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Identification and antifungal susceptibility pattern of *Candida* species isolated from patients with nosocomial candiduria

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Abstract:

Introduction: Nosocomial candiduria could be due to cystitis, pyelonephritis, or fungus ball in the urinary tract system. Several reports have shown candidemia and upper urinary tract involvement as the complications of candiduria. The aim of this study was to assess nosocomial candiduria; identify *Candida* isolates and determine their drug susceptibility pattern. **Materials and Methods:** Urine samples of 115 hospitalized patients were collected during a period of five months. *Candida* species were isolated and identified using conventional and molecular (PCR-RFLP) diagnostic methods. Antifungal susceptibility profiles for amphotericin B and fluconazole were performed using broth microdilution method, based on the Clinical and Laboratory Standards Institute (CLSI) M27-A2 guideline. **Results**: Nosocomial candiduria was diagnosed in 5 (4.3%) patients. The isolated *Candida* species were identified as *Candida albicans* (n: 4) and *C. glabrata* (n: 2). Two strains of *C. albicans*, and *C. glabrata* were resistant to fluconazole. **Conclusion:** Similar to several reports, the results of this study show that *C. albicans* is the main *Candida* species causing nosocomial candiduria and drug resistant *Candida species* are causative agents of candiduria in hospitalized patients.

Keywords: *:* *Candida* species, candiduria, fluconazole, nosocomial, PCR-RFLP.

Introduction

Among the various pathogenic and opportunistic fungal species causing urinary tract infections (UTIs), *Candida* species are the most common causative agent, particularly in hospitalized patients (Yashavanth *et al*., 2013). Although nosocomial candiduria is usually considered as benign lower urinary tract colonization or urine contamination, it may represent cystitis, pyelonephritis or fungus ball in the

urinary tract system (Singla *et al*., 2012). On the other hand, candidemia and upper urinary tract involvement are some complications of candiduria (Behzadi *et al*., 2015; Lundstrom and Sobel, 2001). Disseminated candidiasis due to candiduria occurs infrequently in patients with neutropenia, low-birth weight neonates, renal recipients, individuals with urinary tract obstruction and ICU patients (Lundstrom and Sobel, 2001; Bakhary, 2008; Guler *et al*., 2006; Fraisse *et al*., 2011; Nayman *et al*., 2011). Generally, differentiation of colonization from UTI is difficult and there is no suitable protocol for management of candiduria (Yashavanth *et al*., 2013; Lundstrom and Sobel, 2001).

Although *C. albicans* (52%) is the most common etiologic agent of candiduria, *non-albicansCandida* (NAC) species can also be related to UTIs and in 10% of cases, different *Candida* spp. may be isolated from a urine sample. The resistance of *Candida* spp., especially NAC spp. to antifungal drugs has increased in recent years. Also, the drug susceptibility patterns are different in various geographic regions (Behzadi *et al*., 2015; Dismukes *et al*., 2003).

Due to the increased prevalence of nosocomial candiduria in recent years, drug resistance of the causative agent and considerable complications of infection, early diagnosis and treatment of UTIs are important. The aim of this study was to diagnose candiduria in hospitalized patients, identify etiological agents and determine the antifungal susceptibility profile.

Materials and Methods

This cross-sectional study was conducted on 187 patients referred to the Hashemi Nejad Hospital in Tehran, from March to August 2014. Informed consent to participate in this study was signed by all patients, after which the study was approved by the Ethics Committee of Tehran University of Medical Sciences. Urine samples were first collected at the time of admission and patients without candiduria were followed. The second and third urine samples were collected 2 and 7 days after admission. A total of 10 µl of the sample was cultured on CHROM agar Candida medium (CHROM agar, France) before and after centrifugation. The culture media were incubated at 35°C for 48 h and evaluated based on color and number of growth colonies. If no growth was observed, the media were incubated for several additional days. In addition, isolated colonies were cultured on cornmeal agar medium (Micromedia, Hungary) supplemented with Tween 80 for identification of *Candida* species based on their morphology. In this study, urine wet-mount examination was performed to detect fungal elements in the urine sediment.

PCR-RFLP method was performed for definite identification of species. All isolated strains subcultured on Sabouraud dextrose agar medium (Sigma, USA), and genomic DNA were extracted by the phenol-chloroform method. PCR amplification was performed using the ITS1 (forward: 5´-TCC-GTA-GGT-GAA-CCT-GCG-G-3´) and ITS4 (reverse: 5´-TCC-TCC-GCT-TAT-TGA-TAT-GC-3´) primers. *Msp*I restriction enzyme was used for digestion of PCR products, and restriction fragments were separated by 2% agarose gel electrophoresis.

