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Rapid Detection of Candida species Isolated from Denture Stomatitis Patients using Phenotypic methods and Chromogenic agar media

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الخلاصة

الاهداف : الهدف من هذه الدراسة هو كشف وتمييز أنواع المبيضات المعزولة من المرضى الذين يعانون من حالات التهاب الفم المصاحب لقاعدة الطقم باستخدام طرق التشخيص المظهرية التقليدية . **المواد وطرائق العمل** : تم عزل ما مجموعهُ ٦٢ عزلة من المبيضات من عينات سريريه لـ٤٣ حالة من أشخاص يعانون من التهاب الفم المصاحب لقاعدة الطقم،والذين يراجعون قسم صناعه الاسنان في المستشفى التعليمي لطب الأسنان في جامعه الموصل والتي خضعت لطرق التشخيص التقليدية مثل صبغه كرام . تكوين الأنبوب الجرثومي، تكوين الابواغ الكلاميدية إضافة إلى زراعتها على وسط كروم أكار ووسط سابروبد دكستروز اكار، اختبار اليوريز وفحص تخمر السكريات . جميع العزلات تم تشخيصها بدقة على مستوى النوع بواسطة مختلف الطرق التقليدية . **النتائج**: إن من بين ما مجموعه ٦٢ عزلة كانت Candida albicans هي النوع السائد وشكلت نسبة٤٦,٨ % تلاها النوعCandida glabrata وشكلت نسبة ٣٣,٩ % ومن ثم النوع Candida tropicalis وشكلت نسبة ١٧,٧ % وأخيرا النوع Candida krusei وشكلت نسبة٦، ١%. **الاستنتاجات**: خلصت الدراسة إلى أن المبيضات البيضاء هي الأكثر انتشارا بين الأنواع المعزولة من حالات التهاب الفم المصاحب لقاعدة الطقم بشكل خاص وان استخدام الكروم أكار كان من أفضل الطرق في التحري السريع عن لانواع المختلفه من المبيضات

ABSTRACT

Aims: The purpose of this study was Isolation and phenotypic identification of different candida species isolated from denture stomatitis patients. **Materials and Methods** : Over all 62 isolates of Candida species yielded from 43 patients attending prosthodontic department\College of Dentistry\Mosul university\Dental teaching Hospital. Clinically, the samples processed by traditional methods including culture characteristics, gram staining reaction , germ tube, chlamydospore formation, culture on CHROM agar Candida , Sabouraud Dextrose agar, urea's test and Carbohydrates fermentation test. **Results**: All isolates were accurately diagnosed to the species level by various traditional methods , among the total of 62 Candida species , the predominant type was the Candida albicans which accounted for (46.8%) followed by Candida glabrata (33.9%), Candida tropicalis (17.7%), finally the species Candida krusei accounted for (1.6%). **Conclusions**: Candida albicans is highly spreading among patients wearing dentures particularly those suffering from denture stomatitis in which CHROM agar Candida medium is best method for rapid identification and documentation of different Candida species .

Key words: Candida species, Denture stomatitis, Phenotypic methods, CHROM agar Candida medium.

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INTRODUCTION

Candida species are regarded the most common isolated and the frequent etiological factor of denture stomatitis. [1] Approximately 300 *Candida species* are documented ,10 % can cause infections in human[2]. Different species of Candida are described as a communal microorganisms, they exist in 30-60 % of healthy people and 60-100% of patient with denture stomatitis [3,4], non albicans species including *Candida glabrata, Candida tropicalis, Candida parapsilosis*, and *Candida krusei* etc .also appeared as etiological agents in oral candidiosis either as a co-infection with *C.albicans* or as a sole pathogenic microorganism[4,5]. there are different conventional methods for identification of yeast such as: culture on sabouraud dextrose agar (SDA) media ,corn meal agar (CMA), germ tube test, carbohydrate fermentation test ,and in order to accelerate detection of candida CHROM agar are used as a selective and differential media which inhibit the growth of any microorganisms except candida [6]. The component of this media ,yeast extract,chromopepton ,chromogen mixture ,chloramphenicol and agar allowing identification of candida isolates by their color and colony aspect , special colony with different colors appear on this media due to chromogenic substrates which react with enzymes produced by candida therefore , chrmogenic media has the ability to differentiate mixed candida and yeast infections [6] .

MATERIALS AND METHODS

1- Sample collection :

patient selection in this study including: elderly adult patients ,suffering from stomatitis . Their ages range (50-85)years old, wearing complete denture, the age of denture range(5-20) years , patients who have taken antifungal drugs were excluded from the samples.,at least three month ago . Swaps were gained from 43 patients who attending Dental teaching Hospital \ College of Dentistry \Mosul university.

Specimens were collected after clinical diagnosis by scraping sterile swabs across the palatal mucosa in contact with the denture and the inner surface of denture , then swabs were transferred into 2ml sterile nutrient broth vials.

