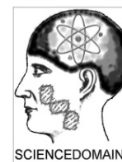
British Journal of Medicine & Medical Research
15(9): 1-15, 2016, Article no.BJMMR.25940
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org



Nosocomial Candiduria in Critically Ill Patients Admitted to Intensive Care Units in Menoufia University Hospitals, Egypt

Amira A. Alkilani[1], Abeer H. El Shalakany[1], Eman A. El-Masry[2*], Elham T. Awad[3] and Eman A. Mohamad[4]

[1]Department of Clinical Pathology, Faculty of Medicine, Menoufia University, Egypt.
[2]Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt.
[3]Microbiology Unit, Shebin El-Kom Teaching Hospital, Egypt
[4]Department of Medical Microbiology and Immunology, Faculty of Medicine for Girls, Al Azhar University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors 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. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/25940
Editor(s):
(1) S. U. Fuhong, ICU Laboratory, Erasme Hospital, Free University Brussels, Brussels, Belgium.
(2) Nurhan Cucer, Erciyes University, Medical Biology Department, Turkey.
(3) Chan Shen, Department of Biostatistics, MD Anderson Cancer Center, University of Texas, USA.
Reviewers:
(1) Paul E. Imade, University of Benin Teaching Hospital, Nigeria.
(2) Ronald Bartzatt, University of Nebraska, USA.
Complete Peer review History: http://sciencedomain.org/review-history/14603

Original Research Article

Received 27th March 2016
Accepted 3rd May 2016
Published 13th May 2016

ABSTRACT

Aims: Detect the incidence of urinary tract infection caused by *candida species* and to determine their antifungal susceptibility, biofilm formation and its minimal biofilm inhibitory concentration. In addition, detect the importance of multiplex nested polymerase chain reaction (PCR) in detection of candidemia in serum of patients with candidurea.
Methodology: Study was carried out by collecting urine samples from 200 patients admitted in the

*Corresponding author: E-mail: emanshma@yahoo.com;

intensive care unit inMenoufia university hospitals and suspected to have hospital acquired urinary tract infection. Isolation, identification and antifungal susceptibility testing were done. Biofilm formation and Minimum biofilm inhibitory concentration testing were detected. Patients with positive candiduria were tested for the presence of *candida* in serum by multiplex nested PCR.

Results: *Candida* spp. were isolated from urine of 38(19%) patients, 78.9% of them were catheterized, *C. albicans* was isolated from 18(47.3%) samples as detected by Analytical profile index (API system). Antifungal susceptibility show that Flucytosmine, Amphotericin B, Voriconazole were more effective antifungal agents against *Candida spp* (100%, 84.2% and 84.2% respectively). A total of 26 (%68.4) out of 38 *Candida* species isolates produced biofilm. 72.2% of the tested *C. albicans*, were resistant to fluconazole and had MBIC > 640 µg/mL while only 27.8% were sensitive to fluconazole and had MBIC < 10 µg/mL. 26.3% out of 38 patients with candiduria had *candida*emia as detected by multiplex nested CR.

Conclusion: *Candida albicans* is the most common *Candida* spp that show biofilm production. There is increased in the percentage of the resistance to fluconazole in *candida* isolates in this study. The incidence of candidemia among patients with candidurea was high in our study.

Keywords: Candiduria; biofilms; MBIC; antifungal susceptibility; multiplex nested PCR.

1. INTRODUCTION

Nosocomial candidal infections had emerged as an increasing problem in the last years [1]. *Candida spp* rarely encountered in urine is in healthy people with structurally normal urinary tract. However, It is of common occurrence in hospitalized patients [2,3].

Candida spp are opportunistic mycoflora found frequently in genitourinary tract of humans especially in the immunocompromised, diabetics or pregnant women. If left untreated, this may lead to systemic candidiasis, multiple organ failure or death [4].

In the Intensive Care Units (ICU), there are many risk factors that can leads to increasing candiduria, such as use of indwelling urinary devices, frequent use of antibiotics, diabetes mellitus, immunosuppressive therapy, severity of the underlying illness, extreme of age, female sex, and prolonged hospitalization [5].

The genus *Candida* includes several species implicated in human pathology such as *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. lusitaniae, C. kefyr, C.guilliermondii and C. dubliniensis* [6]. *Candida albicans* is by far the most common cause of mucosal yeast infection.

Candida expresses a variety of virulence factors that contribute to its pathogenesis for persistent infection and tissue damage of the host when immunity is debilitated [7,8]. Major virulence factors of *Candida* are its ability to adapt to a variety of habitats of the body (oropharyngeal, gastrointestinal and female genitalia), adherence to host cells, the ability to switch between the yeast form and filamentous (pseudo hyphae formation), biofilm formation and production of hydrolytic enzymes such as proteinases, phospholipases, lipases and other factors play a major role in successful colonization and subsequent infection of *Candida* [8].

The important factors contributing to the virulence of *Candida* is the formation of surface attached microbial communities known as "biofilms" [9]. 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 [10]. Biofilm formation helps the organism to evade host defenses, exist as a persistent source of infection and develop resistance against antifungal agents [11,10]. The resistance of biofilm forming *Candida* spp. to antifungal agents represents a major challenge especially in the design of therapeutic and prophylactic strategies [12]. The detachment of cells from an adherent biofilm on a catheter can give rise to a septicemia that may respond to conventional drug therapy. However, biofilm cells are not killed by such treatment and remain as a reservoir of infection until the implant is removed [13].

Candidemia is late-onset ICU-acquired infection associated with high mortality [14].

It is associated with a considerable increase in hospital costs and length of hospital stay [15].

The evidences to determine a proper correlation between candiduria and invasive candidiasis is

not yet clear or well established, due to publication of conflicting results and the lack of consensus [16].

2. MATERIALS AND METHODS

This study was done in intensive care unit at Menufiya University Hospitals, Egypt between October 2014 and April 2015 included 200 patients admitted in the intensive care unit and suspected to have hospital acquired UTI. Detailed informations, including age, gender, use of catheter, antibiotics, diabetes mellitus.

Urine specimens were collected as midstream morning sample or from the port of the catheter and processed form microbiological examinations, culture, identifications and antimicrobial sensitivity.