Susceptibility of *Candida* isolates to fluconazole (Avesina, Iran), and amphotericin B (Cipla, India) was evaluated using broth micro-dilution method according to the Clinical and Laboratory Standards Institute (CLSI)-M27-A2 guideline. Stock solutions were prepared in dimethyl

sulfoxide. Further dilutions of each antifungal agent were prepared with RPMI 1640 medium. The drug dilutions were dispensed into 96-well microdilution plates. The final concentrations of the antifungal agents ranged from 0.3125 - 64 µg/ml for fluconazole and 0.3125 - 16 µg/ml for amphotericin B.

The yeast inoculum was provided to a concentration of 0.5 to 2.5 × 10^3 cell/ml in the RPMI 1640 medium, and 100 µl of inoculum was added to each well of the microdilution plates.

These plates were incubated at 37°C for 24-48 h and the MIC endpoints were visually determined. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality controls.

Data analysis

Statistical analysis was carried out using Fisher's exact test.

Results

In the present study, a total of 115 patients which included 75 (65.2%) males and 40 (34.8%) females aged 5-85 years, were followed for nosocomial candiduria. The urine cultures of 5 (4.3%) patients were positive and by morphological method, the *Candida* isolates were identified as *C. albicans* (n: 4) and *C. glabrata* (n: 2), (Fig. 1).In this study, the urine culture of a patient with history of renal transplantation and recurrent UTIs, revealed both C. *albicans* and C. *glabrata* species. Direct examination of urine samples showed yeast budding cells in the urine sediments of two patients with candiduria (Fig. 2).

In the present study, by PCR-RFLP method, six isolated yeasts were identified as *C.albicans* (n: 4) and *C.glabrata* (n: 2) (Fig. 3) which confirmed the results of the conventional method (Table. 1).

In this study, a total of 5 cases of nosocomial candiduria were identified, all in female patients aged 18 - 34 years. In none of them candiduria was accompanied by vaginal candidiasis. By applying Fisher's exact test, the correlation between candiduria and sex was found to be statistically significant ($P<0.05$). However, there was no significant correlation between candiduria and age or underlying diseases of patients.

The results of antifungal susceptibility testing for fluconazole and amphotericin B showed that two *C. albicans* strains were susceptible to fluconazole (MIC≤8), while two other *C. albicans* strains and both strains of *C. glabrata* were resistant to fluconazole (MIC≥64). All isolated *Candida* spp. were susceptible to amphotericin B (MIC≤1).

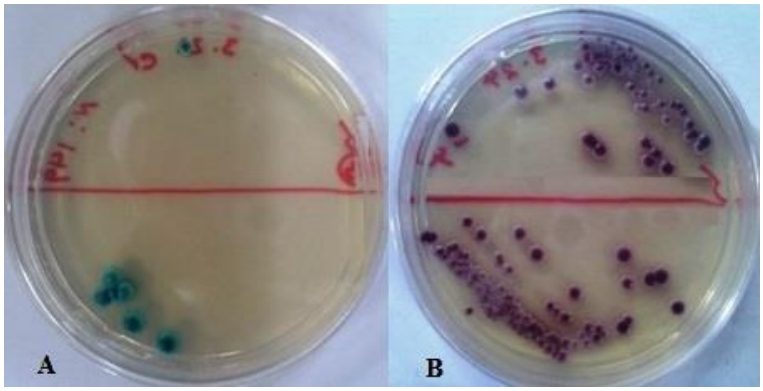

Fig. 1. C. *albicans* (A) and C*. glabrata* (B) isolated from urine culture (CHROMagar Candida medium)

