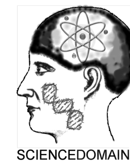
British Journal of Medicine & Medical Research
7(5): 410-418, 2015, Article no.BJMMR.2015.347
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org



Genetic Diagnosis and Prevalence of Urinary Tract Fungal Pathogen with Antifungal Susceptibility Pattern in Iraq

Zaidan Khlaif Imran[1*] and Suha Hamid Abuad[1]

[1]*Department of Biology, All Women College of Science, Babylon University, Babylon Province, Hilla, Iraq.*

Authors' contributions

This work was carried out in collaboration between both authors. Author ZKI designed the study, managed the literature searches read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/12559
Editor(s):
(1) Nurhan Cucer, Erciyes University, Medical Biology Department, Turkey.
(2) Kate S. Collison, Department of Cell Biology, King Faisal Specialist Hospital & Research Centre, Saudi Arabia.
(3) Jimmy T. Efird, Department of Public Health, Epidemiology and Outcomes Research East Carolina Heart Institute, Brody School of Medicine, Greenville, North Carolina, USA.
Reviewers:
(1) Nwako Okechukwu Francis, College of Medicine, Madonna University, Rivers State, Nigeria.
(2) AngèleThéodora Ahoyo, Department of Human Biology, EPAC university of Abomey-Calavi, (UAC), Benin.
(3) Anonymous, Nigeria.
(4) Anonymous, Brazil.
(5) Anonymous, Italy.
(6) Veronica A. Gaona Flores, Infectology Hospital, National Medical Center "La Raza", Instituto Mexicano del Seguro Social, México.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=945&id=12&aid=8291

Original Research Article

Received 7th July 2014
Accepted 12th September 2014
Published 27th February 2015

ABSTRACT

Background: Urinary tract infection (UTI) is considered as one of the frequent diseases affecting humans. Usually, emphasis has been on bacteriological etiologies but this study focuses on fungal etiologies.
Aims: The aim of this study is isolation and characterisation of fungal spp. from urine of patients diagnosed of urinary tract infections genetically and phenotypically. Secondly, to evaluate antifungal sensitivity of isolated fungi against 5 antifungals in Iraq.
Methods: A total of 150 urine specimens were collected from 150 UTI suspects patients attending Marrjan hospital in Babylon province from October 2012 to October 2013. Fungal organisms were isolated, virulence of *Candida* was detected and both genetic analysis of isolates and antifungal susceptibility test, using 5 antifungals were performed on the most frequent isolate.

Corresponding author: Email: zaidan_omran@yahoo.com;

Results: *Candida albicans* (32.7%) and *Aspergillus fumigatus* (10.9%) were the most frequent fungal isolates. Other isolated fungi from the urinary tract of patients include: *Acremonium polychromium*, *Penicillium digitatum*, *A. fumigatus*, *A. niger* and some other *Candida* species including *C. albicans*, *Geotrichum capitatum*, *C. glabrata, C. guilliermondii* and *C. famata.* Most *Candida* spp. produced proteinase and lipase. *Candida* spp. has the ability to ferment most of sugar types under interest except lactose. Clotrimazole, Fluconazole, Ketoconazole, and Amphotercin B showed high activity while Miconazole showed low activity to the most frequent isolate of *C. albicans*, *C. guilliermondii*, *C. famata*. The range of antifungal activity concentration for Amphotericin B was 10-100 mg while for Fluconazole, Miconazole and Ketoconazole was 100-1000 mg. Females (39 cases) aged 18-30 years showed frequency of 26% while males (21cases) aged 30-40 years showed frequency of 14%. Eighteen isolates were genetically analyzed and identified as *Candida* genus with PCR product size of 210 bp.

Conclusion: Fungal organisms are common etiological agents of UTI patients. *Candida* spp. and *Aspergillus* spp. showing highest frequency in our study. All isolates of yeast were genetically diagnosed as *Candida* except *Geotrichum* isolates. The common antifungals like Clotrimazole, Fluconazole, Ketoconazole and Amphotericin B are still effective in eradicating these pathogens in Iraq.

Keywords: UTI; fungi; genetic analysis; anti-susceptibility test; Iraq.