2.1 Culturing on Sabouraud's Dextrose Agar (SDA)

All samples were cultured onto Sabouraud's Dextrose Agar (SDA) (HiMedia, Mumbai, India) plates supplemented with 0.05% (W/V) chloramphenicol [17]. Cultures were incubated at 37ºC for 24-48 hours .

2.2 Culturing on CHROM Agar *Candida*

Chrom agar contain chromogenic substrates which react with enzymes secreted by the target microorganisms to yield colonies of varying colours [18]. In this study, we used CHROMagar *Candida* from (CHROM agar Company, Paris, France). Separate colonies proved to be *Candida* by growth on Sabouraud's dextrose agar and gram stain were used to grow on chrom agar.

Presumptive identification was done based on colony color of the growing *Candida* strains.

C. kruseiATCC® 14243 ....pink and fuzzy

*C. albicans*ATCC® 60193 .... green

C. tropicalisATCC® 1369 .... metallic blue

C. glabrataATCC® 2001 .... mauve

E. coli ATCC® 25922 ....inhibited

2.3 Germ-Tube Test

Small inoculum of suspected *Candida* cultures that had been confirmed by chrom agar were inoculated into 1 ml of human serum in a small tube and incubated at 37ºC for 2 hours. The germ tube is indicative of *C. albicans* and *C. dubliniensis*. This test is negative with other *Candida* species. [17].

2.4 Culturing on Corn Meal Tween 80 Agar (CMA)

As recommended by [19,20], chlamydospore formation by certain *Candida* species (*C. albicans* and *C. dubleniensis*) is an encouraged by culturing on CMA.

2.5 API Identification System

Integral system yeasts Plus (Liofilchem, s.r.l., Italy), for Identification and Antifungal Susceptibility Testing of the main pathogenic yeasts from Clinical Specimen. For Yeast Identification, the kit contain 12 sugar assimilation tests and 1 new chromogenic test. For Antifungal Susceptibility Testing, 10 standardized antifungal agents (Nystatin, Amphotericin B, Flucytosine, Econazole, Ketoconazole, Clotrimazole, Miconazole, Itraconazole, Voriconazole, Fluconazole)

2.6 Antifungal Susceptibility Test

The disc diffusion test was performed according to the procedure described in the Clinical and Laboratory Standard Institute [21]. The turbidity was adjusted to yield 0.5 McFarland standard. Sex kinds of antifungal agents obtained from HiMedia Company in India were tested. The interpretative breakpoints of these antifungal agents were done according to [22] as shown in Table 3.

Candida isolates were stored in distilled water at room temperature and subcultured on Sabouraud dextrose agar 48 hours prior to further study [23,24].

2.7 Biofilm Formation

A total of 38 *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 resuspended in 5 ml of sterile saline, and turbidity adjusted to 3 of the McFarland scale. To each well of the microtiter plates (flat bottom) 180 µl of SDB with 8% glucose were added, then inoculated with 20 µl of the above yeast cell suspension. Yeast free medium controls were also included. The plates were then incubated for 48 h at 37ºC.

Table 1. Interpretative breakpoints of antifungal agents

Antifungal agents (Abbreviation)	Concentration /disc	Zone of activity in mm		
		Sensitive	Intermediate	Resistant
Nystatin (NYS)	100U	≥15	10 - 14	<10
Amphotericin-B (AM-B)	100U	≥15	10 - 14	<10
Ketoconazole (KET)	10ug	≥28	21 - 27	≤20
Clotrimazole (CLO)	10ug	≥20	12 - 19	≤11
Itraconazole (ITR)	10ug	≥23	14 - 22	≤13
Fluconazole (FLU)	25ug	≥19	15 - 18	≤14

After incubation, plate growth was assessed by measuring the absorbance at 490 nm. The medium in wells was removed and washed three times with sterile phosphated buffer solution (PBS). Microplates were stained with 200 µl 1%crystal violet for 5 minutes and the plate was allowed to stand for 20 minutes at room temperature and then read at 405 nm by an ELISA reader The percent transmittance (%t) was measured.All tests were done in triplicates Finally, adherent biofilm layers were scored as either negative; weak (+) (percentage transmittance (%T ≤ 20)); moderate, (++) (%T = 20-35); strong (+++) (%T = 36-50) and very strong (++++) (%T ≥ 50) [25].

2.8 Minimum Biofilm Inhibitory Concentration Testing (MBIC)

The MBIC testing was performed by using the calorimetric indicator resazurin. Fresh *Candida* colonies were resuspended in 0.9% NaCl and opacity adjusted to 3 McFarland. The stock solution of resazurin dye was prepared by diluting the resazurin sodium salt in distilled water at 0.01% (w/v) which was filter sterilized and then added to RPMI medium (buffered to pH 7.0 using 165 mmol/L3 - N-morpholino) propanesulfonic acid) in a 1:10 ratio (0.001%) [26].

Biofilms were formed on presterilized, polystyrene, flat-bottom 96-well microtiter plates. Biofilms were formed by pipetting 90 µl of Sabouraud's dextrose broth supplemented with 8% glucose and 10 µl of standardized cell suspensions (prepared as above) into wells of the microtiter plate and incubating them for 48 hours at 37°C. After biofilm formation, the medium was aspirated, and thoroughly washing the biofilms three times in 0.9% sterile saline, then the antifungal agents were added.

Different concentrations of fluconazole (1 to 1280 µg/ml) were made from stock solutions using RPMI medium with 0.001% resazurin dye. 100µl of each dilution of the antifungal agent was aseptically added to the wells and incubated for a further 48 hours at 37°C to detect the viability of biofilms. A series of wells without the antifungal agent and un inoculated wells served as positive and negative controls, respectively. MBIC was determined as the lowest concentration of the antifungal agent maintaining the blue color of calorimetric medium [26].

