Phenotypic and genotypic characteristics of *Candida* species isolated from some domestic insects in Diwaniya city / Al – Qadisiya governorate

Azhar Noory Hussein
Al-Qadisiya University - Pharmacy College

الخصائص المظهرية والوراثية لأنواع جنس المبيضات المعزولة من بعض الحشرات المنزلية في مدينة الديوانية \ محافظة القادسية

أزهار نوري حسين
جامعة القادسية – كلية الصيدلة

المستخلص

اجريت هذه الدراسة لتشخيص الأنواع الشائعة لجنس المبيضات من السطوح الخارجية للصر صر الأمريكي والذباب المنزلي باستخدام وسط كروم المبيضات كوسط تفريغي وتكوين أنبوب الإنبات وإنتاج السبورات الكلاميدية وتمثيل الكاربوهيدرات ، وأكد التشخيص عن طريق الطراز الوراثي باستخدام تقنية تفاعل البلمرة المتسلسل .

اظهرت نتائج هذه الدراسة ان الانواع الاكثر شيوعا من المبيضات هي : *C. albicans* (44.6%) و *C. glabrata* (23%) و *C. krusei* (19.6%) و *C. tropicalis* (7.4%) و *C. dubliniensis* (3.6%) و *C. parapsilisis* (1.8%) . كل العزلات الـ 27 العائدة لنوعي *C. albicans* و *C. dubliniensis* تم تأكيد تشخيصها بتفاعل البلمرة المتسلسل واكد عائدية 25 عزلة لنوع *C. albicans* وعزلتين للنوع *C. dubliniensis* . اظهرت النتائج عدم وجود اختلاف بين الانواع المعزولة من الذباب المنزلي والصرصر الامريكي باستثناء النوعين *C. parapsilisis* و *C. dubliniensis* تم عزلهما من الصرصر الامريكي فقط . عكس فحص الحساسية للمضادات الفطرية الفعالية العالية لمضادي النستاتين (98%) و الفلوكونيزول (89.3%) تجاه جنس المبيضات ، من ناجية أخرى نلاحظ أن أعلى نسبة مقاومة كانت لمضاد الكيتوكونيزول (16%) وكانت طريقة التركيز المثبط الأدنى هي أفضل طرية لتحديد مستويات نمط الجرعة لكل الأنواع مقارنة بطريقة الانتشار بالأقراص .

Abstract

This study was conducted to identify and characterize the *Candida* species isolates from external surfaces of American cockroaches and houseflies by using Hichrome Candida agar as differential culture medium, germ tube , chlamydospore production and carbohydrate assimilation test. Genotypic confirmation was performed by PCR. The results of this study showed that the predominant *Candida* species included *C. albicans*(44.6%), *C. glabrata*(23%), *C.krusei*(19.6%), *C. tropicalis*(7.4%), *C. dubliniensis*(3.6%) and *C. parapsilisis* (1.8%) . All 27 strains indicative of *C. albicans* and *C. dubliniensis* were confirmed by PCR as *C.albicans* (25 isolates) and *C. dubliniensis* (2isolates). The study should no difference between the species of *Candida* strains from American cockroaches and houseflies except that *C. parapsilisis* and *C.dubliniensis* which were isolated from American cockroaches. Antifungal susceptibility revealed that nystatin (98%) and fluconazole (89.3%) were highly effective against *Candida* species , on the hand , the highest resistance was observed in the case of ketoconazole (16%) and MICs method was excellent for determining the levels of dosage pattern for each species compare with disc diffusion method.

Introduction

Insects are some of the most successful organisms on earth. They occur in great diversity and high density and they occupy many niches. The remarkable success of insects has been attributed to their small size, reproductive success and exoskeleton modifications, including prevention of desiccation and development of wings (1). American cockroaches (*Periplaneta Americana*. L: Orthoptera , Blattidae) and housefly (*Musca domestica* L : Diptera , Muscidae) are medically important as many diseases and health problems have been associated with them because their ability to function as mechanical vectors of various pathogens that may cause disease in humans such as poisoning, diarrhea and dysentery (2,3). These insects may be consider as one possible source of food contamination that could be dissemination of the pathogens to food and lorutensils of catering centers through them because they live closely to humans urban environments (4). Various investigation around the world revealed that cockroaches and housefly living close to human dwellings were important carriers of etiologic agents belonging to all groups of potential pathogens: viral, bacterial, fungal and protozoan (5, 6, 7).

Candida spp. is one of the most important microorganisms which were isolated from the gut or surface of insects that feed on variety materials (1). *Candida* is a gram positive, oval, budding yeast cell that produces pseudohyphae both in culture and in tissues and exudates (8).

Candida is a member of the normal flora of mouth and gastrointestinal tract of human body, but they can cause different clinical manifestations of candidiasis when they are opportunist (9) . The association of *Candida* spp. and domestic insects (cockroaches and houseflies) have been verified by several authors (10, 7, 3) . Among of *Candida* spp . *C. albicans* is still the most frequently isolated species which was isolated from cockroaches (10) and houseflies (3), followed by *C. glabrata*, *C. magnoliae*, *C. famata* and *C. parapsilosis* (11). These insects feed on garbage, sewage, secretions, excretions and other human wastes and are able to transport pathogenic organisms from infected materials to human (12) It has been demonstrated that some microorganisms my live inside and / or on its body surfaces from 5-6 h. up to 35 days (13).

The objective of this study was to isolate and identify *Candida* spp. on the external surfaces of cockroaches (*Periplaneta Americana*) and adult housefly (*Musca domestica*) by using Hicrome Candida agar. This medium contains enzymatic substrates which are linked to chromogenic substrates (14).

