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RESEARCH ARTICLE

Isolation and identification of Candida species from vaginal, urine and oral swabs by chromagar Candida

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Abstract

A total of 150 samples (50 vaginal swabs , 50 urine samples and 50 oral swabs) of patients at many age group range from 1to 50 year old and for both gender were collected from patients suffering from vaginal candidiasis, oral thrush and urinary tract infection who attending the Samawah Teaching Hospital for pediatrics and Gynecology of AL-Muthanna and AL-Diwanyia governorates ; Through the period which extended from October 2010 to May 2011.

The isolation and identification methods of yeast isolates were followed upon the morphological, cultural and biochemical characteristics, in addition, the confirmative systems such as CHROMagar Candida and Api Candida were done for differentiation among Candida species. The phenotypic results showed that the isolation percent of Candida albicans was 63%, while the other species such as Candida glabrata, Candida krusei and Candida tropicalis were isolated with the ratios (14.2%, 8.69%, 7.6%) respectively. On the other hand, the percentage of yeasts such as Trichosporon sp. and the Geotrichum sp. were 5.43% and 1.08% respectively.

INTRODUCTION

Candida species is a part of human microflora and it becomes pathogenic when certain conditions are present and cause an opportunistic infections (Ryan and Ray, 2004). The major etiological agent is Candida albicans, whereas different Candida species can cause a variety of infections including C. tropicalis ,C. dubliniensis, C. parapsilosis, C. krusei , C. guillermondii , C. glabrata, and C. kefyer which represent many clinical forms of candidiasis. Some of these species are encountered as secondary infections to another species, for example; C. parapsilosis is secondary infection only when C. albicans as a cause of Candida endocarditis (Amy, 2000). Still other species of Candida have been occasionally isolated from clinical isolates like C. catenulate , C. intermedia, C. lambica and C. zeylanoides. These species are therefore not considered as agents of opportunistic infections. (Randhaw and Sharma, 2004).

Materials and Methods
Media

Sabouraud's Dextrose Agar (SDA)

Sixty five grams of this media was dissolved in one liter of distilled water and mixed well by exposing it to heat, it was sterilized by autoclave ,the pH of this medium is (6.8) . Chloramphenicol was added to this medium after sterilization and cooling to 50 C◦ (Collee et al. ,1996).

CHROMagar Candida :

It was prepared according to the manufacturer's instructions by suspending 47.7 g of powder in 1000 ml distilled water, do not autoclave.

Each isolate was cultured on Sabouraud's Dextrose Agar at 30ºC for 48 h. After this, they were seeded on CHROMagar Candida and incubated at 30ºC for 48 h. The CHROMagar Candida allows selective yeast isolation, identifying colonies of C. albicans, C. dubliniensis, C. tropicalis and C. krusei by morphology and color reaction. The strains were identified according to the manufacturer's instructions, which differential media C. albicans or C. dubliniensis as green colonies, C. tropicalis as metallic blue colonies, C. krusei colonies as showing pink color and rough aspect, and the other species as developing colonies from white to mauve (Hospenthal et al., 2006).

Yeast extract Agar (YEA)

It was prepared according to the manufacturer's instructions by suspending 23 g of YEA powder in 1000 ml distilled water and sterilized by autoclave. It is used for culture of Candida spp. (McGinnis, 1980).

Patients group

This research enrolls 150 patients who attending Samawah Teaching Hospital for pediatrics and Gynecology in AL-Muthanna and AL-Diwanyia governorates from October 2010 to May 2011 and labeled before brought to laboratory for processing according to the standard methods . All patients were (1 -50) years for both genders and clinically diagnosed as suspected urinary tract infection , vulvovaginal candidiasis and oral candidiasis . A total of 50 urine samples, 50 vaginal swabs and 50 oral swabs are collected .

Control group

The control group consists of 50 apparently healthy volunteers. Their ages between 1-50 years.

