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# Identification of *Candida* Species Screened from Catheter Using Patients with PCR-RFLP Method

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**ABSTRACT**

*Candiduria or presence of infecting Candida species in urinary tract is one of the usual hospital infections. Factors increasing susceptibility of patients to such infections are including high utilization of antibiotics, long hospitalization periods, higher ages, diabetes mellitus, sexuality, female sex and use of immunosuppressive therapy. This study focuses not only on diagnosis of candiduria and its causes using phenotype and genotype analyzing methods, but also discusses on frequencies of Candiduria in catheter using patients. 250 samples of catheter using patients were collected and cultured in biological media. Identifying the yeasts, initially some phenotypic experiments were carried out such as germ tube, the production of hyphae or pseudohyphae and clamydospores in CMA+TW80 medium, and CHROMagar Candida. Genomic DNA of all species were extracted and analyzed with PCR and subsequent Polymerase Chain Reaction - Restriction Fragments Length Polymorphism (PCR-RFLP) methods. Analyzing urination of 95 female and 155 male, 40 samples were recognized as infected that demonstrates 55% and 45% Candiduria infection in females and males. Frequency of C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei are 45%, 32.5%, 15%, 5%, and 2.5%, respectively. Candiduria frequency is measured to be about 16%.*

**Key words:** Candiduria, *Candida albicans*, phenotype, genotype, CHROMagar *Candida*.

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## INTRODUCTION

*Candida* species are the main cause of most fungal infections. *Candida albicans* is the most abundant infection agent [1]. Candiduria is the main hospital infection that afflicts the urinary tracts [2]. This kind of disease is so rare and often happens in hospitalized patients, but in recent years it has been widely seen because of mentioned high risk factors [3]. The most important risk factors which help emergence of Candiduria are high utilization of antibiotics, long hospitalization periods, higher ages, diabetes mellitus, sexuality, female sex and use of immunosuppressive therapy [3, 4]. *Candida* is the pathogen causing urinary tract infections in 27% of utilizations of indwelling catheters [5]. Catheters are like entrance tracts for pathogenic bacteria into urinary system where they can colonize after long presence [2, 6]. Most experimental studies on Candiduria are highlighting dominance of *Candida*

*albicans* and *Candida glabrata* with 51% and 16% presence in infections, respectively [7, 8]. Diversity of infecting *Candida* species depend on environment. Non-albicans *Candida* spp have more incidence in throat and vagina [9, 10]. Choosing anti fungal agent depends on clinical status of patient, infection area, pharmacodynaics, and pharmacokinetics factors. fluconazole is highly preferred against urinary tract infections and has wide activity against *Candida* species [11]. There have been many methods for diagnosis of yeast and specially *Candida* species. They could be divided into two genotypic and phenotypic groups. Phenotypic methods are including: yeast colony morphologies on Malt extract agar and sugar absorption and fermentation tests and utilization of commercial kits like API and etc [12]. Genotypic methods are various and different such as specific primers in Polymerase Chain Reaction (PCR) and Multiplex PCR [13], specific probes for each specie [14], Polymerase Chain Reaction - Restriction Fragments Length Polymorphism (PCR-RFLP) [15, 16], sequencing of specific regions of genome [17], and Real-time PCR [18]. Each mentioned method has its advantages and disadvantages. This study focuses on diagnosis on *Candida* species based on phenotypic and genotypic approaches and analysis of frequency of Candiduria in catheter using patients.

## MATERIALS AND METHODS

250 samples were collected from patients of Shohada-e-Tajrish hospital, Tehran, that were using catheters for more than 10 days. Samples were cultured in CHROMagar *Candida* (biomeriux, France) for morphologic analysis such as color and shape of colonies, CMA+TW80 (Merck, Germany) for hyphae, pseudohyphae, and clamydospore presence. Ability to generate germ tube in fresh serum was used to distinguish yeast species. According to previously described method [19], by a bacteriological loop, about 10 $mm^3$ of a fresh colony was transferred to a 1.5 ml eppendorf tube and then 300 µl of lysis buffer (100 mM Tris pH 8, 10 mM pH 8, 100 mM NaCl, 1% SDS, Triton 2% X-100), 300 µl of phenol: chloroform (1:1) and 200 µl of glass beads, with a diameter of 1 mm, were added and the tube was vigorously shaken [20]. Then, the sample was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean tube and 400 µl of chloroform was added. After centrifuging as the previous conditions, the aqueous phase was transferred to a clean tube and then 1 volume of cold isopropanol and 5 of 3M sodium acetate (pH: 5.2) were added and was kept at -20 °C for 10 minutes. After that, the sample was washed by 70% ethanol. Then 50 µl distilled water was added and the sample was kept at -20 °C. PCR was performed to amplify ITS1-5.8S-ITS2 segment in ribosomal DNA. For this, ITS1-5.8S-ITS2 universal primers were used. The sequences of ITS1 and ITS4 were 5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATATGC-3' respectively [21, 22]. The components of PCR reaction were as follows: 2.5 µl of 10x PCR buffer, 1.5mM MgCl2, 0.5 µl of 10 mM dNTPs, 0.4µM Primers, 1.25 units of Taq polymerase (Sinagene, Iran), 1 µl of template DNA and molecular grade dH2o up to 25 µl. The temperature cycles were as follows: initial denaturation at 95 °C for 6 min, 30 cycles of 30 sec at 94 °C, 45 sec at 56 °C and 1 min at 72 °C and finally 72°C for 7 min. After confirming PCR products on agarose gel, they were digested by *MspI* (Roche Molecular, Germany). The components of digestion reaction were as follows: 10 µl of PCR products, 1.5 µl of digestion buffer, 5 units of *MSP*1 enzyme, and dH2o up to 15 µl. The prepared sample then was placed at 37 °C for 3 hours. Enzymatic digestion of PCR products of different yeasts will produce different patterns. The digested fragments were then electrophoresed through 1.8% agarose gel and then visualized by ethidium bromide staining [23].

