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Molecular Identification of Yeast *Candida glabrata* from Candidemia Patients in Iraq

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Abstract

This study aimed for isolation and identification of *Candida glabrata* from specimens of blood collected from national center of hematology /Al - mustansiriya University of immunocompromised patients infection of candidemia after diagnosis by doctor. Results showed the morphological features on many media SDA and Corn meal and differential CHROMagar. Colonies appear small and pink pale to dark. Microscopic exam of show that *C. glabrata* not form pseudohyphae and not produce germ tubes, the cell size 2 - 3 microns, on the other hand, Biochemical test of *C. glabrata* have high ability for fermentation of glucose within three hours and trehalose given false positive within two hrs., and the Urease test given negative result, on the other hand, The results indicated that the DNA of isolated strains was extracted successfully. The results of PCR amplification which was performed on that DNA extracted from all isolates were confirmed by the electrophoresis analysis. The primer amplified of the ribosomal DNA in all strains tested, resulting in amplified products of approximately 610 bp in *C. glabrata*.

Keywords: *Candida glabrata*, candidemia.

التشخيص الجزيئي للخميرة *Candida glabrata* المعزولة من مرضى داء مبيضات الدم في العراق

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الخلاصة

هدفت هذه الدراسة الى تشخيص خميرة *Candida glabrata* من عينات الدم التي تم جمعها من المركز الوطني للأمراض الدم / الجامعة المستنصرية لمرضى نقص المناعة المصابين candidemia (المبيضات في الدم) بعد التشخيص من قبل الطبيب. أشارت النتائج تشخيص الصفات المرفولوجية على عدد من الأوساط سابرود اكار ووسط طحين الذرة ووسط CHROM التفرقي ظهرت مستعمرات صغيرة ووردية شاحبة إلى سوداء. أما الفحص الميكروسكوبي *C. glabrata* لاتملك هايفات كاذبة وليس لها القدرة على تكوين أنبوية إنبات كما أن حجم الخلايا 2-3 مايكرون. من جانب اخر اظهر الفحص البايوكيميائي *C. glabrata* لها القدرة العالية على تخمير الكلوز خلال 3 ساعات و trehalose اعطى فحص موجب كانب خلال ساعتين. وفحص اليوريز اعطى نتيجة سالبة، من ناحية أخرى، أظهرت النتائج ان استخراج الحمض النووي من السلالات المعزولة تم بنجاح. وقد أكدت نتائج PCR التضخيم التي أجريت على الحمض النووي المستخرج من جميع العزلات التي اكدت بالتحليل الكهربائي. التضخيم التمهيدي لحمض النووي Ribosomal DNA في جميع السلالات المختبرة، النتائج في المنتج المتضخم كان تقريبا 610 bp للخميرة *C. glabrata*.

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Introduction

Infections caused by yeast are increasing through last decade, many species of yeast *Candida* especially *Candida albicans*, *C. glabrata* and *C. tropicalis* are responsible of candidiasis [1, 2]. *Candida glabrata* is responsible of many hospital infections, it does little harm, however rarely, *C. glabrata* is rich to blood stream and develop septicemia. It could be harmful only in immunocompromised persons, such as cancer and AIDS and diabetes patients [3-5]. Yeast infections to human caused by many factors firstly that environmental of host including heat and humidity and immune defense of host [6]. On the other hand, Virulence factors of *C. glabrata* have few studies has been conducted through adherence is important that cell surface hydrophobicity (CSH) can effect to interaction of adhesion receptors [7]. Proteinase the extracellular enzyme associated with pathogenicity but it was not specified and about 41% of isolated *C. glabrata* from blood infections are able to produce phospholipases, that good indicates the importance or role of this enzyme in causing this type of infections [8]. *C. glabrata* colonies on Sabouraud Dextrose Agar (SDA) are white to cream-coloured, smooth, glabrous yeast-like, Ovoid to ellipsoidal budding blastoconidia, 3.4 x 2.0 µm in size. No pseudohyphae or chlamydo spores produced [9]. Therefore, this study aimed to molecular identification of *Candida glabrata* from blood specimens.

