Jundishapur J Microbiol. 2013;**6**(1): 24-28. DOI: 10.5812/jjm.4633

Jundishapur Journal of Microbiology
www.jjmicrobiol.com

Antifungal Susceptibility of *Candida* Species Isolated From Candiduria

Ali Zarei Mahmoudabadi[1,2], Majid Zarrin[1*], Maryam Beheshti Fard[1]

[1] Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
[2] Infectious Diseases and Tropical Medicine Centre, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 28 Feb 2012
Revised: 15 Apr 2012
Accepted: 28 Apr 2012

Keywords:
Antifungal Agents
Susceptibility
Candiduria
Candida Species

ABSTRACT

Background: Candiduria is one of the most common symptoms of urinary tract infections caused by several species of Candida spp.. Several antifungals are available to treat such candidal infections. During the last decades, resistance to antifungal especially to non-albicans species has increased.
Objectives: The present study aimed to evaluate the susceptibility to antifungal drugs of *Candida* species isolated from candiduria in Ahvaz.
Materials and Methods: Ninety three species of yeasts and yeast like organisms isolated from urine samples [*Candida albicans* (58), *C. glabrata* (25), *C. tropicalis* (4), *C. krusei* (1), unknown *Candida* species (4) and *Geotrichum* species (1)] were used for susceptibility tests. All species were re-identified based on standard mycological methods. Then a suspension of each isolate of overnight cultures was prepared in 1ml of sterile PBS and adjusted to 0.5 McFarland turbidity standards. In the present study several antifungal drugs (fluconazole, amphotericine B, ketoconazole, econazole, itraconazole) were used for susceptibility test using disk diffusion method.
Results: In the present study all tested isolates were sensitive/dose dependent to amphotericine B and nystatin, whereas only one isolate of *C. glabrata* was resistant to both antifungals. Resistance against fluconazole (48.4%) and ketoconazole (26.9%) were observed among tested isolates. Resistance against fluconazole was detected among all tested organisms, 34.4% of *C. albicans*, and 7.5% of *C. glabrata*. On the other hand, all isolates were sensitive to econazole (93.5% sensitive, 6.5% dose dependent).
Conclusions: It was concluded that *Candida* species isolated from candiduria in hospitalized patients had excellent *in vitro* sensitivity against econazole. Other suitable antifungal drugs were amphotericine B and nystatin, itraconazole. Whereas, resistance against ketoconazole (26.9%) and especially fluconazole (48.4%) was significant.

Implication for health policy/practice/research/medical education:
Candiduria is a common infection of the urinary tract. Evaluation of the susceptibility to antifungal drugs could be used for treatment and control of infection.

Please cite this paper as:
Zarei Mahmoudabadi A, Zarrin M, Beheshti Fard M. Antifungal Susceptibility of *Candida* Species Isolated From Candiduria. *Jundishapur J Microbiol.* 2013;**6**(1):24-8. DOI: 10.5812/jjm.4633

* *Corresponding author:* Majid Zarrin, Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran. Tel: +98-6113330074. Fax: +98-6113332036, *E-mail:* mjzarrin@yahoo.co.uk

DOI: 10.5812/jjm.4633

1. Background

Candiduria is one of the most common symptoms of urinary tract infections caused by several species of Candida, which is a normal flora of human body. *Candida albicans* has played an important role in candiduria (1, 2), however during the last decades non-albicans, such as *C. glabrata*, and *C. tropicalis*, have gradually increased in the incidence of nosocomial infections (1, 3-6). Old age, long stayin hospital, using broad spectrum antibiotics, and renal defects are the most important predisposing factors for candiduria (1, 5, 7, 8). Several reports have indicated that candiduria is a very common infection in hospitalized patients and its incidence is linked to antibiotic usage, long stay in hospitals, old age etc (2, 6, 9).

There are several valuable antifungals, such as amphotericine B, itraconazole, fluconazole, ketoconazole, econazole and nystatin, that are effective against *Candida* species. Some of these agents (fluconazole, amphotericine B, ketoconazole, econazole, itraconazole) are systemically used to treat urinary tract infections (UTI) (4, 10). Several reports have demonstrated that antifungal fluconazole has been effective for short-term eradication of candiduria (1, 11). On the other hand, some researches have found that the susceptibility degree of *Candida* species vary towards the used antifungal drugs (6, 7). For example, *C. krusei* and *C. glabrata* are resistant and less susceptible to fluconazole, respectively (7, 8, 12). Recent reports from different countries and hospitals have indicated that there has been an association between non-albicans and the rate of fluconazole resistance (12-14).

