

Molecular Detection of *Candida glabrata* Isolated from Denture Stomatitis Patients

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الخلاصة

الأهداف: تهدف الدراسة إلى كشف وتمييز المبيضات *C. glabrata* المعزولة من مرضى التهاب الفم المصاحب لقاعدة الطقم باستخدام تقنيته تفاعل البلمرة المتسلسل. **المواد وطرائق العمل:** تم عزل 62 عزلة من المبيضات من عينات المسحات الفموية لـ 43 حالة من أشخاص يعانون من التهاب الفم المصاحب لقاعدة الطقم والذين يراجعون قسم صناعه الأسنان في المستشفى التعليمي لطب الأسنان بجامعة الموصل والتي شخّصت على مستوى النوع باستخدام طرق التشخيص التقليدية كزراعتها على وسط سابرويد دكستروز أكار ووسط كروم أكار والفحص المجهرى واختبار تكوين الأنبوب الجرثومي. تم استخلاص الحامض النووي DNA من عزلات *C. glabrata* على الوسط الزرعى السائل وبعدها استخدمت تقنيته تفاعل البلمرة المتسلسل للتحري عن جين الفوسفولايبيز B في هذه العزلات **النتائج:** كانت *Candida albicans* هي النوع السائد من بين مامجموعه 62 عزله وشكلت نسبة 46.8% تلاها النوع *Candida glabrata* بنسبة 33.9% ، من ثم النوع *Candida tropicalis* بنسبة 17.7% وأخيرا النوع *Candida krusei* بنسبة 1.6%. ، وظهرت النتائج ان وسط الكروم اكار لا يستطيع تشخيص المبيضات بسهولة حيث اعطى في بعض الاحيان نتائج خاطئة في تشخيص *C. glabrata* على انها *C. parapsilosis* كما وان نتائج تفاعل البلمرة المتسلسل اعطت حزما عند الموضع 404bp على هلام الاكاروز. **الاستنتاجات:** خلصت الدراسة الى ان *C. glabrata* مثلت ثاني اهم نوع من المبيضات تم عزله . كما قدم جين الفوسفولايبيز B كهدف حاسم من الممكن استخدامه لتشخيص *C. glabrata* بتقنية تفاعل البلمرة المتسلسل في العينات السريرية

ABSTRACT

Aims: This study aimed to detect and identify *Candida glabrata* isolated from denture stomatitis patients by using polymerase chain reaction(PCR). **Materials and Methodes:** Total of forty three of oral swabs samples were obtained from patients suffering from denture stomatitis attending prosthodontic department(College of Dentistry /Mosul university/ Dental teaching Hospital. Clinically ,62 isolates of *Candida* spp. were identified to species level by standard culture methods using Sabouraud Dextrose agar(SDA) , HiCrome™ *Candida* Differential Agar followed by microscopic examination, and germ tube test. DNA extraction of *Candida glabrata* from broth cultures was carried out ,then molecular identification with PCR using specific primers targeting phospholipase B gene (PLB) were done to confirm *C. glabrata* diagnosis. **Results:** Among 62 isolates of *Candida* species , the predominant type was the *Candida albicans* which accounted for 29(46.8%) followed by *Candida glabrata* 21(33.9%), *Candida tropicalis* 11(17.7%), finally *Candida krusei* accounted for only 1 (1.6%) . HiCrome™ *Candida* Differential Agar do not easily recognize *Candida* spp. Sometimes *C. glabrata* was falsely identified as *C. parapsilosis* on HiCrome™ *Candida* Differential Agar. The result showed that the PCR products for the specific primer gave bands on agarose gel at the position 404 bp . **Conclusion :** *Candida glabrata* is emerging as the second most spreading among the isolates. Detection of PLB gene using PCR provides a definitive target that could be used for the identification and detection of *Candida glabrata*. from clinical samples .

Key words: *Candida glabrata* , Denture stomatitis, Polymerase Chain Reaction (PCR)

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INTRODUCTION

Denture stomatitis is defined as a common condition usually occurs in people who wear a complete or partial denture , so it is called denture induced stomatitis (DIS). ,Candida- related DS is usually caused by *C. albicans* ⁽¹⁾ However , recent study showed that non albicans *Candida spp.* is increased and reported in many studies as a large source of infection and markedly increased worldwide ^(2,3,4) In general *C. glabrata* is reported as the second prevalent which lead to invasive fungal infection species recovered from denture and from mucosal surface of patients who suffer from DIS in 44% of cases worldwide ^(5,6,7) both *C. albicans* and *C. glabrata* are usually co-isolated from candidiasis of mucosal tissue , a pathogenic role of *C. glabrata* in denture stomatitis remains unknown⁽¹⁾ . As the CHROM agar used for presumptive yeast detection do not easily recognize *C. glabrata* from other species, recommended confirmation of this species by another test, several techniques were developed to readily detect this species⁽⁸⁾ Polymerase chain reaction PCR is one of the most important techniques were used for this purpose which had a huge impact on detection ,identification ,diagnosis of microorganism ⁽⁸⁾ , phospholipase B gene (PLB) which are present in *C. species* have been used as a novel target gene shows a high variability among different *C. species* . the variability of nucleotide sequence in different species of candida may be reach to 95% ⁽⁹⁾ the species- level distinction among Candida depends on the special profiles of the PCR amplicons

,which displays sequence difference within the target regions between different *Candida spp.* ⁽¹⁰⁾.