Materials and Methods

Collection of samples

Cockroaches and adult houseflies were collected from different sites: Toilets, parlours, Kitchens and bedrooms in the houses which were chosen from randomly selected residential areas (Al Hakeem, Al Moalemeen and Al Oroba districts) in the Al-Diwaniya city of Al- Qadisiya governorate between March and April 2012. Each insect was placed in sterile test table. After capture, the tubes containing insects transported with in one h. to the Biology Department\ College of Education\ Al- Qadisiya University. In the Laboratory all specimens killed by pillaging them into a freezer set at 0 ^{o}C for 5min. (11).

Isolation of *Candida* spp.

After immobilization, two milliliters of sterile normal saline (0.9%) was added to each test tube containing housefly and 5 milliliters of the same buffer to each test tube containing cockroach and through shaker for 2 min . 0.1 ml of the washing were cultured on sabouraud dextrose agar (SDA) medium containing chloramphenicol to inhibit bacterial growth and Hichrome candida agar (Himedia, India) (3,2). The plates were incubated at 37 ^{o}C and reed after 24 and 48h. of incubation (15).

Identification of *Candida* spp.

Candida spp. were identified on Hichrome Candida agar by morphology and color of the colonies as per the manufacturer's instructions (Table 1).

Table (1): Color colonies of *Candida* spp. on Hichrome Candida agar

Species of *Candida*	Color on HiChrome Candida agar
C. albicans	Light green
C. dubliniensis	Dark green
C. glabrata	Dark pink
C. krusei	Pale pink with white edge rough and spreading
C. tropicalis	Dark blue color
C. parapsilisis	Pale cream color

Candida isolates also were identified on routine microscopy and by growth pattern on sabouraud dextrose agar for species typing of the isolates germ tube and chlamydospore production test were performed according to Badiee and Al borzi (16). The assimilation test was taken as the best standard for differentiation of *Candida* spp. The carbohydrate assimilation patterns of all the isolates were studied using the KBOO6 HiCandida^{TM} identification Kit (Himedia, India) according to the manufacturer's procedure.

Molecular identification

C. albicans were recognized by molecular assay from *C. dubliniensis* because these species have the same morphological and physiological characteristics such as both have the same growth on the HiChrome Candida agar, germ tube positively , the ability to form chlamydospore in cornmeal agar and the same pattern for carbohydrate assimilation (17).

A- Genomic DNA extraction

Genomic DNA extraction and purification were performed using an EZ-10 Spin Column yeast Genomic DNA purification Kit (BioBasic, Canada) according to the manufacturer's instructions.

B- Polymerase chain reaction (PCR).

PCR was performed using the PCR premix Kit (Accupower Pioneer, Korea) with a total reaction volume of a 20 µl containing Accupower TM PCR premix, 10 pmol \ µl each of primer 1 µl of DNA extracted from culture of 5 µl of DNA extracted and 17 µl of PCR water. The primers used in the reaction were synthesized at Pioneer Company, Korea. The pair of primers 5-TGTTGCTCTCTCGGGGGCGGCCG-3 and 5-AGATCATTATGCCAACATCCTAGCTTAAA-3 specific for the amplification of a

fragment of the 25S rDNA gene of *C. albicans* described by Marinho *et al*. (2010) was used to confirm phenotypic identification of *C. albicans* and *C. dubliniensis*. PCR amplification process was carried out with an eppendorf thermal cycler (TECHNE, USA) under the following condition: Initial denaturation at 94 ^{o}C for 5 min followed by 35 cycles of denatartion at 94 ^{o}C for 30 s, Annealing at 57 ^{o}C for 1 min and extension at 72 ^{o}C for 30 s with a final extension at 72 ^{o}C for 4 min (18). PCR Products were analyzed by electrophoresis thorough a 2% agarose gel containing 5 µg \ml ethidium bromide. The gel was exposed to UV light and photographed. The size of amplified DNA fragments were identified by comparison with molecular size marker DNA (100 bp DNA Ladder). The positive result was that PCR produces a DNA fragment of 175 bp.

Antifungal susceptibility testing

The test was carried out using the disk diffusion method following the M44- A2 National committee for clinical laboratory standards (NCCLS) guidelines using fluconazole (10 mcg \disc) itraconazole (10 mcg\ disc), ketoconazole (10 mcg \disc), clotrimazole (10mcg\disc) and nystatin 100 units \disc (Himedia, India) (19).

Inoculum was prepared by picking 5 colonies of approximately 1mm in diameter from 24h. old culture of *Candida* spp. Colonies were suspended in 5ml of sterile saline and its turbidity was adjusted visually with the transmittance that produced by McFarland standards supplemented Muller Hinton agar(Muller Hinton agar + 2% glucose and .5 µg \ml methylene blue dye (GMB. Medium) was used for performing with a sterile cotton swab dipped in to the suspension. The dried surface of agar plates was inoculated by evenly streaking the swab over the entire agar surface. Antifungal discs were placed onto the surface of inoculated agar plates. Plates were inverted and incubated at 37 ^{o}C with in 15 min. after the antifungal discs were applied. The plates were examined after 20 – 24h. of inculcation. The zone of inhibition was measured and the result was recorded as Susceptible and resistant.