Materials and Methods

Specimen's collection

Eighteen specimens of blood collected from national center of hematology / Al - mustansiriya University of immunocompromised patients infection of candidemia after diagnosis by doctor, the specimens were taken to the laboratory for isolation and identification.

Sabouraud's dextrose agar (SDA)

Sabourauds Dextrose agar (40 g dextrose, 10 g peptone, 20 g agar, 1000 ml distilled water) Sabouraud' Dextrose agar. Is the main culture medium used in current study for being recommended for the examination of colony morphology, added chloramphenicol 250 mg/1000 ml, and cycloheximid 0.4g [9]. Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

Corn meal with Tween 80

This medium was prepared according to [10], with modeling by dissolved 45 gm of flour extract corn in 100 ml of distilled water and was boiled at 60°C for one hour and added to 20 gm peptone, 20 gm glucose and 15 gm then volume was completed to 1000 ml and added to 0.05 gm of chloramphenicol, then add 200 ml of tween 80 after sterilization 20 min in autoclave this medium using to diagnosis yeasts and used to observed chlamydo spores.

CHROM Agar *Candida* medium

The media were selective and differential CHROMogenic medium that has been shown to be useful for identification of *Candida* species. All media were incubated at 30C⁰ for 1-7 days each plate was read on 1 to 4 days with and observed the colony color, size, texture, and presence of color diffusion into the surrounding agar [1].

Fermentation sugar media and Biochemical test

Medium Prepared by dissolving 10gm peptone, 5 gm NaCl, 5 gm Yeast extract, 0.2% Bromthymol blue, the volume was completed to 100 ml after sterilization 20 min in autoclave [11].

Taken suspend of yeast colonies by transfer part of colonies growth on SDA and put in vial contain distal water, then add 0.2 ml of yeast suspended to media that contain sugar that want testing , incubated tube in Durham tube at 37 C⁰ then seen change of the medium color and gases product through 3 days [11, 12].

Urease test solution

This medium was prepared according to [13], by using 29gm of Urea agar base which dissolved in 100 ml of distilled water and sterilized by Millipore filtration. A 15gm of agar was suspended in 900 ml of distilled water, heated with gentle mixing to boiling, and autoclaved, sterilized urea agar base was added to the cooled (50 °C) agar and poured in sterile tubes. This medium used to test the ability of microorganism to produce urease enzyme by inoculating a urea medium with a loop of the yeast isolate, incubated at 30 C⁰ for 72 hr. the change of the medium color into pink is indicated by a positive result.

Identification of *Candida glabrata* isolates

Candida isolates were identified depending on the morphological features on culture medium and Microscopic Examination as follow:

- Morphology

All isolates were grown on SDA and Corn meal with Tween 80 to examine their general feature including shape, size, color through 24-48 hrs., and distributed to the differential medium Chrom Agar incubated for 24-48 hrs., at a temperature hrs., at 37 C° using this medium as a compromise diagnostic by default of the types of the fungus *Candida* species [1].

- Microscopic Examination

Microscopic examination of their by taken small amount from culture of *Candida glabrata* and placed on a slide with drops lactophenol cotton blue and overlaid with cover slip then the slide gently heated and examed under the compound light microscope under 40x lens[14].

Germ tube formation testing

This testing was carried out according [15, 16], small amount of the inoculum yeast were suspended in a 0.5ml of human serum in a small tube centrifugation at 1500 rpm for 15mins and the serum were obtained, then tubes was incubated at 37°C for 2-3hrs. after that examine germ tubes formation by taken drop of the incubated serum was placed on a slide and examined by microscopic.

Chlamydospores formation test

This test was carried out to determine the ability of yeast to form Chlamydospores, Pseudohyphae and truehyphae by using corn meal agar, inoculum the plate by colony of yeast by a sterile needle and spread on the surface of the media in the Petri dish and keep for a period of 1-3 days of 25C° then examined under microscope to observed chlamydospores [15].