2.9 Detection of *Candida* DNA in Serum by Multiplex Nested PCR

2.9.1 DNA extractions [27]

Sample preparation:

1- 500 µl EDTA anticoagulated blood were mixed with 1500 µl blood lysis solution and were incubated on ice for 10-15 minutes.
2- The mixture was centrifuged at 3000 rpm for 10 min and the supernatant was discarded.
3- Steps 1 and 2 were repeated, then step 4 is continued.
4- The cell pellet was resuspended in 1 ml Nucleolysis solution and was incubated at 65°C for 45 min.
5- Then centrifugation was done at 5000 rpm for 10 min and the supernatant was discarded.
6- 500 µl lyticase solution were added and incubated at 37°C for 30 min to produce spheroplasts
7- Centrifugation was done at full speed for 10 min and the supernatant is discarded.
8- Tissue protocol is then continued from Mammilian tissue Genomic DNA Purification Protocol.

Table 2. Primers and there product size (pb)

Primer	Sequence (5- -3-)	Product size (pb)
ITS 1/4	F- TCCGTAGGTGAACCTGCGG R- TCCTCCGCTTATTGATATGC	variable
Candida albicans	F-TTTATCAACTTGTCACACCAGA R- ATCCCGCCTTACCACTACCG	272 bp
Candida tropicalis	F- CAATCCTACCGCCAGAGGTTAT R- TGGCCACTAGCAAAATAAGCGT	357 bp
Candida krusi	F- ACTACACTGCGTGAGCGGAA R- ACTACACTGCGTGAGCGGAA	362 bp

2.9.2 PCR primers

- The fungus-specific universal oligonucleotides ITS1 and ITS4 were used as outer primers. In the second amplification, the previously described inner primers for *C. albicans*, , C. tropicalis, and C. krusei were used.: [28]

2.9.3 Amplification

The total reaction volume for the first – round PCR amplification was 25 µl.

i) **First – round PCR amplification**: an initial denaturation step of 5 min at 95℃ followed by 35 cycles of 45 s at 95℃, 45 s at 50℃, and 45 s at 72℃, with a final extension of 5 min at 72℃. The reactions were carried out in a thermocycler.(peco, made in Germany).

   N.B The second round of amplifications were performed in two separate assays: assay 1, containing primers C. tropicalis, *C. albicans*, at concentrations of 0.12 µM, 0.2 µM, respectively; and assay 2, containing primers C. kruzi, at concentrations of 0.2 µM, In both assays 2 µL of a 1:100 dilution of the ITS PCR product was used as the DNA template [29].

   The total reaction volume for the second – round PCR amplification is 20 ul.

ii) **Second – round PCR amplification**. The amplifications were carried out in the same thermocycler (peco, made in Germany) under the following conditions: an initial denaturation step of 5 min at 95℃, 10 cycles of 45 s at 95℃, 45 s at 67-58℃ (touchdown), and 45 s at 72℃ followed by 20 cycles of 45 s at 95℃, 45 s at 58℃, and 45 s at 72℃, with a final extension of 5 min at 72℃ [29].

In all experiments, negative controls containing sterile water instead of genomic DNA and positive controls containing *Candida* DNA were tested

2.9.4 Detection

The nested PCR products were detected on 2.5% agarose gels stained with ethedium bromide and visualised under a UV trans illuminator apparatus (UVITEC, Cambridge, UK).

3. RESULTS

Candiduria was proved in 38 out of 200 urine samples (19%), bacterial infection was 85 out of 200(42.5%),other fungi was 2(1%), while 75 (37.5 %) showed no growth.

Age > 50, female gender, stay above 10 days in ICU, previous antibiotic therapy and urinary catheterization were the main risk factors for developing candiduria.

Total no. of *Candida* was 38 as detected by API, 18(47.3%) of them was *Candida albicans*, 19(50%) of them was *Candida* tropicalis, and 1 (2.6%) of them was *candida* kruzi.

Flucytosmine, Amphotericin B, Voriconazole were the most effective antifungal against *Candida* spp (sensitivity 100%, 84.2% and 84.2% respectively).

All isolates of *C. albicans*, c. tropicalis and c. krusei were sensitive to Flucytosamin,

26(68.4%) of total isolates of *Candida* were biofilm producers, 15(83.3%) of *C. albicans* were positive biofilm producers, 11(57.9%) of C. tropicalis were positive biofilm producers and no one of C. kruzi produce biofilm.

Table 3. Demographic profile and various risk factors in patients with candiduria

Risk factor	No. of specimen		*Candida* infection		Other fungi		Bacterial infection		No growth	
	No.(200)	**%**	**No.(38)**	**%**	**No.(2)**	**%**	**NO.(85)**	**%**	**NO.(75)**	**%**
Age										
17-50	80	40%	16	42.1%	0	0%	28	32.9%	36	48%
>50	120	60%	22	57.9%	2	100%	57	67.1%	39	52%
Sex										
Males	125	62.5 %	18	47.4%	2	100%	48	56.5%	57	76%
Females	75	37.5%	20	52.6%	0	0%	37	43.5%	18	24%
Duration of ICU stay										
3-10 days	48	24%	10	26.3%	1	50%	20	23.5%	17	22.7%
above 10 days	152	76%	28	73.7%	1	50%	65	76.5%	58	77.3%
Antibiotic therapy										
Yes	140	70%	38	100%	2	100%	65	76.5%	35	46.7%
No	60	30%	0	0%	-	0%	20	23.5%	40	53.3%
Diabetes mellitus										
Yes	35	17.5%	12	31.5%	0	0%	23	27%	0	0%
No	165	82.5%	26	68.4%	2	100%	62	73%	75	100%
Urinary catheterization										
Yes	113	56.5%	30	78.9 %	1	50%	77	90.5%	5	6.7%
No	87	43.5%	8	21.1%	1	50%	8	9.5%	70	93.3%
total	200	100%	38	19%	2	1%	85	42.5%	75	37.5%

Table 4. Identification of C. *Spp* by Chromagar, germ tube test, Corn-meal agar Tween 80 mediaand API

Media	*C. albicans*		*C. tropicalis*		*C. krusei*	
	No	%	No	%	No	%
Chrom agar	15/38	39.4	17/38	44.7	6	15.7
Corn-meal agar Tween 80	18	47.3	15	39.4	5	13.1
Germ tube test	18	47.3	0	0	0	0
API	18	47.3	19	50	1	2.6%