Urine samples collection

Midstream urine samples (50) were collected from patients and instructed on hand to collect the midstream urine into sterile bottles. The samples were then transported to the laboratory with ice packs in sterile container. After that the samples were centrifuged at 2500g for 10 min. Four plates of Sabouraud's dextrose agar with the addition of 0.05 g/L chloramphenicol were inoculated :two plates were incubated at 25C° for 48 h and the other two at 35C° for 48 h (Ellis, 1994).

Vaginal Swabs

A total of 50 vaginal swabs are obtained from females. All swabs were subjected to culture for detection of Candida Sp. Patients mainly include women from different age groups with excessive vaginal discharge, pruritis vulva, dysuria, irritation, pregnant and non-pregnant women. Specimens are taken using sterile bivalve speculum and sterile swabs, and then transported to the laboratory for diagnosis. (Koneman and Roberts, 1985).

Oral swabs

A total of 50 oral swabs were obtained from children suffering from oral candidiasis and septicemia with oral thrush. Specimens were taken using sterile swabs, and then transported to the laboratory for diagnosis.

Results and discussion

Isolation percent of yeast infections:-

The results revealed that the percent of yeast isolates were (52% , 58%,74%) from oral thrush , urine and vaginal swabs respectively. The results of statistical analysis test showed significant differences ($P<0.05$) between percentage of samples (Table 1). This result was also found by Khudor (1998) who reported that out of 334 examined vaginal swabs 96 (27.1%) are cultured positive for yeasts and Darogha (2005) who found that the highest infection rate with vaginitis belongs to C. albicans with 31.1%. The results of studies indicate that, Candida albicans is the causative fungus for 50% to 70% of all candiduria isolates (Zarei et al ,2009 ; Lagrotteria et al ,2007) .

The percent of isolation revealed that the most prevalent yeast isolates from vaginal swabs, urine samples and oral thrush samples are C. albicans or C. dubliniensis 58/92 (63%) , C. glabrata 13/92 (14.2%), C. krusei 8/92 (8.69%), C. tropicalis 7/92 (7.6), Trichosporon sp. 5/92 (5.43%) and Geotrichum sp. 1/92 (1.08) (table 2). This referred by AL-bakri (1981), which isolate of Candida albicans by (84.7%). While the result of Abbas, (2001) revealed that the percentage of infection with C. albicans is (68.6%) compared with another species, (14%) for C. glabrata. In addition Al- Barzanjy (2002) found that the highest percentage of vulvovaginal infection with C. albicans is (55.7%) compared to (9.4%) for C. glabrata. Spinillo et al., (1997) reported that the non-C. albicans spp. is the causative agents of 17% of vaginal candidiasis patients, because C. albicans contain virulence factors such as binary form that can transition from spore form to filament form , which begins filament growth and colonization of the surface of the vagina, as well as the ability to attach and it can attach with the epithelial cells of the vagina with a high degree when compared with other species (Granger, 1992), in addition to their ability to secrete enzymes such as enzyme Phospholipiase, resistance to anti-fungal, as associated with resistance to strains of Candida albicans to the anti-fungal with the effectiveness of enzymatic high and chlamydospores production (De-Bernardis et al., 2001) .

Candida glabrata is the second cause of inflammation of the vagina , it is infected in the current study ,13/92(14.2%) (Table 2), which was located within the referred by Redondo-lopes et al. , (1990) revealed that the proportion of Candida glabrata isolate the women suffer from symptoms of inflammation of the vagina is (16.7%) . While Candida tropicalis was isolated 7/92 (7.6%), which was comparable to the total mentioned by the research in other parts of the world, it is due to this species of complicated cases and recurrent disease because resistance to drugs which block the Candida albicans (Horowitz et al., 1985)

Table (1): Number and percent of isolation of Candida species from different clinical samples (n=150) .

Clinical samples	No. of tested specimens	No. of +ve samples	%	No. of –ve samples	%
Oral thrush	50	26	52	24	48
Vaginal swabs	50	37	74	13	26
Urine	50	29	58	21	42
Total	150				
$X^2_{calculated} = 12.88$ d.f =2 p<0.05 $X^2_{tabulated} = 5.99$ significant					

Table (2): Distribution of isolated yeast species in urine ,vaginal swabs and oral specimens (n=150) .