Determination of minimal inhibitory concentrations (MICS) of antifungal

MTC is the lowest concentration of antifungal that completely inhibits 100% the growth of test fungi (16). The MIC was performed by using E - test (Himedia MIC Hicombs strips; Himedia, India) according to the manufacturer's instructions. Briefly GMB agar plates was prepared and inoculated with as previously. the Hicomb strep for fluconazole , ketoconazole and itraconazole applied to the agar surface with MIC scale facing upwards plates were incubated in37 ^{o}C for 18-24h. MIC values determined as the value at which the zone of inhibition around the strep convenes the comb like projections of the strips and not at the handle. the MIC values were measured in the range of 0.016 – 256µg for fluconazole, 0.016 -32 µg for ketoconazole and 0.002 -32 µg for itraconazole the interpretive susceptibility criteria used for ketoconazole itraconazole and fluconazole were the same as those specified by the (16) for the resistant breakpoints for antifungal are fluconazole $\geq$ 64; itraconazole $\geq$ 1.0 and ketoconzole $\geq$ 4.0 µg\ml.

Statistical analysis

Statistical analysis was performed by using SPSS 11.5 Windows software . Rates were compared using the ANOVA test. P>0.05 was considered to be statistically significant.

Results and discussion

A total of 40 domestic insects (20 American cockroaches and 20 houseflies) were studied in this work. Approximately 56 *Candida* isolates were obtained from the external surface of the American cockroaches and houseflies. 14 *Candida* isolates were isolated from fouseflies while 42 isolates were obtained from American cockroaches (Table 2).

Table (2): Distribution of *Candida* isolates between domestic insects (American cockroaches and houseflies)

Species of *Candida*	**American cockroaches** Number (%)	**houseflies** Number (%)	**Total of *Candida* Isolates** Number (%)
C. albicans	17 (46.5)	8 (57)	25 (44.6)
C. glabrata	11 (26)	2 (14.5)	13 (23)
C. krusei	8 (19)	3 (21)	11 (19.6)
C. tropicalis	3 (7)	1 (7.5)	4 (7.4)
C. dubliniensis	2 (5)	_ (0)	2 (3.6)
C. parapsilisis	1 (2.5)	_ (0)	1 (1.8)
Total (%)	42 (75)	14 (25)	56

The results were reported considering Hichrome Candida agar as a primary medium for differentiating between various *Candida* species based on their colony colon and morphology (Table 3).

Table (3): Identification of *Candida* species by germ tube, chlamymdospore formation, culture in Hichrome Candida agar and carbohydrate assimilation test

Species of *Candida*	Color on Hichrome Candida agar	G.T	C.F	Carbohydrate assimilation											
				Melibiose	**Lactose**	**Maltose**	**Sucrose**	**Galactose**	**Cellobiose**	**Inositol**	**Xylose**	**Dulcitol**	**Raffinose**	**Trehalose**
C. albicans	Light green	+	+	-	-	+	-	+	-	-	+	-	-	+
C. glabrata	Dark pink	-	-	-	-	-	-	-	-	-	-	-	-	+
C. krusei	Pale pink with white edge rough and spreading	-	-	+	-	-	-	-	-	-	-	-	-	-
C. tropicalis	Dark blue color	-	-	-	-	+	+	+	+	-	+	-	-	+
C. dubliniensis	Dark green	+	+	-	-	+	-	+	-	-	+	-	-	+
C. parapsilisis	Pale cream color	-	+*	-	-	-	-	-	-	-	+	-	-	+

C.F = Chlamymdospore formation**; G.T** = Germ tube **; * = Pine forest appearance**

Based on the colony color developed on Hichrome Candida agar, All the *C. albicans* isolates (n=25) formed light green colonies after incubation for 48 h. (figure 1- A) .this hue was distinctive for this species and among the other species tested only *C. dubliniensis* gave a similar color (figure 1- B) . Isolate of *C. dubliniensis* (n=2) developed dark green colony and other isolate could not be distinguished from *C. albicans*. Therefore as shown previously for HiChrome Candida agar, a dark green appearance on HiChrome Candida agar may be taken as an indication of the presence of *C. dubliniensis* but should not be used as the sole criterion (20). All *C. Krusei* (n=11), *C. glabrata* (n=13), *C. tropicalis*(n=4) and *C. parapsilosis* (n=1) formed typical colonies that were easily differentiated from those of other yeasts. *C. Krusei* formed dry, pale pink with white edge, rough and spreading (figure 1- C) . *C. tropicalis* formed dark blue colonies (Figure 1- D) and *C. parapsilisis* formed pale cream colonies (figure 1- E) . These findings indicate that HiChrome Candida agar allows presumptive identification of *C.albicans, C. Krusei, C. glabrata, C. tropicalis* and *C. parapsilisis* with high sensitivity and specificity particularly after incubation at 37 C^{o} for 48h. (21). *Candida* isolates were further identified by germ tube,chlamydospore formation and assimilation of sugar (Table 3). This results reflected that in spite of its great cost (in comparison with SDA), Hichrome Candida agar medium was found to be extremely helpful, allowing direct identification of *C. albicans* colonies as well as Identification of the most common yeasts (especially C. *krusei, C. glabrata, C. tropicalis* and *C. parapsilisis*) and the easy recognition of associations of multiple yeast species were taken from either American cockroaches and houseflies. Thus this easy to use and time saving medium appears to be well suited for routine use in clinical mycology laboratories.

