Biofilm Formation and Antifungal Susceptibility of Candida Isolates from Various Clinical Specimens

Sahar Ali Mohamed¹ and Ziab Zakey Al-Ahmadey²

¹Microbiology and Immunology Department, Faculty of Medicine, Menofiya University, Egypt.
²Al-Ansar hospital, General Directorate of Health Affairs in Al-Madinah, Ministry of Health, Al-Madinah, Saudi Arabia.

Authors’ contributions

This work was carried out in collaboration between the two authors. Author SAM planned and designed the study, wrote the protocol, collected the samples, performed the practical laboratory activities, participated in the interpretation of the results and analysis, drafted and critically revised the manuscript. Author ZZA participated in planning and designing the study, sample collection, performing the practical laboratory activities, participated in the interpretation of the results. Both author read and approved the final manuscript.

ABSTRACT

Background: Candida species are now recognized as major causative agents of hospital-acquired infection.

Aims: To evaluate the species distribution, biofilm formation, and antifungal susceptibility (amphotericin B, ketoconazole, and fluconazole) of Candida isolates.

Place and Duration of Study: This is a Six-months Cross sectional study conducted in Al-Ansar hospital, Al-Madinah, Saudi Arabia.

Methodology: One hundred and three isolates of Candida spp. were cultured on Sabouraud dextrose agar (SDA). Candida spp. were identified by four standard methods, CHROMagar candida, cornmeal agar, germ tube test and API 20C. Detection of Biofilm formation was done by microtitre plate and antifungal susceptibility testing was done by disc diffusion.

Results: C. albicans was the most common species 61%, followed by C. tropicalis 25%, C. lusitanae 5%, C. parapsilosis 4%, C. glabrata 4% and C. famata 1%. Biofilm formation was found to occur most frequently among non-albicans spp. (70%) than C. albicans (46%).

*Corresponding author: Email: saharmali2004@yahoo.com;
isolates were sensitive to amphotericin B and ketoconazole. Resistance to fluconazole was found in 22.5% of non-albicans spp. and 5% of C. albicans isolates.

**Conclusion:** The present study proved that C. albicans is still the major isolate from urinary, vaginal and respiratory samples but non-albicans spp. predominate in the blood samples and from plastic devices. The non-albicans spp. were more biofilm - producers compared to C. albicans and C. tropicalis showed the highest score of biofilm intensity (grade 4+). The species isolated are less susceptible to fluconazole.

**Keywords:** Candida spp.; non-albicans spp.; biofilm formation; antifungl.

**ABBREVIATIONS**

SDA: Sabouraud’s Dextrose Agar; SDB: Sabouraud Dextrose Broth; CLSI: Clinical and Laboratory Standards Institute; S: susceptible; S-DD: susceptible dose dependent; R: resistant; spp: Species and HVS: High Vaginal Swab.

**1. INTRODUCTION**

*Candida* spp. are the most common cause of fungal infections leading to a range of invasive life threatening to non-life-threatening diseases. *Candida* spp. has been recognized as the fourth commonest cause of nosocomial invasive infections [1]. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immune-compromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation. Among *Candida* spp., *C. albicans* is the most common frequent causes of infection, but non-albicans spp. Infections are increasing [2].

The important factors contributing to the virulence of candida is the formation of surface-attached microbial communities known as “biofilms” [3]. Biofilms are attached to a surface and encased in a matrix of exopolymeric material. A typical laboratory fungal model of biofilm formation involves three operational steps: (a) adhesion, (b) biofilm growth, and (c) maturation [4]. Biofilm formation helps the organism to evade host defenses, exist as a persistent source of infection and develop resistance against antifungal agents. *Candida* spp. are frequently found in the normal microbial flora of humans, which facilitates their encounter through implanted biomaterials and host surfaces [5]. The resistance of biofilm forming *candida* spp. to antifungal agents represents a major challenge especially in the design of therapeutic and prophylactic strategies [6].