1. INTRODUCTION

Fungal infections of the urinary tract are common and the most common pathogen is *Candida albicans* [1]. Many fungi are implicated in urinary tract complications and are known to lead to systemic candidiasis [2]. *Candida* spp. associated UTI causes candiduria and its frequency has been on the rise during the past two decades [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, including disseminated disease, multiple organ failure or death [4]. Many fungal UTI has been associated with the use of urinary catheter [5]. Fungal balls could develop in the urinary tract, leading to obstructive uropathy (a condition in which the flow of urine is blocked and hydronephrosis occurs. It is not a disease itself) [6]. The most common yeasts causing both complicated and uncomplicated UTI are *C. albicans* and *C. glabrata* [7-11]. However, treatment with antifungal agents like fluconazole will reduce disease progression and morbidity associated with these fungal pathogens. Many of such fungi, including *Candida* spp. were isolated from UTI patients in a study viz *C. tropicalis* and *C. glabrata*, *C. kefyr*, *C. guilliermondii*, and *Rhodotorula* species [12]. Most UT patients suffering from fungal infections may disseminate these infections if considered that the fungal presence is only due to normal colonization [13]. Therefore, a precise assay to diagnosis UT infectious agents can reduce the risk of complications in patients [14].

Molecular assays are more reliable methods than conventional assays for screening microbial pathogens, reduce contamination and time, especially for patients suffering from complicated infections, and they also reduce the burden and provide precise diagnosis for deciding the treatment. Molecular identification of *Candida* with specific primers has facilitated the diagnosis of *Candida* isolates and also can be used to demonstrate the absence of *Cryptococcus* sp. in any samples under interest [15]. The aim of this study is isolation and characterisation of fungal spp. from urine of patients diagnosed of urinary tract infections genetically and phenotypically. Secondly, to evaluate antifungal sensitivity of isolated fungi against 5 antifungals in Iraq.

2. MATERALS AND METHODS

2.1 Collection of Patients Samples

150 urine specimens were collected from 150 patients (87 females and 63 males) aged 8- >40 years who attended Marrjan hospital in Babylon province and other four provinces (part of Baghdad, Najaf, Karbala and Al-Diwania provinces) served by this hospital in Iraq between October 2012 to October 2013.

2.2 Collection and Processing of Urine Specimens

Collection of mid-stream urine specimen: Females and males were advised to wash their

hands with soap and water. Dry-wipe them. Take the clean collection container, and avoid touching the inside with their fingers. Washed their outer genitals with a sterile water without using any soap. Dry-wipe. When urinating, let the first portion pass into the toilet. Collect 50-100ml urine/patient from the mid-portion in the container. Allow any excess urine to pass again into the toilet. After urination, dry-wipe the outer surface of the container, secure the lid and transfer the urine to the tubes provided, and write patient names and the date and time on label, then fixed the label on the container [16].

One ml of urine from each sample was directly inoculated onto Sabouraud Dextrose Agar (SDA) mixed with chloramphenicol and streptomycin (50:50 ug/ml) to inhibit the growth of bacteria, in a Petri dish and isolates were grown and detected after 24-96 h for *Candida* isolation, and after 5-7 days for filamentous fungi isolation, incubation at 28ºC. Tiny inoculum from each *Candida* colony was streaked on CHRO Magar candida for Identification of *Candida* spp. A loop full of yeast cells suspension was inoculated into 0.5 ml of human serum and incubated at 37°C for 3 h. After incubation period, it was examined under field microscope. Germ tube was considered as a lateral tube without septum and had no constriction at initiating site, which is a positive test for *C. albicans* [17-19].

2.3 Proteinase Production

Proteinase (gelatenase) production was determined for 18 isolates of *Candida* using Nutrient Gelatin Agar plates. Disc inoculum of tested isolate and control *Candida* isolates - 5 mm Disc inoculum of yeast cells were spot-inoculated onto the plates. The gelatin agar plates were incubated at 37ºC for 48 h. Plates production of the enzyme was detected as zone of clearance after addition of Frazier's reagent HgCl2, 12 g; distilled water, 80 ml; concentrated HCl, 20 ml/plate) showing gelatin hydrolysis after incubation period at 20ºC for 20 min. The proteolytic activity was detected on the basis of a clear zone appearing around the colonies flooded with Frazier's reagent (in mm) was measured [20].