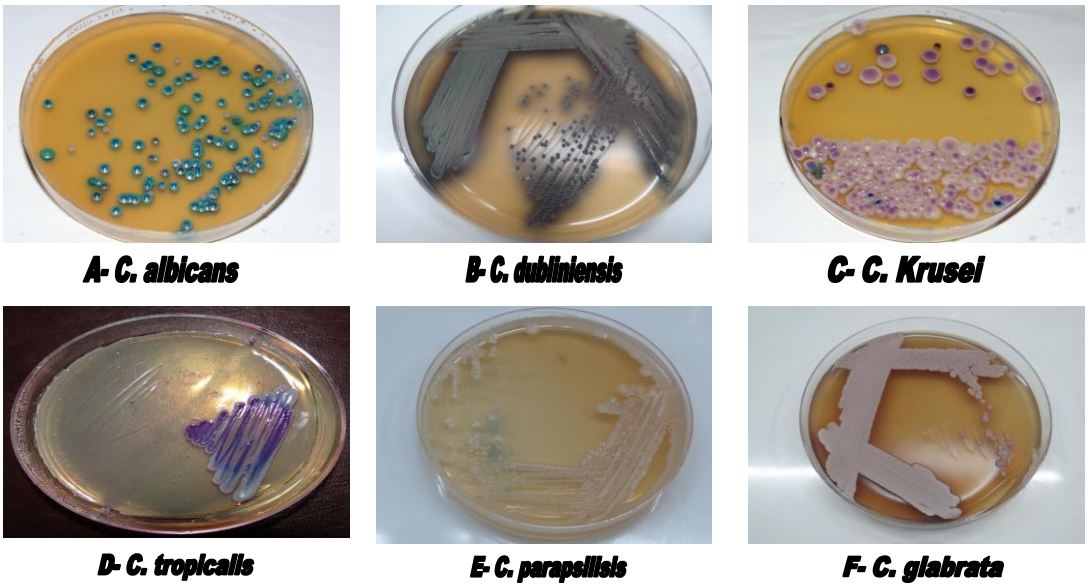


Figure (1): Appearance of yeast colonies on HiChrome Candida agar

Molecular identification

A total of (27\56) 48.2% of *Candida* spp. were able to produce germ tube and chlamydospore production were observed in 27 of the tested strains and these strains

showed green colonies in HiChrome Candida agar medium indicating *C. albicans* or *C. dubliniensis* (Table 2,3). To confirm these tests the carbohydrate assimilation test was used in all 56 *Candida* isolates and 27 strains were indicative of *C. albicans* or *C.dubliniensis* . all 27 strains were characterized phenotypically as *C. albicans* or *C. dudbliniensis* were tested by PCR. The PCR produced a DNA fragment of 175 bp when testing with *C. albicans* and did not yield amplification product when the lyses cells of *C. dubliniensis* were used. 25 strains being identified as C. *albicans* and two out of these strains failed to produce a DNA fragment of 175 bp (Figure 2).

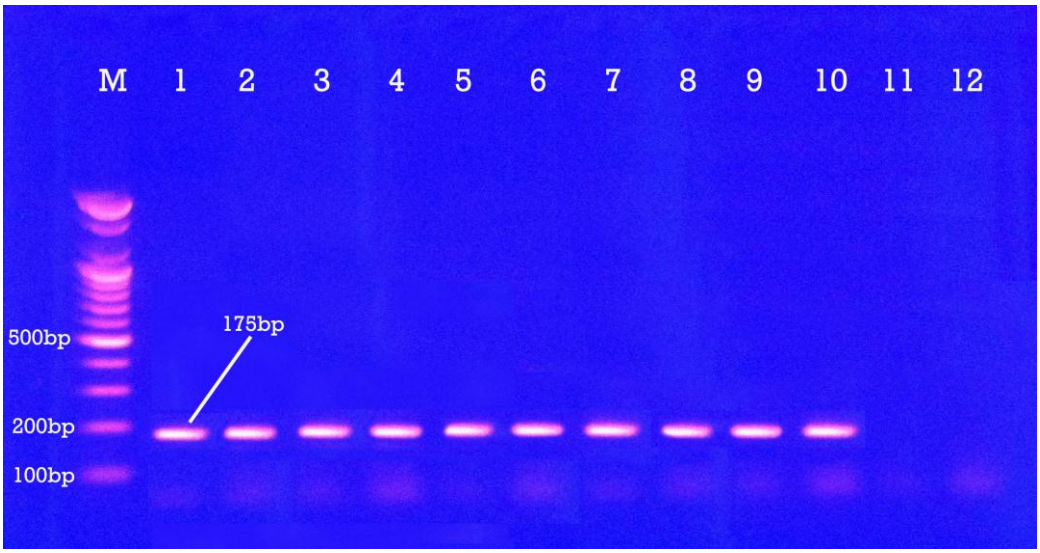


Figure (2): PCR identification of C. *albicans* and C. *dubliniensis* . PCR products were analyzed by 2% agarose gel electrophoresis. The size of amplified DNA fragments were identified by comparison with molecular size marker DNA(100 bp DNA Ladder) . the positive result the PCR produces a DNA fragment of 175 bp . Lane 1- 8 show *C. albicans* and 9-10 show C. *dubliniensis*.

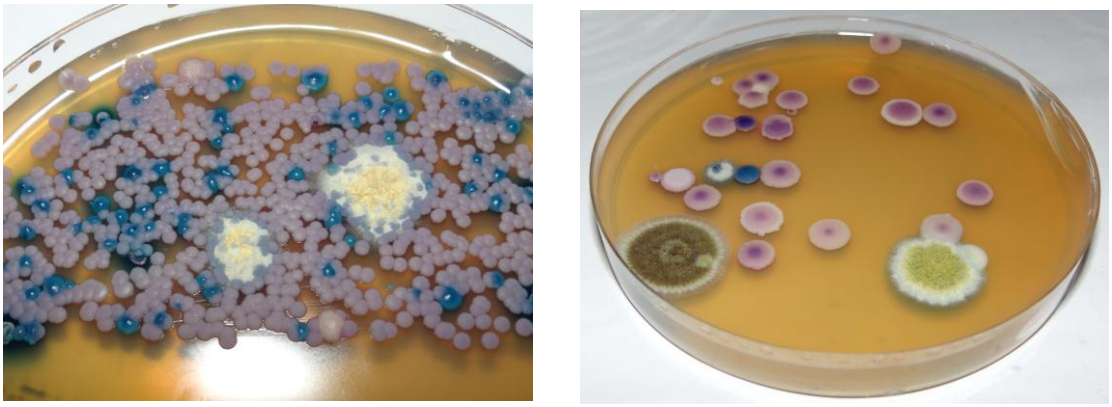
No single phenotypic test has been proved to be highly effective in the distinction between *C. albicans* and *C. dubliniensis*. According to Milan and Zaror (22) there is a difference in the intensity of color of colonies between *C. albicans* and *C. dubliniensis*. *C. albicans* colonies are green pale and *C. dubliniensis* dark green. Yet, it is important to observe that this ability is lost after freezing the samples and it did not show reproducible results.