Amphotericin B, a polyene fungicidal agent, has been standard treatment for candidal infections for decades, but the toxicity of the conventional form and the coast of its lipid forms limit its use. More recently, azole antifungal compounds, with lower toxicity and perfect efficacies, have emerged as the principal drugs used in treatment of candidal infection. Disk diffusion has served as simple, rapid, and cost-effective method for screening the susceptibility patterns of the yeasts [7].

The aim of this study was identification of *Candida* spp. isolated from various sources, detection of biofilm formation among isolates and in-vitro antifungal susceptibility of the isolates to amphotericin B, ketoconazole, and fluconazole.
2. MATERIALS AND METHODS

2.1 Isolation and Identification of the Candida Isolates

A total of 103 non repetitive different clinical samples were collected from patients being treated in Al-ansar hospital, Al-Madinah, Saudi Arabia from August 2012 till February 2013. Isolates were from different sites of the body including respiratory tract (n= 41, 40%), blood (n=14, 13%), urine (n=26, 25%), genital tract (n=10, 10%), plastic devices (n=8, 8%), and other sources (n=4,4%). all samples were cultured onto Sabouraud Dextrose Agar (SDA) (Hi-media, Mumbai, India). Isolates were identified by four standard methods, CHROMagar Candida (CHROMagar company, BD), cornmeal agar, germ tube test (Table 1 and Fig. 1) and API 20C (Bio-Merieux, France). API 20C strip (20 cupules) were inoculated according to the manufacturer’s instructions, incubated at 30ºC and observed at 48 and 72h. Reactions were read by comparing the strips to growth controls and identification was obtained by referring to the analytical profile index. C. albicans ATCC 10231 was used for quality controls [8]. The collected Candida species strains were stored in 10% glycerinated water at −20ºC until biofilm was performed.

2.2 Antifungal Susceptibility Testing

The in vitro activity of antifungal agents (amphotericin B, ketoconazole, and fluconazole) was measured by disk diffusion method according to the procedure described in the clinical and laboratory standard institute [9]. The plates were incubated at 35ºC, and inhibition zone diameters were measured after 24 and 48 h especially for C. glabrata. The interpretive criteria for the disk test were as follow: amphotericin B: dz ≥15mm, susceptible; 14≤dz≤10mm, susceptible dose dependent and dz≤9mm, resistant. Fluconazole: dz ≥19mm, susceptible; 15≤dz≤18mm, susceptible dose dependent and dz≤14mm, resistant. As for ketoconazole: dz≥20mm, susceptible; 10<dz<20mm, susceptible dose dependent and dz≤10mm, resistant [10].

2.3 Biofilm Formation

A total of 103 Candida spp. isolates were grown in Sabouraud Dextrose Broth (SDB) at 30ºC for 18 h. Centrifugation was done for 5 minute at 4000 rpm and washed twice with saline. The pellet was then re suspended in 5ml of sterile saline, and turbidity adjusted to 3 of the McFarland scale. To each well of the microtiter plates (flat bottom) 180µl of SDB with 8% glucose were added, then inoculated with 20µl of the above yeast cell suspension. Yeast-free medium controls were also included. The plates were then incubated for 48 h at 37ºC. After incubation, plate growth was assessed by measuring the absorbance at 490nm. The wells were washed thrice with sterile saline. A volume of 200µl of 1% crystal violet (Sigma, USA) was added to each well, and the plate was allowed to stand for 20 minutes at room temperature. The wells were subsequently washed thrice with sterile saline. A volume of 200µl of ethanol was added to each well and absorbance was measured at 630nm with a microtiter plate reader PEBIII (Dade Behring). The values above negative control were considered as biofilm producers[11]. The percent transmittance (%t) was measured, the %t value for each test sample was subtracted from the %t value for the reagent blank to obtain a measure of the amount of light blocked when passing through the wells (%t_bloc). Biofilm production by each isolate was scored as either negative (%t_bloc. < 5), 1+ (%t_bloc. 5 to 20), 2+ (%t_bloc. 20 to 35), 3+ (%t_bloc.35 to 50), or 4+ (%t_bloc. >50) [12].
2.4 Statistical Analysis

The results were expressed as percentages for the description of Candida isolates according to species and various clinical samples. Data were statistically analyzed using the chi-squared test. A value of \( p < 0.05 \) was considered significant.