2.4 Lipase Production

Lipase production was assayed for 18 isolates of *Candida* using an egg yolk agar plate method [21]. SDA plates containing 1 M NaCl, 0.005 M $CaCl_2$ and 8% sterile egg yolk emulsion were used. Standard inoculum of test and control *Candida* isolates (5 µl containing 10^8 yeast cells were spot-inoculated on the plates. A further 5 µl saline without yeast cells was also spot-inoculated as control treatment. The plates were incubated at 37ºC for 5 days after which the diameter of the zone of precipitation around the colony (in mm) was determined. [22].

2.5 Antifungal Susceptibility

Susceptibility test for the fungal isolates was performed by the disk diffusion method; an agar based testing method, using five drugs Clotrimazole, Fluconazole, Amphotericin B, Ketoconazole, and Miconazole against *Candida*. Incubation period was 12- 120 hours at 28±2ºC [23-25].

The frequency of a fungus is denoted by the number of sampling in which a fungus is recorded against the total by

Frequency (%) = No. of observation in which colony appear / total number of observation recorded x 100 [26].

2.6 Genotypic Identification of *Candida albicans*

2.6.1 Extraction genomic DNA

Eighteen isolates of *Candida* spp. were subjected for DNA extraction and PCR assays. Yeasts were individually grown on Sabouraud's Dextrose agar and incubated at 37ºC for 2 days. A small amount of *Candida* colony was pickup and suspended in 400µl of lysis buffer (400 mM Tris-HCl, 20 mM EDTA, 150 mM NaCl, and 0,5% SDS adjusted 8.5 pH). Incubated for 10 min at 85ºC in water bath, the suspension was vortex for 2 min .150 µl of phenol-chloroform, vortex for 2 min, centrifuged at 5000 rpm for 2 min, the supernatant was decanted into new sterile tubes, and mixed gently with 500 µl isopropanol for one min and centrifuged at 12000 rpm for 12 min. Dropped the isopropanol and washed the DNA pellet with 70% Ethanol and centrifuged at 5000 rpm for 2 min, dried the DNA pellet and then suspended with 75 µl elution buffer and preserved at – 20ºC until use [27].

2.6.2 PCR assays

The final volume of PCR mixture was 25 µl {12 µl green master mix from promiga company ,10 µl deionizer water ,1 µl genomic DNA ,1.2 µl (10 pemole for both primer)}was amplified by thermal cycler PCR System (Labnet, USA) .The primer pairs CAINB Forward Primer: (5'-GAGGGCAAGTCTGGTG- 3') and CAINB Reverse Primer: (5'CCTGCTTTGAACACTCTAA-3') [15]. The PCR conditions for CAINB primers were 95ºC for 3 min followed by 30 cycles 94ºC for 1 min. annealing temperature 55ºC for 1 min. Extensions temperature 72ºC for 1 min. followed by final extension temperature 72ºC for 7 min. The PCR products for each targeted region were run on 1.2% agarose gel (Bio Basic Canada Inc.) electrophoreses performed at 100 V. in TBE buffer. The gel was pre-stained with 0.05% ethidium bromide.

3. RESULTS

3.1 Identification and Fungal Frequency

36.7%(55/150) urine samples from suspected UTI patients gave positive evidence for molds and yeast infections in consecutive patients , 14 (25.5%) isolates of filamentous fungi gave positive results including *Acremonium polychromum*, *Penicillium digitatum*, *A. fumigatus* and *A. niger.* The high frequent filamentous fungi was *A. fumigates* 10.9%, while 41(74.54%) isolates of yeast which gave positive results included: *C. albicans*, *G. capitatum*, *C. glabrata, C. guilliermondii* and *C. famata*. *C. albicans* (32.7%) *and G. capitatum* (20%) were the common yeast in urine samples (Table 1).