The susceptibility degrees of *Candida* species towards the used antifungal drugs vary and due to the growing use of these antifungals, resistance to these agents has increased during the last decades (14).

2. Objectives

The present study aimed to determine the susceptibilities to antifungal drugs of *Candida* species isolated from candiduria in hospitalized patients of educational hospitals in Ahvaz.

3. Materials and Methods

3.1. Tested Yeasts and Identification

In the present study, 92 *Candida* spp. isolates ,and one *Geotrichum* spp. isolate were used for susceptibility tests. All species had been previously isolated from urine samples of hospitalized patients in the two educational hospitals in Ahvaz and identified by routine methods. Tested Candida isolates were included, *C. albicans* (58, 62.3%), *C. glabrata* (25, 26.8%), *C. tropicalis* (4, 4.3%), *C. krusei* (1, 1.1%), and *Candida* spp. (4, 4.3%). In addition, one isolate (1.1%) of *Geotrichum* spp. was also used for susceptibility tests. All strains were preserved in sterile distilled water at refrigerator temperature. The isolates were first subcultured on CHROMagar Candida (CHROMagar Candida®, France) plates and incubated at 37°C for 24h, aerobically to check for purity. All isolates were re-identified based on standard mycological methods, morphology on CHROMagar Candida, morphology on cornmeal agar, germ tube production and growth at 45°C. Then a suspension of each isolate of overnight cultures was prepared in 1ml of sterile PBS and adjusted to 0.5 McFarland turbidity standards.

3.2. Susceptibility Method

two sterile swabs were dipped into the suspension and rolled separately on the surface of two series of plates containing Sabouraud dextrose agar SDA (Merck, Germany) as lawn (17). The inoculated plates were dried in laminar hood at ambient temperature for 15mins. Paper disks of antifungals were placed on plates (three antifungal disks for each plate) by forceps and incubated at 37°C for 24h, aerobically. Antifungal disks were nystatin (100U), amphotericine B (20μg), fluconazole (100μg), ketoconazole (10μg), itraconazole (50μg) and econazole (10μg). All antifungal disks were purchased from Liofilchem Bacteriology Products (Italy). After 24h, the zone diameter around each antifungal disk was manually measured by ruler and recorded.

4. Results

4.1. Interpretive Criteria for Susceptibility of Antifungals

The interpretive criteria for the fluconazole, nystatin, amphotericine B, ketoconazole, itraconazole and econazole disks were indicated in *Table 1* (15, 16).

Table 1. Interpretive Criteria of Susceptibility and Resistance of Used Antifungal Disks

	Zone Diameter in mm		
	Sensitive	**Dose Dependent**	**Resistance**
Amphotericine B	> 15	10-14	< 9
Nystatin	≥ 25	17-24	< 16
Fluconazole	≥ 19	15-18	≤ 14
Itraconazole	> 16	10-15	< 9
Ketoconazole	≥ 30	29-23	≤ 22
Econazole	> 16	10-15	< 9

4.2. Susceptibility to Amphotericine B

In the present study 54.8% and 44.1% were dose dependent and sensitive to amphotericine B, respectively (*Table 2*). 44.8% of *C. albicans* isolates were dose dependent and the rest of them (55.2) were sensitive to amphotericine B. One isolate (4%) of *C. glabrata* was resistant to amphotericine B, whereas 15 (60%) and 9 (36%) were dose dependent and sensitive to amphotericine B, respectively.