Genotypic tests may be necessary for definitive identification. The combination of some phenotypic methods can be useful for the presumptive identification of these species but they would require a pure culture and 3-5 days or longer for differentiation between *C. albicans* and *C. dubliniensis* isolates (18). Finally, detection and identification of *C. albicans* and *C. dubliniensis* could be established by molecular methods . This method can give definitive identification with one day results and do not need previous cultures (23).
Results of yeast identification showed that *Candida* spp. present in the American cockroaches samples were also present in houseflies with exception of *C. parapsilisis* and *C. dubliniensis* which were isolated from American cockroaches, but were not present in

the houseflies samples (table2). Among these 40 samples of domestic insects , 18 samples (45%) yielded mixed growth and *C. albicans* was the common spears in all mixed growth (table4) and figure (3) . This result is similar to other worked by Mulu *et al*. (24) who reports the high frequency (42%) of mixed colonization, usually *C. albicans* with other *Candida* spp.

Table (4): Distribution of mixed isolates and their percentage between domestic insects (American cockroaches and houseflies)

Species of *Candida*	**American cockroaches** Number (%)	**houseflies** Number (%)
C. albicans + *C. krusei*	7 (46.7)	1 (33.3)
C. albicans + *C. parapsilisis*	1 (6.7)	- (0)
C. albicans + *C. tropicalis*	2 (13.2)	1 (33.3)
C. albicans + *C. glabrata*	3 (20)	1 (33.3)
C. albicans + *C. dubliniensis*	1 (6.7)	- (0)
C. albicans + *C. krusei* + *C. tropicalis*	1 (6.7)	- (0)
Total	**15**	**3**



C. albicans + C. *glabrata* *C. albicans* + *C. Krusei*

Figure (3): Mix species isolated from two domestic insects

The most frequent *Candida* isolates from American cockroaches and houseflies coming from three districts of the city were *C.albicans*, *C. Krusei* and *C. glabrata* (44.6%, 23% and 19% respectively), while the least present were *C. parapsilisis* (1.8%) (table2). The results showed there was a significant difference between the amounts of the *Candida* spp. between American cockroaches and houseflies. These results are in accordance with other reports which highlight the importance of American cockroaches and houseflies in carrying various species of *Candida* (25,26). Furthermore, this study found that American cockroaches harbored more species of *Candida* than the houseflies. Although American

cockroaches is seven to ten folds larger than houseflies in length, the capability of harboring microorganisms in insects is not only related to their size but may also depend on the association of these insects with unsanitary conditions of the environment (25). Therefore these two species of insects are the most common ones in Iraq because of the favorable environmental and climatic conditions and this study has established that American cockroaches and houseflies carry *Candida* species in 3 districts of Al – Diwaniya city, therefore , they have to be controlled and density of their population should be reduced under taken different vector control approaches, especially in hospitals , where immunocompromised people are move likely to be exposed to opportunistic infections.

Antifungal drug susceptibility and MICs

Invitro antifungal drug susceptibility and MICs testes were done on all isolates of *C. albicans* (n=25), *C. glabrata* (n=13), *C. krusei* (n=11), *C. tropicalis*(n=4), *C.dubliniensis*(n=2) and *C. parapsilisis*(n=1) as shown in tables (5,6) and figures (4, 5). *C. albicans* the most isolated species was sensitive to nystatin, ketoconazole, fluconazole, itraconazole and clotrimazole with 96%. , 80%, 84%, 92% and 88% respectively. Of the 13 *C. glabrata* strain 13 (100%) were sensitive to nystatin, 12(92%) to ketoconazole and fluconazole and 11(85%) for both itraconazole and clotrimazole . Among the 11 *C. krusei* , the third isolated species, 11(100%) isolates were found to be sensitive to nystatin , 10(90%) to ketoconazole and fluconazole and 9(82%) for itraconazole and clotrimazole . *C. tropicalis* species had the least sensitivity to ketoconazole and itraconazole and high sensitivity (100%)to fluconazole,nystatin and clotrimazole. *C. dubliniensis* species showed high sensitivity rate (100%) to all antifungal agents except the ketoconazole (50%). *C. parapsilosis* species showed sensitive to nystatin, ketoconazole and fluconazole1 (100%), while resistance to itroconazole and clotrimazole 1(100%) (table5) .The results of the present study , generally indicate that the important *Candida* spp. appear to be highly sensitive to azole groups and polyene (ketocoazole, fluconazole, itraconazole,clotrimazole and nystain). In this study all *Candida* isolates were susceptible to nystatin in agreement with previously reported data (27, 28). Even though polyenes were widely used for more than 40 years in many fungal infections, the incidence of resistance in *Candida* spp. is rare (29). But, this result does not agree with Khan and Baqai (30) who found that the rate of resistance to nystatin 63.8% in *Candida* isolates. Moreover, resistance of *Candida* spp. to azoles continues to increase in response to the widespread application of triazole therapeutics, although few ketocoazole, fluconazole, itraconazole and clotrimazle resistant strains have been isolated from domestic insects in agreement with previous studies (16 ,31) . While this result disagree with the study by Khan and Baqai (30)who found that 30% and 63.8% of *Candida* isolates were resistant to clotrimazole and fluconazole respectively.

