

Full Length Research Paper

# Identification of *Candida* species isolated from hospital acquired infections cases and hospital indoor environments

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In this study it has been shown that hospital sources of *Candida* species could cause human infections, via identification of the yeasts isolated from cases and also environmental specimens, staff, visitors and patient's micro flora. Microscopic direct examination was used for detection of *Candida* pathogenic forms. Differential cultures and molecular tests including; PCR and RFLP were performed for the identification of *Candida* species. From the isolated fungi, we obtained *Candida* 36 (66.6%), *Aspergillus* 17 (31.4%) and *Alternaria* species only one case. *Candida* species isolated from clinical samples included: *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida guilliermondii* and *Candida parapsilosis*. Among all environmental isolated yeasts; *Candida albicans* (60%), *Candida krusei* (17%), *Candida glabrata* (14.3%), *Candida tropicalis* (0.7%) and *Candida parapsilosis* (3%) were identified. We believe that *Candida* contamination of environmental surfaces and hand touches can facilitate the occurrence of several *Candida* infections predominantly in patients with immune compromised system.

**Key words:** Hospital, infection, *Candida*, source.

## INTRODUCTION

Nosocomial infections have tremendous health and financial costs with an estimated incidence of 2,000,000 infections per year, 20,000 deaths per year, and added costs of 2 billion per year (Pfaller and Diekema, 2007). During times of prolonged antibiotic treatment and severe immunosuppression, fungi as opportunistic organisms can cause hospital acquired infections. This group includes *Candida albicans*, *Aspergillus* spp., *Cryptococcus*

*neoformans*, *Cryptosporidium*, *Malassezia* spp., and *Saccharomyces cerevisiae*. There is an increasing incidence of life-threatening systemic fungal infections in immune-compromised patients, especially fulminate infections with *Candida* spp. Those who are immune-compromised as a result of HIV infection, organ transplantation, patients from intensive care units, and premature infants can be affected, but at highest risk for these severe infections are those with prolonged use of immunosuppressive drugs and also hospitalization. These include patients with acute leukemia during induction therapy or after transplantation of allogeneic hematologic stem cells (Vidal et al, 2008). Also there has been much debate over hospital increasing numbers of hospital acquired infections (HAIs). The morbidity rate of 8% was reported for *Candida* infection so that they are 4<sup>th</sup> among the HAI causing agents.

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**Abbreviations:** PCR, Polymerase chain reaction; RFLP, restriction fragment length polymorphism.

Effective infection control programs are essential to controlling and preventing nosocomial infections. It is important that the sources of HAI isolates such as *Candida* spp. be searched, identified and controlled. In this study it has been tried to find hospital sources of *Candida* species which could be causing agents of human infections by using molecular methods for the identification of clinical and environmental *Candida* isolates. In this study it has been shown that hospital sources of *Candida* species could be causing agents of human infections by using molecular methods for the identification of clinical and environmental *Candida* isolates.

## MATERIALS AND METHODS

### Subjects

This study was performed at Medical Mycology Lab and Molecular Biology Center, Urmia University of Medical Sciences. Our study cases were the patients with clinical disorders in single or multiple organs who included no symptoms at the time of reception in hospital. Clinical specimens including; BAL, sputum, sinus discharge, urine and others were transported to the mycology lab. Environmental sampling was performed only after approving a fungal nosocomial infection, and the specimens were collected with sterile swabs from the Air, and solid surfaces including walls and curtains, beds and blankets, trolleys, air condition, medical devices near the patient's site and also finger touch samples of the cases, personnel and visitors. All swab samples were inoculated into the tubes containing sabouraud glucose agar 4% (SGA 4%) medium to transport to Medical Mycology Center, UMSU

### Culture and identification

A primary diagnosis on clinical specimens was performed using microscopic features of yeasts in direct preparations and morphological characteristics of colony on differential media: corn meal agar (CMA) and CHROM agar *Candida* (Diba, 2007). *Candida* infection was proved by microscopic observation of *Candida pseudohypha* or filamentous blastoconidia in direct preparations. This microscopic feature could be a probable reason for pathogenic form of *Candida* in tissue. The primary results of microscopy and culture were necessary for designing an environmental sampling protocol as mentioned before. Morphologic identifications were used for the environmental specimens as well.