MATERIALS AND METHODS

1-Sampling and cultural diagnosis :

The criteria for patient selection in current study include elderly patient from both genders suffering from denture stomatitis wearing complete denture. Patients age between (50-85)years old .excluded criteria including patients do not received antifungal drugs., at least for three months ago. Swabs were obtained from 43 patients attending Dental Teaching Hospital /College of Dentistry / University of Mosul .

All Samples were collected, by scraping sterile cotton swabs across the palatal mucosa which contact with denture surface and the inner surface of denture then directly immersed into 2ml sterile nutrient broth vials .

Then streaking on Sabouraud dextrose agar(SDA) with chloramphenicol 50mg/L , the plates incubated at 37 C° for 24-48 hrs .each isolate cultured on HiCrome™ Candida Differential Agar (Hi media, B/4-6 ,M.I.D.C., India, No: 00-91-22-61169797) and incubated at 37 C° for 24-48 hrs^(11,12) the chromogenic media provides selective yeast isolation ,identifying colonies of *C. species* according color reaction and morphology⁽¹³⁾ .

2-Molecular diagnosis of *C. glabrata* :

A- DNA extraction

DNA extraction of *C. glabrata* was applied from each sample by picking single colony

using sterile loop, the colonies were inoculated into sterile tubes containing 5ml of nutrient broth, incubated at 37°C for 24 hr. Following the manufacture information, Candida DNA for each isolate was extracted using candida DNA preparation kit (i-genomic BYF, DNA extraction Mini Kit, iNtRON Biotechnology, South Korea)

B- polymerase chain reaction (PCR) :

The primers employed for the amplification PLB gene : F5'TCTCACAC TCCATTGTCTCA-3' and R 5'-AGCAGGTTTACCATCAGAA-3' (14) were provided in lyophilized forms by (IDT, USA). Working stock were prepared by adding PCR grade water to required concentration according to the supplier recommendation. The PCR mixture were prepared in 20µl according to (Table 1).

Table (1): Reaction mixture of PCR for amplification of PLB gene *Candida glabrata*

| Component | Volume | Final concentration |
|--------------------|--------|---------------------|
| 2 x Taq master mix | 10µl | 1x |
| PCR grade water | 6 µl | — |
| F (10µm) | 1µl | 1 µm |
| R (10µm) | 1µl | 1 µm |
| DNA (100 ng/µl) | 2 µl | 2 ng/µl |
| Total | 20 µl | |

The PCR was done using thermal cycler (Optimus 96G, QLS, UK) the cycling conditions including temperature and time were appeared in (Table 2). DNA sample were observed by using agarose gel electrophoresis (Jena Bioscience, Germany) prepared as reported by Maniatis et al (15). 1x TBE buffer (Genet Bio, Korea) and 100 bp DNA ladder (Jena Bio-

science, Germany) as standard molecular weight marker. The electrophoresis was performed using power supply MP 300V (Major Science, UK), then the agarose gel was placed in documentation system (Bio Doc Analyze, Germany) and examined under UV light for documentation and determination of expected bands.

Table (2): Cycling condition of PCR for amplification of PLB gene of *Candida glabrata*

| No. | Step | Temp. | Time | Cycle |
|-----|-----------------------|-------|--------|----------|
| 1 | Polymerase activation | 94 C' | 5min | 1x |
| 2 | Denaturation | 94 C' | 1 min | |
| 3 | Annealing | 50 C' | 45 Sec | 35 Cycle |
| 4 | Extension | 72 c' | 45 Sec | |
| 5 | Final Extension | 72C' | 5min | 1x |
| 6 | Hold | 4 C' | | ∞ |

RESULTS

The diverse species of *Candida* revealed dissimilar colors of colonies on HiCrome™ *Candida* Differential Agar Figure (1). Out of 62 isolated *Candida spp.*, 29(46.8%) isolates were detected as *C.albicans* showed green colonies and have the ability to form germ tube, but the rest species failed to form it. 21(33.9%) isolates as *C.glabrata* showed pale pink colonies and sometimes also appeared in ivory

color, Figure(2), 11(17.7%) isolates as *C.tropicalis* showed steel blue with violet shade colonies or dark blue colonies and only 1(1.6%) isolate as *C.krusei* pink with white borders rough colonies. The extracted DNA for all suspected isolates of *C.glabrata* were subjected to PCR for amplifying PLB gene. The results confirm the presence of 404bp PCR products when compare with DNA ladder, Figure (3).