Yeast species	No. of isolates (+ve)	% of isolation
C. albicans & C. dubliniensis	58	63
C. glabrata	13	14.2
C. krusei	8	8.69
C. tropicalis	7	7.6
Trichosporon spp.	5	5.43
Geotrichum spp.	1	1.08
Total	92	100

Phenotypic characteristics of Candida species and other yeasts:-

Colonies Characteristics

The suspected colonies of Candida sp. on the Sabouraud's dextrose agar are characterized by cream colored, and smooth, at 30 C◦ (Figure 1) and developed to wrinkle whitish creamy colonies after further incubation for 7 days and odor of yeast like. While , Candida albicans colonies appear in the yeast extract agar rapidly during (1-2) day are soft, smooth, creamy to white color and the smell of ferment , as shown in figure (2) .These characteristics were identical to those described by (Bodey, 1993), and on chromagar media , the results revealed that a total of 63.04% (58/92) of isolates showed green colonies in chromogenic medium, indicating C. albicans or C. dubliniensis (table 1) . Whereas 8 isolates (8.69%) of colonies which are able to form pink colonies on CHROM^{TM} Candida, were identified as C. krusei. Seven isolates from 92 (7.6%) were indicative of C. tropicalis due to metallic blue colonies. While 19 (20.65%) are belong to other species as C. glabrata, Trichosporon sp. or Geotrichum sp. developing colonies from white to mauve, as shown in figure (3). According to Milan and Zaror (2004), there is a difference in the intensity of color of colonies between these species; C. albicans colonies are green-blue pale, and C. dubliniensis dark-green. Yet, it is important to observe that this ability is lost after freezing of the samples, and it did not show reproducible results . Furthermore, some authors described that the dark-green color on CHROMagar Candida as phenotypic marker for C. dubliniensis cannot be generally adopted as criterion, and may be limited to primary cultures (Tintelnot et al. 2000). Moreover, C. albicans colonies can grow sharing a variation of green color, ranging from light-green to dark green, depending on growth density and incubation period (10), often with the periphery of the colonies having a color distinctly different from that of the rest of the colony

(Stenderup, 1998). However, Mesa et al. (2004) observed that among 55 strains identified as C. albicans tested by CHROMagar medium, none showed the dark-green color typical of C. dubliniensis, indicating this medium as a good phenotypic criterion to differentiate both species.

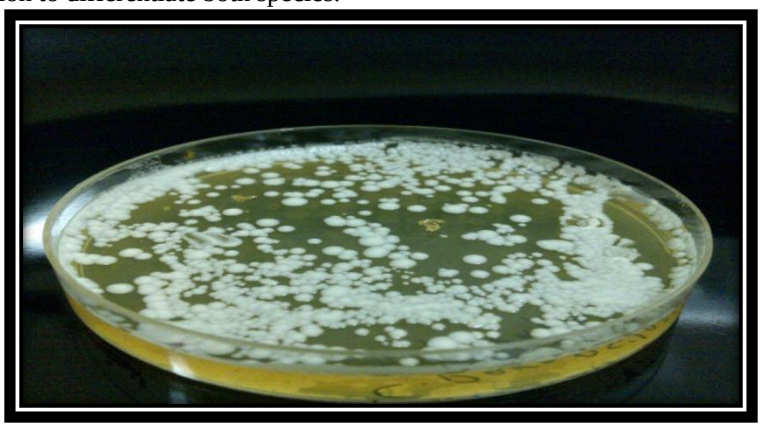

Figure (1): Candida albicans isolates on Sabouraud's dextrose agar showing round, smooth and creamy colonies .

