Jundishapur J Microbiol. 2015 March; 8(3): e24298. DOI: 10.5812/jjm.24298

Published online 2015 March 21. **Research Article**

The Susceptibility Patterns of *Candida* Species Isolated From Urine Samples to Posaconazole and Caspofungin

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Received: October 1, 2014; **Revised:** November 22, 2014; **Accepted:** December 5, 2014

Background: Candiduria is a rising condition among hospitalized patients and *Candida albicans* is the most common recovered agent. However, non-*albicans Candida* species (NACs) such as *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* are also important. Although most *Candida* species especially *C. albicans* are sensitive to routinely used antifungals, an increasing trend in resistance has been observed among NACs.

Objectives: The aim of the present study was to detect the susceptibility of *Candida* strains recovered from candiduria in hospitalized patients against posaconazole and caspofungin.

Materials and Methods: A total of 120 urine samples were taken from patients hospitalized in Intensive Care Units (ICUs) (65) and urology (55) wards. All recovered yeasts were differentiated by using CHROMagar Candida medium and routine tests for identification of *Candida* species. Minimal inhibitory concentrations (MICs) of all isolates towards posaconazole and caspofungin were determined using the microdilution method with serial dilutions from 8 to 0.0625 μg/mL (posaconazole) and 4 to 0.03125 μg/ mL (caspofungin).

Results: In total, 41.7% of urine samples were positive for *Candida* isolation, including *C. albicans* (46%), *C. glabrata* (24%), *C. tropicalis* (16%) and *C. krusei* (14%). The MIC of caspofungin for 90% of the tested isolates was lower than 2 μg/mL. Furthermore, 94% of the tested isolates were inhibited by posaconazole at lower than 2 μg/mL after 24 hours, whereas 6% of isolates had MICs of more than 4 μg/mL.

Conclusions: This study demonstrates the importance of *Candida* species in urine samples from hospitalized patients in ICUs and urology wards. It showed that both tested antifungals had excellent effects on different species of *Candida*, however the strains from ICUs were found to be more sensitive to caspofungin than posaconazole.

Keywords: Candida; Intensive Care Units; Posaconazole; Caspofungin

1. Background

There has been an increasing trend in the prevalence of candidiasis during the last two to three decades. Long stays at hospitals, antibacterial therapy, immunocompromised disease, instrument therapy, corticosteroid therapy and organ transplantation have been considered as important factors for invasive candidiasis. The urinary tract is the most common site of infection among hospitalized patients. However, the majority of urinary tract infections (UTIs) are bacterial and fungal infections account for only up to 10% (1). Although, several fungi such as saprophytic molds are associated with fungal UTIs, *Candida* species are the most prevalent fungal isolates (2). Candiduria is a general term for the presence of *Candida* species in the urinary system with different forms, asymptomatic candiduria to clinical sepsis. Candiduria is a common contamination among hospitalized patients (Intensive Care Unit (ICU) and neonatal intensive care unit (NICU)) and its mortality rate is higher among immunocompromised patients.

Candida albicans is the most common agent for candiduria, while non-*albicans Candida* species (NACs) such as *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* are also important due to increasing resistance to antifungal agents (3, 4). Although most species of *Candida*, especially *C. albicans*, are sensitive to routinely used antifungals increasing resistances is observed among NACs. Resistance to fluconazole among NACs is an important problem for clinicians during therapy and prophylaxis (3, 5, 6).

Two new antifungal agents, caspofungin (class echinocandins) and posaconazole (class triazoles), have shown excellent activities against *Candida* species *in vitro* (7, 8). Caspofungin (Cancidas) acts against fungal growth via inhibition of 1,3-β-D-glucan synthase. The broad-spectrum of fungicidal activity against *Candida* species, low side effects, and favorable drug interaction profile has made caspofungin as a first-line therapy for different types of candidiasis (9). On the other hand, posaconazole (Noxafil) is an approved triazole that is usually used for the treatment of fungal UTIs (10).