## RESULTS

Total number of collected samples was 250 and positively infected samples were 40. Incidence frequency of Candiduria in this study is about 16%. Figure 1 and 2 shows the growth of *Candida albicans* colonies and *Candida tropicalis* on CHROMagar *Candida* medium. Figure 3 and 4 show PCR and PCR-RFLP of different *Candida* spp, respectively. Table 1 shows PCR product sizes of ITS1-ITS4 for different standard species of *Candida* before and after restriction digestion with *MspI*. Studied groups and frequency of infections are mentioned in Table 2. Part and partial frequencies of *Candida* species with in patients are mentioned in Table 3 and represents total dominance of *Candida albicans* specie.

**Figure1. *Candida albicans* colonies in CHROMagar *Candida* media.**

**Figure2. *Candida tropicalis* colonies in CHROMagar *Candida* media.**

**Table1. Size of ITS1-ITS4 PCR products of *Candida* species before and after digestion with *MspI* [20].**

| *Candida* species | Size of ITS1-ITS4 | Sizes(s) of restriction product(s) | Accession number |
|---|---|---|---|
| *C. albicans* | 535 | 297, 238 | L47111 |
| *C. glabrata* | 871 | 557, 314 | AF167993 |
| *C. tropicalis* | 524 | 340, 184 | L47112 |
| *C. krusei* | 510 | 261, 249 | L47113 |
| *C. parapsilosis* | 520 | 520 | L47109 |

**Table2. Part and partial frequencies of studied cases based on gender and Candiduria incidences.**

| Studied groups | Males | Females |
|---|---|---|
| Total Number (No/%) | 155 (62%) | 95 (38%) |
| Candiduria incidences (No/%) | 18 (16.61%) | 22 (23.15%) |

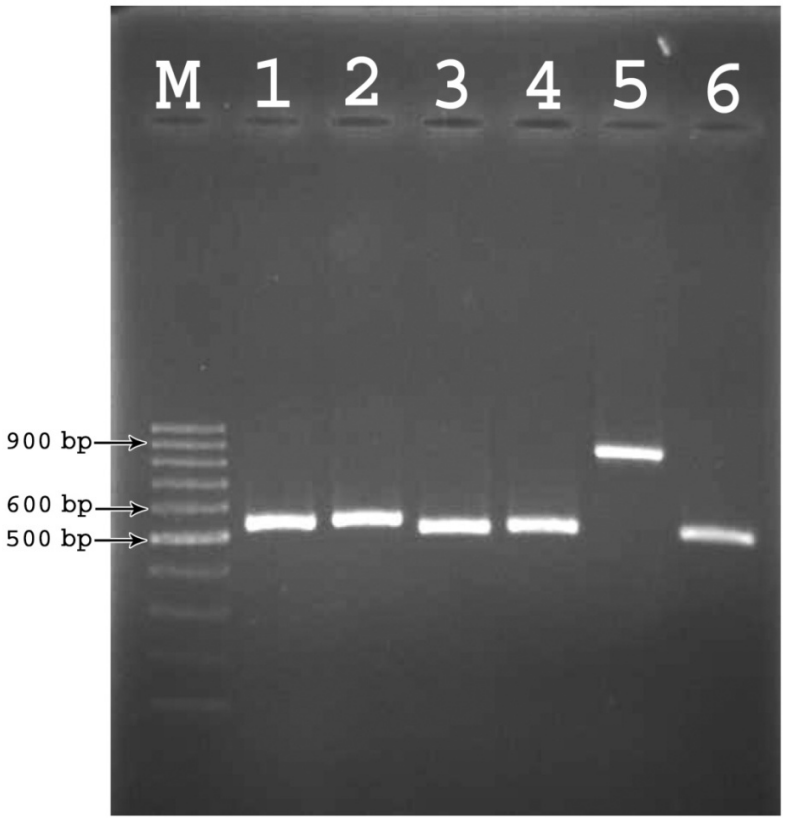


**Figure3. Electrophoresis of PCR products, 1: *C. tropicalis*, 2: *C. albicans*, 3: *C. parapsilosis*, 4: *C. parapsilosis*, 5: *C. glabrata*, 6: *C. krusei*.**