Table 5. Sensitivity to antifungal drugs among *Candida* species

	Sensitivity to antifungal drugs N = 38					
	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
Nystain (NY)	3	7.8	9	23.6	26	68.4
Amphotericin (AMB)	32	84.2	4	10.5	2	5.2
Flucytosmine (FCX)	38	100	0	0	0	0
Econazole (ECN)	5	13	1	2.6	32	84.2
Ketonozole (KCA)	25	65.8	13	34.2	0	0
Clotrimoxzole (CLO)	5	13	11	29	22	57.8
Micoconazole (MIC)	3	7.9	1	2.6	34	89.4
Itraconazole (ITR)	26	68.4	10	26.3	2	5.2
Voriconazole (VOR)	32	84.2	4	10.5	2	5.2
Fluconazole (FLU)	10	26.3	8	21	20	52.6

Table 6. Antifungal susceptibility tests among *Candida* species of patients with candiduria by using integral system yeasts plus

Antifungal agents	*C. albicans* (No= 18)			*C. tropicalis* (No = 19)			*C. krusei* (No = 1)		
	SS	DD	R	SS	DD	R	SS	DD	R
Nystatin (NY)	2	5	11	1	3	15	0	1	0
Amphotericin(AMB)	15	1	2	16	2	1	1	0	0
Flucytosamin(FCX)	18	0	0	19	0	0	1	0	0
Econazole (ECN)	4	1	13	1	0	18	0	0	1
Ketoconazole(KCA)	9	9	0	15	4	0	1	0	0
Clotrimoxzole (CLO)	2	8	8	3	3	13	0	0	1
Micoconazole (MIC)	1	1	16	1	0	18	1	0	0
Itraconazole (ITR)	10	5	3	16	1	2	0	1	0
Voriconazole (VOR)	16	2	0	15	2	2	1	0	0
Fluconazole (FLU)	3	3	12	7	5	7	0	0	1

Table 7. Biofilm production by *candida* species

Candida species (38)	Positive biofilm		Negative biofilm	
	No	%	No	%
C. albicans=18	**15**	**83.3**	**3**	**16.7**
C. tropicalis =19	11	57.9	8	42.1
C. krusei=1	0	0	1	100
Total (26)	26	68.4	12	31.6

There was statistically significant difference regarding biofilm formation among catheterized than non-catheterized patients (P=0.003).

72.2% of the tested *C. albicans*, were resistant to fluconazole and had MBIC > 640 µg/mL while only 27.8% were sensitive to fluconazole and had MBIC < 10 µg/mL, 100 % of the tested C.

tropicalis, were resistant to fluconazole and 100 % of the tested C. kruzi, were resistant to fluconazole >1280 µg/mL.

26.3% out of 38 candiduric patients had *Candida* in their blood, 60% of them were *C. albicans* and 40% were *C. tropicalis*

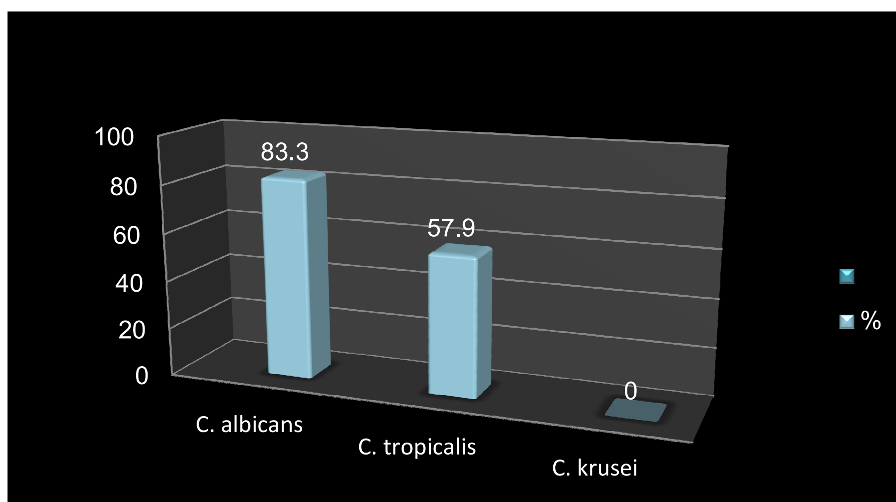


Fig. 1. Biofilm production by *Candida* species

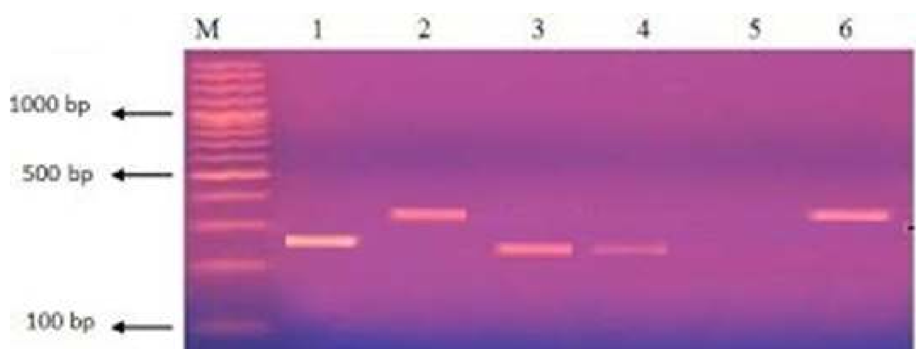


Fig. 2. Agarose gel electrophoresis showing the nested multiplex PCR products obtained from patients serum: Lane M, GeneRuler^{TM} 100 pb plus DNA ladder (thermoscientific, fermentas, UE), Lanes 1,3, and 4 showing *C. Albicans* (272 bp), Lanes 2 and 6 showing *C. tropicalis* (357 bp), Lane 5 showing negative control

Table 8. Frequency of biofilm formation among candiduric patients according to presence or absence of a catheter

	Positive biofilm Formation		Catheterized patients		Non catheterized patients		P value
	No	%	No	%	No	%	
C. albicans (No= 18)	15	83.3	12	80	3	20	0.047
C. tropicalis (No =19)	11	57.9	8	72.7	3	27.2	0.040
C. krusei (No = 1)	0	0	0	0	0	0	
38	26	68.4	20	76.9	6	23.1	0.003

Table 9. Minimal biofilm inhibitory concentration (MBIC) of *Candida* species against different concentrations of Fluconazole, µg/mL