![Fig. 1. Different candida spieces on CHROMagar](image)

**A:** C. albicans with green color Colonies, **B:** C. glabrata with dark pink color colonies, **C:** C. parapsilosis with white & pale pink color colonies and **D:** C. tropicalis with fuzzy blue color colonies.

| Candida spp. | Color on CHROMagar       | Germ tube test | Chlamydosporie |
|--------------|---------------------------|----------------|----------------|
| C. albicans  | Light green               | +              |                |
| C. tropicalis| Fuzzy blue                | -              | -              |
| C. lusitaniae| Pink                      | -              | -              |
| C. parapsilosis| White, pale pink       | -              | -              |
| C. glabrata  | Dark pink                 | -              | -              |
| C. famata    | Pink                      | -              | -              |

3. RESULTS

Distribution of Candida isolates from different departments: A total of 103 isolates were collected from patients admitted in Al-ansar hospital, Saudi Arabia. The maximum number of Candida isolates were isolated from Intensive Care Units (ICUs) 59/103 (57%), followed by Surgical departments 23/103 (22%), Medical departments 14/103 (14%), Other Departments 7/103 (7%) (Fig. 2).
Fig. 2. Distribution of candida a isolates from different departments

Candida spp. isolated from various clinical samples: The present study showed the distribution of Candida spp. in different clinical samples and the predominance was for C. albicans 61%(63/103) with statistical significant difference than non-albicans spp. (P=0.002). The most common isolate from non-albicans spp. was C. tropicalis 25%(26/103) followed by C. lusitaniae 5%(5/103), C. parapsilosis 4%(4/103), C. glabrata 4%(4/103), and C. famata 1%(1/103). The predominant species recovered from respiratory tract samples was C. albicans 73.2%(30/41). Urine samples yielded C.albicans (69%); followed by C. lusitaniae (11%). Also, HVS samples revealed C. albicans as a predominant isolates 70%(7/10) while the predominant species isolated from the blood samples were non-albicans spp. and mainly C. tropicalis 43%(6/14). Plastic devices like endotracheal tube, suction tip, and catheter tip were collected from patients. Their cultures yielded C. albicans and C. tropicalis equally (Table 2 and Fig. 3).

Biofilm formation by various Candida spp. Out of 103 Candida spp. tested 56 (54%) were found to be biofilm producers. Biofilm formation was found to occur most frequently among non-albicans spp. 28/40 (70%) than C. albicans 29/63(46%) with a statistical significant difference between two groups (P=0.02). Among the non-albicans spp., C. parapsilosis 4/4 (100%) was the highest biofilm producer followed by C. tropicalis 19/26 (73.1%), C. lusitaniae 3/5 (60%) then C. glabata 1/4 (25%). Only one isolate of C. famata was detected with positive biofilm formation. In our study the biofilm scoring from +1 to +4 among C. albicans and non-albicans spp. isolates was done and revealed that the score for C. albicans isolates were [6(+1), 10(+2), 11(+3), and 2(+4)] while for non-albicans spp. were [1(+1), 7(+2), 6(+3), and 14(+4)]. The biofilm formation by C. albicans was less frequent (46%) than that by non-albicans spp. (70%) (Table 3 and Fig. 4).

Biofilm formation in various clinical samples: The results of biofilm formation were also analyzed with respect to the site of infection, and revealed that the biofilm positivity was observed more with plastic devices 6/8 (75%) and bloodstream isolates (71%), urine samples were moderately biofilm producers 15/26 (58%) and the least isolates for biofilm production were from HVS 3/10 (30%) and respiratory tract 21/41 (51%) (Table 4).