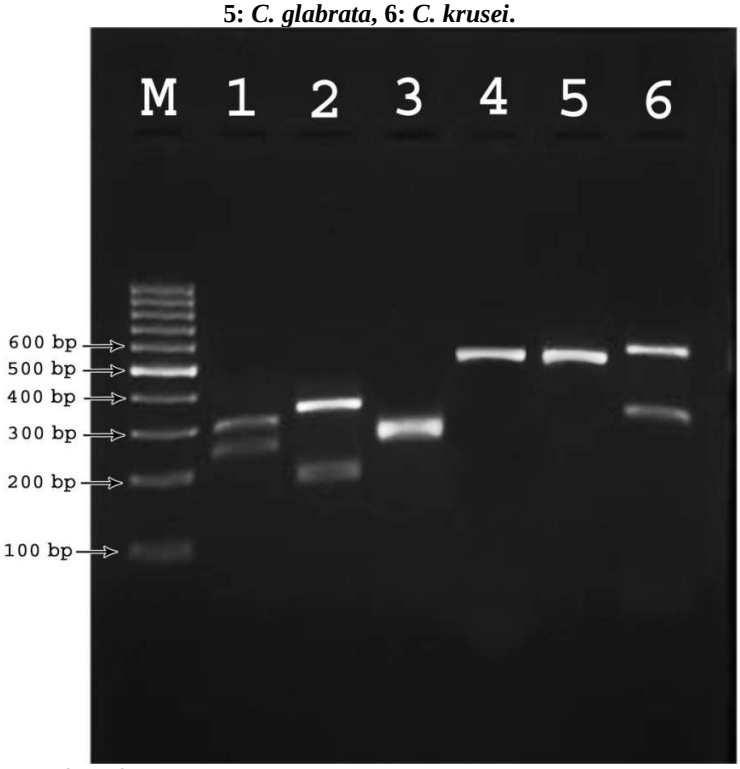


**Figure4. PCR-RFLP profile of samples, 1: *C. albicans*, 2: *C. tropicalis*, 3: *C. krusei*, 4: *C. parapsilosis*, 5: *C. parapsilosis*, 6: *C. glabrata*.**

**Table3. Part and partial frequencies of *Candida* species of patients.**

| Yeast spp | Frequencies | Number |
|---|---|---|
| *C. albicans* | 45% | 18 |
| *C. glabrata* | 32.5% | 13 |
| *C. tropicalis* | 15% | 6 |
| *C. parapsilosis* | 5% | 2 |
| *C. krusei* | 2.5% | 1 |
| Total | 100% | 40 |

## DISCUSSION

In this study frequency of Candiduria infection is reported about 16%. The color of *Candida albicans* colonies on CHROMagar *Candida* was green. Candiduria epidemiology in kidney transplanted patients studied at Wisconsin University within 8 years; the most abundant specie isolated from 51% of patients was *Candida glabrata*, most patients did not show any symptoms of infection. Risk factors in this study were defined as: Female sexuality (OR: 12.5%), hospitalization in ICU (OR: 8.8%), antibiotics utilization (OR= 3.8%), bladder catheter (OR= 4.4%), diabetes mellitus (OR= 2.2%), Neurologic Bladder (OR= 7.6%), and malnutrition (OR= 2.4%) [24]. In other report frequency of Candiduria is mentioned as 11% [25]. A meaningful increase in frequency of Candiduria infection happens along with increase in periods of catheter and antibiotics usage and hospitalization [26]. Risks for Candiduria infections within surgeries, diabetic patients, corticosteroids and immune suppressant drug treatments, catheterization, and antibiotics exploitation are 4, 2, 1.4, 12, and 6 folds higher, respectively. *Candida albicans* is the most abundant isolated microorganism from Candiduria patients with frequency of 68.62% [27]. *Candida albicans* is the most dominant specie in reports with 58.6% [25] and 56.7% [28] frequencies. PCR-RFLP data and sizes which are mentioned in table provide us usable information for determination of unknown yeast based on molecular sizes of known yeasts. In this study we verified the abundance of *albicans* specie and claimed higher incidence of infections in females. This shows an increase in number of incidences of infection in hospitalized patients who use urinary catheter.

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