### Molecular identification

All clinical and environmental isolates of *Candida* species were tested by molecular method of PCR-RFLP, as following steps.

### DNA extraction

Genomic DNA were extracted by Glass beads / Phenol-Chloroform method, (To make a lysis solution with 1mM EDTA, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl and 2% Triton X-100, in a total volume of 25 ml, It was added 250 µl of 1 M Tris, 100 µl of 1 M EDTA, 2500 µl of NaCl and 500 µl of Triton X-100 to 20 µl of distilled water, adjusted PH to 8.0 and then brought the volume to 25 ml). Isolation of DNA was followed by DNA purification and gel electrophoresis (Yamada et al., 2002).

### PCR amplification

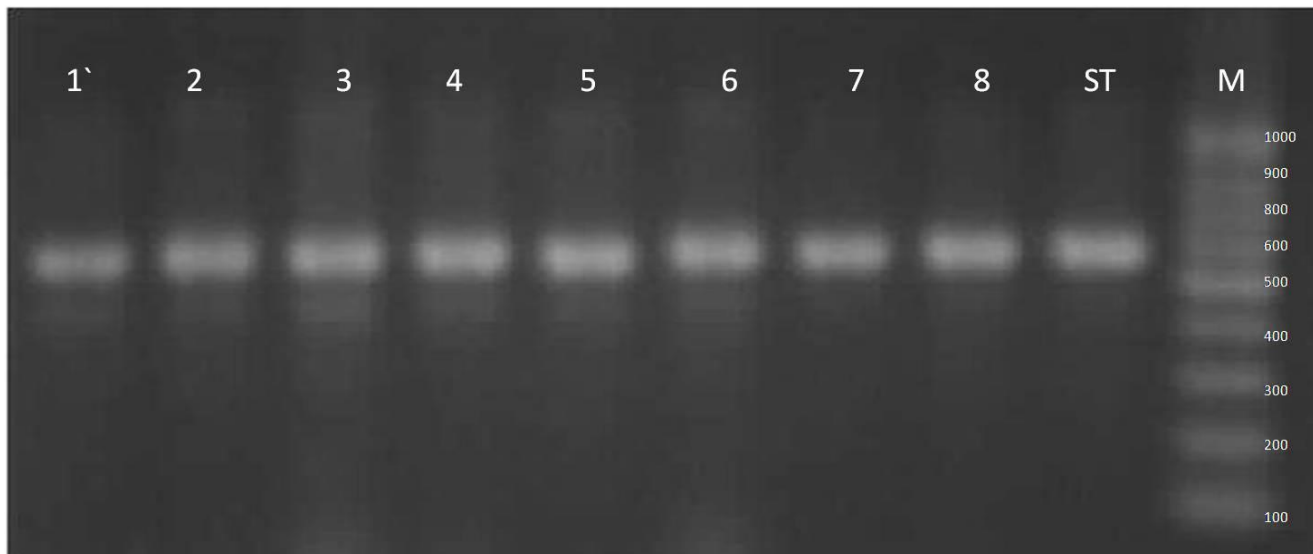
There are two important non coding regions as internal transcribed spacer 1 and 2 with a variable sub region inside and a conservative sub region outside the ITS fragments are located between the 18 and 28s rRNA gene of *Candida* species as well as other microorganisms. The variability of ITS regions has made it useful for the phylogenic studies of many organisms. The PCR assay was performed using 5 µl of the DNA template in a total reaction volume of 50 µl (consisting of PCR buffer (20 mM Tris- HCL at pH 8.0), 50 mM KCL, 0.1 mM each of forward (ITS: 5-TCC GTA GGT GAA CCT GCG G-3) and reverse (ITS-4 5-TCC TCC GCT TAT TGA TAT GC-3) primers (purchased from Mirhendi Molecular Biology Center, TUMS), and 1.5 U of Taq DNA polymerase. We used universal primers for the amplification of *Aspergillus* ITS regions (Forward Primer: 5'- TCC GTA GGT GAA CCT GCG G - 3' Reverse Primer: 5'- TCC TCC GCT TAT TGAT TAT GC - 3') (Henry, 2000). The reactions were performed in a Thermocycler model XL (Bioer, China). Thirty amplification cycles were performed in the thermo cycler after initial DNA denaturation at 95 °C for 5 min. Each cycle consisted of a denaturation step at 95°C for 30 s, an annealing step at 55°C for 30 s, and an extension step at 72°C for 1 min, with a final extension at 72°C for 5 min following the last cycle (Walsh et al., 1995). PCR products included 500 to 600 bp fragments were shown using a 1.5% agarose gel electrophoresis. After a post staining in 0.50 mg/ml of ethidium bromide solution, the results of gel electrophoresis were documented (Figure 1) by using a gel doc system (Bioer China) (Orita et al., 1989).