3.2 Profile Phenotypic and Enzymatic Production of *Candida* spp.

The results showed that only *C. albicans* had ability to produce chlamydospore and germ tube formation (Table 2). Proteinase and lipase activity was detected in all *C. albicans* (14/18 isolates), *C. glabrata* 2/18, *C. famata* 1/18 isolates (Table 2). The isolates of *Candida* were classified based on CHROMagar into 4 species: *C. albicans* revealed green color while other 3 species revealed pink to white pink color (Table 2).

Table 1. List of UTI fungal clinical isolates and their frequency in percentage

Fungi	% Frequency	No. of isolates
Acremonium	3.64	2
Penicillium digitatum	5.4	3
A. fumigatus	10.9	6
A. niger	5.4	3
C. albicans	32.7	18
G. capitatum	20	11
C. glabrata	10.9	6
C. guilliermondii	5.4	3
C. famata	5.4	3
Total	≈100%	55

3.3 Capability of *Candida* spp. for Sugars Fermentation

The result showed *C. albicans*, *C. glabrata* and *C. guilliermondii*. had ability to fermented most of sugar types under interest except lactose, while *C. famata* fermented only glucose (Table 3).

3.4 The Relation between Positive Candiduria with Both Age and Gender

Table 4 revealed the relation between Candiduria with both age categories and gender, the results showed that 18-30 years of women age more sensitive for infection with Candiduria while the age of men occur in between 30-40 years.

Table 2. Microscopic characters, CHROMagar color and enzymes production for *Candida* spp. isolated from UTI samples

Candida spp.	Chlamydospore	Germ tube	Proteinase	Lipase	CHROMagar
C. albicans	+	+	+	+	Green
C. glabrata	_	_	+	+	Red-pink
C. famata	_	_	+	+	White-pink
C. guilliermondii	_	_	_	_	Pink

Table 3. Sugars fermentation for *Candida* spp. isolated from UTI samples

Sugar	*C. albicans*	*C. famata*	*C. guilliermondii*	*C. glabara*
Glucose	+	+	+	+
Lactose	_	_	_	_
Sucrose	+	_	+	_
Maltose	+	_	+	+
Galactose	+	_	+	+
Xylose	+	_	+	_

Table 4. Relationship between age and gender with frequency of *Candida* spp. isolated from UTI samples

Age category	No. of UTI from females	No. of UTI from males
8-18	7 (4.6%)	10(6.7%)
>18-30	39(26%)	15(10 %)
>30-40	22(14.7 %)	21(14 %)
>40	19(12.7%)	17(11.3 %)
Total	87(56.1%,)	63(42%)

3.5 Antifungal Susceptibility

The results of antifungal susceptibility show activity with Clotrimazol, Fluconazol, Amphotericin B, Ketoconazole more effective antifungal against *Candida* spp. (Table 5).

Table 5. Antifungal sensitivity against *Candida* isolated from UTI Infection

Antifungal fung	C L	FL	MI	AMB	KE
C. albicans	+	±	+	+	+
C. guilliermondii	+	+	-	-	-
C. famata	+	+	+	+	-
C. glabrata	-	+	+	+	-

+ =Sensitive, - =Resistance, ± =Moderately sensitive
Abbreviations: CL=Clotrimazol, FL= Fluconazol, AMB= Amphotericin B, KE =Ketoconazole, MI= Miconazole.

3.6 Molecular Diagnosis of *Candida* spp. by Specific Primer Pair

The results of PCR products by specific primer pairs CAINBF and CAINBR for *Candida* form genus was produce amplicon size 210 bp as genotyping for 18 isolates of *Candida* isolates refer to *Candida* genus (Fig. 1).

4. DISCUSSION

The most frequent causative agent of UTIs in this study occurred in women more than men, was found to be *C. albicans* (32%) followed by *G. capitatum* (20%), *C. glabrata* and *A. fumigatus* (10.9% each), these result may be explained based on 1) the age of pregnancy and abortion for women (2) drug intake which causes hormonal imbalance and 3) comorbidities like other diseases. In men, Candiduria may be related with diseases or sex fatigue. Our results supported a work done in Iran,[28] *C. albicans* was the most common species among the isolates (93.9%) followed by *C. glabrata* (2%), *C. dubliniensis* (2%), and *Candida* species (2%).Our results showed that *C. albicans, C. famata, C. guilliermondii* and *C. glabrata* were suspect with UTI complications. Unfortunately, no data is available on UTI prevalence in Iraq, the present study is compared with the available data from the neighboring countries, recent study in Iran [28,29] and Saudi Arabia [30-32] shows the prevalence of UTI in children and reported approximately one-third of hospitalized patients with urine cultures giving positive tests . Many previous studies attempted identifying *Candida* in urine by phenotypic and molecular assays [10,33-34].