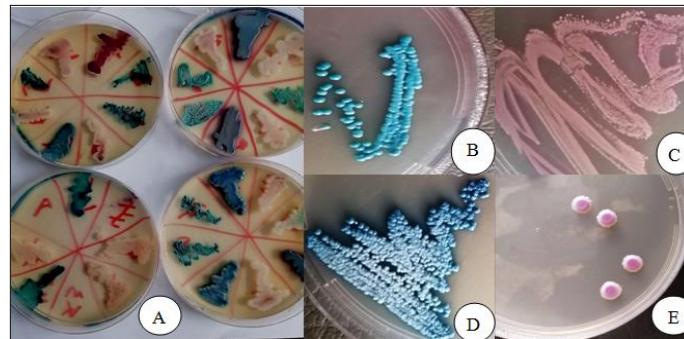


Figure (1) : (A) *Candida species* , (B) *C. albicans* (C) *C.glabrata* , (D) *C.tropicalis* , and (E) *C. krusei* on Hi Crome™ *Candida* Differential agar



Figure (2) : *Candida glabrata* on HiCrome™ agar ,(A) Colonies appear as a pink color , (B) Colonies appear as an ivory color

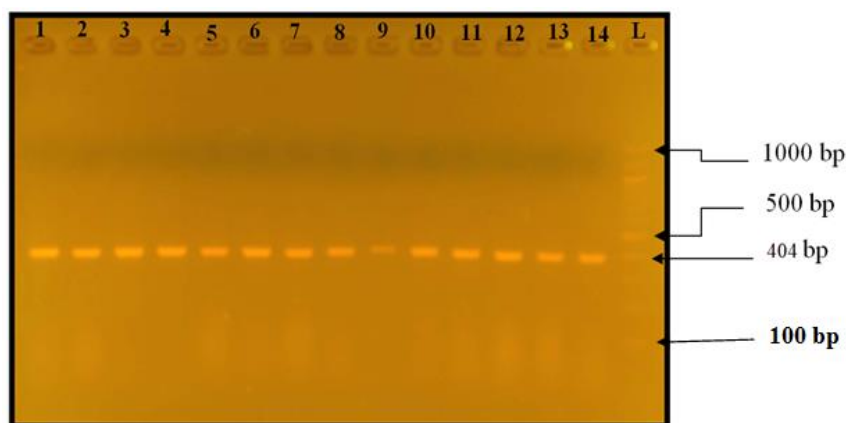


Figure (3): Lanes 1-14 represent PCR amplicon of PLB gene with molecular 404bp For 14 isolate of *C. glabrata* / L: DNA ladder (100bp)

DISCUSSION

The results of the current study agreed with several reports which observed that *C. glabrata* can be recognized from other *Candida spp.* by the formation of lavender to pale pink colonies on HiCrome™ Candida Differential Agar⁽¹⁶⁾ However, in this study the colonies of *C. glabrata* strains exhibited significant variability when they appeared as ivory or white large colonies, this result agreed with Hospenthal (2006)⁽¹⁷⁾ noted that most non-*C. albicans* strains are ivory, pink, lavender on HiCrome™ Candida Differential Agar. previous study conducted by Sagar et al proved that *C. glabrata* falsely identified as *C. parapsilosis* on Hicrome agar⁽¹⁸⁾. Because the lack of consensus for the ability of HiCrome™ Candida Differential Agar to identify *C. glabrata* based on pale pink to purple colonies after 48hr incubation⁽⁸⁾. It was necessary to use molecular methods for definitive identification of this species of candida, nowadays, PCR is one of the most important tools that employ for

identification of fungal species in a poly population or complex sample such as clinical specimens due to high specificity and its versatility⁽¹⁹⁾. The phospholipase extracellular enzyme is produced by different species of candida and play an active role in controlling of candida growth, spreading in host tissues by hydrolysis phospholipids of host cell membrane.⁽²⁰⁾ literature reports showed that 30-100% of the oral isolates produced phospholipase with variable degree of enzymatic activity⁽²¹⁾.

Thus, employing specific primer associated to target sequence of phospholipase B gene (PLB gene) and also using conventional PCR assay as an accurate, rapid assay for amplification of PLB gene of candida glabrata is very useful to detect this species in clinical samples⁽²²⁾, therefore PCR outperformed other phenotypic assay as it could be achieved in a short time, less than 5 hours while other phenotypic method required at least 48 hrs. or about 72hr.⁽²³⁾ and no single phenotypic

technique has assured to be highly effective to distinct *C. glabrata* from other species of *candida* .

CONCLUSIONS

C.glabrata is emerging as the second most prevalence among patients wearing dentures and suffering from denture stomatitis. Molecular method using PCR targeting phospholipase B gene provided definitive identification of *C. glabrata* and differentiate it from other *Candida* spp.

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