for human

health and survival, Diagnosis and therapy can be challenging because the microorganisms are responsible for them (fungi) mimic the metabolism and cellular activity of host cells (human or animal) [2]. The fungus *Candida albicans* causes most human diseases, and several species within the genus *Candida* have been related to human infection [3].

C. albicans stands out as the most dangerous pathogen in this group. *Candida* species other than albicans have been blamed for outbreaks in recent years [3]. This study was aimed to evaluate the prevalence of *Candida* spp. among Thalassemia patients in Al-Najaf Provence throw conducting the following objectives: Oral swaps collected from Thalassemia patients and isolation of fungal species after culturing on sabouraud dextrose agar (SDA), Identification of fungal species after culturing on chromagar *Candida* media, Study some virulence factors of fungal species (germ tube test, biofilm formation, Phospholipase production test), and Study the effects of some antifungal agents susceptibility on the growth of *Candida* species.

2. Methodology

Collection of samples

50 oral swab samples were taken from patients at AL-Zahraa Teaching Hospital in A.L. Najaf from October to November 2022. Only 14 samples showed positive growth; all models were grown in the medical technology Faculty microbiology lab at the Islamic University of Najaf. Two types of media were used, SDA and chromagar medium, to identify Candida species.

Identification of fungal isolates

The shape, size, color, and border of yeast isolates were analyzed after 24-48 hours of growth in SDA media. Candida species were identified by color using a chromagar test, single cells colony were isolated from yeast cultures growing on SDA, and cultivation were spread by using a loop technique then kept for 24-48 hours at 37 degrees Celsius [4].

Virulence factors detection

Germ tube test

Isolated yeast cell inoculums were cultured and incubated in 3-5 ml of human serum (Serum was extracted from the blood by centrifuging it at 1500 speed for 15 minutes.), After 2–3 hours incubation at 37 ° C. A drop of the incubated serum was put on a slide, covered with a cover slip, and observed under a microscope to determine whether or not germ tube formation [5].

Biofilm formation test

All the isolates and the reference strains had their biofilm formation quantified using a technique suggested by [6]. A loopful of colonies were used to inoculate 10 mL of Sabouraud's liquid medium with glucose. (Final concentration of 8%). After 24 hours of incubation at 37 °C, the tubes were emptied of their broth and stained with [7].

Phospholipase production test

Candida isolates were tested for external phospholipase action by observing the extent of the precipitation zone following development on agar plates containing egg yolk. [8]. The test briefly as follows; the yolk was centrifuged at 5000 rpm for 10 minutes, then control *Candida* isolates (5 ml, with 10^8 yeast cells [ml saline]$^{-1}$) and 20 ml of supernatant were plated out onto a substrate made from egg yolk agar., along with distilled water as the negative control. After 48 hours of incubation at 37 degrees Celsius, the size of the precipitation zone (Pz) around each cell was measured to assess phospholipase activity [9].

Antifungal susceptibility

This test was performed by using of the well diffusion technique on Muller Hinton agar at 37 C for 2-4 days, the antifungal properties

of Nystatin and amphotericin B against the growth of Candida spp. were examined. At the end of 2 days of incubation, the circumference of inhibition was calculated in millimeters [10].

4. Results and discussion

Collection of samples

50 thalassemia patients' samples were collected. Only 14 were considered positive growth, which included *Candida* species.

Frequency of *Candida* species

The majority of the isolates were *C. albicans* 10 (71.4%), followed by *C. parapsilosis* 3(21.4%) , *C. tropicalis* 1(7.1%)., as show in **(Fig 4.1)**.

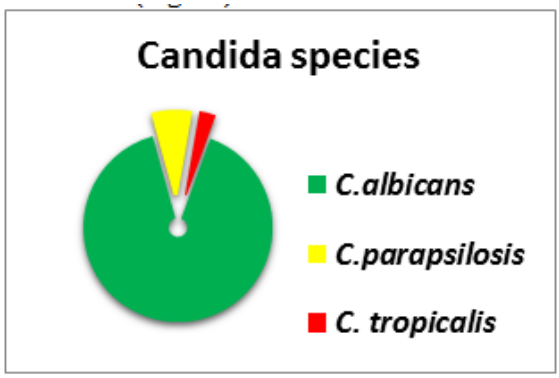

Fig1. percentages (%) of distribution *Candida* spp. Isolated from thalassemia patients.

Identification on SDA medium

All of the samples were cultured on SDA, where the Candida spp. Colonies were a creamy to yellowish color, grew quickly to maturity in 24 to 48 hours at 37°C, and had a smooth, shiny, or dry texture. **(Fig.2)**. These results were agreed with [11].

