



## Isolation and Detection of Yeast Biofilms From Urine Catheters of Infectious Patients

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### ABSTRACT

**Background:** Recent evidences have revealed that in more than 65% of microbial infections, biofilms have critical roles. The ability of yeasts to form biofilms on surface of medical devices such as urine catheters is an important reason of the capability of these microorganisms to cause human disease.

**Objectives:** The aim of this study was to isolate and identify yeasts on the surfaces of urine catheters and to investigate their ability to form biofilms.

**Patients and Methods:** In this research, 55 urine catheters from patients of ICU ward of Army Family Hospital in Tehran (Iran) were assayed. The catheters were taken aseptically to the laboratory for studying biofilms. Then they were sonicated and cultured on Sabouraud dextrose agar, CHROM agar and Corn meal agar. Examination of germ tube, sugar absorption and PCR method by two primers of ITS1 and ITS4 were used to identify pathogenic yeasts.

**Results:** According to the classical methods and molecular techniques, *Candida albicans*, *C. krusei*, *C. glabrata* and *C. tropicalis* were identified.

**Conclusions:** The results show that yeast biofilms can form on the surface of catheters. *Candida* species are the yeasts which were isolated from these surfaces of infectious patients. *C. tropicalis* was the most abundant species which was isolated from the patients of this study.

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### ► Implication for health policy/practice/research/medical education:

As a medical point of view, the findings can help to detect yeast infections and to control these kinds of infections.

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## 1. Background

The most important fungal pathogens are yeast species belonging to the genus *Candida*. *Candida* species are opportunistic pathogens that can cause superficial mucous membrane infections in life – threatening systemic diseases. Although *Candida albicans* remains the most frequently cause of Candidiasis, the incidence of the disease caused by non-*albicans* species has been increased steadily. A major

virulence factor of *Candida* species is its ability to form biofilm. Biofilms are a complex of microorganisms surrounded by a self-production matrix. In fact, biofilms include microbial cells and extra cellular polymeric substances (EPS) (1, 2). Biofilms can organize in most types of surfaces such as rocks and industrial line production; some parts of body like teeth and medical devices (3, 4). They are responsible for a wide range of infections and mortality especially in hospital infections. In the United States more than 45 million

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medical implant devices are used every year (5). Relevant infection to these devices occurs in 1-60% of patients. It was reported that *Candida* species cause 20% of infections. Infections associated with medical devices cause more than 50% of hospital infections (5). Biofilms exhibit increased resistance against most antimicrobial agents and that is an important problem in treatment of infections related to biofilms (1-6). To treat patients with these kinds of infections, at the first step, it is necessary to identify causes of infection. For this reason, isolation and characterization of yeast from the surface of catheters was carried out.

## 2. Objectives

The aim of this search was to isolate and identify yeasts from the surfaces of urine catheters of infectious patients in ICU ward of Army Family Hospital in Tehran. In addition the ability isolated yeasts to form biofilms was also investigated.

## 3. Patients and Methods

In this research, 55 urine catheters from patients of ICU ward of Army Family Hospital in Tehran were assayed. The catheters were taken aseptically to the laboratory for studying the biofilms. These catheters were sonicated by sonicator (Elma, Germany) in 60 hertz for 2 minutes. Then cell suspensions were cultured on Sabouraud dextrose agar (Merck, Germany) including 0.05 g/L chloramphenicol to eliminate bacterial growth. After that, isolated yeast was cultured on Chrome agar (Himedia), Corn meal agar (Merck). Production of germ tube, assimilation of sugar and PCR method were carried out to identify yeasts at this step. First, yeasts were cultured into sabouraud dextrose broth (Merck, Germany). Next, suspensions of yeasts including  $1 \times 10^6$  cells/ml were transferred into medium containing (67gr/L) yeast nitrogen base (Himedia, india) medium including 2% agar. Then blank disks were submerged on 20% of sugar solution of glucose, galactose, maltose, lactose, raffinose, terehalose, sucrose, cellobiose and xylose. After that, the disks were placed on the media and production of halo was investigated during 24-48 hours.

For germ tube test, a little of a pure culture of yeast was inoculated to 0.5ml of human serum and incubated for 2 hour. At 10 minutes intervals, a drop of the yeast-serum mixture was placed on a clean microscope slide, covered by a cover slip and observed through 10X and 40X objective lenses of microscope. The appearance of small filaments projecting from the cell surface confirmed formation of germ tubes. The earliest time of each germ tubes production was noted for each serum test (7).