In vitro antifungal susceptibilities of various Candida spp. Antifungal susceptibility testing in our study showed that all isolates were susceptible to amphotericin B and ketoconazole.
Resistance to fluconazole was found in 22.5% of non-albicans spp. and 5% of C. albicans isolates with statistical significant difference between two groups (P= 0.01) (Table 5).

**Table 2. Candida spp (n=103) isolated from various clinical samples**

| Candida spp. Isolated | Blood | Respiratory tract | Urine | Plastic devices pus | HVS | Others |
|-----------------------|-------|-------------------|-------|---------------------|-----|--------|
| C. albicans (63)      | 4     | 30                | 18    | 2                   | 7   | 2      |
| C. tropicalis (26)    | 6     | 9                 | 2     | 6                   | 1   | 2      |
| C. glabrata (4)       | 2     | 0                 | 0     | 0                   | 2   | 0      |
| C. lusitanae (5)      | 0     | 2                 | 3     | 0                   | 0   | 0      |
| C. parapsilosis (4)   | 2     | 0                 | 2     | 0                   | 0   | 0      |
| C. famata (1)         | 0     | 0                 | 1     | 0                   | 0   | 0      |
| Total non-albicans spp. (40) | 10   | 11                | 8     | 6                   | 3   | 2      |

(Comparing the percentage of C. albicans and non-albicans spp. isolated from various clinical samples $\chi^2 = 9.28$ and $P = 0.002$)

**Fig. 3. Percentage of Candida species isolated**
Table 3. Biofilm formation by various Candida species

| Candida spp.       | No (%) of biofilm negative | No. of biofilm positive | Total |
|--------------------|---------------------------|-------------------------|-------|
| C. albicans (63)   |                           |                         |       |
| C. tropicalis (26) |                           |                         |       |
| C. glabrata (4)    |                           |                         |       |
| C. lusitanae (5)   |                           |                         |       |
| C. parapsilosis (4)|                           |                         |       |
| C. famata (1)      |                           |                         |       |
| Non-albicans spp.  |                           |                         |       |
| Total non-albicans spp. (40) | 12 (30) | 1 | 7 | 6 | 14 | 28 (70) |

(Comparing the C. albicans and non-albicans spp. regarding the biofilm positivity $X^2 = 4.77$ and $P = 0.028$)

Fig. 4. Biofilm formation among C. albicans and non-albicans spp.

Table 4. Biofilm formation in various clinical samples

| No. of Candida species isolated | No. (%) of biofilm negative | No. of biofilm positive | Total |
|---------------------------------|-----------------------------|-------------------------|-------|
| Blood (14)                      | 4 (29)                      | 1 1 5 3 10 (71%)        |       |
| Respiratory tract (41)          | 20 (49)                     | 0 6 9 6 21 (51%)        |       |
| Urine (26)                      | 11 (42)                     | 3 8 0 4 15 (58%)        |       |
| Plastic devices (8)             | 2 (25)                      | 2 0 1 3 6 (75%)         |       |
| HVS (10)                        | 7 (70)                      | 1 2 0 0 3 (30%)         |       |
| Others (4)                      | 2 (50)                      | 0 0 2 0 2 (50%)         |       |
Table 5. In vitro antifungal susceptibilities of Candida spp. against fluconazole, ketoconazole and amphotericin B