2. Objectives

The aim of the present study was to detect the frequency of candiduria in patients hospitalized at the ICU and urology wards of hospitals in Ahvaz, Khuzestan, Iran. In addition, the susceptibility of recovered agents was evaluated against two new antifungals, posaconazole and caspofungin.

3. Materials and Methods

The study and sampling from patients was approved by the Ethics committee of the Ahvaz Jundishapur University of Medical Sciences. A total of 120 urine samples were taken from patients hospitalized at ICUs (n = 65, 54.2%) and urology (n= 55, 45.8%) wards, including 74 (61.7%) males and 46 (38.3%) females. Samples were collected in sterile containers and immediately transferred to the medical mycology laboratory, affiliated to the Ahvaz Jundishapur University of medical sciences and then 10 µL of each urine sample was inoculated on CHROMagar Candida (CHROMagar Microbiology, Paris, France) as lawn. Cultures were aerobically incubated at 35-37°C for 24 - 48 hours. The colored colonies on culture media were initially determined as yeast using a light microscope. Final identification of the isolated yeasts was carried out by classical methods including germ-tube formation, growth at 42 - 45°C, and microscopic morphology on Cornmeal agar (High Media, India) with 1% Tween 80 (11).

Colony counts were also applied for all cultures and total CFU per mL was calculated. The minimal inhibitory concentrations (MICs) of the isolates against posaconazole and caspofungin (Sigma, USA) were determined for all recovered isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M27-A3 document) (12). In the present study 50 strains of *Candida* species including; *C. albicans* (23 strains), *C. glabrata* , (12 strains) *C. tropicalis* (8 strains) and *C. krusei* (7 strains) were tested for antifungals susceptibility. Briefly, for each antifungal a serial dilution from 8 to 0.0625 µg/mL (Posaconazole) and 4 to 0.03125 µg/mL (Caspofungin), was prepared using RPMI 1640 (Bio IDEA, Iran) in 96-well microplates (SPL, Korea). Then a standard suspension of yeast was inoculated in each well and read using the Enzyme Linked Immunosorbent Assay (ELISA) Reader (Bio Lab, USA) at 530 nm wavelength after 24 and 48 hours.

4. Results

Primary screening by CHROMagar Candida and further conventional methods yielded the isolation of *Candida* strains in 50 (41.7%) urine samples of which 28 (56%) were from ICU (male/female = 13/15) and 22 (44%) from urology wards (M/F = 10/12). *Candida albicans* (n = 23, 46%) was the most frequently recovered isolate from both sampled wards, followed by *C. glabrata* (n = 12, 24%), *C. tropicalis* (n = 8, 16%) and *C. krusei* (n = 7, 14%). As shown in Table 1 *C. glabrata* and *C. tropicalis* were mainly isolated from ICU wards. The colony counts of isolated *Candida* from ICU were more than 104 CFU/mL in 78.6% of positive cultures (Table 2). The results of colony count in urology ward samples are shown in Table 2. As shown, 54.5% of the samples yielded more than 104 CFU/mL.

Table 1. The Frequency of *Candida* Species Isolated From Intensive Care Unit and Urology Wards [a]

Organisms	Urology Ward	ICU	Total
C. albicans	12 (24)	11 (22)	23 (46)
C. glabrata	4 (8)	8 (16)	12 (24)
C. tropicalis	1 (2)	7 (14)	8 (16)
C. krusei	5 (10)	2 (4)	7 (14)
Total	22 (44)	28 (56)	50 (100)

[a] Data are presented No. (%).