Phenotypic assay based on the CHROMagar candida was preliminary presumptive test, but are not accurate and precise for *Candida* identification; earlier studies noticing the same results [34-38].

Suspect the presence of renal failure and complication in the presence of *C. albicans* and *G. capitatum* yeast in urine samples as most of our patients with this condition were positive for these organisms. These results were also shown in a study [5,12]. Sugar fermentation abilities by *Candida* spp. explain the role of predisposing factors like diabetes in predisposing them to *Candida* colonization of the vulvovaginal area by enhancing urinary fungal growth in the presence of glycosuria [39].

Miconazole has been used by patients with many infections. It has a broad spectrum of activity,

Table 6. Antifungal concentrations activities against *Candida* isolated from UTI Infection

Antifungal	AMB			FL			KE			MI			CL		
(Mg/ml) Isolate No.	10	100	1000	10	100	1000	10	100	1000	10	100	1000	10	100	1000
Ca_1	±	+	+	±	+	+++	+	+	++	±	+	+++	+	+	++
Ca_2	±	+	+	+	+	+++	±	+	+++	±	+	++	+	+	+++
Ca_3	+	+	+	±	+	++	+	+	++	±	+	+++	+	++	+++
Ca_4	+	+	+	+	+	+++	±	+	++	+	±	+++	+	++	+++
Ca_5	+	+	+	+	+	+++	+	++	+++	+	+	++	+	+	+++
Ca_6	+	+	++	±	+	+++	+	+	+++	+	+	+++	+	++	+++
Ca_7	+	+	++	±	+	++	+	+	++	+	+	+++	+	+	+++

Inhibition zone size (centimeter): +++ =2.6-3.3 , ++ =1.5-2.5.+ = 0.8-1.45, ± =0.2-0.75. Abbreviations: CL=Clotrimazol, FL= Fluconazol , AMB= Amphotericin B, KE =Ketoconazole, MI= Miconazole.

Fig. 1. Gel electrophoresis of PCR product size 210 bp as genotype for 18 isolates of candida isolates from urine samples.1-18 isolates of Candida isolates , M=molecular marker 100 bp

including against *Aspergillus* spp. and *Candida* spp. Systemic miconazole, however, is associated with significant toxicity and has resulted in undetectable concentrations in the cornea, Fluconazole is active against *Candida* species; the low sensitivity of some antifungal under interest in this study (Table 5) may be due to development of gene resistance against some fungal isolates when compared with Econazole, Miconazole and Ketoconazole. Our results agree with [12,40], when they reported that Amphotericin B has relatively narrow range for some fungi *Aspergillus* and *Fusarium*. *A. niger*, the low concentration of AM (10 mg) compared with higher concentration of others antifungals (1000 mg) (Table 6 see above) may be explained based on activity and cell toxicity. The active antifungal concentration 10-100 mg for Amphotericin B while100-1000 mg for Fluconazol, Miconazole and Ketoconazole against *C. albicans in vitro*. [12]. Molecular identification with specific primers is sufficient for the characterization of *Candida* isolates and demonstration of absence of *Cryptococcus* sp. in urine samples under interest, and need no additional biochemical tests like staining with Indian ink to distinguish between the duo. This result is in concordance with earlier studies about the importance of PCR assays for the detection of *Candida* spp. [15,28,41].