### Digestion with restriction enzyme

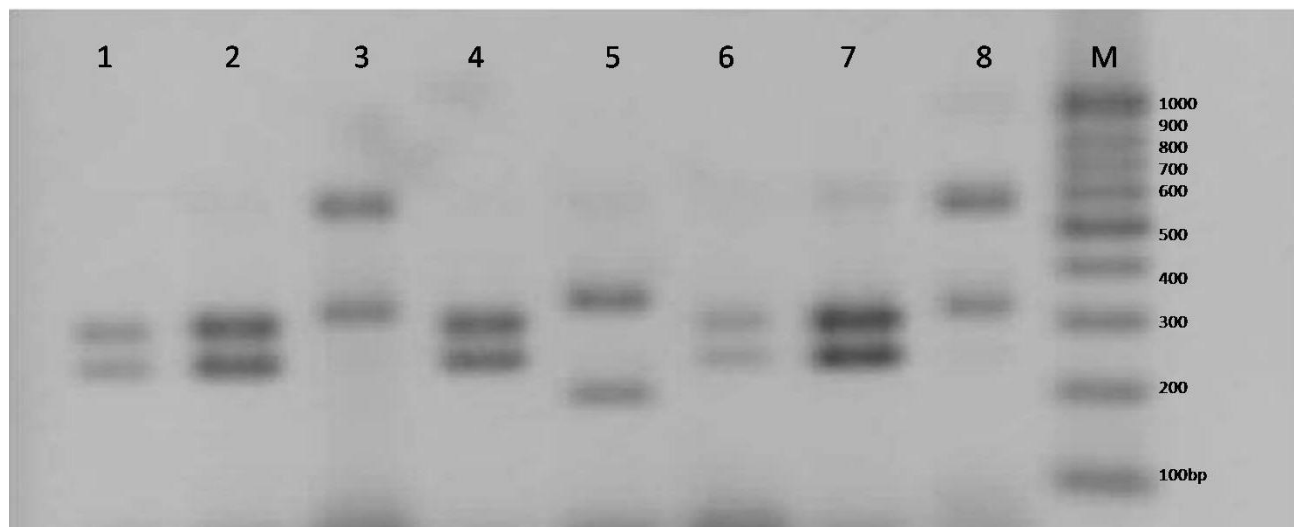
Restriction Fragment Length polymorphism differential pattern were used for the identification of *Candida* isolates. Restriction enzyme *MspI* were used for cutting the amplified DNAs of *Candida* spp (Mirhendi, 2006). Digestion of amplified ITS fragments produced different size fragments in a differential pattern so that we used them to identify some *Candida* species isolated from the clinical and environmental specimens. For the restriction digestion, 13 µl of each PCR product was directly digested with by 5 U (0.5 µl) of the restriction enzyme *MspI*, 1.5 µl of the related buffer, and incubated at 37°C for 180 min. Digested PCR products were subjected to electrophoresis in a 2% agarose gel and visualized with trans-illuminator (Moody, 1990) (Figure 2).

