Journal of Scientific Research in Medical and Biological Sciences
ISSN 2709-0159(print) and ISSN 2709-1511 (online)
Volume 6, Issue 1 Article 8
DOI: https://doi.org/10.47631/jsrmbs.v3i3.531

EVALUATION OF FUNGAL ISOLATES FROM ORAL SAMPLES IN PATIENTS WITH THALASSEMIA

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ARTICLE INFO

Received: 26 Feb 2025
Revised: 10 May 2025
Accepted: 16 May 2025

Keywords:
Thalassemia Disease, Fungal Isolates, SDA Media, Virulence Factors

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ABSTRACT

This research was carried out for biochemical diagnosis of fungal species taken from Thalassemic subjects in AL-Najaf City and assessment of some antifungal agents (Amphotericin B and Nystatin). From January through December 2023, fifty samples were obtained from Al-Sadr Medical City in Al_Najaf City, and only fourteen samples showed positive growth. All isolates were cultivated in on chromagar medium after culture on Sabouraud dextrose agar for identification of different species of Candida by color of the colonies. We can consider they yeast species as the most common fungal etiological agent of life threatening invasive infections in Thalassemia patients who are severely immunocompromised and treatment which requires extended stay in intensive care units Results in this study confirmed the presence of 10 colonies of C. tropicalis1(7.1%), C. parapsilosis 3(21.4%), and C. albicans 10(71.4%), and finally. In this study, C. albicans was investigated for the production of some pathogenesis factors such as biofilms and germ tubes and enzymes-phospholipase during culture of Candida spp. on several cultures. Cultivation of all samples on Sabouraud dextrose agar SDA and then on CHROMagar medium for the purpose of diagnosing the species of Candida depending on the color of the colonies. Findings of fungal growth exhibit that C. albicans forms all pathogenic factors including phospholipase enzyme, germ tube, and biofilms. The finding of this research concerning antifungal activity for (Nystatin and Amphotercin B), shows the impacts on Candida spp. by utilizing the disk diffusion method. Findings also exhibit that Amphotercin B exhibited more antifungal activity than Nystatin against Candida spp. he results were diagnosed on CHROMagar medium as follows: C.albicans 10(71.4%), followed by C. parapsilosis 3(21.4%), C. tropicalis1(7.1%).In this study, the ability of C.albicans was tested to produce some virulence factors such as germ tube, biofilms and production of enzymes phospholipases when culturing Candida spp. on different media..

INTRODUCTION

Thalassemia is a blood disease that is inherited by the offspring from parents and caused by inadequate production of hemoglobin, a major protein component in red blood cells. Inadequate production results in diminished numbers and shorter lifespans of effective red cells in circulation, as they would eventually be lost when their lifespan is reached [1, 2].

Fungal infections are considered major diseases with considerable influence on human health mainly because the organisms (fungi) share a likeness in metabolism and cellular functions with host cells (animal or human). This complicates diagnosis, treatment, and identification of the pathogenic species involved for healthcare providers [3].

The importance of fungal pathogens is increasing in terms of their etiological role in both nosocomial and community-acquired infections. Whether as a genus Candida are the most clinically important fungi, there are over 200 species of Candida and only a few have been correlated with human diseases. Of this C. albicans is the most serious infectious fungus and is considered as a major general health problem subsequently detailed in this paper while 5 Candida species other than albicans are also involved [4]

The aim of the study as follow:

1. Collect the samples from oral thalassemia patients and bring them out to the laboratory.
2. Separation of fungi species after cultivation on SDA
3. Detection of fungi species after cultivation on chromogenic Candida agar media
4. Investigation of some pathogenic factors of fungi species (biofilm formation, germ tube test, N-acetylglucosaminidase test)
5. Investigation of the impacts of two antifungals on culture of Candida species.

MATERIALS AND METHODS

Collection of samples

From January to December 2023, a sample from fifty patients was taken from Al-Sadr Medical City in Al-Najaf City: swabs from the oral cavity. Only fourteen samples showed fungal culture.

All samples were cultured on SDA, at the Micrology Department Faculty of Medicine/ Kufa University then Chromagar medium for identification of the species of Candida according to the colonies color.

Identification of Fungal Isolates

For yeast isolates, the following characteristics were checked on SDA media after an incubation period of 24–two days: appearance, color, size, shape, and edge. The Chromagar test was used with aid based on color to identify species of Candida. A cell was picked from the yeast growth on SDA and streaked for isolation using the loop method, followed by incubation for 24–two days at 37°C [4].