Identification of *Candida* spp. by PCR technique

Preparing the primers

The primers which used in PCR for detection *Candida* spp. Were designed in this study by using NCBI-Gen bank and primer3 plus design online, fungal incubated 37°C for 24 and 48 h for *Candida* species then cultured in Sabouraud dextrose broth (SDB) for 24 and 48 h before proceeding to the DNA extraction by Kit s used to extract the DNA equipped by the company Promega U.S. the Amplification reaction mixture PCR master mix was prepared by using (AccuPower PCR Premix Kit) and this master mix was done according to company instructions. Finally, The PCR products were analyzed by agarose gel electrophores method according to [17].

Results and Discussion

Identification of *Candida glabrata* isolates

Morphological and culturing features

The colonies of *C. glabrata* appear white (cream) colored on SDA within three days converted to yellow, smooth, glossy, convex, glistening [9], but when cultured On Corn meal *C. glabrata* appear groupings not separations packed and close on the other compare with *Candidia* species, when *C. glabrata* growth on CHROM agar medium, differential colonies appear small and pink pale to dark and purple - conqueror often with dark center, this media chromogenic substrates that react with enzymes secreted by yeast to media product pigmentation through media that important factor to identify microorganisms [18, 19].

C. glabrata isolates color variation during 24 h. of incubation, waning over the following 7 days, eight isolates of *C. glabrata* from 50 strains of *Candida* did diffusion of pigment into the surrounding agar during 72 h. but nine from it not develop color until 96 h. [1].

Microscopy identification

Candida glabrata is not dimorphic in contrast to other *Candida* species, size cell about 2 - 3 microns in diameter, not produce germ tubes [20]. Cell were oval blastoconidia 1 to 4 µm and exist as small under conditions for environmental does not form pseudohyphae at temperatures above 37°C, lack pseudohyphae production reason to classified *C. glabrata* in the genus *Candida* [21].

Biochemical test

Candida glabrata can ferment glucose rapidly that turn the color from blue to yellow, this considering on differential properties among other yeasts were ferment media through three hours Table-1. All other carbon source (sugar) given negative test, but trehalose given false positive through two hours. Results agreement with [20], improve positives glucose compare with other sugar types in present study.

Kwon-Chung and Bennett [21], refer that *C. glabrata* ferments and assimilates only glucose and trehalose. In contrast to *C. albicans*, which ferments and / or assimilates a number of sugars.

On the other hand , Urease test given negative was no color change this result were improve by [22], the Urease test used to detect presence of urease enzyme that change color from yellow to red produced by different *Candida* species [23].

Table 1- Biochemical tests of sugar fermentation of *C. glabrata*

Sugar Type	Test Result
Glucose	+
Maltose	-
Galactose	-
Sucrose	-
Trehalose	false positive
Starch	-
Lactose	-

Extraction of genome DNA of *Candida glabrata* and Amplification of target DNA using PCR:

PCR products were separated and the isolation of genomic DNAs was verified with electrophoresis on 2% agarose gels (w/v) and run in 1x Tris-borate buffer, pH 8.3. Genomic DNAs were seen as a smear of different molecular weight DNAs under UV light. The amplified DNA bands were visualised after ethidium bromide staining and photographed under ultraviolet light. The results show the DNA of isolated strains was extracted successfully. The results of PCR amplification which was performed on the DNA extracted from all isolates were confirmed by the electrophoresis analysis.

The primer amplified of the ribosomal DNA in all strains tested, resulting in amplified products of approximately 610 bp in *Candida glabrata* Figure-1. PCR technology is simple, rapid, and able to generate species-specific DNA polymorphisms with many dermatophyte species on the basis of characteristic band patterns detected by agarose gel electrophoresis [24].