5. CONCLUSION

This study concluded that many filamentous fungal species like *Aspergillus* spp., *Penicillium* spp. and yeast like *C. albicans and Geotrichum* were common in urine samples of UTI patients when specifically searched for. These fungi were high suspect especially in the presence of kidney failure cases and complications. Ketoconazole ,Miconazole and Econazol were more effective antifungal against *Candida* spp. Molecular identification of *Candida* genus with specific primers has facilitated the diagnosis of *Candida* isolates and may be useful in demonstrating absence of *Cryptococcus* sp. in urine samples under interest.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGMENTS

This study was conducted in Biotechnology Lab. Biology department, all women college of Science, Babylon University, Iraq. The authors would like to thank Marrjan hospital staff for their logistic support and their help in sampling.

COMPETING INTERESTS

Authors have declared that no competing interests exist and no financial disclosure to be made.

REFERENCES

1. Kale H, Narlawar RS, Rathod K. Renal fungal ball: An unusual sonographic finding. J. Clin. Ultrasound. 2002;30:178–180.
2. Dembner AG, Pfister RC. Fungal infection of the urinary tract: Demonstration by anterograde pyelography and drainage by perentaneous nephrostomy. AJR Am. J. Roentgenol. 1977;129:415–418.
3. Álvarez-Lerma F, Nolla-Sallas J, Palomar M, Jordá R, Carrasco N, Bobillo F. Candiduria in critically ill patients Admitted to intensive care medical units. Inten Care Med. 2003;29:1069-1076.
4. Hazen KC. New and emerging yeast pathogens. Clin. Microbiol. Rev. 1995;8: 462–478.
5. Badawi H, Kamel AI, Fam N, El-Said M, Elian S. Candida urinary infections: emerging species, anti- fungal Susceptibility trends and antibody response, Egypt J. Med. Microbiol. 2004; 13(1):1-14.
6. Febré N, Silva V, Medeiros EAS, Wey SB, Colombo AL, Fischman O. Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catherization. J Clin Microbiol. 1999;37:1584-1586.
7. Bakir M, Cerikciogˇlu NC, Tirtir A, Berrak S, Zek EO¨, Canpolat C. *Pichia anomala* fungaemia in immunocompromised children. Mycoses. 2003;47:231–235.
8. Bukhary ZA. Candiduria: A review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19:350-360.
9. 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;2:84-93.
10. Ang BSP, Telenti A, King B, Steckelberg JM, Wilson WR. Candidemia from a urinary tract source: Microbiological aspects and clinical significance. Clin. Infect. Dis. 1993; 17:662-666.
11. Ayeni O, Riederer KM, Wilson FM, Khatib R. Clinicians' reaction to positive urine culture for Candida organisms. Mycoses. 1999;42:285-289.
12. Ellis D. Antifungal susceptibility testing. (Neo- Sensitab and E-test methods. Notes on disc diffusion and E- test methods). Mycology Online. The University of Adelaide CRICOS Provider No. 00123M; 2011.
13. Zarei MZA, Keradmand AR , Enayatollahi N. Frequency of candiduria in inpatients and outpatients in Department of Urology, Golestan Hospital, Ahvaz, Iran. IJKD. 2009;3:114-115.
14. Nucci M. Candiduria in hospitalized patients: A review. Braz J Infect Dis 2000;4:168-172.
15. Luo G, Mitchell TG. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. J. Clin. Microbiol. 2002;40:2860-32865.
16. Aspevall O, Hallander H, Gant V, Kouri T. European guidelines for urinalysis: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in collaboration with ESCMID European Society of Clinical Microbiology and Infectious Diseases. CMI. 2001;7(4):173–1787 .
17. Williams DW, Lewis MAO. Isolation and identification of Candida from the oral cavity. Oral Diseases. 2000;6(1):3–11.