Microbiological work

All samples were primarily cultured on sabouraud dextrose agar(SDA) adding chloramphenicol 50mg/L to inhibit bacterial growth , and incubated at 37 C^{o} for 24-48 hrs, to ensure that all *Candida spp* in samples were isolated, The identification to species level was carried out :

A- Gram staining reaction for all specimens .

B- Germ tube production (GT) .

To differentiate between *C. albicans* from non albicans groups, human blood was

placed in test tube and left in rack to clot, after clotting the sample centrifuged at 3000 rpm for 10 min, serum was separated carefully and placed in sterile test tube and then colony was picked up with sterile loop and was placed in 0.5 ml of serum, this mixture was placed in an incubator at 37 Cº for 3-4 hrs. Adrop of this mixture was placed , put on a clean slid, overlaid with a clean cover slip and examined by high power objective lens of microscope (40x) [7]

C- Chlamydospor production :

The colony was picked up from selective media and then was cultured on 1cm x shape in corn meal agar, covered with sterile cover slip the plate incubated at room temperature for 48-72 hrs. then examined under 40x [8] .

D- Chromogenic agar medium :

All samples were cultured on to CHROM agar Candida (Hi media, M.I.D.C., India) ,then the plates were incubated at 37 Cº for 24-48 hrs. [9,10]

E- Carbohydrate fermentation test :

This test based on sugars fermentation and prepared according to Knenman et al. [11]

F- Ureas's test : The isolated colonies was inoculated on urea base agar within test tubes and after incubation the color of indicator (phenol red) was changed from yellow to pink color as a positive result [9,12] .

The results were analyzed statistically by using Q square ,all data analyzed statistically significant value at $p < 0.05$ was considered by using sigma sat program.[13]

RESULT

The distribution of *Candida species* in this study exhibited no significance in both sex male and female (p=0.388), Figure (1).

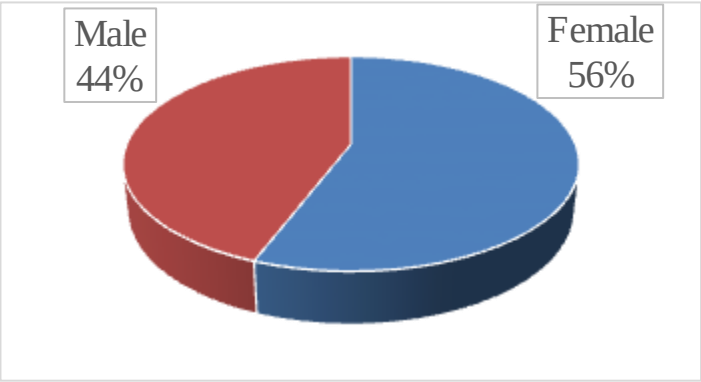

Figure (1): distribution of Denture Stomatitis in male and female

This study was achieved to identify different species of candida in clinical samples. Twenty nine isolates of *C.albicans* identified by germ tube test ,while other *Candida specie*s were negative. Twenty nine species of *C.albicans* produce chlamydospore and only two species of *C.tropicalis* gave positive result (Table1).

Table (1) : Identification of *Candida species* by germ tube and chlamydospore formation

Candida species	Total	Germ tube formation		Chlamydosopres formation	
		positive	Negative	Positive	Negative
Candida albicans	29	29	0	29	0
Candida glabrata	21	0	21	0	21
Candida tropicalis	11	0	11	2	9
Candida krusei	1	0	1	0	1

Out of 62 isolated Candida , 29(46.8%) isolates were detected as *C. albicans* by CHRom agar, 21(33.9%) isolates as *C.glabrata*, 11(17.7%) isolates as *C.tropicalis* and only 1(1,6%) isolate of *C.krusei* (Table 2) the distribution of *Candida species* were shown in Figure (2) . gram's staining findings , the shape and the size of blastoconidia have been shown in (Figure 3). Germ tube and were chlamydospore formation tests were used for identification of *Candida spp*. (Figure 4) .

Table (2) : Identification of *candida species* by using CHRom agar

Candida species	Colony characteristic on CHRomogenic agar	No. of isolates total (62)	Percentage
Candida albicans	Light smooth green colonies	29	46.8 %
Candida glabrata	White large glossy pale pink colonies	21	33.9%
Candida tropicalis	Steel blue with violet shade	11	17.7 %
Candida krusei	Pink with white borders rough colonies	1	1.6%