Table (5): Antifungal susceptibility pattern of isolates *Candida* species using disc diffusion method

species of *Candida*	Nystatin		Ketoconazole		Fluconazole		Itranconazole		Clotrimazole	
	Sensitive number (%)	resistance number (%)	Sensitive number (%)	resistance number (%)	Sensitive number (%)	resistance number (%)	Sensitive number (%)	resistance number (%)	Sensitive number (%)	resistance number (%)
C. albicans (N=25)	24 (96)	1 (4)	20 (80)	5 (20)	21 (84)	4 (16)	23 (92)	2 (8)	22 (88)	3 (12)
C. glabrata (N=13)	13 (100)	0 (0)	12 (92)	1 (8)	12 (92)	1 (8)	11 (85)	2 (15)	11 (85)	2 (15)
C. krusei (N=11)	11 (100)	0 (0)	10 (90)	1 (10)	10 (90)	1 (10)	9 (82)	2 (18)	9 (82)	2 (18)
C. dubliniensis (N=2)	2 (100)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)
C. tropscalis (N=4)	4 (100)	0 (0)	3 (75)	1 (25)	4 (100)	0 (0)	3 (75)	1 (25)	4 (100)	0 (0)
C. parapsilisis (N=1)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
Total	55 (98)	1 (2)	47 (84)	9 (16)	50 (89.3)	6 (10.7)	48 (85.7)	8 (14.3)	48 (85.7)	8 (14.3)

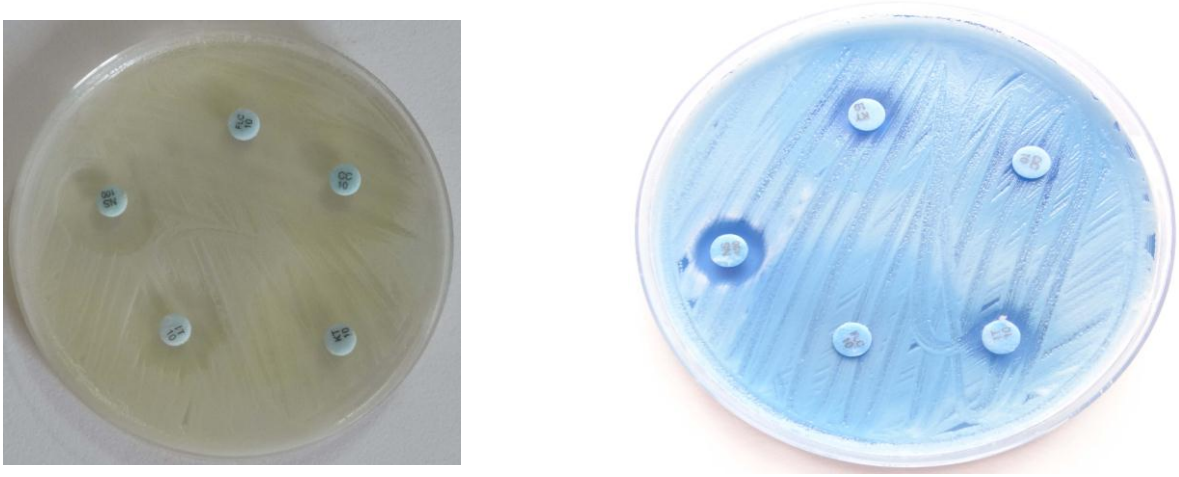


Figure (4): Invitro susceptibility of Candida species to five antifungal agents by using disc diffusion method

Table (6) and figure (5) presents the minimal inhibitor concentration testing of *Candida* isolates to antifungal agents. Comparing the MIC, of species the lowest MIC was observed for itraconazole (0.002 µg\ml). The range of MICs for all *Candida* spp. was 0.004- 16 µg \ml, 0.002-8 µg \ml and 0.016-64 µg \ml for ketoconazole, itraconazole and fluconazole respectively. According to the breakpoints for these antifungals, almost *Candida* spp. were sensitive to these antifungals except 16%. , 14.3% and 2% of *Candida* isolates were resistant to ketoconazole, itraconazole and fluconazole respectively.

Recently an increase in the isolation of azole- resistant *Candida* strains have been reported (9). This result showed that fluconazole was most effective (2%) as compare to ketoconazole (16%) and itraconazole (14.3%). This result is in agreement with previous studies (30, 32).

Table (6): Minimal inhibitor concentrations profile of *Candida* isolates from domestic insects

Antifungal agent	Ketoconazole		Itraconazole		fluconazole	
species of Candida	MIC range µg/ ml	Resistance number (%)	MIC range µg/ ml	Resistance number (%)	MIC range µg/ ml	Resistance number (%)
C. albicans (N=25)	0.016-16	5 (20)	0.008-4	2 (8)	0.016-32	0 (0)
C. glabrata (N=13)	0.008-8	1 (8)	0.032-8	2 (15)	0.016-32	0 (0)
C. krusei (N=11)	0.032-6	1 (10)	0.002-8	2 (18)	0.256-64	1 (8)
C. dubliniensis (N=2)	0.004-8	1 (50)	0.002-32	0 (0)	0.256-1.024	0 (0)
C. tropscalis (N=4)	0.004-8	1 (25)	0.002-8	1 (25)	0.128-2.048	0 (0)
C. parapsilisis (N=1)	0.032	0 (0)	4	1 (100)	2.048	0 (0)
Total	0.016-16	9 (16)	0.002-8	8 (14.3)	0.016-32	1 (2)