For molecular identification it is necessary to extract DNA of yeasts. Genomic DNA was extracted using the glass bead disruption method. Then PCR was done by a pair of primers ITS1 and ITS4 (Sinagene, Iran). The sequences of the upstream and downstream primers are

5'-TCCGTAGGTGAACCTGCGG 3' (ITS1) and 5'-TCC TCC GCT TAT TGA TAT GC-3' (ITS4), respectively.

PCR amplification was carried out in a final volume of 25 $\mu$ l. Each reaction contained 2  $\mu$ l of template DNA, 12/5 $\mu$ l master mix, 7/5  $\mu$ l water, 1/5  $\mu$ l forward primer (ITS1) and 1/5  $\mu$ l reverse primer (ITS4). An initial denaturation step at 94 °C for 5 min was followed by 34 cycles of denaturation at 94 °C for 45s, annealing at 56 °C for 45s, extension at 72 °C for 55s and final extension step of 72 °C for 10 min. Amplified products were visualized by 1/5 % (W/V) agarose gel electrophoresis in TBE buffer, stained with ethidium bromide and photographed (8, 9).

In this study, the MspI enzyme was able to identify and differentiate six species of all yeasts. The reaction mixture and enzyme digestion was prepared as described in Table 1. All tests were performed at room temperature.

Microtubes were incubated for 2 hour at 37 °C. Restriction fragments were separated on 1/8% agarose gel through electrophoresis for 45 min at 100 V. The gel was stained with ethidium bromide (10).

**Table 1.** Component of the MspI Enzyme Digestion Reaction

Components	
Deionized water	17
X10Buffer	2
PCR product	10
MspI enzyme	1
Total volume	30

## 4. Results

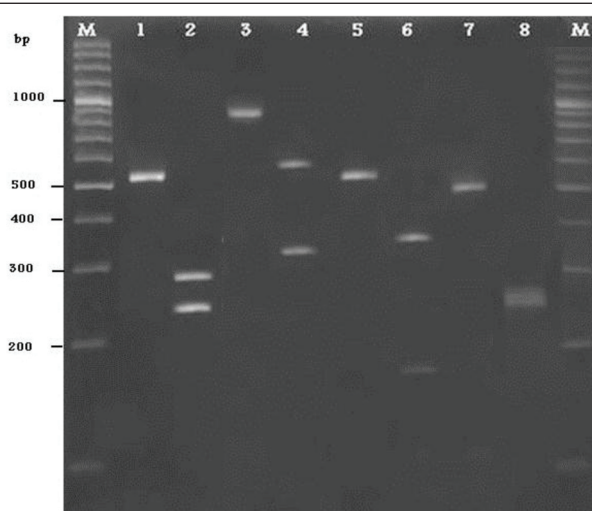
55 catheters were collected and assayed in this study. 29% of organisms which were isolated from urine catheters surface, were yeasts. 10 out of 16 of isolated yeasts were achieved from female patients (62.5%). It means only 6 yeasts were isolated from male patients (37.5%).

Glucose, galactose, maltose and terehalose showed the most amount of absorption and were used by all of yeasts. Among these sugars, lactose and raffinose were quantitatively adsorbed less. Results of culturing on Chromagar, Corn meal agar and germ tube technique was summarized in Table 2. As shown in this table, producing of germ tube and presence of chlamydiospore on Corn meal agar was investigated. The color of colony of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* on chrom agar medium were Clear green, blue - Blue-green, pink and purple, respectively.

Yeast DNA was amplified by ITS1/ITS4 primers. According to molecular techniques, such as DNA extraction, PCR amplification and sequencing of the intragenic transcribed spacers (ITS) the isolated yeasts were identified as *Candida albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata*. Among 16 yeasts, 4 out of all isolates were identified as *C. albicans* and 7, 4, 1 as a *C. tropicalis*, *C. krusei*, and *C. glabrata*, respectively (Figure). In this study, from catheters of patients, 4 different species of *Candida* were characterized.