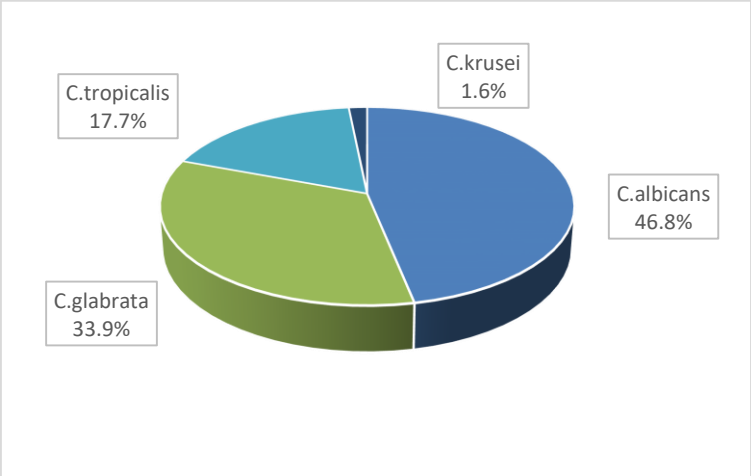

Figure (2): Distribution of *Candida spp*

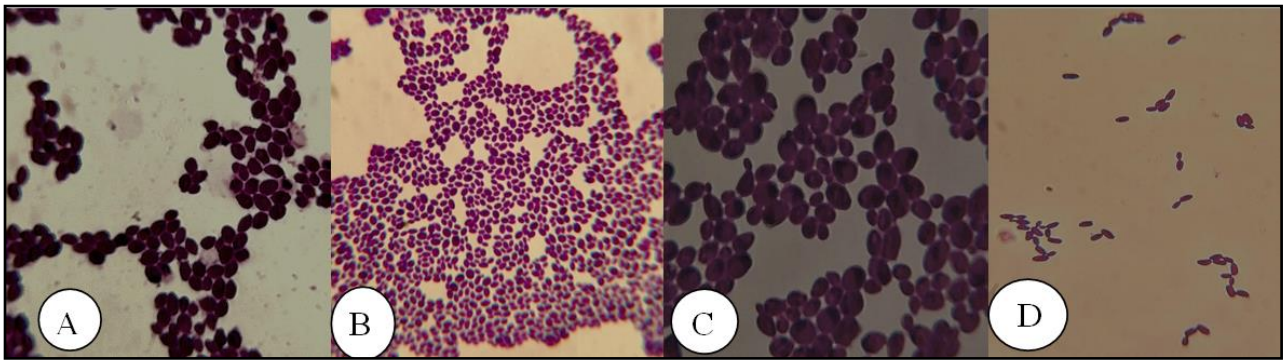

Figure (3): Gram stain of *Candida species* A- *C.albicans* B- *C.glab*rata C- *C.tropicalis* D- *C.krus*ei

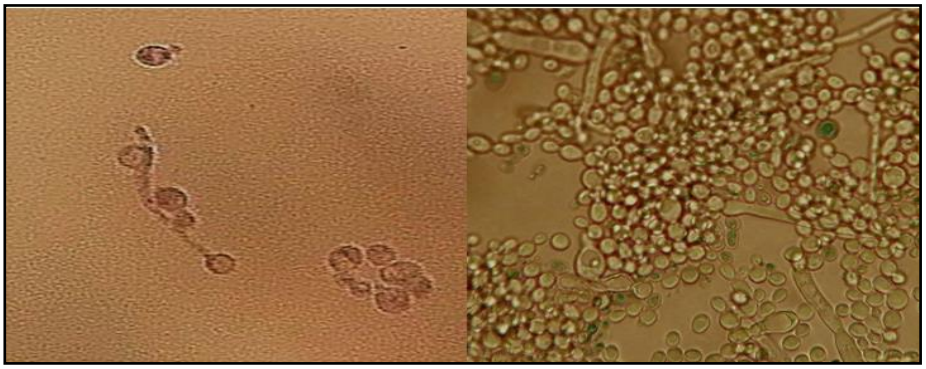

Figure (4): Detection of *Candida spp*. By Germ tube(left) and Chlamydospore formation (right)

Different species of Candida were detected by culturing on SDA and CHROM agar Candida medium (Figure 5). On CHROM agar, the diverse species of *Candida* revealed different color scolonies (Table 2). Carbohydrate fermentation and ureas test that were used to confirm the growth of candida isolates were shown in (Table 3) and only *Candida krusei* changed the urea base agar to pink color as a positive result of ureas test (Figure 6)

Table (3): Biochemical reaction of isolated *Candida species*

Organisms	Fermentation of charbohydrat						Ureas's test
	Glu	Mal	Suc	Lac	Gal	Treh	
Candida albicans	F*	F	–	–	F	F	–
Candida glabrata	–	–	–	–	–	F	–
Candida tropicalis	F	F	F/V*	–	F	F	–
Candida krusei	F	–	–	–	–	–	+