Fig2. Macrograph showing *Candida* spp.colon ies grown on SDA for 24-48 hrs at 37Cº .

Identification of *Candida* spp. on chromagar medium

This study demonstrated that *C.albicans* colonies develop on chromagar Candida. This differential agar appeared a light green color with smooth colonies, *C.parapsilosis* appeared white, pale pink, and *C.tropicalis* appeared dark blue **(Fig.3)**. These results were consistent with [12,13] who found the exact charact - eristics of colonies of *Candida* spp. that appeared on chromagar. After injection and incubation, chromogenic media produce a distinct color that can be used to quickly and accurately diagnose which species of Candida has been introduced; the medium significantly simplifies the detection of specimens containing mixtures of yeast species by producing a color change due to the reactions of species-specific enzymes with a proprietary chromogenic substrate. [13]. After 48 hours of incubation at 37°C, as directed by the manufacturer, all of the yeast isolates examined grew on chromagar *Candida.*

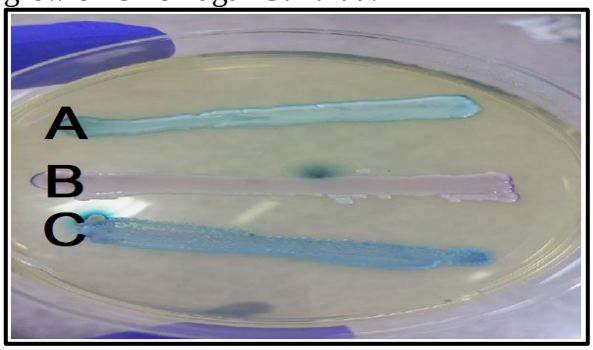

Fig3. Macrographs showing *Candida* spp. Colonies grown on chromagar for 24-48 at hr. 37 Cº, A: *C. albicans* , B: *C. parapsilosis*, C: *C. tropicalis*

Virulence factors

Test of germ tube

This was a quick way to identify specific species of Candida based on their characteristic of producing structures identical to tubes called germ tubes after being cultured in serum for 2–4 hours at

37C. Germ-tube forming and non-germ forming Candida spp. strains are displayed in the findings of this test. **(Fig.4)**. The observer's ability to tell the germ tube apart from the pseudohyphae will be tested. Germ tubes were meant was meant when the phrase was used to describe offspring cells that had grown in length from their mother cells without any origin constriction, in contrast to the pseudohy -phae that formed from the mother cells' origin constriction. [14]. *C. albicans* has a higher ability to create germ tubes. These results agreed with [13.15].

Biofilm formation

The results Some Candida species were found to affect biofilm formation significantly. *Candida albicans* yeast tested positive [14]. A significant virulence component in the pathogenesis of infections is due to biofilms formation., because biofilm associated microor -ganisms show an innate resistance to antibiotics, disinfectants and clearance by host defence mechanisms [15] The ability of C. albicans to form biofilms on abiotic or biotic surfaces is an important virulence factor [16] Recent studies have confirmed biofilm growth in the majority of diseases caused by Candida spp [17] As shown in (Fig.5). These results were agreed with [18].

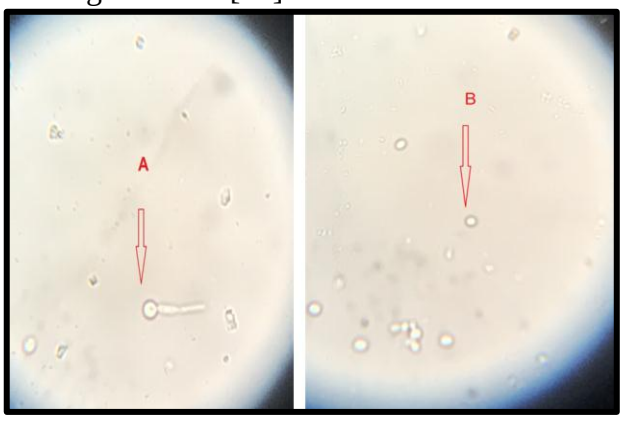

Figure (4): A- Germ tube formation of some of *C. albicans*. B-Non germ tube formation of other *C andida* spp.)

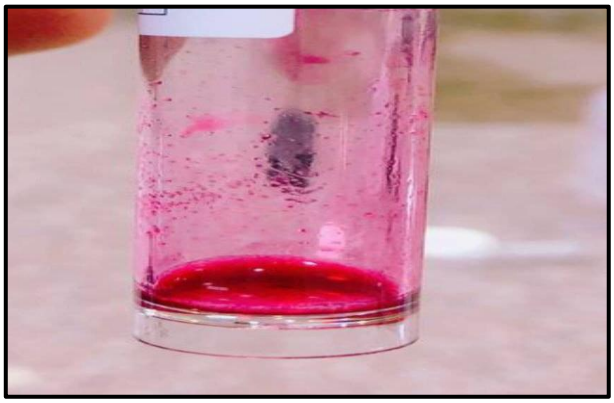

Figure (5): Biofilm formation of *C. albican* (positive).