Fluconazole concentration ug/ml	*C. albicans*	*C. tropicalis*	*C. krusei*	Total No %
>1280	7(38.8)	10 (52.6)	1(100)	18(47.3)
1280-640	6(33.3)	7(36.8)	0	13(34.2)
639-320	0	2(10.5)	0	2(5.3)
319-160	0	0	0	0
159-80	0	0	0	0
79-40	0	0	0	0
39-20	0	0	0	0
19-10	0	0	0	0
<10	5(27.7)	0	0	5(13)
Total	18(100)	19(100)	1(100)	38(100)

Table 10. Results of Nested PCR on serum of patients with candiduria

Candiduria		Nested multiplex PCR candidemia	
No	**%**	**No**	**%**
38 /200	19	10/38	26.3
C. albicans (18/38)	47.3	*C. albicans* (6/10)	60%
C. tropicalis (19/38)	50	*Tropicalis*(4/10)	40%
C. krusei (1/38)	2.6	*C. krusei*(0)	0

4. DISCUSSION

Urinary tract infections (UTIs) account for 20–50% of all hospital-acquired infections occurring in the Intensive Care Unit (ICU). In some reports, UTI found to be more frequent than hospital-acquired pneumonia and intravascular device bacteremia, with a greater incidence in developing countries [30,31].

Results of our study showed that, *Candida spp*. were isolated from urine of 38(19%) patients out of 200 hospitalized ICU patients. In similar studies, the mean incidence of candiduria was 44.4% [32], and 32.26% [1].

Age of the patients with candiduria in this study ranged between 17 and 74 years with a mean age 51±11, and Candiduria was more frequent in patients above 50 years (22 patients, 57.9%). This finding was in agreement with results obtained by [33,32,5]. This could be due to lowered host defenses in these patients.

As regard sex, candidurea was more common in female (52.6%) than male patients (47.4%). Candiduria was more frequent in females because they are more liable to develop ascending infection [2]. Some reasons for higher prevalence of candiduria in females compared with males may be their shorter urethral length, transmission from the genital tract to the urinary tract, and the anti-*Candida* activity of prostatic fluid in male [34]. On the other hand, candiduria was more common in males (61.4%) [5].

The duration of ICU stay in the patients ranged from 3 to 18 days (mean, 11±6). Twenty-eight (73.7%) of patients had a history of ICU stay >10 days (Table 3). Extended ICU stay may increase the risk for ICU-acquired UTIs [1]

Indwelling urinary catheter is one of the most imperative risk factor contributing to the overgrowth of *Candida* spp. [1,26]. Among the investigated patients with candidurea in this study, 78.9%were urinary catheterized. Hundred (100%, 92.6 and 84.4) of patients with candidurea were catheterized. [34,32,35]

High percent of candiduria in catheterized patients in this study may be because the catheter act as foreign body on which *Candida* can colonize and form biofilm the catheter itself can introduce infection and favor epithelial surface fungal colonization. The urinary catheter connects the heavily colonized perineum with the normally sterile bladder, and it provides a route for bacterial and *Candida* entry along both its external and internal surfaces. Urine often pools in the bladder or in the catheter itself, and urinary stasis encourages pathogen multiplication. Obstruction of the catheter can lead to over distension and ischemic damage of the bladder mucosa, thus, increasing its susceptibility to bacterial and *Candida* invasion. The catheter also damages the bladder mucosa by triggering

an inflammatory response and by mechanical erosion. Monthly urine cultures for patients with long-term indwelling catheters show that the bacterial flora is constantly shifting and changing, regardless of antibiotic use [36].

Our study showed that 100% of patients with positive candidurea were under antibiotic therapy. The risk of candiduria was highest after treatment with Imipenam/Meropenam group of drugs (75%), followed by cephalosporin (57%) [26].

The broad-spectrum antibiotics play a critical role in the pathogenesis of candiduria by suppressing susceptible endogenous bacterial flora in the gastrointestinal and lower genital tracts. Antibiotic favors epithelial surface fungal colonization of the urinary tract especially in the presence of indwelling bladder catheter through impairing phagocytic activity and antibody synthesis [37].

Diabetes is a well-known risk factor for developing nosocomial UTI due to *Candida spp* [26].

Results of the present study (Table 3) showed that 12(31.5%) of *Candida* infected patients were diabetic Risk factors for nosocomial candiduria showed that candiduria was increased by 2-fold in the presence of diabetes mellitus [38,39]. This is because diabetes lowers host resistance to invasion by fungi through impairment of phagocytic activity of macrophages and promotes stasis of urine in neurogenic bladder. [26,1].

In our study, *C. albicans* were isolated from 18 (47.3%) samples of candiduria as detected by API system .Similar to our results [40,41] also reported the most commonly isolated species was *C. albicans* (61% and 61.36%). On the other hand, a study conducted by [42] showed non-albicans *Candida,* predominated (54.1%) over *C. albicans* (45.9%). Lower prevalence rates (39.5%) of *C. albicans* were reported [43].

Among non albicans(NAC) spp, *C. tropicalis* was the major isolate (50%) followed by *C. krusei*. In addition, among NAC spp. *C.tropicalis* was the major isolate [44].

Results of antifungal susceptibility in our study show that Flucytosmine, Amphotericin B, Voriconazole were the most effective antifungal against *Candida spp* (sensitivity 100%, 84.2% and 84.2% respectively). Similar to these results all *Candida* spp isolates were susceptible to amphotericin B and ketoconazole and voriconazole [40,45].

Resistance of *Candida* to fluconazole in the present study was 52.6%, this differ from results in [40] study, the resistance of all the isolated *Candida spp* to fluconazole was 11%, and [46] reported that the rate of resistance to fluconazole among *Candida spp*. ranged from null to the 15%. Furthermore, our data on the fluconazole against *C. albicans*, revealed that 16.6% of tested strains were susceptible to fluconazole. This sensitivity rate is more or less comparable with those rates of 95% and 89.5% previously reported by [40,47] respectively.

This increased rate of resistance to multiple azoles explained also by an upreglutation of CDR genes that encode the CDR efflux pumps [37]. The possibility of increased in the percentage of the resistance to fluconazole in *candida* isolates in this study, may due to widespread use of antifungal drugs, long-term use of suppressive azoles, and the use of short courses of antifungal drugs.