Table 2. Details of Colony Counts in Positive Samples Recovered From Intensive Care Unit and Urology Wards [a]

Organisms	≤ 500	501 - 1000	1001 - 5000	5001 - 10000	> 10000	Total
C. albicans						
Urology	3 (13.6)	2 (9.1)	1 (4.5)	1 (4.5)	5 (22.7)	12 (54.5)
ICU	0 (0)	0 (0)	4 (14.3)	0 (0)	7 (25)	11 (39.3)
C. tropicalis						
Urology	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	1 (4.5)
ICU	0 (0)	0 (0)	1 (3.6)	0 (0)	6 (21.4)	7 (25)
C. glabrata						
Urology	0 (0)	0 (0)	0 (0)	0 (0)	4 (18.2)	4 (18.2)
ICU	0 (0)	0 (0)	1 (3.6)	0 (0)	7 (25)	8 (28.6)
C. krusei						
Urology	0 (0)	0 (0)	1 (4.5)	1 (4.5)	3 (13.6)	5 (22.7)
ICU	0 (0)	0 (0)	0 (0)	0 (0)	2 (7.1)	2 (7.1)
Total						
Urology	3 (13.6)	3 (13.6)	2 (9.1)	2 (9.1)	12 (54.5)	22 (100)
ICU	0 (0)	0 (0)	6 (21.4)	0 (0)	22 (78.6)	28 (100)

[a] Data are presented No. (%).

In view of susceptibility testing, 90% of isolates were sensitive to caspofungin; MIC of 0.03125 μg/mL for 24% of tested isolates followed by 0.0625 μg/mL for 16%, 0.125 μg/mL for 8%, 0.25 μg/mL for 18%, 0.5 μg/mL for 16%, 1 μg/mL for 6% and 2 μg/mL for 2%; and only 10% of isolates including two (4%) *C. glabrata* and three (6%) *C. albicans* were resistant to this drug (MIC = 4 μg/mL). The details of MIC for each species are shown in Table 3. Posaconazole inhibited the growth of 74% of tested isolates at a concentration lower than 0.5 μg/mL after 24 hours, while 20% of the isolates were dose dependent (MIC = 1-2 μg/mL). Resistance to posaconazole was detected in three (6%) of the *C. albicans* isolates after 24 hours, however this rate increased to six (12%) after 48 hours of incubation (Table 4).

Table 3. Susceptibility of *Candida* Isolates to Caspofungin After Twenty-Four Hours

Susceptibility	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*	Total
Sensitive[a, b]					
0.03125	5 (10)	2 (4)	2 (4)	3 (6)	12 (24)
0.0625	3 (6)	2 (4)	3 (6)	0 (0)	8 (16)
0.125	2 (4)	0 (0)	1 (2)	1 (2)	4 (8)
0.25	6 (12)	1 (2)	1 (2)	1 (2)	9 (18)
0.5	3 (6)	5 (10)	0 (0)	0 (0)	8 (16)
1	1 (2)	0 (0)	0 (0)	2 (4)	3 (6)
2	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Resistance [a,c]					
4	3 (6)	2 (4)	0 (0)	0 (0)	5 (10)
MIC 50 [d]	0.25	0.5	0.0625	0.125	0.25
MIC 90	4	4	2	1	2
Total [a]	23 (46)	12 (24)	8 (16)	7 (14)	50 (100)

[a] Data are presented No. (%).
[b] Concentration (μg/mL) ≤ 2.
[c] Concentration (μg/mL) > 2.
[d] Abbreviation: MIC, minimum inhibitory concentration.

Table 4. Susceptibility of *Candida* Isolates to Posaconazole After Twenty-Four and Forty-Eight Hours