Virulence Factors

1. Germ Tube Test

Inoculums of a yeast cell from a single colony were cultured in 0.5 ml human serum in a small tube and incubated at 37ºC for 2-3 hours. This incubation should not be more than 3 hours as some other yeast species will begin forming germ tubes. A drop of the incubated serum was placed on a slide, covered with a coverslip, and examined microscopically for germ tubes [5].

2. Biofilm Formation Test

Test the ability to form a biofilm in standard strains and all isolates using the method of [6]. A loop is taken with the SDA colonies and put into 10 ml of liquid Sabouraud medium supported with sugar at a final concentration of 8%. Incubate the tubes for 24 hours at 37°C, after which remove the broth and stain the walls of the tubes with safranine. Gently rinse off excess safranin stain under running tap water. Stand at an angle to air-dry completely [7].

3. Phospholipase Production Test

The isolates of Candida were assessed for the activity of phospholipase extracellular by the area of the precipitation zone after their growth on egg yolk agar medium [8]. It was prepared based on the procedure identified by Sav et al. with slight changes. The formulation contained 13.0 g of Sabouraud dextrose agar (SDA, Himedia, Mumbai, India), 11.7 g of NaCl, 0.11 g of CaCl2, and 10% sterile egg yolk (all in 184 ml distilled water). The medium, without the egg yolk, is first prepared by mixing these components and sterilizing them. The egg yolk is then centrifuged at 500 g for 10 minutes at 25 C until the supernatant is separated. 20 ml of the supernatant, presented the medium to be sterilized. Egg yolk agar plates are prepared by pouring a basal layer of egg yolk agar into each plate and allowing it to solidify. Standard inocula of test and control cultures of Candida isolates (10^8 fungal cells [ml saline]^-1) were then put on the surface of the agar medium, which was allowed to dry at room temperature. An additional 5 ml of 0.9% saline solution (devoid of fungal cells) was then overlaid on the agar surface and allowed to be dried at 25 C°. Incubated at 37°C for two days, after which Pz size was measured [9].

Antifungal Susceptibility

Two antibiotics: Nystatin and amphotercin B were active against the growth of Candida spp. and were tested, applying a good diffusion method in Muller Hinton agar and put at 37 c for 2-4 days. The inhibition diameter was determined in millimeters after 2 days of incubation [10].

RESULTS AND DISCUSSION

1. Isolation and Identification

a. Collection of samples

The current study consisted of a total sample of 50 swabs from thalassemia patients. Only 14 were diagnosed as fungal culture specifically as Candida species.

2. Frequency of Candida Species

The main species were C. tropicalis 1(7.1%), C. parapsilosis 3(21.4%), and C. albicans 10(71.4%), as show in (Fig 1).

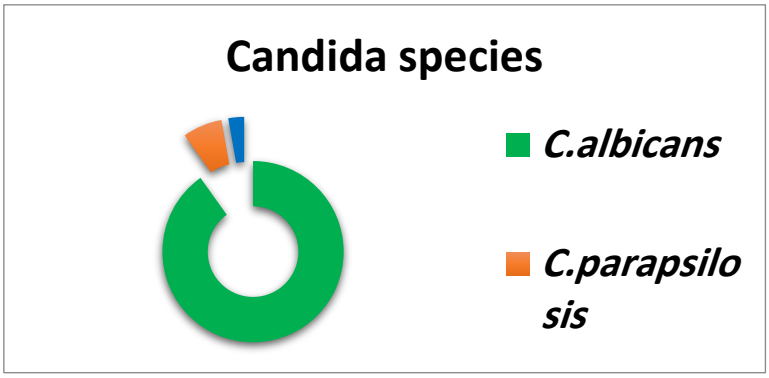

***Fig**. 1. Showing proportion (%) of Candida spp. Diagnosed from thalassemic subjects*

4. Morphological Identification

a. Identification of SDA Medium

Samples were grown on Sabouraud dextrose agar. Candida spp. Colonies were yellowish to cream in color. These colonies are rapid growers and can mature within one to two days at 37°C, having a dry, glistening, or smooth texture, (Fig 2). These results were agreed with [11].