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 [40].

In the present study, 26(%68.4) out of 38 *Candida* species isolates obtained produced biofilm. Also, (50.8%, 56.7% and 61%) of *Candida* species were biofilm producers [48-50].

There was statistically significant difference regarding biofilm formation among catheterized than non-catheterized patients (P=0.003). The difference between the biofilm forming ability of isolates from catheter associated UTI patients and UTI without indwelling catheter patients was pointed out by [51].

The reason that biofilm is so prevalent on urinary catheters is that it conveys a survival advantage to the microorganisms; for this same reason urinary catheter biofilm is difficult to eradicate. Organisms in a biofilm function as a community and communicate closely with one another. Survival advantages conferred by the biofilm community include resistance to being swept away by simple shear forces, resistance to phagocytosis, and resistance to antimicrobial agents [36].

Biofilm formation was detected in 83.3% of *C. albicans* and 57.9% of *C. tropicalis*. While all *C.krusei* isolates were negative. *C. albicans* produces quantitavely more biofilm than other *Candida* species [52]. On the other hand, results of [50,53,45,42] stated that non-albicans *Candida* predominated over *Candida albicans* regarding biofilm production. Biofilm formation is more important for non-albicans spp. and *C. albicans* possess mechanisms other than biofilm formation to establish infections, he found that *C. tropicalis* showed the highest score of biofilm intensity of grade (+4) [40].

This difference in results than multiple studies may be attributed to that the biofilm,in vitro can be affected by external factors such as coinfection with other pathogens, glucose concentration, antibiotic treatment, and pH. In addition to the presence of a surface such as a urinary catheter. Hence, host factors and specific conditions in the local microenvironment of the bladder may select for strains with high BF and constitute a risk factor for persistent candiduria. In addition, BF is an inherent and stable characteristic of a *Candida* strain. Supportive of this is molecular typing data, 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 [54].

Routine antifungal tests usually detect resistance/sensitivity to planktonic forms and there are only a few studies that have evaluated antifungals against biofilms. The viable cells in the biofilms after its exposure to antifungals can be conveniently measured by calorimetric methods, which are easy to read because of clear-cut end points. The most commonly used method, e.g., reduction of tetrazolium to formazan, is laborious and expensive; other disadvantages include toxicity due to the chemicals and requirement of addition of an electron-coupling reagent [26].

Others researchers had also shown the usefulness of resazurin/alamar blue as a cell viability indicator in antifungal susceptibility testing. The use of resazurin dye test in antifungal susceptibility testing of biofilm-forming cells has benefits like simplicity, low cost, lack of toxicity, and easy determination of ends [55].

In this study, The MBIC (minimal biofilm inhibitory concentration) testing was performed by the technique using the calorimetric indicator resazurin .Our results indicated that 72.2% of the tested *C. albicans*, were resistant to fluconazole and had MBIC > 640 μg/mL while only 27.8% were sensitive to fluconazole and had MBIC < 10 μg/mL. (Table 10) similar results, 59.2% of the tested *C. albicans* were resistant to fluconazole and had MBIC > 640 μg/mL while only 26.7% were sensitive to fluconazole and had MBIC < 10 μg/mL [55] and [56] who found (65.7%) of *C. albicans* isolates were resistant (>640 μg/mL) to fluconazole. All resistant *Candida* species to fluconazole were biofilm producers [45]. Biofilms from all *C. albicans* strains tested were intrinsically resistant to fluconazole [57].

Our results reported that 100% of the tested *C. tropicalis* and *C. kruzi*,, were resistant to fluconazole (Table 9) this is similar to results who found that among the 50 isolates tested by MBIC, all isolates were found to be resistant [26].

The mechanisms by which these forms exhibit increased resistance are not fully understood, but is believed to be due to complex mechanisms such as reduced diffusion of antimicrobial agents through biofilm layer, slow growth rate, and surface-induced expression of resistant genes [26].

The slow growth rate of organisms in biofilms are probably the major factor in conferring resistance. In addition, the juxtaposition of microorganisms of 1 or more species within a biofilm facilitates the transfer of antimicrobial resistance genes [26].

Candidemia is a late-onset ICU-acquired infection associated with high mortality [14, 58]. Candidemia in ICUs is associated with a considerable increase in hospital costs and length of hospital stay [15]. Broad-spectrum antibiotics, a prolonged ICU stay for >20 days, the use of corticosteroids, diabetes and mucosal colonization with *Candida*, age < 1 year > 64 years, all are risk factors for invasive candidiasis [59].

In our study, 26.3% out of 38 patients with candidurea had *Candida* in their blood. Species identification *Candida* isolates in blood was identified to the species level by multiplex nested PCR was *C. albicans and C. tropicalis* at frequencies of 60% and 40% respectively (Table 10).

A study found that the *C. albicans* were the commonest species isolated (50% and 41.3%)

[60,61] While in another study [62], *Candida tropicalis* (74.35%) was the most common isolate The evidences to determine a proper correlation between candiduria and invasive candidiasis is not yet clear or well established, due to publication of conflicting results and the lack of consensus [16].

In this study, we tried to find correlation between candiduria and *candida*emia in ICU patients depending on evidences from several studies [14], there were no difference in susceptibility and genetic background were found between blood and urine strains of *Candida* species. Multilocus sequence typing of *C. albicans* isolates revealed that in eight of nine patients with *C. albicans* the infecting fungal strain was identical in blood and urine Molecular typing of the urinary and blood samples collected from this patient exhibited similar pattern, suggesting that all isolates belonged to the same strain [63].

Candiduria has been found to be a risk factor for candidemia and an indicator of impending sepsis with *Candida* species in patients admitted to hospitals, especially those in ICUs and as many as 10% of all candiduria cases may be associated with candidemia [64]. In the presence of associated risk factors, as CVCs, surgical intervention or procedures involving the urinary tract, presence of urinary catheters, there is a definite risk of invasive candidiasis following candiduria [26,64]. The theory of occurrence of candidemia as a complication of candiduria can be explained by that 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 [40].

Our hypothesis supported by [65] who stated that, candiduria can be reliably considered as a marker of high density of colonization. Diverse *Candida* colonization anatomical sites were potential sources for candidaemia. She used a combination of restriction endonuclease analysis (REAG) and randomly amplified polymorphic DNA(RAPD) methodologies to prove this concept [66].