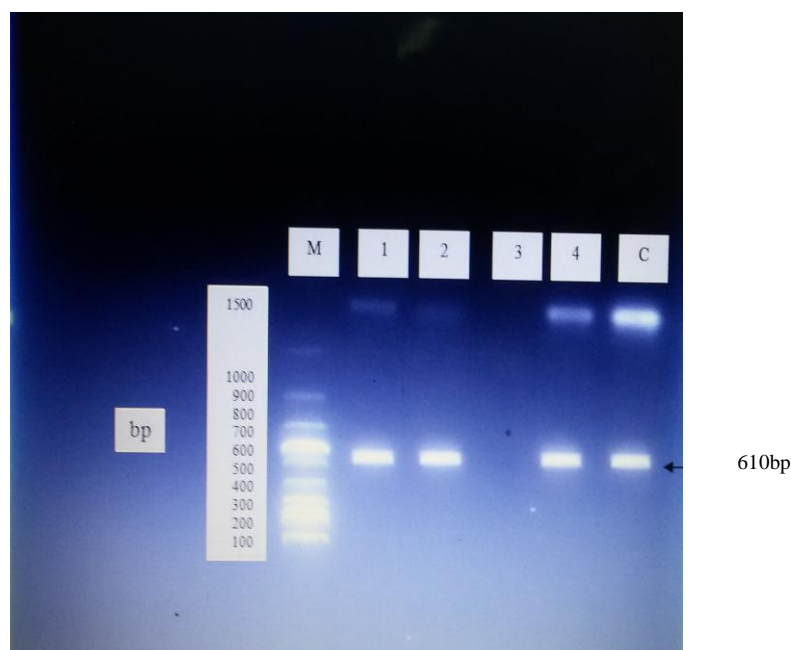


Figure 1- Molecular identification of *Candida glabrata*. 2% Agarose gel electrophoresis showed amplification of 610 bp fragments of the four (M: molecular weight PCR marker, 1-4 *Candida glabrata* isolates, C: negative control).

Identification of the fungal species is essential for appropriate clinical decision making concerning both the significance of a particular isolate and the dosage and duration of antifungal therapy. Culture and microbiological determination of the species of fungi from clinical material usually require up to several days, DNA extraction and amplification of the PCR products and determination of the fungal species can be performed within 12 h. [25].

PCR demonstrated a high specificity for screening blood samples [26]. The largest criticism directed against the use of PCR to detect fungal DNA is the concern that, since molds are so prevalent in the environment and are such common contaminants, they may invalidate the use of PCR because of the risk of contamination. [27].