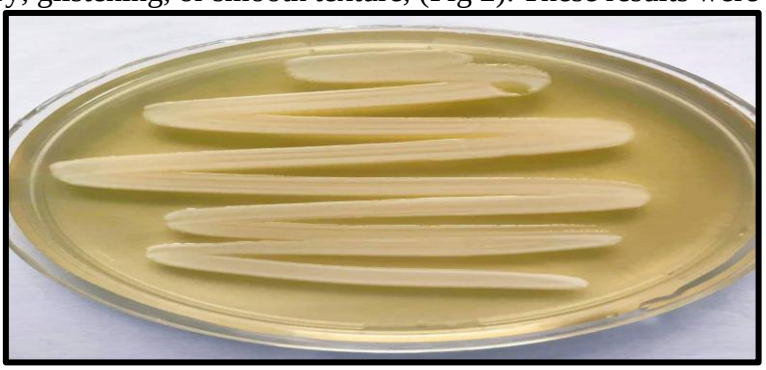

***Fig. 2.** Colonies Candida Spp. Grown on Sabouraud Dextrose Agar*

b. Diagnosis of Candida Species by Chromagar Medium

The current study indicated that Chromagar Candida as differential agar for Candida colonies appears as C. albicans characterized by C. parapsilosis appear white pale pink, smooth light green color colonies, and C. tropicalis appear dark blue (Fig 3). These findings supported by [12, 13] who reported similar results on Chromagar.

Chromogenic media provide a rapid and effective approach for identifying Candida species based on the color changes observed following inoculation and incubation, particularly when compared to conventional culture techniques. The medium significantly aids in detecting specimens that contain a variety of yeast species due to the color change resulting from species-specific enzymes reacting with a proprietary chromogenic substrate [14]. After 2 days of incubating at 37°C, all yeast isolates examined showed growth on chromagar Candida, with most yeasts flourishing as outlined in the manufacturer's guidelines. Some other things turns out uninterpretable as they were not what the reader should do, but what was recommended to happen in the text.

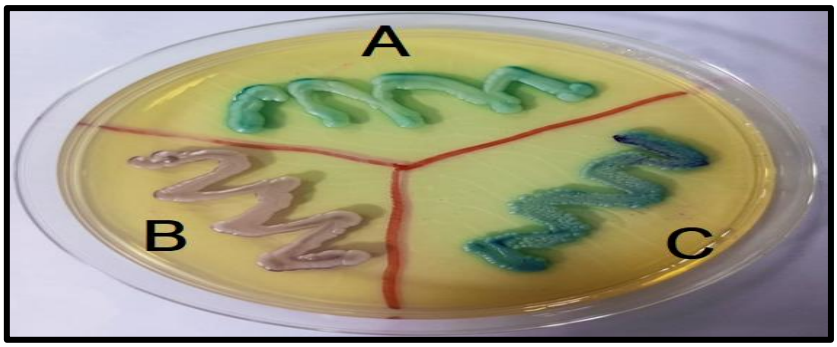

***Fig. 3.** Colonies Candida spp. Grown on chromagar. C: C.tropicalis , B: C.parapsilosis , A: C.albicans*

5. Virulence Factors

a. Test of germ tube

For rapid testing of certain Candida spp, serum is incubated at 37°C for a period ranging from 2 to 4 hours, leading to the development of germ tubes that are slender and short-tube-like structures. As shown in Fig 4, a different strains of Candida spp will either form germ tubes or not, and the observer needs to distinguish between germ tubes and pseudo hyphae. Germ tubes are defined as daughter cells elongating from the parent cell without constriction at their origin, whereas pseudohyphae are characterized by the constriction present at the origin of the mother cells. It is worth noting that C. albicans most strongly expresses germ tubes [15]. These findings come in agreement with [16] and [15, 17].