Resistance is defined as the following MIC µg/ ml Flu≥64 , keto≥4.0 and Itra ≥1.0

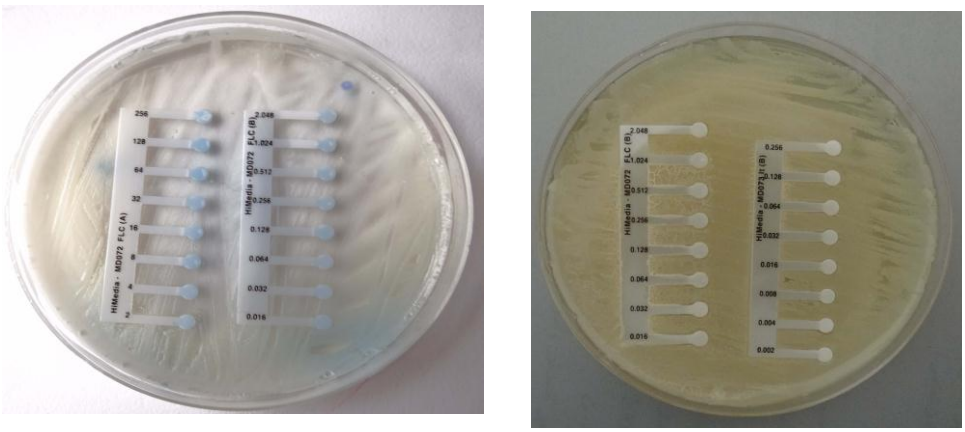


Figure (5): In vitro susceptibility of *Candida* species to five antifungal agents by using E- test

Antifungal choice is first based on *Candida* spp. identification but antifungal susceptibility testing will play an increasingly important role when selecting antifungal drug dosage to use (31). In this study, the result showed that minimal inhibitor concentration method was excellent for determining the levels of dosage pattern of each species compare with disc diffusion method. This result is in agreement with Saroj *et al*.

(21) and Rudensky *et al*.(33) who found that MIC method was better than the other method were used for determining *Candida* susceptibility.

Reference

1- **Nguyen, N.H. ; Suh, S.O. and Blackwell, M.(2007).**Five novel *Candida* species in insect-associated yeast clades isolated from Neuroptera and other insects. Mycol.;99(6):842-858.

2- **Feizhaddad, M. ; Kassiri,H. ; Sepand, M. and Ghasem, F. (2012).**Bacteriological survey of American cockroaches in hospitals. Middle-East J. Sci. Res.; 12 (7): 985-989.

3- **Srivoramas, T. ; Chaiwong, T. and Sanford , M.(2012).** Isolation of fungi from adult house fly; *Musca domestica* and the blow fly *Chrysomya megacephala* in ubon ratchathani province, northeastern thailand. Inter. J. Parasitol. Res. 4(1):53-56

4- **Tachbele, E.; Erku, W.; mGebre-Michael,T. and Ashenafi, M.(2006).** Cockroach-associated food-borne bacterial pathogens from some hospitals and restaurants in Addis Ababa, Ethiopia: Distribution and antibiograms. J.Rur.Trop.Pub. Heal. 5: 34-41.

5- **Zarrin,M.; Vazirianzadeh,B.; Solary,S. S.;Mahmoudabadi, A. Z. and, Rahdar,M. (2007).**Isolation of fungi from housefly(musca domestica) in ahwaz, Iran. Pak. J. Med. Sci. 23(6):917-919.

6- **Vazirianzadeh, B.; Solary,S. S. ; Hajhossien, R. and Mehdinejad, M.(2008).** Identification of bacteria which possible transmitted by *Musca domestica* (Diptera: Muscidae) in the region of Ahvaz, SW Iran . Jundishapur J.Microbiol.; 1(1): 28-31

7- **Davari, B.; Khodavaisy, S. and Ala F. (2012).**Isolation of fungi from housefly (*Musca domestica* L.) at slaughter house and hospital in Sanandaj, Iran. J. Prev. Med. Hyg. ;53(3):172-174.

8- **Jha, B.J.; Dey, S.; Tamang, M.D.; Joshy, M.E.; Shivananda, P.G. and Brahmadatan, K.N. (2006).** Characterization of Candida species isolated from cases of lower respiratory tract infection. Kathmandu univ. Med. J. , 4(3):290-294.

9- A**baci, O. and Haliki-Uztan, A.(2011).**Investigation of the susceptibility of *Candida* species isolated from denture wearers to different antifungal antibiotics. Afr. J. Microbiol. Res. 5(12): 1398-1403.

10- **Adeleke,M. A.; Akatah, Hi. A.; Hassan, A. O. and Adebimpe, W. O.(2012).**Microbial load and multiple drug resistance of pathogenic bacteria isolated from feaces and body surfaces of cockroaches in an urban area of southwestern Nigeria. J.M.B.F.S. ,1(6):1448-1461.

11- **Saichua, P. ; Pinmai, K. ; Somrithipol,S. and Tor-Udom, S.(2008).** Isolation of medically important fungi from cockroaches trapped at Thammasat Chalermprakiat Hospital. Thammasat Med. J.,8(3):345-351.

12- **Nazni, W.A.; Seleena, B.; Lee, H.L. ; Jeffery, J.T. ; Rogayah. T.A. and Sofian, M.A.(2005).** Bacteria fauna from the house fly, *Musca domestica* (L.). Trop. Biomed. ;22(2):225-231.

13- **Graczyk, T.K.;, Knight, R.; Gilman, R.H. and Cranfield, M.R. (2001).** The role of non-biting flies in the epidemiology of human infectious diseases. Microbes Infect. ; 3(3):231-235.