| No. of candida species isolated | No. (%) of isolates | Fluconazole | Ketoconazole | Amphotericin B |
|-------------------------------|---------------------|-------------|--------------|---------------|
|                               |                     | S | S-DD | R | S | R | S | R | R |
| C.albicans (63)               | 58(92)              | 2(3) | 3(5) | 63(100) | 0 | 63(100) | 0 |
| C.tropicalis (26)             | 21(81)              | 3(11) | 2(8) | 26(100) | 0 | 26(100) | 0 |
| C.glabrata (4)                | 1(25)               | 0 | 3(75) | 4(100) | 0 | 4(100) | 0 |
| C.lusitaniae (5)              | 4(80)               | 0 | 1(20) | 5(100) | 0 | 5(100) | 0 |
| C.parapsilosis (4)            | 1(25)               | 0 | 3(75) | 4(100) | 0 | 4(100) | 0 |
| C.famata (1)                  | 1(100)              | 0 | 0 | 1(100) | 0 | 1(100) | 0 |
| Total non-albicans spp. (40)  | 28(70)              | 3(7.5) | 9(22.5) | 40(100) | 0 | 40(100) | 0 |

S, susceptible; S-DD susceptible dose dependent; R, resistant.

(Comparing the C. albicans and non-albicans spp. regarding the resistance to fluconazole $X^2 = 5.61$ and $P = 0.017$)

4. DISCUSSION

Candida organisms are commensals; and to act as pathogens, interruption of normal host defenses is necessary. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation [2].

Our study indicated an increased prevalence of the Candida spp. In ICUs (57%) and surgical wards (22%). Similar results were reported by Sheevani et al, 2013. This can be explained on the basis of the fact that the use of invasive devices are common in these wards and that the immune status of the patients are also compromised to the maximum as compared to that of the patients of other wards [15].

The present study showed the distribution of Candida spp. In different clinical samples and the predominance was for C. albicans 61% (63/103) with statistical significant difference than non-albicans spp. (P=0.002). Similar to our results, C. albicans was the most common species isolated from different clinical sources [13, 14]. On the other hand lower prevalence rates (39.5% and 25%) of C. albicans was reported by Mokaddas et al,2007 and Chakrabarti et al, 1996, respectively [16, 17].

Our study showed that C. albicans was the predominant species recovered from respiratory tract samples, Urine samples and HVS. These findings are consistent with those previously reported by other researchers [2, 18, 19]. In a recent study C. albicans was reported as the major agents of candiduria [20]. However other study showed that non-albicans spp., especially C. tropicalis and C. glabrata predominate in urine samples in many regions [21]. Emam et al, 2012 reported C. albicans as a predominant species isolated from pregnant women and non-pregnant women with vulvovaginitis [22].

Meanwhile our results showed that the predominant species isolated from the blood and plastic devices were non-albicans spp. and mainly C. tropicalis. These results are nearly similar to those reported by Hasana et al, 2009 [23]. Most catheter-related septicemias are caused by microorganisms that invade the intracutaneous wound during catheter insertion or
thereafter [12]. The proportion of such infection due to non-albicans spp. is persistently rising [24,25].

However other studies reported that the common isolate from the blood stream infection was C. albicans[26] and others reported C. parapsilosis rather than C. tropicalis as a predominant non albicans spp. isolates from blood stream [27] and reported that C. parapsilosis followed C. albicans as the most frequently isolate from candidemia clinical cases. Other studies on the epidemiology of candidaemia, have stressed the importance of the speciation of Candida, as it provides accurate information on the disease incidences and the trends in most of the infections [28].Kothavade et al, (2010) reported that C. tropicalis as the most prevalent pathogenic yeast species of the non-albicans spp.[29]

A biofilm is a community of microorganisms and their extra cellular polymers that are attached to a surface [30]. The ability to form biofilms is associated with the pathogenicity and as such should be considered as an important virulence determinant during candidiasis. Biofilms may help to maintain the role of fungi as commensal and pathogen, by evading host immune mechanisms, resisting antifungal treatment, and withstanding the competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat [31].

Our study revealed that a statistically significant difference (P=0.02) regarding the biofilm formation was found among non-albicans spp. 28/40 (70%) than C. albicans 29/63 (46%). These results are nearly similar to those reported by many other researchers [32,33]. Mohandas and Ballal, 2011 concluded that the biofilm formation is more important for non-albicans spp. and C. albicans possess mechanisms other than biofilm formation to establish infections[2].