Susceptibility	*C. albicans*		*C. glabrata*		*C. tropicalis*		*C. krusei*		Total	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Sensitive[a, b]										
0.0625	6 (12)	3 (6)	2 (4)	2 (4)	2 (4)	1 (2)	1 (2)	1 (2)	11 (22)	7 (14)
0.125	2 (4)	1 (2)	3 (6)	1 (2)	3 (6)	2 (4)	1 (2)	1 (2)	9 (18)	5 (10)
0.25	1 (2)	1 (2)	3 (6)	2 (4)	2 (4)	2 (4)	0 (0)	0 (0)	6 (12)	5 (10)
0.5	7 (14)	6 (12)	1 (2)	0 (0)	0 (0)	1 (2)	3 (6)	3 (6)	11 (22)	10 (2)
Dose dependent [a,c]										
1	3 (6)	7 (14)	2 (4)	4 (8)	0 (0)	1 (2)	1 (2)	1 (2)	6 (12)	13 (2)
2	1 (2)	0 (0)	1 (2)	2 (4)	1 (2)	1 (2)	1 (2)	1 (2)	4 (8)	4 (8)
Resistance [a,d]										
4	2 (4)	3 (6)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	4 (8)
8	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	2 (4)
MIC 50 [e]	0.5	1	0.25	1	0.125	0.25	0.5	0.5	0.25	0.5
MIC 90	4	4	1	2	2	2	2	2	2	4
Total [a]	23 (46)		12 (24)		8 (16)		7 (14)		50 (100)	

[a] Data are presented No. (%).
[b] Concentration (μg/mL) ≤ 1.
[c] 1 < Concentration ≤ 2.
[d] Concentration (μg/mL) ≥ 4.
[e] Abbreviation: MIC, minimum inhibitory concentration.

5. Discussion

The presence of *Candida* species in urine, candiduria, is an asymptomatic condition that results from contamination during urine collection in patients with bladder colonization or upper urinary tract infection and haematogenous spread from other sites (13). The incidence rate of candiduria is variable and several reports have shown different frequencies. It corresponds to more predisposing factors including, long stays at hospitals (especially in ICU and NICU), urinary indwelling catheters, abnormality in urinary tract, immunosuppressive therapy in immunocompromised patients, renal transplantation, broad spectrum antibacterial therapy and hemodialysis (6, 14-17). In addition, candiduria was commonly found in elderly people aged 85 years and older (18).

The incidence of candiduria was estimated as 25,000 cases per year in the USA during 2004 (15). Multiple studies indicate that 10 - 22% of hospital acquired UTIs are caused by *Candida* species (14, 15, 19, 20). Other studies have indicated the high rates of *Candida* colonization ranging from 12.7% to 70.6% (5, 17, 21) in urine samples of ICU patients. Similarly, 56% and 44% of our patients hospitalized in ICU and urology wards, respectively, were contaminated with different species of *Candida*. The higher frequency of candiduria in females from both groups of patients (ICU = 53.6% and urology ward = 54.5%) is comparable with other studies, which concluded that females are at greater risk for development of candiduria (4, 10, 17).

Candida albicans has historically been reported as the predominant cause of funguria, however in the two recent decades a paradigm shift occurred toward NACs, particularly *C. glabrata*, *C. tropicalis* and *C. krusei* (5, 14, 17, 22). In some reports, *C. tropicalis* was ranked as the most important NACs (3, 17), while others reported that *C. glabrata* was the predominant species (16). In our study *C. albicans* was the predominant agent recovered from both groups, however in ICU patients, *C. glabrata* (28.6%) and *C. tropicalis* (25.0%) were the second and third-most frequent agents, respectively. In contrary to the study by Sobel et al. who believed that *C. glabrata* adapts well to some urine features like osmolality, substrate availability and pH, in the current survey *C. krusei* (22.7%) and *C. glabrata* (18.2%) were the most prevalent species isolated from urine in urology ward patients (15). Kauffman et al. (4) believed that in persistent candiduria, as low as 10,000 CFU/mL may mean infection, whereas 10,000 to $\geq$ 100,000 CFU/mL may result colonization. In our study, colony counts of $\geq$ 10,000 CFU/mL were seen in 54.5% of ICU and 78.6% of urology patients.