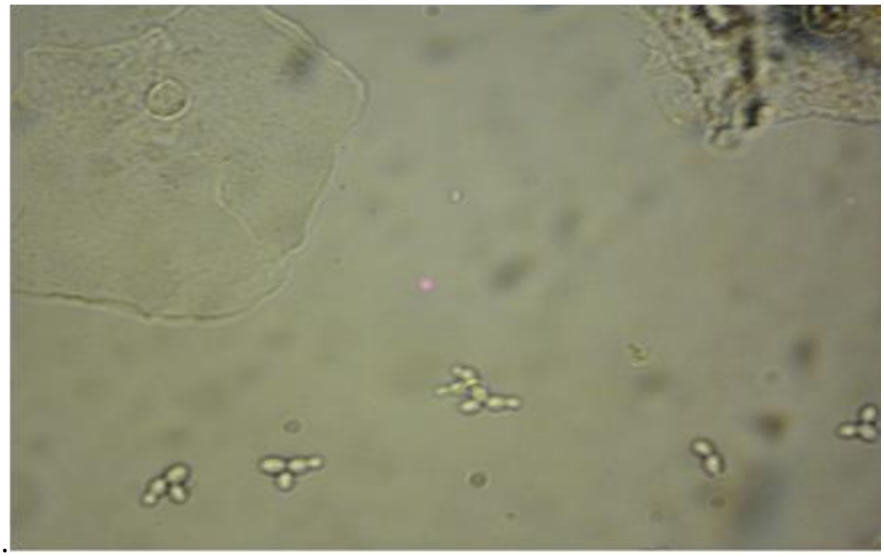

Fig. 2. Budding yeast cells in direct examination of urine sediment (×400)

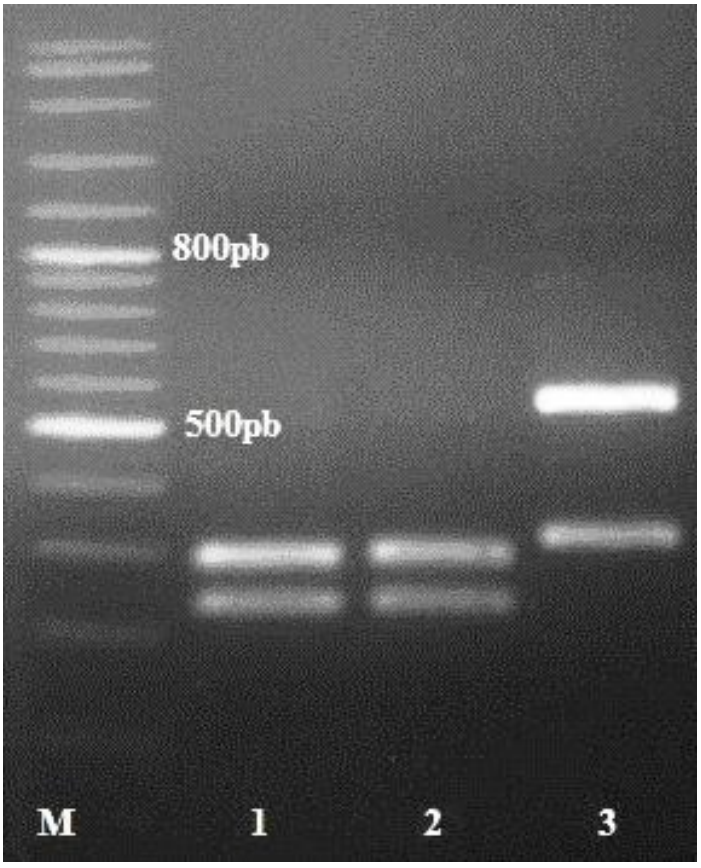

Fig. 3. PCR product patterns of *Candida* isolates after digestion with the restriction enzyme *Msp*I. Lane 1 and 2: *C. albicans* , lane 3: *C. glabrata*, Lane M: 100 bp DNA ladder

Table 1. The results of conventional and molecular methods in diagnosis of candiduria in hospitalized patients in the Hashemi Nejad Hospital -Tehran

No.	Direct examination	Colony No/1ml whole urine sample	Colony No/1ml urine sediment sample	CHROMagar Candida medium (color of colony)	CMA+tween 80 medium	PCR-RFLP
1	-	100	1000	*C. albicans* (Green)	*C. albicans*	*C. albicans*
2	Budding yeast	300	1300	*C. albicans* (Green)	*C. albicans*	*C. albicans*
3	Budding yeast	3000	5000	*C.glabrata* (Purple)	NAC species	*C.glabrata*
4	-	0	100	*C.glabrata* (Purple)	NAC species	*C.glabrata*
5	-	100	200	*C. albicans* (Green)	*C. albicans*	*C. albicans*
6	-	100	600	*C. albicans* (Green)	*C. albicans*	*C. albicans*

Discussion

The frequency of UTIs due to *Candida* spp. is becoming increasingly common, especially in hospitalized patients. The presence of *Candida* spp. in urine may indicate contamination, colonization or infection. It is difficult to differentiate UTI from colonization (Lundstrom and Sobel, 2001). Most cases of candiduria are considered as benign asymptomatic infection, but may be associated with candidemia and renal infection. Indeed, candiduria could result in pyelonephritis or disseminated infection (Bakhary, 2008).