Our study showed that among the non-albicans spp., the biofilm positivity occurred most frequently among isolates of C. parapsilosis (100%), followed by C. tropicalis (73.1%) also C. tropicalis showed the highest score of biofilm intensity 12/18 (66.6%) was of grade (+4). C. tropicalis have also been recognized as strong slime producers in many other studies[2,13,25]. However, Kuhn et al, (2002) showed that C. albicans produces quantitatively more biofilm than other Candida spp., but in that study the assessment of biofilm was based on quantitation and fluorescent microscopic examination proving that the biofilm formed by pathogenic C. albicans was a complex phenomenon composed of blastospore layer covered by a thick biphasic matrix, consisting of a dense extracellular component comprised of cell wall-like compounds and abundant hyphal elements composed of polysaccharide elements[34].

Our data provide evidence that the majority of Candida spp. recovered from the plastic devices (75%) and bloodstream isolates (71%) have higher capacity to produce biofilm. Similar results were obtained by other studies[6,35]. Kuhn et al, (2002) reported that Invasive C. albicans isolates form more biofilm than noninvasive isolates [34]. Candida spp. are frequently found in the normal microbial flora of humans, which facilitates their encounter through implanted biomaterials and host surfaces [5]. The devices become colonized by Candida which forms biofilm, the detachment of which can result in candidemia. Indwelling catheters therefore, represent a major risk factor associated with nosocomial candida infections[32]. On the other hand the least biofilm producer isolates were observed in HVS 3/10 (30%) and respiratory tract 20/41 (48.7%). These findings were in concordance with studies conducted by Golia et al., 2011 [6]. It could readily be hypothesized that for certain
body sites, colonization requires a particular phenotype with respect to biofilm formation. Such a biofilm phenotype might be genetically rather than environmentally governed [36].

In this study the resistance of all the isolated *Candida* spp. to fluconazole was 11%. The study by Nemati et al (2008), reported that the rate of resistance to fluconazole among *Candida* spp. ranged from null to the 15% [37]. Furthermore, our data on the fluconazole against *C. albicans*, revealed that 95% of tested strains were susceptible. This sensitivity rate is more or less comparable with those rates of 87.5% and 89.5% previously reported by Citak et al, (2005) and Badiee and Alborzi (2011), respectively [38,39]. In agreement with the study of Sabatelli et al. (2006)[40], most of the detected resistant strains belong to non-albicans spp. (22.5%), emphasizing, its greatest potential to acquire resistance to fluconazole. Also, in agreement with the finding of Ng et al (2000) who reported, amphotericin B and ketoconazole susceptibility data and showed that all yeast isolates were susceptible. The resistance to antifungal agents such as fluconazole has been noted among non-albicans isolates. The possibility of increase in the percentage of the resistance to antifungal agents among non-albicans spp. Is due to widespread use of antifungal drugs, long-term use of suppressive azoles, and the use of short courses of antifungal drugs [10].

5. CONCLUSION

The present study proved that *C. albicans* is still the major isolate from urinary, vaginal and respiratory samples but non-albicans spp. predominate in the blood samples and from plastic devices. Biofilm positivity occurred most frequently in isolates of *C. parasilosis* followed by *C. topicals*, *C. lusitaniae*, *C. albicans*, and *C. glabrata*. Non-albicans spp. commonly resistant than *C. albicans* to fluconazole agents.