**Table 2.** Results of Producing Germ Tube, Observing of Chlamydospore on Corn Meal Agar Were Listed in Table.

	Germ Tube	Corn Meal Agar
1	+	Producing Chlamydospore
2	+	Producing Chlamydospore
3	-	Not producing Chlamydospore
4	-	Not producing Chlamydospore
5	-	Not producing Chlamydospore
6	+	Producing Chlamydospore
7	-	Not Producing Chlamydospore
8	-	Not producing Chlamydospore
9	-	Not producing Chlamydospore
10	-	Not producing Chlamydospore
11	-	Not Producing Chlamydospore
12	+	producing Chlamydospore
13	-	Not producing Chlamydospore
14	-	Not producing Chlamydospore
15	-	Not producing Chlamydospore
16	-	Not producing Chlamydospore

**Figure.** Patterns of PCR Products of *Candida* Isolates Before and After Digestion with the Restriction Enzyme *MspI*.

1) PCR product of *C.albicans*, 2) PCR product of *C.albicans* after digestion with the restriction enzyme *MspI*, 3) PCR product of *C.glabrata*, 4) PCR product of *C.glabrata* after digestion with the restriction enzyme *MspI*, 5) PCR product of *C.tropicalis*, 6) PCR product of *C.tropicalis* after digestion with the restriction enzyme *MspI*, 7) PCR product of *C.krusei*, 8) PCR product of *C.tropicalis* after digestion with the restriction enzyme *MspI*.

## 5. Discussion

*Candida* is a fungus that inhabits in half of oral cavities of human (10). A major virulent factor of *Candida* species is its ability to adhere and form surface attached microbial communities known as biofilms (4, 10, 11). *Candida* biofilms are more resistant than their planktonic counterparts to different antimicrobial agents (6, 11). Resistance of *Candida* biofilms to antifungal agents was first demonstrated in 1995 (2). They have crucial role in

causing medical devices infections such as stents, shunts, implants and various types of catheters and hospital infections (6, 11). *Candida* infections of the urinary tract are strongly linked to the presence of urinary catheter (3). In contrast to 13% of non-catheter associated infections, the National Nosocomial Infections Surveillance (NNIS) data indicated that *C. albicans* caused 21% of catheter associated urinary tract infections (2). Biomaterials infections are a growing alarming problem because they are highly recalcitrant to antimicrobial therapy (11). Recently studies have shown that many of *Candida* species are able to attach to polymeric surfaces such as urine catheters and produce biofilms.

In this study abundant of isolated yeast species is 25% *C. albicans*, 25% *C. krusei*, 6.25% *C. glabrata* and 43.75% *C. tropicalis*. Molecular techniques and classical methods to identify isolates are in accordance except of some sugar's absorption. RFLP-PCR method is a rapid, easy, and reliable technique. This method can also be used in clinical laboratories to identify clinically important *Candida* spp. (12). Mirhendi and *et al.* in 2006 used RFLP-PCR for identifying six medically *Candida* species (13). Ayatollahi Mousavi *et al.* identified *Candida* species isolated from oral colonization in Iranian HIV-positive patients by RFLP-PCR method (14). Furthermore, Mohammadi *et al.* isolated and identified *Candida* species in patients with various forms of *Candidiasis* by using this molecular method. Results of all mentioned researches were accordant with our findings (12).

Febre *et al.* reported that *C. albicans* with 46.15% frequency was the most abundant fungus followed by *C. glabrata* (30.77%) and *C. krusei* (7.7%) from urine specimens (15). These results indicated that *C. tropicalis* has been replaced with *C. albicans* and *C. tropicalis* has the major role to cause yeast infections in form of biofilm. Also in other studies the presence of yeasts in 18.6% of urine specimens of patients with indwelling urinary catheters were observed (15). The results of this study revealed the presence of yeasts in 32% of patients with urinary catheters which indicates the fungal infections 'increasing. Pakshir was shown in patients with urine catheters involving both bacterial and *Candida* infections with abundance of 50.4% and 28.7%, respectively (16). Febre *et al.* have reported, women in comparison to men, are more susceptible to expose yeast infections (15). Pakshir has reported that abundance of *Candidiasis* in women and men was 68.9 % and 31.1 %, respectively (16). These findings are in accordance with our results. It can be concluded that yeast infections were increased in recent years and the crucial problem of this fungal structure is their resistant to antimicrobial agents because of biofilm formation. Further investigation will be carried out to assay antibiofilm agents against *Candida* species isolated from surface of urine catheters.

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