Caspofungin resistance in *Candida* species is known to be uncommon but some species e.g. *C. tropicalis* have been reported to be resistant. Pasquale et al. (21) documented the first case of *C. tropicalis* infection clinically resistant to caspofungin with MIC of 4 μg/mL. Caspofungin resistances in *C. parapsilosis* and *C. tropicalis* isolates have been reported from China and Malaysia (24, 25). Moreover, Krogh-Madsen et al. (22) revealed that caspofungin resistance could occur in *C. glabrata* during antifungal therapy. Our study showed that only five isolates (10%), including three *C. albicans* (two isolates from urology ward and one from ICU) and two *C. glabrata* strains (one strain from each ICU and urology ward) were resistant to caspofungin (MIC > 2 μg/mL). Non-*albicans Candida* species are usually resistant to most used antifungals (3, 6, 10). Fekkar et al. (23) detected FKS gene mutations that increased the resistance of *Candida* species to caspofungin.

Posaconazole is a triazole antifungal with a spectrum of activity that extends to some yeasts, many saprophytic and some endemic fungi. In a murine model of hematogenous renal candidiasis posaconazole was demonstrated to be efficacious (24). It is known to be active against *C. albicans* and a variety of NACs, including *C. krusei* and *C. glabrata* (10). However, its *in vitro* activity against *Candida* species is not static (25). Consistent with this fact, in our study, resistance of *C. albicans* and *C. glabrata* isolates to posaconazole increased from 6% after 24 hours to 12% after 48 hours of incubation while no resistance was found among *C. tropicalis* and *C. krusei* strains.

The present study demonstrates the importance of *Candida* species in urine samples from hospitalized patients in ICU and urology wards. Our study shows that both tested antifungals had excellent effects on different species of *Candida*, however most strains from the ICU were more sensitive to caspofungin than posaconazole.

Acknowledgements

We thank the Jundishapur Infectious and Tropical Diseases Research Centre for their cooperation with this study. This article was extracted from an MSc thesis (Fataemeh Ghanavati).

Authors' Contributions

Ali Zarei Mahmoudabadi developed the original idea and the protocol, edited the final manuscript, and was the guarantor. Fataemeh Ghanavati contributed to the development of the data and analyzed the data. Ali Rezaei-Matehkolaei contributed to the development of the protocol and edited the draft of the manuscript.

Funding/Support

This study was extracted from an MSc thesis (Fataemeh Ghanavati) supported by a grant (No: 91131) from the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran.

References

1. Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, Karchmer AW, et al. Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. *Clin Infect Dis*. 2000;**30**(1):19–24.
2. Ahmadzadeh A, Valavi E, Shamsizadeh A, Zarei Mahmoudabadi A, Hydari M, Ahmadzadeh A. Fungal urinary tract infection in