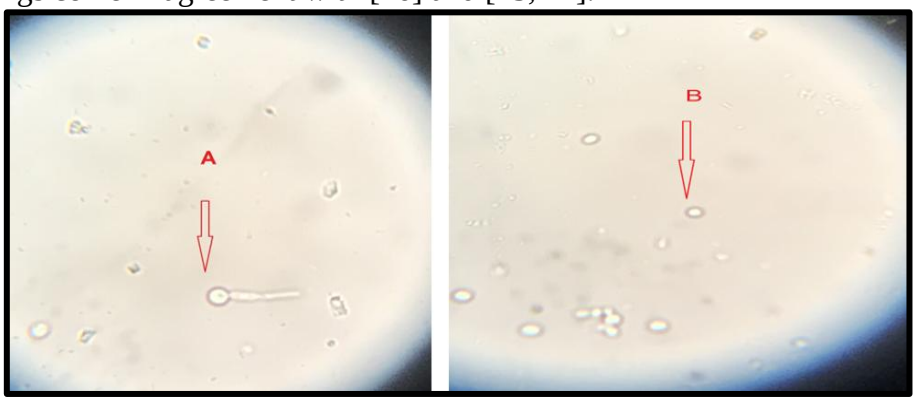

***Fig. 4. A**- Formation of Germ tube for C. albicans, **B**- Formation of Non germ tube of other Candida spp*

6. Biofilm Formation

Results indicate some kinds of Candida concerning their biofilm-forming ability. The test was positive in the case of Candida albicans [18]. Biofilms have long been viewed as a critical virulence factor in the pathogenesis of infections due to the intrinsic resistance that biofilm-associated microorganisms display towards disinfectants antibiotics, and elimination by human immune mechanisms [19]. Its formation by C. albicans biofilm on both biotic and abiotic surfaces acts as a virulence factor [20]. Recent research has confirmed that biofilm formation occurs in almost all Candida spp.-related diseases. As illustrated in Fig 5. These findings come in line with [13, 17].

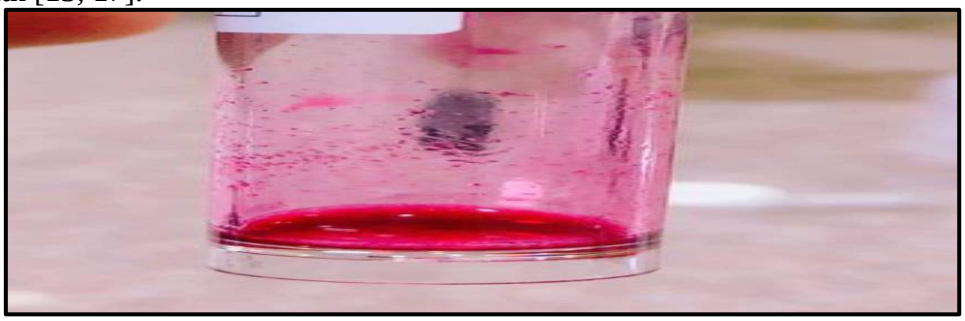

***Fig. 5.** Formation of Positive Biofilm of C. Albicans*

7. Phospholipase Activity Test

Positive test results indicate the capability of certain Candida spp. to form the enzyme phospholipase. A distinct precipitate zone surrounding the colonies indicated phospholipase enzyme production by the isolate on Egg yolk agar, detected using the method described by [21, 22]. The phospholipase activity was diagnosed as positive when there was a precipitation zone on the periphery of the colonies on the plate [23]. All isolates of C. albicans tested positive for phospholipase formation [24]. These findings come in line with [13,17]. As shown in Fig. 6.

Fig. 6. Phospholipase Enzyme Activity for C.Albicans.

8. Antifungal Susceptibility

Antifungal agents fall into two classes regarding their mechanism of action: nystatin and amphotericin B, both reacting with sterols in fungal membranes in a physicochemical manner. These results suggest that amphotericin B exhibited more antifungal activity than nystatin on Candida spp. As shown in (Fig 7), (Fig 8) and (Fig 9).