ETHICAL APPROVAL

Ethical committee of Al-ansar hospital approved the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jacqueline M, Achkar, Bettina C. Candidal Infections of the Genitourinary tract. Clin. Microbiol. Rev. 2010;23(2):253-72.
2. Mohandas V, Ballal M. Distribution of Candida Species in Different Clinical Samples and Their Virulence: Biofilm Formation, Proteinase and Phospholipase Production: A Study on Hospitalized Patients in Southern India. J. Glob. Infect. Dis. 2011;3(1):4–8.
3. Seneviratne CJ, Jin L, Samaranayeke LP. Biofilm lifestyle of Candida: A mini review. Oral. Dis. 2008;14:582-590.
4. Aparna MS, Yadav S. Biofilms: Microbes and Disease. The BrazJ.Infect. Dis. 2008;12:526-530.
5. Dominic RM, Shenoy S, Baliga S. Candida biofilms in medical devices. Evolving trends. Kath. Univ. Medical. J. 2007;5(3):431-436.
6. Golia S, Hittinahalli V, Sangeetha KT, Vasudha CL. Study of biofilm formation as a virulence marker in candida species isolated from various clinical specimens. JEMDS. 2011;1:1238-1246.
7. Aoki T, Moro H, Koshio N, Tamura T, Tanabe Y, Kawai H, Matsuto T, Okada M. Results of antifungal susceptibility testing of Candida species and trends of antifungal use in Niigata university medical and dental hospital, RinshoByori. 2010;58(7):658-63.
8. Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S. Culture and Candida albicans from Vaginal Ulcer an lase on SDS-PAGE. Interna. J. Biol. 2010;2:84.
9. CLSI, Clinical and Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing of yeasts: Approved guideline M44-A, Clinical and Laboratory Standards Institute, Wayne, PA, USA. 2004:65-74.
10. Ng KP, Saw TL, Na SL, Soo-Hoo TS. Systemic Candida infection of University Hospital 1997-1999: the distribution of Candida biotypes and antifungal susceptibility patterns. Mycopathologia. 2000;149(3):141-146.
11. Ruzicka F, Hola V, Votava M, Tejkalova R. Importance of biofilm in Candida parapsilosis and evaluation of its susceptibility to antifungal agents by colorimetric method. Foha. Microbiol. 2007;52(3):209-214.
12. Shin JH, Kee SJ, Shin MG, Kim SH, Shin DH, Lee SK. Biofilm production by isolates of Candida species recovered from nonneutropenic patients: Comparison of blood stream isolates from other sources. J. Clin. Microbiol. 2002;40:1244-1248.
13. Dag I, Kiraz N, Yasemin OZ. Evaluation of different detection methods of biofilm formation in clinical Candida isolates. Afri. J. Microbiol. Res. 2010;4(24):2763-2768.
14. Nerurkar A, Solanky P, Chavda N, Baria H, Desai B. Isolation of Candida Species in Clinical Specimens and its Virulence Factor. The Biofilm. Int J Med Sci Public Health. 2012;1(2):97-100.
15. Sheevani, Sharma P, Aggarwal A. Nosocomial Candida Infection in a Rural Tertiary Care Hospital. J. Clin. Diagn. Res. 2013;7(2):405-406.
16. Mokaddas EM, Al-Sweih NA, Khan ZU. The species distribution and the antifungal susceptibility of Candida bloodstream isolates in Kuwait: A 10 year study. J Med Microbiol. 2007;56:255-9.
17. Chakraborti A, Ghosh A, Batra R, Kaushal A, Roy P, Singh H. Antifungal susceptibility patterns of the non-albicans Candida species and the distribution of the species which were isolated from candidaemia cases over a 5 year period. Indian J Med Res 1996;104:171-6.
18. Zarei-Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh B. Candiduria in hospitalized patients in teaching hospitals of Ahvaz. Iran. J. Microbiol. 2012;4(4):198–203.
19. Alhussaini MS, El-Tahtawi NF, Moharram AM. Phenotypic and molecular characterization of Candida species in urine samples from renal failure patients. Sci. J. Clin. Med. 2013;2(1):14-25.
20. NaymanAlpat S, Özguneş I, Ertem OT, Erben N, DoyukKartal E, Tözun M. Evaluation of risk factors in patients with candiduria. Mikrobiyol. Bul. 2011;45(2):318–24.