an infant with posterior urethral valves. *Jundishapur J Microbiol.* 2011;**4**(1):71–6.
3. Ozhak-Baysan B, Ogunc D, Colak D, Ongut G, Donmez L, Vural T, et al. Distribution and antifungal susceptibility of Candida species causing nosocomial candiduria. *Med Mycol.* 2012;**50**(5):529–32.
4. Kauffman CA, Fisher JF, Sobel JD, Newman CA. Candida urinary tract infections–diagnosis. *Clin Infect Dis.* 2011;**52 Suppl 6**:S452–6.
5. Singla N, Gulati N, Kaistha N, Chander J. Candida colonization in urine samples of ICU patients: determination of etiology, antifungal susceptibility testing and evaluation of associated risk factors. *Mycopathologia.* 2012;**174**(2):149–55.
6. Seifi Z, Azish M, Salehi Z, Zarei Mahmoudabadi A, Shamsizadeh A. Candiduria in children and susceptibility patterns of recovered Candida species to antifungal drugs in Ahvaz. *J Nephropathol.* 2013;**2**(2):122–8.
7. Tobudic S, Kratzer C, Lassnigg A, Graninger W, Presterl E. In vitro activity of antifungal combinations against Candida albicans biofilms. *J Antimicrob Chemother.* 2010;**65**(2):271–4.
8. DiDone L, Oga D, Krysan DJ. A novel assay of biofilm antifungal activity reveals that amphotericin B and caspofungin lyse Candida albicans cells in biofilms. *Yeast.* 2011;**28**(8):561–8.
9. Thompson GR, 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS, 2nd, Patterson TF. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to Candida glabrata infection. *Antimicrob Agents Chemother.* 2008;**52**(10):3783–5.
10. Fisher JF, Sobel JD, Kauffman CA, Newman CA. Candida urinary tract infections–treatment. *Clin Infect Dis.* 2011;**52 Suppl 6**:S457–66.
11. Salehei Z, Seifi Z, Zarei Mahmoudabadi A. Sensitivity of Vaginal Isolates of Candida to Eight Antifungal Drugs Isolated From Ahvaz, Iran. *J Microbiol.* 2012;**5**(4):574–7.
12. Clinical and Laboratory Standards Institute.. *Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard.* 3 edWayne: Clinical and Laboratory Standards Institute; 2008.
13. Kauffman CA. Candiduria. *Clin Infect Dis.* 2005;**41 Suppl 6**:S371–6.
14. Zarei-Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh B. Candiduria in hospitalized patients in teaching hospitals of Ahvaz. *Iran J Microbiol.* 2012;**4**(4):198–203.
15. Sobel JD, Fisher JF, Kauffman CA, Newman CA. Candida urinary tract infections–epidemiology. *Clin Infect Dis.* 2011;**52 Suppl 6**:S433–6.
16. Bukhary ZA. Candiduria: a review of clinical significance and management. *Saudi J Kidney Dis Transpl.* 2008;**19**(3):350–60.
17. Jain M, Dogra V, Mishra B, Thakur A, Loomba PS, Bhargava A. Candiduria in catheterized intensive care unit patients: emerging microbiological trends. *Indian J Pathol Microbiol.* 2011;**54**(3):552–5.
18. Fraisse T, Crouzet J, Lachaud L, Durand A, Charachon S, Lavigne JP, et al. Candiduria in those over 85 years old: a retrospective study of 73 patients. *Intern Med.* 2011;**50**(18):1935–40.
19. Zarei Mahmoudabadi A, Keradmand AR, Enayatollahi N. Frequency of candiduria in inpatients and outpatients in department of Urology, Golestan Hospital, Ahvaz, Iran. *Iran J Kidney Dis.* 2009;**3**(2):114–5.
20. Kobayashi CC, de Fernandes OF, Miranda KC, de Sousa ED, Silva Mdo R. Candiduria in hospital patients: a study prospective. *Mycopathologia.* 2004;**158**(1):49–52.
21. Pasquale T, Tomada JR, Ghannoun M, Dipersio J, Bonilla H. Emergence of Candida tropicalis resistant to caspofungin. *J Antimicrob Chemother.* 2008;**61**(1):219.
22. Krogh-Madsen M, Arendrup MC, Heslet L, Knudsen JD. Amphotericin B and caspofungin resistance in Candida glabrata isolates recovered from a critically ill patient. *Clin Infect Dis.* 2006;**42**(7):938–44.
23. Fekkar A, Dannaoui E, Meyer I, Imbert S, Brossas JY, Uzunov M, et al. Emergence of echinocandin-resistant Candida spp. in a hospital setting: a consequence of 10 years of increasing use of antifungal therapy? *Eur J Clin Microbiol Infect Dis.* 2014;**33**(9):1489–96.
24. Andes D, Marchillo K, Conklin R, Krishna G, Ezzet F, Cacciapuoti A, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother.* 2004;**48**(1):137–42.
25. Cuenca-Estrella M, Gomez-Lopez A, Cuesta I, Zaragoza O, Mellado E, Rodriguez-Tudela JL, et al. Frequency of voriconazole resistance in vitro among Spanish clinical isolates of Candida spp. According to breakpoints established by the Antifungal Subcommittee of the European Committee on Antimicrobial Susceptibility Testing. *Antimicrob Agents Chemother.* 2011;**55**(4):1794–7.