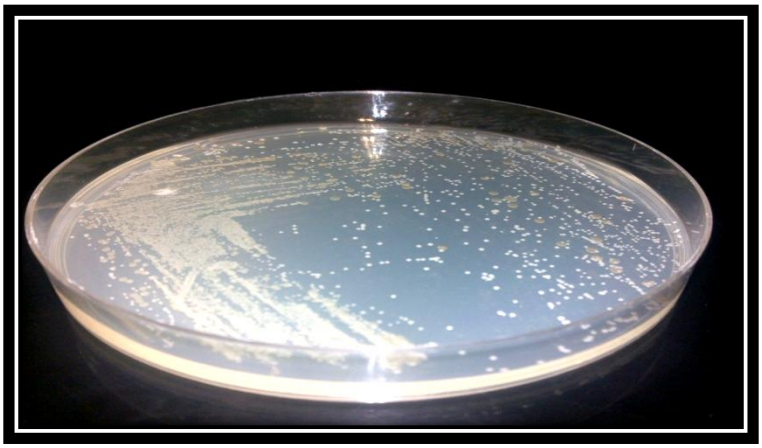

Figure (2): Candida albicans isolates on yeast extract agar showing small, soft, and white color.

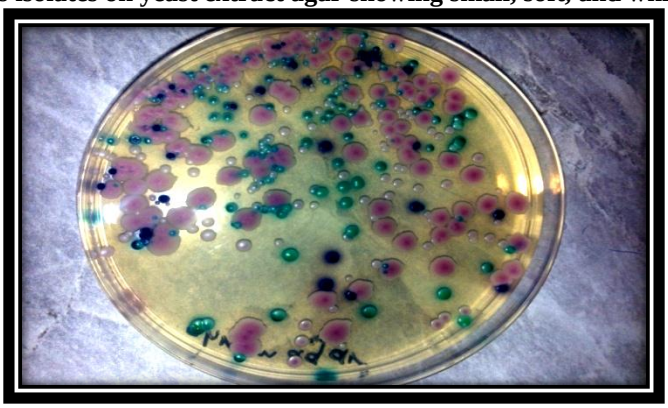

Figure (3): Candida species isolates on CHROMagar Candida showing color colonies (green = C. albicans or C. dubliniensis , C. krusei =pink colonies, C. tropicalis = metallic blue colonies and other species as C. glabrata , Trichosporon spp. or Geotrichum spp. = white to mauve.

Microscopic examination of Candida species

Microscopic examination of yeast cells for 92 isolates grown on yeast extract agar showed that 58 isolates appeared mostly spherical to oval or elongated oval or cylindrical and positive for Gram's stain as shown in figure (4). The presence of clusters is relatively sensitive and highly specific for C. albicans, and application of these results could provide useful preliminary information for guiding diagnosis , as shown in figure (4) .

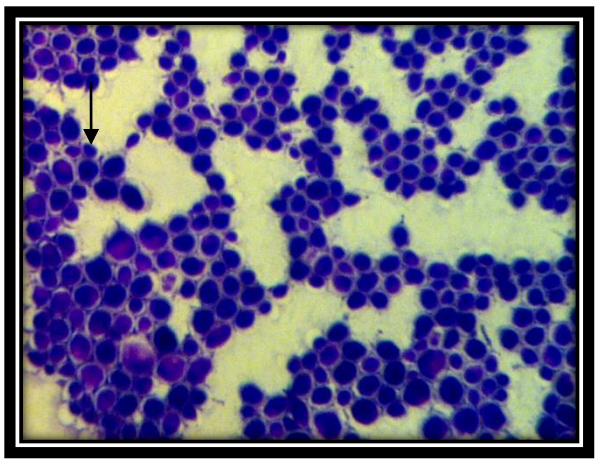

Figure (4): Gram's stain smear of Candida albicans (100 X) showing spherical to oval yeast cell with budding (The arrow).