18. Abu Daia JM, Al-Aaly MA, De Castro R. Urinary tract infection in childhood. Saudi Medical J. 2000;21(8):711-714.
19. Nadeem SG, Hakim ST, Kazm SU. Use CHROMagar candida medium for the presumptive identification of *Candida* species directly from clinical specimens in resource –limited setting. Libyan J Med. 2010;5:1-6.
20. Hankin L, Anagnostakis SL. The use of solid media for detection of enzyme production by fungi. Mycologia. 1975;67: 597-607.
21. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, other authors. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006; 34:1589–1596.
22. De Luca C, Guglielminetti M. Ferrario A. Calabr M, Casari E. Candidemia: species involved, virulence factors and antimycotic susceptibility. New Microbiologica. 2012;35:459–468.
23. Kirkpatrick WR, Turner TM, Fothergill AW, McCarthy DI, Redding SW, Rinaldi, MG, Patterson, TF. Fluconazole disk diffusion susceptibility testing of *Candida* species. J. Clin. Microbiol. 1998;36:3429-3432.
24. Barry AL, Brown SD. Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. J. Clin. Microbiol. 1996;34:2154-2157.
25. Ellis D. Antifungal susceptibility profile (Australi- an antifungal susceptibility data for Candida isolates from recurrent volvovaginal candidiasis (2007-2009) using the CLSI M44-A2 disc susceptibility standard for yeasts). Mycology Online. The University of Adelaide. CRICO Provider N. 00123M. 2011.
26. Adhikari A, Sen MM, Gupta - Bhattacharya S, Chanda S. Airborne viable, non-viable and allergenic fungi in a rural agricultural area of India: a 2- year study at five outdoor sampling stations. Sci. Total Environ. 2004;326: 123 - 141.
27. Harju T, Petre I, Rozenberg G. Two models for gene assembly. In: J. Karhumaki, G. Paun, G. Rozenberg (Eds.), Theory is Forever, LNCS. Springer. 2004;5113: 89-101.
28. Mahmoudabadi ZA, Najafyan M, Alidadi M. Clinical study of *Candida* vaginitis in Ahvaz, Iran and susceptibility of agents to topical antifungal. Pak J Med Sci. 2010;26(3):607-610.
29. Mohammadi P, Shoaie N. Mohammadi SR. Isolation and detection of yeast biofilms from urine catheters of infectious patients. Jundishapur J Microbiol. 2012;5:533-536.
30. Abu Daia JM, Al-Aaly MA, De Castro R. Urinary tract infection in childhood. Saudi Medical J. 2000;21(8):711-714.
31. Guler S, Ural O, Findik D, Arslan U. Risk factors for nosocomial candiduria. Saudi Med J .2006;27:1706–1710.
32. Bukhary ZA. Candiduria: A review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19:350-360.
33. Fidel PL, Vazquez JA, Sobel JD. *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to *C. albicans*. Clin Microbiol Rev. 1999;12(1):80–96.
34. Ergon MC, Gulary Z. Molecular epidemiology of *Candida* species isolated from urine at an intensive care unit. Mycoses. 2005;48:126-131.
35. Ahmed S, Khan Z, Mustafa AS , Khan ZU. Semi-nested PCR for diagnosis of candidemia: Comparison with culture, antigen detection and biochemical methods for species identification. J Clin Microbiol. 2002;40:2483-2489.
36. Kobayashi CC, de Fernandes OF, Miranda KC, de Sousa ED, Silva MR. Candiduria in hospital patients: A study prospective. Mycopathologia. 2004;158:49-52.
37. Campbell CK, Holmes AD, Davey KG, Szekely A, Warnock DW. Comparison of a new chromogenic agar with the germ tube method for presumptive identification of *Candida albicans*. Eur J Clin Microbiol Infec Dis. 1998;17:367-368.
38. Beheshti F, Smith AG, Krause GW. Germ tube and chlamydospore formation by *Candida albicans* on a new medium. J Clin Microbiol. 1975;2(4):345-348.
39. Obiogbolu CH, Okonko IO, Anyamere CO, et al. Incidence of Urinary Tract Infections (UTIs) among pregnant women in Akwa metropolis, Southeastern Nigeria. Sci Res Essays. 2009;4:820–824.
40. Arikan S, Paetznick V, Rex JH. Comparative evaluation of disk diffusion

with microdilution assay in susceptibility testing of caspofungin against *Aspergillus* and *Fusarium* isolates. Antimicrob Agents Chemother. 2002;46:3084.

41. Khlif M, Sellami H, Sellami A, Makni F, Cheikhrouh- hou F, Chelly H. *Candida dubliniensis*: First identification in Sfax hospital, Tunisia. Mycoses. 2007;52(2): 171-175.

© 2015 Imran and Abuad; 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://www.sciencedomain.org/review-history.php?iid=945&id=12&aid=8291