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

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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 wards were more than 104 CFU/mL in 78.6% of positive cultures. Table 1. The Frequency of Candida Species Isolated From Intensive Care Unit and Urology Wards

| 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) |

Data are presented No. (%).

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

| 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 | 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) |

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 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**                   |              |              |                |            |       |
| 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**                  |              |              |                |            |        |
| 4                               | 3 (6)        | 2 (4)        | 0 (0)          | 0 (0)      | 5 (10)  |
| MIC 50                         | 0.25         | 0.5          | 0.0625         | 0.125      | 0.25    |
| MIC 90                          | 4            | 4            | 2              | 1          | 2       |
| **Total**                      | 23 (46)      | 12 (24)      | 8 (16)         | 7 (14)     | 50 (100) |

| Data are presented No. (%). |
|-----------------------------|
| Concentration (µg/mL) ≤ 2.  |
| Concentration (µg/mL) > 2.  |

| 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 |
| **Sensitive**                   |              |              |                |            |       |
| 0.0625                          | 6 (12)       | 3 (6)        | 2 (4)          | 1 (2)      | 11 (22) |
| 0.125                           | 2 (4)        | 1 (2)        | 3 (6)          | 2 (4)      | 6 (14)  |
| 0.25                            | 1 (2)        | 1 (2)        | 3 (6)          | 2 (4)      | 5 (10)  |
| 0.5                             | 7 (14)       | 6 (12)       | 1 (2)          | 0 (0)      | 11 (22) |
| **Dose dependent**              |              |              |                |            |        |
| 1                               | 3 (6)        | 7 (14)       | 2 (4)          | 4 (8)      | 13 (2)  |
| 2                               | 1 (2)        | 0 (0)        | 1 (2)          | 0 (0)      | 4 (8)   |
| **Resistance**                  |              |              |                |            |        |
| 4                               | 2 (4)        | 3 (6)        | 0 (0)          | 1 (2)      | 6 (12)  |
| **MIC 50**                      | 0.5          | 0.25         | 0.125          | 0.25       | 0.5    |
| **MIC 90**                      | 4            | 4            | 1              | 2          | 4      |
| **Total**                      | 23 (46)      | 12 (24)      | 8 (16)         | 7 (14)     | 50 (100) |

| Data are presented No. (%). |
|-----------------------------|
| Concentration (µg/mL) ≤ 1.  |
| Concentration (µg/mL) ≤ 2.  |
| Concentration (µg/mL) ≥ 4.  |
| 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). Kaufman et al. (4) believed that in persistent candiduria, as low as 10,000 CFU/mL may mean infection, whereas 10,000 to ≥ 100,000 CFU/mL may result colonization. In our study, colony counts of ≥ 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 hematoegous 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 resistance could occur in *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.

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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 protocol and edited the final manuscript, and was responsible for the development of the data and the protocol, edited the draft of the manuscript.

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