F* = Fermenter V* = variable

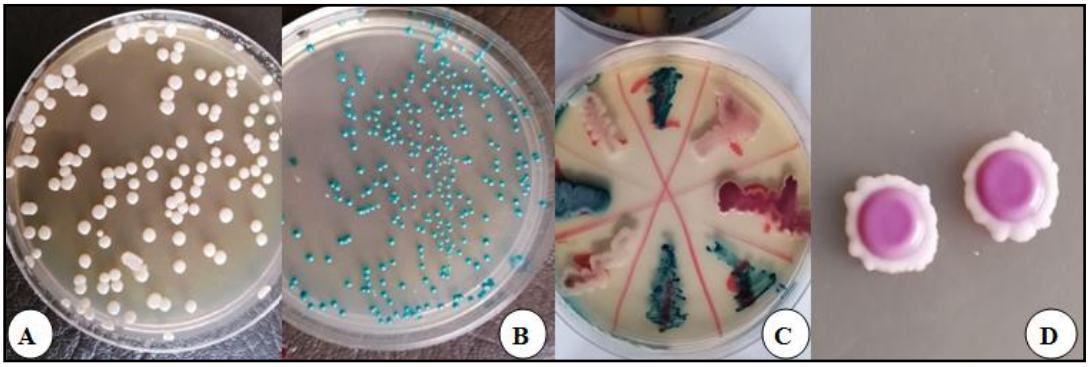

Figure (5) : (A) The colony of Candida spp. on SDA agar, (B) *Candida albicans,(C)all Candida species*,(D)*Candida krusei* on CHRom agar

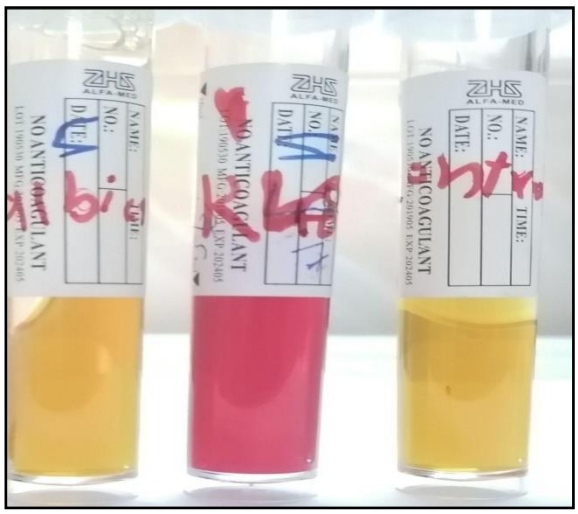

Figure (6) : *Candida species* on urea base agar

DISCUSSION

In present study *C.albicans* appeared as a predominant isolates in patients with DS agreed with other studies that found the same result [14,15,16]. The results of this study exhibited that there is no significant distribution of candida between male and female. all *C. albicans* strains and few strains of *C.tropicalis* form chlamydospore on nutritionally insufficient media such as corn meal agar. Thus this method can be used to determine these species and this result agreed with other studies ,[2,9] and all isolates of *C.albicans* form germ tube while the other species cannot produce it [9] .depending on several studies carried out in universities and hospitals, 65% of denture users suffer from problems caused by candida albicans [17]

CHROM agar Candida medium is present as a novel medium which helped in isolation and differentiation of different species of Candida according on different colors and morphology of colonies[4,9,18]. CHROM agar *Candida* medium in each liter contained peptone (10 g), glucose (20 g), agar (15 g), and chloramphenicol (0.5 g) and Chromogenic mixture.(2 g), while pH of the medium was maintained at 6.1according to instructions of manufacturer, for this reason this medium aided in differentiation between different species of Candida and this data was confirmed by another study carried out by Horvath et al [18]. in this study all 62 isolates which obtained from 43 patients produced distinct colors on CHROM agar and were grown well on this medium and this agrees with the truth that this medium act as a good differential medium , having good performance, less time consuming , having sensitivity for isolation and determination of *Candida species* [19] . Other study conducted by Lynn et al. noted that CHROM agar quickly identify C. *albicans*

,*C.glabrata* , *C.tropicalis and C. krusei* based on different colors and morphological features of colonies CHROM agar candida gave a fast recognition repeatedly found yeast species which would usually be missed during traditional plating on solid media[18] . Among 43samples grown on CHROM agar Candida medium plates ,twenty nine isolates of *C.albicans* showed Light smooth green colonies, twenty one isolates of *C.glabrata* showed White large glossy pale pink colonies, eleven isolates of *C.tropicalis* appeared as Steel blue with violet shade or as a dark blue colonies and one isolate of *C. kruzei* showed as pink with white borders rough colonies. (Table2) This characteristic of candida colonies on CHROM agar agreed with different study[10,20]

CONCLUSIONS

In conclusion, this study reveals that CHROM agar Candida media having good potential for rapid identification of *Candida species* and this media can be used as a useful adjunctive media in the clinical laboratory for detection of *Candida species.*

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