21. Jain N, Kohli R, Cook E, Gialanella P, Chang T, Fries BC. Biofilm formation by and antifungal susceptibility of Candida isolates from urine. Appl. Environ. Microbiol. 2007;73(6):1697-1703.
22. Emam SM, Abo Elazm AA, Morad AWA Exoenzymes Production and Antifungal Susceptibility of Candida Species Isolated from Pregnant Women with Vulvovaginitis. J. Amer. Sci. 2012;8(12).
23. Hasana F, Xessa I, Wangc X, Jainc N, Friesb BC. Biofilm formation in clinical Candida isolates and its association with virulence. Microbes Infect. 2009;11(8-9):753–7.
24. D’Antonio D, Romani F, Pontieri E, Carruba G. Catheter related candidaemia caused by Candida lipolytica in a patient receiving allogenic bone marrow transplantation. J. Clin. Microbiol. 2002;40:1381-1386.
25. Mohandas V, Ballal M. Proteinase and phospholipase as virulence factors in Candida isolated from blood. Rev. Iberoam. Micol. 2008;25:208-210.
26. Giri S, Kindo. A review of Candida species causing blood stream infection. Indian J of Med Microbiob. 2012;30(3):270-278
27. Trofa D, Ga’cser A, Joshua D. Nosanchuk: Candida parapsilosis, an Emerging Fungal Pathogen; Clinical microbiology reviews, Oct. 2008;21(4):606–625.
28. Dharwad S, Dominic S. Species Identification of Candida Isolates in Various Clinical Specimens with Their Antifungal Susceptibility Patterns. Journal of Clinical and Diagnostic Research. November (Suppl-1) 2011;5(6):1177-1181.
29. Kothavade RJ, Kura MM, Valand AG, Panthaki MH. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to Fluconazole. Med Microbiol. 2010;59:873-880.
30. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar Candida for rapid screening of clinical specimens for Candida albicans, Candida tropicalis, Candida krusei, and Candida (Torulopsis) glabrata. J. Clin. Microbiol. 1996;34:58-61.
31. Baillie GS, Douglas LJ. Candida biofilm and their susceptibility to antifungal agents. Methods Enzy. Mol. 1999;310:644-656.
32. Girish Kumar CP, Menon T. Biofilm production by clinical isolates of Candida species. Med. Mycol. 2006;44:99-101.
33. Muni S, Menon S, Chande C, Gohil A, Chowdhary A, Joshi A. Candida biofilm. Bombay. Hosp. J. 2012;54(1).
34. Kuhn DM, Chandra J, Ghannou MA. Comparison of Biofilms Formed by Candida albicans and Candida parapsilosis on Bioprosthetic Surfaces. Infect Immun. February 2002;70(2):878–888.
35. Singhai M, Malik A, Shahid M, Malik MA, Rowat V. Colonization of peripheral intravascular catheters with biofilm producing microbes: Evaluation of risk factors. Niger. Med. J. 2012;53(1):37–41.
36. Silva S, Henriques M, Martins A, Oliveira R, Williams D &Azeredo J : Biofilms of non- Candida albicans Candida species: quantification, structure and matrix composition. Medical Mycology November 2009;47:681–689
37. Nemati SL, Shams-Ghhfarokhi M, Yadegari MH. Evaluation of disk diffusion and microdilution methods for Fluconazole susceptibility testing in one group of Candida spp. in Tehran. Daneshavar Med. 2008;15:51-58.
38. Citak S, Ozcelik B, Cesur S and Abbasoglu U. In Vitro Susceptibility of Candida Species Isolated from Blood Culture to Some Antifungal Agents. Jpn J Infect Dis. 2005;58(1):44-6.
39. Badiiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. Iran J Microbiol. 2011;3(4):183-8.
40. Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, Loebenberg D, Black TA, McNicholas PM. In vitro activities of Posaconazole, Fluconazole, Itraconazole, Voriconazole, and Amphotericin B against a large collection of clinically important moulds and yeasts. Antimicrob. Agents Chemother. 2006;50(6):2009-2015.

© 2013 Mohamed and Al-Ahmadey: This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=246&id=8&aid=1914