Candiduria is a common finding in patients with predisposing factors such as diabetes mellitus, indwelling urinary catheters, renal transplantation, immunosuppressive therapy and prolonged hospital stay. It was accompanied with renal abscess and fungus ball in more than 40% of very low birth-weight neonates and 85% of patients with candiduria had been previously treated for bacterial infections (Bakhary, 2008; Calderone, 2002; Achkar and Fries, 2010). Treatment of candiduria depends on the clinical status of patients and individuals with symptomatic UTI and underlying diseases should be treated with appropriate antifungal drugs (Sellami *et al*., 2006; Kauffman *et al*., 2000).

In the present study, 4.3% of urine specimens were positive for *Candida* spp. and this rate is low in comparison with some other studies (Zarei *et al*., 2012; Pakshir *et al*., 2004; Fakour *et al*., 2004; Joz *et al*., 2011; Seifi *et al*., 2013). This could be related to different populations of patients, variation in hospital setting and different geographic regions. In our study, candiduria was only seen in women and there was a significant correlation between gender and nosocomial candiduria ($P < 0.05$). In some other studies, candiduria was also found in women (Zarei *et al*., 2012; Pakshir *et al*., 2004). This may be due to shorter urethra and vaginal candidiasis in women or anti-*Candida* properties of prostate fluid in men

(Pakshir *et al*., 2004). None of our patients suffered from vulvovaginal candidiasis and anatomical difference could be responsible for more occurrence of candiduria in female patients.

In direct examination of urine samples, budding yeast cells were seen in only two cases of nosocomial candiduria. This finding suggests that, negative direct examination does not rule out candiduria and both direct examination and culture should be done (Fakour *et al*., 2004). It was also shown that the relatively large lipid contents in cell wall of some *Candida* species caused yeast cells to float in urine (Zaini *et al*., 1993). Therefore, in our study urine samples were cultured before and after centrifugation. But, the cultures of urine sediments yielded much greater numbers of yeast colonies in comparison with whole urine samples. A urine sediment culture in this study also yielded C. *albicans* and C. *glabrata* colonies, whereas from the culture of whole urine sample only *C. albicans* colonies were isolated. These results show the importance of using urine sediment in the isolation of *Candida* spp. from a urine specimen.

Some researchers believe that; 10^3cfu/ml is valuable for diagnosis of UTIs in patients without urinary catheter. In other researches, 10^4cfu/ml in patients with an indwelling catheter was considered as UTIs. However, urinary colonization has been reported as 10^4 to $\geq 10^5$ cfu/ml (Kauffman, 2005). Therefore, unlike bacteria there is no standard colony counting for differentiation of UTI from urine contamination and usually, isolated colonies are interpreted depending on the patient's underlying factors. Although, in our study we could not certainly confirm infection based on colony counting, but the underlying diseases of the patients, including hematologic malignancy, renal failure and renal transplantation, emphasizes follow-up on them.

All *Candida* species are capable of causing UTIs. Although, 50-65% of *Candida* UTIs are caused by *C. albicans*, the prevalence of infections caused by NAC spp. has increased in recent years (Lundstrom and Sobel, 2001; Calderone, 2002; Seifi *et al*., 2013; Goetz *et al*., 2010). Similar to other reports in the present study, *C.albicans* (66%) was the dominant species (Singla *et al*., 2012; Zarei *et al*., 2012; Seifi *et al*., 2013; Padawer *et al*., 2015). Resistance to fluconazole was shown in isolated species and all *Candida* isolates were susceptible to amphotericin B. The antifungal susceptibility pattern in this study was compatible with other reports (Zarei *et al*., 2013; Ozhak–Baysan *et al*., 2012; de Freitas *et al*., 2014; Almeida *et al*., 2015).

Conclusion

Candiduria in hospitalized patients may represent urinary tract infection and requires early diagnosis and treatment. It is difficult to differentiate urinary infection from colonization and nosocomial candiduria caused by drug resistant non-albicans *Candida* species should also be considered.

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