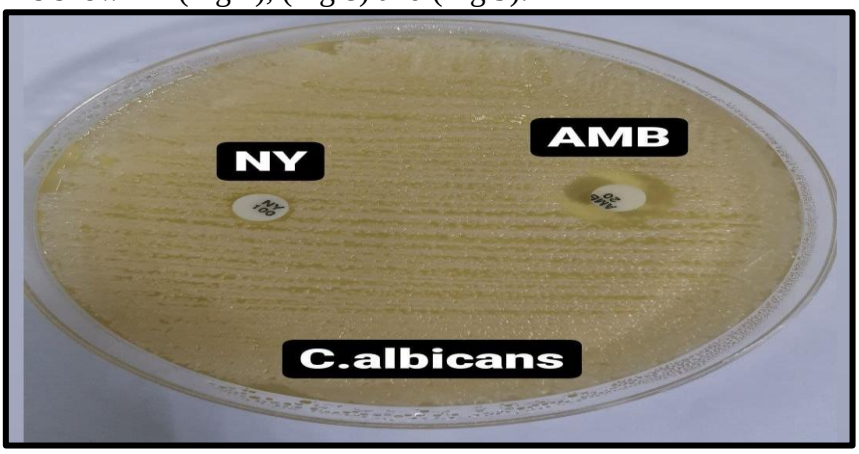

Fig. 7. *The Sensitivity of C.Albicans to NY and AMP*

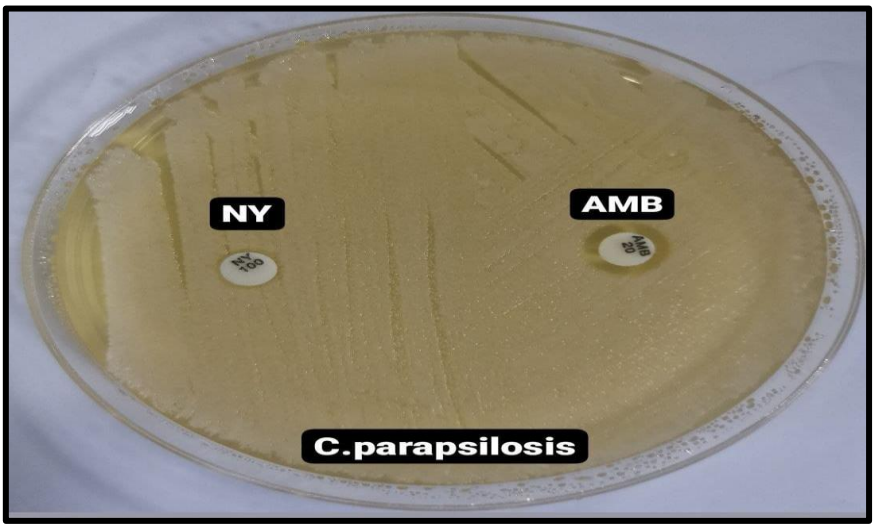

Fig. 8. *The Sensitivity of C.parapsilosis to NY and AMP*

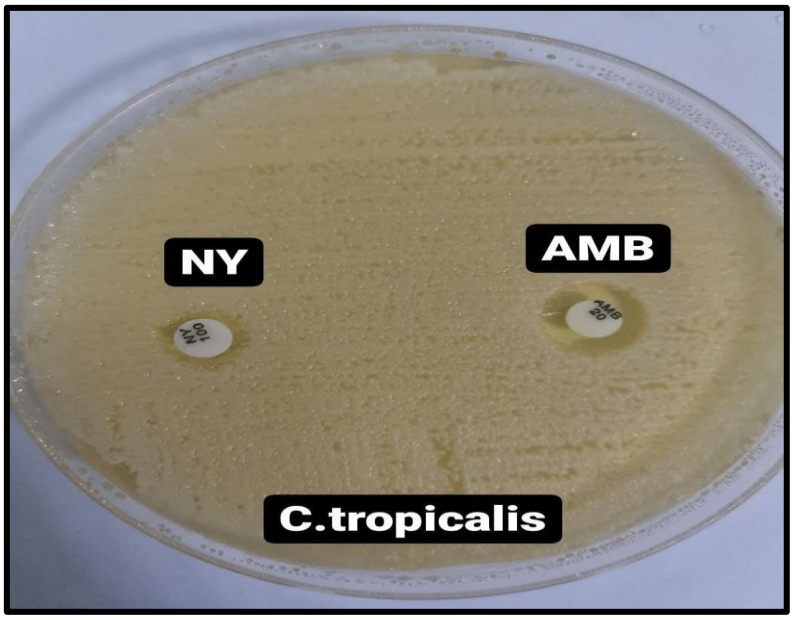

Fig. 9. *The Sensitivity of C. tropicalis to NY and AMP*

CONCLUSION

The results were diagnosed on CHROMagar medium as follows: C.albicans 10(71.4%), followed by C. parapsilosis 3(21.4%), C. tropicalis1(7.1%).In this study, the ability of C.albicans was tested to produce some virulence factors such as germ tube, biofilms and production of enzymes phospholipases when culturing Candida spp. on different media. The results of the culture show that C.albicans produces all virulence factor included germ tube, biofilms and phospholipases enzyme. The result of this study for the antifungal activity for (Nystatin and Amphotericin B), explaining the effects against Candida spp. by using disk diffusion method. These results show Amphotericin B is more effect than Nystatin against Candida spp. We can consider they east species as the most common fungal etiological agent of life threatening invasive infections in Thalassemia patients who are severely immunocompromised and treatment which requires extended stay in intensive care units.

ACKNOWLEDGEMENT

The authors would like to thank all the efforts made by the University of Kerbala/College of Pharmacy for providing the necessary scientific laboratories to complete this scientific effort. The authors would also like to thank all colleagues who provided technical and moral support to make this study a success.

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