## RESULTS

During eighteen months from winter 2008 to autumn 2010 totally 198 samples were obtained from the hospitalized patients and their places. The results of experimental studies on the specimens showed 93(47%) positive for fungi or bacteria and 54(58%) of them had a fungal colonization. From the isolated fungi we obtained *Candida* spp. 36(66.6%), *Aspergillus* spp. 17(31.4%) and *Alternaria* sp. only one. *Candida* species isolated from the cases that was proved with a *Candida* infection and included: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii* and *C. parapsilosis*. Among the isolated *Aspergilli*; *A. flavus*, *A. fumigatus* and *A. niger* were the most frequent species respectively. *Alternaria* (Dematiaceus fungus) was only non *Candida* and non *Aspergillus* isolated fungus in this study which isolated from a case with septic arthritis. All results of *Candida* identifications were confirmed by PCR-RFLP method.



**Figure 1.** Agarose gel electrophoresis picture of amplified ITS fragments of *Candida* species by Taq polymerase, using general primers; ITS1 and ITS2. As it shown, approximately all species make the same size PCR bands in the electrophoresis.



**Figure 2.** Agarose gel electrophoresis of ITS1-ITS2 PCR products from standard *Candida* species after digestion with the restriction enzyme *MspI* Lanes 1,2,4,6,7: *C. albicans* ; 3,8: *C. glabrata* and 5: *C. tropicalis*; Lane M 100 bp ladder.

Sampling from air and indoor surfaces was performed in the hospitals.

Totally 256 specimens were collected from finger and body surface touch samples of patients, personnel, visitors and also swabs of bed, carpet, walls, trolleys, sink and medical devices. Number of each group was shown in the Table 1. Total of all isolates, 35(31.5%) *Candida* species, 48(43.2%) Aspergilli and 28(20.3%) other fungi. As shown in the table, the most frequent probable source of *Candida* is the patient with proved clinical infection, followed by personnel of the ward. From the mentioned

specimens, 110 fungal isolates including *Candida* and *Aspergillus* species and other contaminating moulds, were obtained and morphologically identified. Environmental isolated yeasts included: *Candida albicans* (60%), *C. krusei* (17%), *C. glabrata* (14.3%), *C. tropicalis* (0.7%) and *C. parapsilosis* (3%) were identified. The *Candida* findings were repeated by using molecular method, PCR-RFLP. Use of both methods; morphological and molecular enabled us to precisely identify *Candida* species isolated from clinical and environmental sources each.

Number of each group is shown in Table 1. Total of all

**Table 1.** Isolation of opportunistic fungi including *Candida* species, from hospital environmental sites (indoor and outdoor).

Isolated fungi	Environmental samples											
	Case	Personnel	Visitor	Floor	Walls	Bed and Blanket	Sink	Trolleys	Medical devices	Air	Air condition	Out door
<i>Candida</i>	13	5	1	2	4	4	3	1	0	0	0	2
<i>Aspergillus</i>	0	1	0	9	7	7	2	5	1	5	3	8
Other	0	0	0	3	5	4	1	4	6	4	1	0
Total	13	6	1	14	16	15	6	10	7	9	4	10

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## DISCUSSION

There is plenty of evidence supporting the role of opportunistic fungi as important agents of HAI. During the period 1980 to 1990 *Candida* species emerged as the sixth most common nosocomial pathogens (Almirante, 2005). Bacteria are transmitted between patients: (a) through direct contact between patients hands, saliva droplets or other body fluids, (b) in the air (droplets or dust contaminated by a patient's bacteria), (c) via staff contaminated through patient care (hands, clothes, nose and throat) who become transient or permanent carriers, subsequently transmitting bacteria to other patients by direct contact during care, (d) via objects contaminated by the patient (including equipment), the staff's hands, visitors or other environmental sources (e.g. water, other fluids, food) (Ortega, 2010). A cohort of 36 consecutive patients with nosocomial Candidiasis from a CHROM agar *Candida* culture and also PCR-RFLP program over a period of 18 months