14- **Srinivasulu Reddy, P. and Athuluri, V. (2012).** Definite differentiation of *Candida albicans* from other species by using chrom agar. J. Microbiol. Biotech.Res.92(6):963-941.

15- **Chaudhary, U. ; Deep, A. and Chabbra, N. (2009).** Rapid Identification and Antifungal Susceptibility Pattern of *Candida* isolates from critically ill patients with candiduria. J. Infect. Dis. Antimicrob. agents.26 (2):49-53.

16- **Badiee , P. and Alborzi, A. (2011)**. Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five year study. Iran J. Microbiol.3(4):183–188.

17- **Raut, S. H. and Varaiya, A. (2009).** Differentiation of *Candida dubliniensis* on chrom agar and pals agar . Indian. J. Med. Microbiol., 27(1): 55-58.

18- **Marinho, S. A.; Teixeira, A. B.; Santos, O. S.; Cazanova, R. F.; Ferreira, C. A.S.; Cherubini, K. And Olireira, S. D. (2010).** Identification of *Candida* spp. and PCR. Brazilian J. Microbiol., 41: 286-294.

19- **National Committee for Clinical Laboratory Standards(NCCLS)(2009).** Antimicrobial susceptibility test discs. Approved Guidelines .2ned NCCLS document M44-A2. Wayne.

20- **Mähnβ, B.; Stehr, F.; Schafer, W. and Neuber, K. (2005).** Comparison of standard phenotypic assays with a PCR method to discriminate *Candida albicans* and *C. dubliniensis*. Mycoses 48: 55-61.

21- **Saroj, G. K. ; Mallika, R. K.; Sujatha, K. and Vivek, H. (2013).** Speciation of *Candid*a using chromogenic and cornmeal agar with determination of fluconazole sensitivity Al Ameen J. Med. Sci. 6(2) : 163- 166.

22- **Milan, E. P. And Zarov, L. (2004).** Levenduras: identification laboratorial. In: Sidrim, J. J. And Rocha, M. F. G. (eds) Micologa Medical a luz de autores contemporaneos. Riode. Janero: Guarabara koogan, 89-101.

23- **Ko, Y. J.; Lim, W. B.; Kim, J. S.; Kim, I. A.; Kowon, H. L.; Kim, O. S.L Kim, B.; Kim, S. M.; Youn, S. J.; Kang, B. C.; Lim, H.; Kim, M. S.; Kim, O. J. and Choi, H. R. (2010).** Identification of putatively in gwangju. Kor. J. Maxillofac. Pathol., 34(2):73-82.

24- **Mulu, A. ; Kassu, A. ; Anagaw, B. ; Moges, B. ; Gelaw, A. ; Alemayehu, M. ; Belyhun, Y.; Biadglegne, F. ; Hurissa, Z. ; Moges, F. and Isogai, E. (2013)**. Frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients in northwest Ethiopia. BMC Infect. Dis. 13 (82.):2-10.

25- **Bouamamaa, L.; Sorlozano, A.; Laglaoui; A. Lebbadi, M. Aarab, A. and Gutierrez, J.(2010).** Antibiotic resistance patterns of bacterial strains isolated from *Periplaneta Americana* and *Musca domestica* in Tangier, Morocco. J. Infect. Dev. Ctries. ;4(4):194-201.

26- **Al-galebi, H. H. ; Hussein, H.M. and Hamza A. K.(2011).**Isolation and identification of fungi from outer surface of cockroach *Periplaneta Americana*

collected from AL-Diwaniya educational general hospital and study of some virulence factors. J. Thi-Qar.Sci. , 3(1):23-31.

27- **Gualco, L. ; Debbia, E.A.; Bandettini, R.; Pescetto, L.; Cavallero, A.; Ossi, M.C.; Schito, A.M. and Marchese, A.(2007).**Antifungal resistance in *Candida* spp. isolated in Italy between 2002 and 2005 from children and adults Int. J. Antimicrob. Agents, 29(2):179-184.

28- **Ishida,K.; Ueda-Yamaguchi,M.; Yamada-Ogatta,S. F.; Ueda-Nakamura,T.; Svidizinski, T. I. E. and Nakamura,C. V. (2013).**Characterization of *Candida* spp. isolated from vaginal fluid: identification, antifungal susceptibility, and virulence profile. Acta Scientiarum. Health Sci. , 35(1):1-8.

29- **Ellis, D.(2002).**Amphotericin B: spectrum and resistance. J. Antimicrob. Chemother. ,49 (1):7-10.

30- **Khan, F. and Baqai, R. (2010).** In vitro antifungal sensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age. J. Ayub. Med. Coll. Abbottabad, 22(4): 197-200.

31- **Namkinga, L.A. (2012).**Management of azole-refractory *Candida* species using boric acid preparations: a case study in dar es salaam,Tanzania. Tanz. J. Sci. ;38 (2) :123-137.

32- **Lee, J.; Shin, J. H.; Lee, K.; Kim, M.; Shin, B.; Uh, Y.; Lee, W.; Lee, H.; Chang, C.; Kim, S. S.; Shin, M. G.; Suh, S. P. and Ryang, D. (2007).** Species distribution and susceptibility to azole antifungal of *Candida* bloodstream isolates from eight university hospital in Korea Yonsei. Med. J., 48(5): 779-786.

33- **Rudensky, B.; Broidie, E.; Yinnon, A. M.; Weitzman, T.; Paz, E.; Keller, N. and Raveh, D.(2005).** Rapid flow-cytometric susceptibility testing of Candida species. J.Antimicrob. Chemoth., 55: 106–109.