5. CONCLUSION

Candida albicans is the most common *Candida* spp that show biofilm production. Consequently, biofilm related infections are difficult to treat There is increased in the percentage of the resistance to fluconazole in *Candida* isolates in this study. The incidence of candidemia among patients with candidurea was high in our study. Further studies are required to find correlation between candiduria and candidaemia in ICU patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

Thanks to the staff at Intensive care units in Menufiya University Hospitals

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ghiasian SA, Aghamirian MR, Eshghi GR. Nosocomial candiduria in critically Ill patients admitted to intensive care units in Qazvin, Iran. Avicenna J Clin Microb Infec. 2014;(2):e21622.
2. Bukhary ZA. Candiduria: A review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19:350-60.
3. Kauffman CA. Candiduria. Clin. Infect. Dis. 2005;41:5371-76.
4. Imran ZK, Abuad SH. Genetic diagnosis and prevalence of urinary tract fungal pathogen with antifungal susceptibility pattern in Iraq. BJMMR. 2015;7(5):410-418.
5. Jain M, Dogra V, Mishra B, et al. Candiduria in catheterized ICU patients. Emerging microbiological trends; Indian J Pathol Microb. 2011;54(3):552-55.
6. Sullivan DJ, Westerneng TJ, Aynes H, Ennetka, Oleman DC. *Candida dubliniensis* sp. nov., phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology. 1995;141:1507–1521.
7. Michail SL, Mihai GN. *Candida* and host determinants of susceptibility to invasive

candidiasis. LoS Pathog. 2013;9(1): e1003079.
8. Williams DW, Kuriyama T, Silva S, Malic S, Lewis MA. *Candida* biofilms and oral candidosis: Treatment and prevention. Periodontol. 2011;55(1):250-65.
9. Seneviratne CJ, Jin L, Samaranayeke LP. Biofilm lifestyle of *Candida*: A mini review. Oral. Dis. 2008;14:582-590.
10. Aparna MS, Yadav S. Biofilms: Microbes and disease. The Braz J. Infect. Dis. 2008;12:526-530.
11. Dominic RM, Shenoy S, Baliga S. *Candida* biofilms in medical devices. Evolving trends. Kath. Univ. Medical. 2007;5(3):431-436.
12. 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.
13. Alem MAS, Douglas LJ. Prostaglandin production during growth of *Candida albicans* biofilms. Journal of Medical Microbiology. 2005;54:1001–1005.
14. Bougnoux ME, Kac G, Aegerter P, Enfert C, Fagon JY, CandiRea Study Group. Candidemia and candiduria in critically ill patients admitted to intensive care units in France: Incidence, molecular diversity, management and outcome. Intensive Care Med. 2008;34(2):292-9.
15. Bouza E, Muñoz P. Epidemiology of candidemia in intensive care units. Int J Antimicrob Agents. 2008;32(Suppl 2):S87-91.
16. Pedrina Gonçalves V, Simone Aparecida S, Fernandez MA, et al. Candiduria by *Candida* tropicalis evolves to fatal candidemia. Medical Case Studies. 2011;2(2):22-25.
17. Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S. Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. Interna J Biol. 2010;2:84-94.
18. 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.
19. Koehler AP, Chu KC, Houang ET, Cheng AF. Simple, reliable and cost effective yeast identification scheme for the clinical laboratory. J Clin Microbiol. 1999;37:422–426.
20. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Descriptions of medical fungi 2nd edition. Nexus Print Solutions, Australia. 2007;20-40.
21. 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.
22. Ellis D. Antifungal susceptibility testing. (Neo- Sensitab and E-test methods. Notes on disc diffusion and Etest methods). Mycology Online. The University of Adelaide CRICOS Provider No. 2011; 00123M.
23. Horvath LL, George BJ, Murray CK. Direct Comparison of the BACTEC 9240 and BACT/ALERT 3D automated blood culture systems for *Candida* growth detection. J. Clin. Microbiol. 2004;42(1):115-118.
24. Sims CR, Paetznick VL, Rodriguez JR, Chen E, Ostrosky-Zeichner L. Correlation between microdilution, e-test and disk diffusion methods for antifungal susceptibility testing of posaconazole against *Candida* spp. J. Clin. Microbiol. 2006;44(6):2105-8.
25. 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.
26. Punithavathy PM, Nalina K, Menon T. Antifungal susceptibility testing of *Candida* tropicalis biofilms against fluconazole using calorimetric indicator resazurin. Indian J Pathol Microbiol. 2012;55(1):72–4.
27. Taira CL, Okay TS, Delgado AF, Rivero Ceccon MEJ, Gottardo de Almeida MT, and Barbaro Del Negro GM. A multiplex nested PCR for the detection and identification of *Candida* species in blood samples of critically ill paediatric patients BMC Infectious Diseases. 2014;14:406.
28. Luo G, Mitchell TG. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. J Clin Microbiol. 2002;40:2860–2865.
29. Cleison LT, Thelma SO, Artur FD, et al. A multiplex nested PCR for the detection and identification of *Candida* species in blood samples of critically ill paediatric patients. BMC Infectious Diseases. 2014;14:406.
30. López MJ, Cortés JA. Urinary tract colonization and infection in critically ill