Reference

1. Horvath, L. L., Murray, D. R., C. K. and Doooley, D. P. **2003**. Direct isolation of *Candida* spp. from blood cultures on the chromogenic medium CHROMagar. *Candida. J. Clin Microbiol.*, 41, pp:2629-2632.
2. Basu, S., Chakraborty, D., Kumar Dey, S. and Das S. **2011**. Biological Characteristics of Nosocomial *Candida tropicalis* isolated from Different Clinical Materials of Critically Ill Patients at ICU. *Inter. J. Microb. Res.*, 2(2): pp:112-119.
3. Fidel, P. L. JR, Vazquez, J. A. and Sobel, J. D. **1999**. *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to *C. albicans*, *Clin Microbiol Rev.*, Jan, 12(1), pp: 80–96.
4. Baddley, J. W., Smith, A. M., Moser, S. A. and Pappas, P. G. **2001**. Trends in frequency and susceptibilities of *Candida glabrata* bloodstream isolates at a University Hospital. *Diagn. Microbiol. Infect. Dis.*, 39, pp: 199-201.
5. Siqueira, J. F. and Sen, B. H. **2004**. Fungi in endodontic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.*, 97(5), pp: 632-41.
6. Rippon, J. W. **1988**. *Medical mycology*. Third Edition, W. B. Saunders, Philadelphia, USA. pp: 717-784.
7. Klotz, S. A., Drutz, D. and Zajic, J. E. **1985**. Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun.*, 50, pp: 97–101.
8. Chakrabarti, A., Nayak, N. and Talwar, P. **1991**. In vitro proteinase production by *Candida* species. *Mycopathologia.*, 114, pp: 163–168.
9. Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley R. Adelaide. **2007**. Descriptions of medical fungi, Mycology Unit, Women's and Children's Hospital, and School of Molecular and Biomedical Science, University of Adelaide, p:198.
10. AL-Rubiaa, A. **2001**. Study of fungi that isolate from respiratory tract of patient attends TB center and chest diseases in Basrah. MSc. Thesis, Collage of Science. University of Basrah, Iraq.
11. Kreger-Van Rij, N. J. W. **1984**. *The yeasts*. Ataxonomic study. Third Edition, Elsevier, Amsterdam. The Netherlands.
12. Roberts, G. D. **1990**. *Laboratory method in basic mycology* In: Baily and Scotts: Diagnostic Micro. (ed. E. J. Barren and S. M. Fingolld). The C. V. Mosby Co.
13. Collee, J. G., Fraser, A. G., Marmion, B. P., and Simmons, A. **1996**. *Practical Medical Microbiology*. Fourteenth Edition, v.1. Churchill Livingstone.
14. McGregor, J. A., French, J. I. and Jone, W. **1992**. Association of cervico vaginal infection with increased vaginal fluid phosphates activity. *Am. J. Obst Gyn.*, 167: 1588-1594.
15. Ellis, D. H. **1994**. *Clinical mycology. The human opportunistic mycoses*. Gillingham Printers Pty. Ltd, Australia. 166P.
16. Forbes, B. E., Sahm, D.F. and Weissfeld, A. S. **2007**. *Bailey and Scott's Diagnostic Microbiology*. Twelfth Edition. Mosby Elsevier. Texas, USA.
17. Steffan, P., Boikov, D., Xu, C., Sobel, J. D., Akins, R. A., and Vazquez, J. A. **1997**. Identification of *Candida* species by randomly amplified polymorphic DNA fingerprinting of colony lysates. *J Clin Microbiol.*, 35, pp: 2031–2039.
18. Odds, F. C. and A. Davidson. **2000**. Room temperature use of CHROMagar *Candida*. *Diagn. Microbiol. Infect. Dis.* 38, pp: 147–150.
19. Hospenhal, D. R., C. K. Murray, M. L. Beckius, J. A. Green, and D. P. Dooley. **2002**. Persistence of pigment production by yeast isolates grown on CHROMagar *Candida* medium. *J. Clin. Microbiol.* 40, pp: 4768–4770.
20. Raddi, F. A. **2002**. Search of opportunistic mycoses from Diabetes patients of Babylon Governments. MSc. Thesis, Collage of Science, University of Baghdad, Baghdad, Iraq.
21. Kwon-chung, K. J. and Bennett, J. **1992**. *Medical mycology*. Leaand Fibiger Philadelphia. London.

22. Alghnam, M. H. and AL-Dabbagh, N. Y. **2012**. Diagnostic Efficiency of a New *Candida albicans* Rapid Test Device and the Importance of some Risk Factors of Vulvovaginal Candidiasis in Mosul City, *Raf. J. Sci.*, 23(3), pp:13-22
23. Larone, D. H. **1976**. *Media. In Medically important fungi: A guide to identification*. Harper and Row. Medical Department, London., pp:127-140.
24. Faggi, E., Pini, G., Campisi, E., Bertellini, C., Difonzo, E. and Mancianti, F. **2001**. Application of PCR to distinguish common species of dermatophytes. *J Clin Microbiol.*, 39, pp: 3382–3385.
25. Einsele, H., Hebart, H., Roller, G. and Loffler, J. **1997**. Detection and identification of fungal pathogens in blood by using molecular probes. *J. Clinical Microb.*, 35, pp: 1353–60
26. Ffler, J. LO., Hebart, H., Schumacher, U., Reitze, H. **1997**. Comparison of Different Methods for Extraction of DNA of Fungal Pathogens from Cultures and Blood. *J. Clin. Microbiol*, 35(12), p: 3311.
27. MacKnight, T. K. **2003**. The Polymerase Chain Reaction (PCR): The Second Generation of DNA Analysis Methods Takes the Stand., *Santa Clara High Technology Law J.*, 20(1), p:73.