Biochemical characteristics (Api Candida System)

Based on this system, a total of 58/92 (63%) of the total vaginal swabs , urine samples and oral thrush samples were diagnosed as C. albicans or C. dublinensis while the proportion of other species of Candida which was diagnosed in the Api Candida system showed that C. glabrata 13(14.2%); C. krusei 8(8.69%) ; C. tropicalis 7(7.6%); Trichosporon sp. 5(5.43%); Geotrichum sp. 1(1.08%)., as shown in the table (2, 3) and figure (5). Rippon (1988) lists about 18 species of the genus Candia as being pathogenic, based on their isolation from clinical specimens.

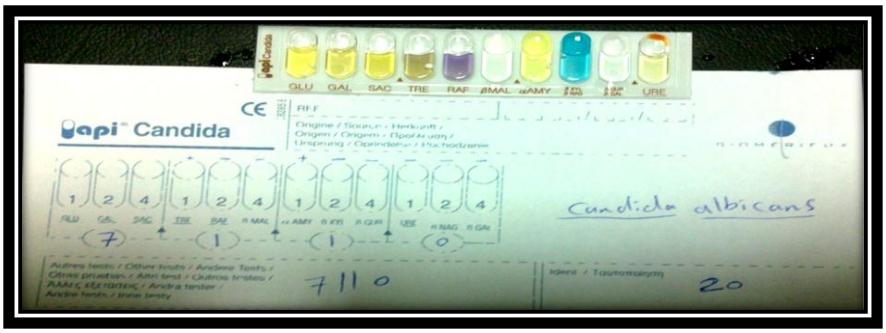

Figure (5): A standard Profile Identification (Api) Candida System. The strip refer to code number of positive and negative biochemical tests of C. albicans.

Table (3): Biochemical characteristics of yeast species isolated from vaginal, urine and thrush samples (Api Candida system).

Yeast species	Api Candida system					
	D-glucose	D-galactose	D-saccharose	D-trehalose	D-raffinose	Urea hydrolysis
C. albicans	+ +	+ +	- +	+ +	- -	-
C. dubliniensis	+ +	+ +	- +	+ +	- -	-
C. glabrata	+ +	- -	- -	+ +	- -	-
C. krusei	+ +	- -	- -	- -	+ -	+
C. tropicalis	+ +	+ +	+ +	+ +	- -	-

Trichosporon species	- +	- +	- +	- +	- +	+
Geotrichum species	+ +	+ +	- -	- -	- -	-

(+): positive result (-): negative result

Virulence factors assay:

The isolates were subjected to some tests shown in table (4) which included germ tube formation that is a characteristic feature of C. albicans and C. dublinensis isolates (Figure 6), chlamydospore (Figure 7), cycloheximide resistance and the ability to grow in 45C◦

Table (4): Virulence factors of Candida species isolated from clinical samples.

Yeast species	Virulence factors			
	Germ tube	Chlamydospores	Cycloheximide resistance	Grow in 45C◦
C. albicans	+	+	+	+
C. dubliniensis	+	+	-	-
C. glabrata	-	-	-	-
C. krusei	-	-	-	-
C. tropicalis	-	-	-	-
Trichosporon species	-	-	-	-
Geotrichum species	-	-	-	-

(+): positive result
(-): negative result

Germ tube formation

Results have shown the possibility of germ tube formation by Candida albicans or C. dubliniensis within 60-120 minutes as previously shown by Richardson et al;(1993). Although the results of the germ tube formation showed that 61(66.30%) isolates were able to produce germ tube, as shown in table (5) while 31 (33.69%) isolates were negative. as shown in figure (6) .Some authors evaluated sensitivity and specificity of the germ tube test, finding results between (93-98.8%) and between (73.3 - 100%), respectively (Gow and Gooday, 1984 and Campbell et al., 1998) and also (Lee et al .,1999) found that sensitivity and specificity of germ tubes production in human serum were 98.1 and 78.6 respectively , while (Sheppard et al ., 2008) noted that direct germ tube test was 87.1% sensitive and 100% specific , and this may explain the failure or inability of some isolates to produce germ tubes .