(2008 to 2010) has been analyzed. According to our results, totally 93 specimens were collected from patients with symptomatic cases. Among all, Candidiasis was detected 66.6% using microscopic features in the direct smears of clinical samples, as mentioned in the methods section. In the yeast isolated series *C. albicans* has been the predominant species followed by *C. glabrata* and other non-albicans *Candida* such as *C. krusei*, *C. tropicalis* and *C. guilliermondii*. However, different species distribution has been reported throughout the world. For example, *C. albicans* and *C. glabrata* were most frequently identified in series from Denmark and the USA, whereas they were uncommon in South America (Pfaller and Diekema, 2007). *C. albicans* also followed by *C. glabrata* and *C. krusei* were the species most frequently isolated in our setting, as previously described [Almirante, 2005; Marco, 2003]. Because of resource limitations, we were unable to determine the species for all *Candida* isolates. We believe that some of the non-albicans isolates for which speciation was not molecularly performed such as *C. parapsilosis*. Therefore, our reported frequency of *C. albicans*, *C. glabrata* and *C. krusei* from clinical samples may represent an under estimation.

According to our results, the most yeast isolates were obtained from cases and personal samples in hospital indoor so that well known sources of

*Candida* contamination were hand touch of the cases followed by hand touch of nurses. As we know *Candida* flora or contamination of injection sites, catheters and sondes can be a source of Candidemia, so that persistent Candidemia in spite of appropriate antifungal treatment was reported in relation to central venous catheter infection in almost a half of cases (46%) (Horn, 2009). Moreover, our findings of environmental isolates showed that more than 30% at all samples positive for *Candida* species. From all of the tested environmental samples, carpets, trolleys and beds were the most contaminated surfaces, totally by isolated fungi but not for *Candida* spp. Walls, beds and washing sinks included most frequency of *Candida* species (Table). Some *Candida* species including *C. glabrata* and *C. krusei* are innately resistant to antifungal drugs such as some kinds of azoles (Wingard et al., 1991). High numbers isolation of these organisms from hospital indoor environments is explained away, considering *Candida* spp as the most frequent isolates from fungal nosocomial infections. *Candida* spp also were obtained from wet surface of washroom sinks commonly used by the patients. In fact contamination of the sinks by *Candida* agents could be explained by wet conditions and also prolonged use in the patients' room. On the other hand, exogenous *Candida* contamination of beds,

walls and trolleys is due to frequently touches of cases, staff and visitors with them. Several studies suggest that different *Candida* species may have similar adherence properties to cotton and other fabrics (Horn, 2009), as well as to plastic and other synthetic materials (Marco, 2003; Horn, 2009). It has been demonstrated in-vitro that once adhered to fabrics and synthetic materials, both *C. albicans* and *C. parapsilosis* can survive for several days. The adhesion capacity of diverse *Candida* species to different surfaces presumably plays a major role in the pathogenesis of human colonization and invasion (Horn, 2009) as well as in the establishment and perpetuation of infection (Almirante, 2005). As a result, different *Candida* species can contaminate similar environmental reservoirs and cause human infection through similar pathophysiological processes. The isolation of *Candida* species from clinical and environmental sources in our study is therefore not in disagreement with available knowledge. In conclusion, we believe that *Candida* contamination of environmental surfaces and hand touches, can facilitate the occurrence of several *Candida* infections predominantly in patients with immune compromised system, although most of *Candida* species isolated from clinical specimens were proved to be found in the environmental sources and also body surface of Patients and staff, but there is no reason that the clinical and environmental *Candida* isolates have a common source. It is necessary to more precise and reliable methods such as RAPD-PCR and pulse field gel electrophoresis to reach the exact sources of clinical isolates within the environment, case and staff materials.

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