patients. Med Intensiva. 2012;36(2):143-51.
31. Binelli C, Moretti M, Assis R, Perry A, Cohen J. Investigation of the possible Association between nosocomial candiduria and candidaemia. Clin. Microbio. Infect. J. 2006;12:538.
32. Passos XS, Sales WS, Maciel PJ, Costa CR, Miranda KC, Lemos Jde A. *Candida* colonization in intensive care unit patients' urine. MemInst Oswaldo Cruz. 2005;100: 925-928.
33. Kauffman CA, Vazquez JA, Sobel JD. Prospective multicenter survilance study of funguria in hospitalized pts. Clin Infect Dis. 2000;30:4-8.
34. Alhussaini MS, El-Tahtawi NF, Moharram. Phenotypic and molecular characterization of *Candida* species in urine samples from renal failure patients. Science Journal of Clinical Medicine. 2013;2(1):14-25.
35. Kobayashi CC, de Fernandes OF, Miranda KC, de Sousa ED, Silva Mdo R. Candiduria in hospital patients: A study prospective. Mycopathologia. 2004;158:49-52.
36. Trautner BW, Darouiche RO. Role of biofilm in catheter-associated urinary tract infection. Am J Infect Control. 2004;32(3): 177–18.
37. Ashour SM, Kheiralla ZM, Maklad SS, Ameen MR, Zaki SS. Relationship between virulence factors of *Candida* species with candiduria and myeloper-oxidase concentrations Int. J. Curr. Microbiol. App. Sci. 2015;4(1):108-123.
38. Guler S, Ural O, Findik D, Arslan U. Risk factors of nosocomial candiduria. Saudi Med J. 2006;27(11):1706-1710.
39. Behiry IK, Hedeki SKE, Mahfouz M. *Candida* infection associated with urinary catheter in critically ill patients. Identification, antifungal susceptibility and risk factors. Res J Medical Science. 2010;5(1):79-86.
40. Mohammed SA, Al-Ahmadey ZZ. Biofilm formation and antifungal susceptibility of *Candida* isolates from various clinical specimens. British Microbiology Research Journal. 2013;3(4):590-601.
41. 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.
42. Vijaya D, Harsha TR, Nagaratnamma T. *Candida* speciation using chrom agar. Journal of Clinical and Diagnostic Research. 2011;5(4):755-757.
43. 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.
44. Sachin D, Santosh S. Evaluation of phospholipase activity in biofilm forming *Candida* species isolated from intensive care unit patients. British Microbiology Research Journal. 2013;3(3):440-447.
45. Khater E Sh, Al-Nory MH. Exoenzymes activity and biofilm production in *Candida* species isolated from various clinical specimens in Benha University Hospital, Egypt. British Microbiology Research Journal. 2014;4(6):654-667.
46. Nemati Shirzi L, Shams Ghahfarokhi M, Yadgari 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.
47. Badiee 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.
48. Amer A. Virulence activities of *Candida* species isolates from patients and healthy subjects. Egyptian Journal of Medical Microbiology. 2009;18:4.
49. Luciana F, Ana F, Fernando C. *In vitro* evaluation of putative virulence attributes of oral isolates of *Candida* species. Obtained from elderly healthy individuals. Mycopathologia. 2001;166:209-217.
50. Omar M, Fam N, El Leithy T, El Said M, El Seidi E, El Etreby T. Virulence factors and susceptibility patterns of *Candida* species isolated from patients with obstructive uropathy and bladder cancer. Egypt. J. Med. Microbiol. 2008;17(2):317-328.
51. Abdallah NMA, Elsayed SB, Mostafa MMY, El-Gohary GM. Biofilm forming bacteria isolated from urinary tract infection, relation to catheterization and susceptibility to antibiotics. Int. J. Biotechnol. Mol. Biol. Res. 2011;2(10):172–178.
52. Kuhn DM, Chandra J, Ghannou MA. Comparison of biofilms formed by *Candida albicans* and *Candida* parapsilosis on bioprosthetic surfaces. Infect Immun. 2002;70(2):878–888.
53. Mohandas V and 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.
54. Jain N, Kohli R, Cook E, Gialanella P, Chang T, Fries BC. Biofilm formation by and antifungal susceptibility of *Candida* isolates from urine. Environ. Microbiol. 2007;73(6):1697-1703.
55. Mahmoudabadi AZ, Zarrin M, Kiasat N. Biofilm formation and susceptibility to amphotericin B and fluconazole in *Candida albicans* Jundishapur J Microbiol. 2014;7(7):e17105.
56. Ozkan S, Kaynak F, Kalkanci A, Abbasoglu U, Kustimur S. Slime production and proteinase activity of *Candida* species isolated from blood samples and the comparison of these activities with minimum inhibitory concentration values of antifungal agents. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2005;100(3):319-324.
57. Ramage G, Stephen S, Thomas D, Lopez-Ribot J. *Candida* biofilms: An update. Eukaryotic Cell. 2005;4:633-638.
58. Andes D, Nett J, Oschel P, Albrecht R, Marchillo K, Pitula A. Development and Characterization of an *in vivo* central venous catheter *Candida albicans* biofilm model. Infect Immun. 2004;72(10):6023–6031.
59. Fatani AJ, El-Hossary D, Meawed TE. Antifungal susceptibility, risk factors and treatment outcomes of patients with candidemia at a university hospital in Saudi Arabia. The International Arabic Journal of Antimicrobial Agents. 2015; 5(2):3.
60. Gupta P, Prateek SH, Chatterjee B, Kotwal A, Singh AK, Mittal G. Prevalence of candidemia in ICU in a tertiary care hospital in North India. Int. J. Curr. Microbiol. App. Sci. 2015;4(6):566-575.
61. Tan BH, Chakrabarti A, Li RY, Patel AK, Watcharananan SP, Liu Z, Chindamporn A, Tan AL, Sun PL, Wu UI, Chen YC. On behalf of the Asia Fungal Working Group (AFWG). Incidence and species distribution of candidaemia in Asia: A laboratory based surveillance study. Clin Microbiol Infect. 2015;21:946–953.
62. Giri S, Kindo AJ, Kalyani JJ. Candidemia in intensive care unit patients: A one year study from a tertiary care center in South India. Postgrad Med. 2013;59(3):190-5.
63. Ruan SY, Chien JY, Hsueh PR. Persistent *Candida* parapsilosis funguria associated with an indwelling urinary tract stent for more than 7 years. J. Med. Microbiol. 2008;57:1585-1587.
64. Giri S, Kindo AJ. A review of *Candida* species causing blood stream infection. 2012;30(3):270-278.
65. Viale P. *Candida* colonization and candiduria in critically ill patients in the intensive care unit. Drugs. 2009;69:51-57.
66. Miranda LN, van der Heijden IM, Costa SF, Sousa API, Sienra RA, Gobara S, Santos CR, Lobo RD, Pessoa Jr VP, Levin AS. *Candida* colonization as a source for candidaemia. J. Hosp. Infec. 2009;72:9-16.

© 2016 Alkilani et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), 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://sciencedomain.org/review-history/14603