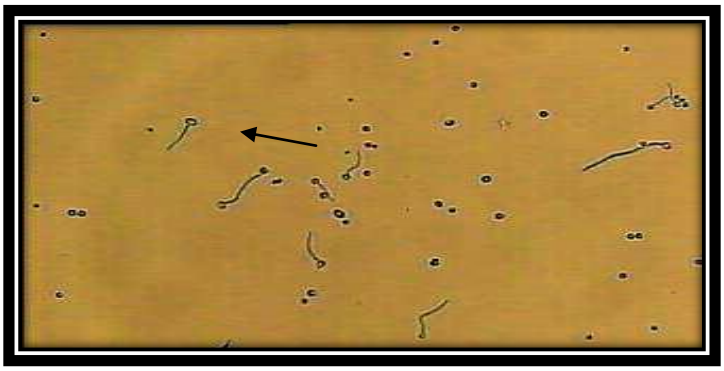

Figure (6): Germ tube formation of Candida albicans (The arrow) (100X) .

Production of chlamydospores :-

Figure (7), shows the presence of filaments and chlamydospores of C. albicans . After stained with lactophenol blue the cells appeared spherical with a thick wall, and where this character dispersed the C. albicans from other species, where they are negative of the chlamydospores. The results showed that 66 isolates

of 92 (71.73%) were positive for the production of chlamydospores , as shown in table (5) and figure (7).Chlamydospores are observed in abundance and often in triplets or in contiguous pairs (Konemon and Roberts., 1985) .

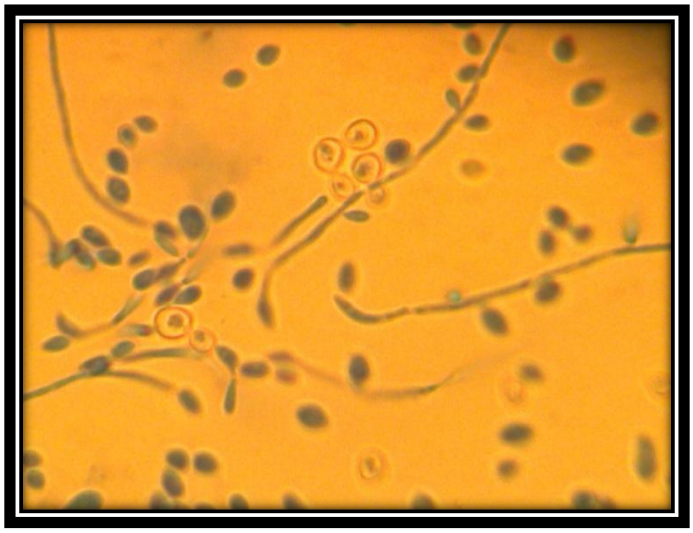

Figure (7) : Production of chlamydospores from Candida albicans (the arrow) (100X).

The Growth at 45 C°

It has been considered that test a diagnositic tool for the differentiation of C. albicans (growth) from C. dubliniensis (no growth).

This research shows that isolates of Candida dubliniensis not show any growth on SDA at 45C◦ through assessment of growth every day at all for 10 days, and the isolates of C. albicans were tolerant and grown in this temperature . Gales et al. (1999) showed that 23 C. dubliniensis isolates was not able to grow at 45ºC, and 66 out of 100 C. albicans isolates were able to grow at this temperature. Pinjon et al. (1998) describe this test as simple, reliable, inexpensive, reproducible, and readily applicable to large numbers of isolates (Sullivan et al, 1995.; Gales et al, 1999).

Table (5): Distribution of Candida species (positive and negative) based on the used techniques (Api Candida, germ tube, chlamydospore and green colony on CHROMagar Candida).

Technique	No.tested	patients			
		Sample- positive		Sample-negative	
		No.	%	No.	%
Api Candida	92*	85	92.39	7	7.60
Germ tube	92	61	66.30	31	33.69
Chlamydospore	92	66	71.73	26	28.26
Green colony on CHROMagar Candida	92	58	63.04	34	36.95

*Mean all the tested isolates of Candida species.

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