Phospholipase production test

The result of this test showed the ability of some of *Candidia* spp to produce phospholipase enzyme. The precipitate zone around the colonies delineated the colonies' phospholipase enzyme synthesis on Egg yolk agar. **(Fig.6)**, It detected the activity of phospholipase of *Candida* spp. from [19]. Method Phospholipase, where the activity of the isolate was considered positive when a precipitation zone was visible around the colony on the plate [20]. All *C.albicans* isolates were positive for phospholipase production. These results were agreed with [21].

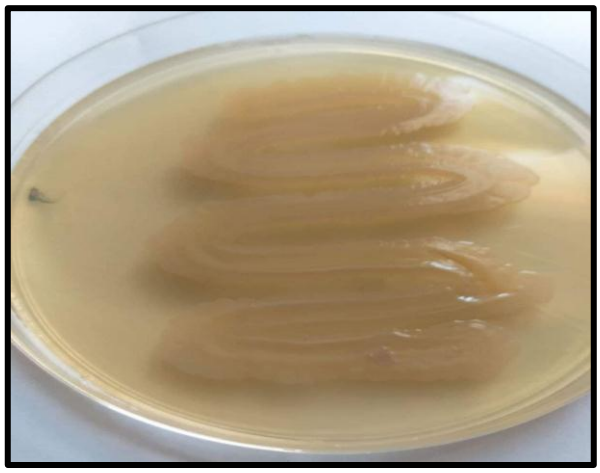

Figure (6): Phospholipase enzyme produ -ction for *C. albicans*.

Antifungal susceptibility

Antifungals can be classified into two groups based on their site of action: nystatin and amphotercin B, which interact with fungal membrane sterols physicochemically. These results show Amphotericin B is more effective than Nystatin against *Candida* spp. [22] As shown in **(Fig7) and (Fig.8)**.

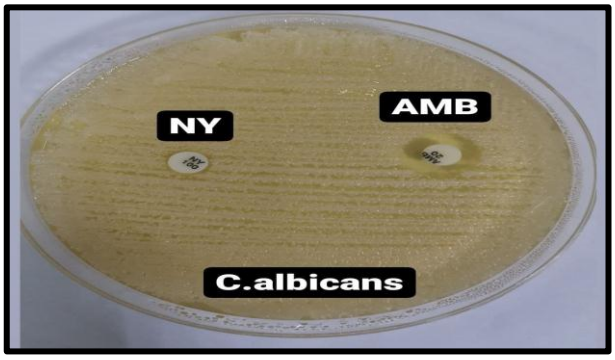

Fig7. The sensitivity of *C.albicans* to nystatin and ampicillin

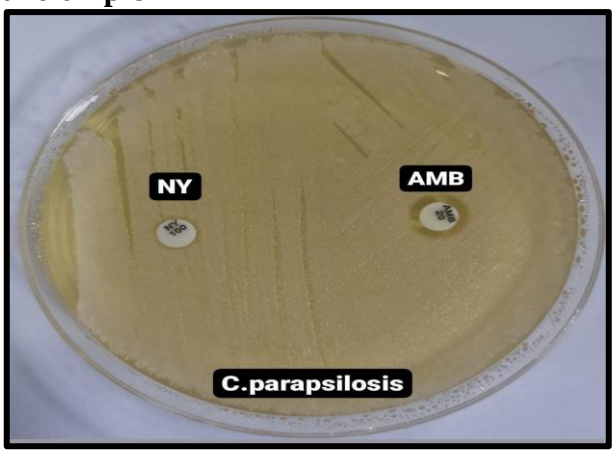

Fig8. The sensitivity of *C.parapsilosis* to nystatin and ampicillin

Conclusion:

The yeasts species were the most prevalent fungal etiological agents of Thalassemia's life-threatening invasive infections. Catheterization, neonatal intensive care, major gut surgery, and liver transplantation are risk factors for disseminated fungal infections for severely immunocompromised patients and treatment requiring extended stay in intensive care units. Fungal infections are the fourth most common cause of nosocomial (hospital-acquired) infections.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Al-Furat Al-Awsat Technical University (2017). Parents and agreement provided verbal and written consent for publication was obtained from both participants and researchers.

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