4.3. Susceptibility to Itraconazole

Susceptibility of tested isolates indicated that only one iso-

Table 2. Susceptibility of *Candida* spp. Isolates to Antifungal Drugs

Amphotericine B	*C. albicans*	*C. glabrata*	*Candida* sp.	*C. tropicalis*	*C. krusei*	*Geotrichum*	Total
Resistance	0 (0.0%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)
Dose dependent	26 (28.0%)	15 (16.1%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	51 (54.8%)
Sensitive	32 (34.4%)	9 (9.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0(0.0%)	41 (44.1%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)
Itraconazole							
Resistance	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)	0 (0.0%)	1 (1.1%)
Dose dependent	52 (55.9%)	24 (25.8%)	4 (4.3%)	4 (4.3%)	0 (0.0%)	1(1.1%)	85 (91.4%)
Sensitive	6 (6.5%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (7.5%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)
Nystatin							
Resistance	0 (0.0%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)
Dose dependent	23 (24.7%)	20 (21.5%)	4 (4.3%)	2 (2.2%)	1 (1.1%)	1 (1.1%)	51 (54.8%)
Sensitive	35 (37.6%)	4 (4.3%)	0 (0.0%)	2 (2.2%)	0 (0.0%)	0 (0.0%)	41 (44.1%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)
Econazole							
Resistance	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dose dependent	4 (4.3%)	2 (2.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (6.5%)
Sensitive	54 (58.1%)	23 (24.7%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	33 (93.5%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)
Fluconazole							
Resistance	32 (34.4%)	7 (7.5%)	2 (2.2%)	2 (2.2%)	1 (1.1%)	1 (1.1%)	45 (48.4%)
Dose dependent	17 (18.3%)	16 (17.2%)	1 (1.1%)	2 (2.2%)	0 (0.0%)	0 (0.0%)	36 (38.7%)
Sensitive	9 (9.7%)	2 (2.2%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	12 (12.9%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)
Ketoconazole							
Resistance	19 (20.4%)	4 (4.3%)	2 (2.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	25 (26.9%)
Dose dependent	31 (33.3%)	14 (15.1%)	0 (0.0%)	4 (4.3%)	1 (1.1%)	0 (0.0%)	50 (53.8%)
Sensitive	8 (8.6%)	7 (7.5%)	2 (2.2%)	0 (0.0%)	0 (0.0%)	1 (1.1%)	18 (19.4%)
Total	58 (62.4%)	25 (26.9%)	4 (4.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	93 (100%)

late of *C. krusei* was resistant to itraconazole. 91.4% of isolates were dose dependent and 7.5% were sensitive to itraconazole (*Table 2*). Overall, it was evident that 89.7% and 96.0% of *C. albicans* and *C. glabrata* were respectively exhibited dose dependent and the rest of them were sensitive.

4.4. Susceptibility to Nystatin

The details of susceptibility tested isolates to nystatin were shown in *Table 2*. As indicated, 54.8% and 44.1% of tested isolates were dose dependent and sensitive to nystatin, respectively. In the present study, only one isolate (1.1%) of *C. glabrata* was resistant to nystatin. Totally 39.7% and 80.0% of *C. albicans* and *C. glabrata* were dose dependent to nystatin, respectively, whereas 60.3% and 16.0% of *C. albicans* and *C. glabrata* were sensitive to nystatin, respectively.

4.5. Susceptibility to Econazole

The results of susceptibilities to econazole indicated that most of the tested isolates (93.5%) were sensitive to econazole and the rest of them were dose dependent (*Table 2*). Besides, 93.1% and 92.0% isolates of *C. albicans* and *C. glabrata* were sensitive to econazole and the rest of them were dose dependent.

4.6. Susceptibility to Fluconazole

Table 2 shows the susceptibility details of 93 tested isolates to fluconazole. As indicated, the zones around 48.4% of isolates were resistant to fluconazole, 38.7% dose dependent and 12.9% sensitive. When looking into *C. albicans*, 32 (55.2%) of isolates were resistant to fluconazole followed by, 17 (29.3%) dose dependent and 9 (15.5%) sensitive. Also, results indicated that 7 (28.0%) of *C. glabrata* were resistant to drug, followed by 16 (64.0%) dose dependent and 2 (8.0%) sensitive.

4.7. Susceptibility to ketoconazole

The susceptibility details of tested isolates to ketoconazole were shown in *Table 2*. As shown 26.9%, 53.8% and 19.4% of isolates were respectively resistant, dose depen-

dent and sensitive to ketoconazole. Totally 53.4% and 56% of *C. albicans* and *C. glabrata* were dose dependent to ketoconazole, respectively. In the present study 32.8% and 13.8% of *C. albicans* were respectively resistant and sensitive to ketoconazole compared to 16.0% and 28.8% of *C. glabrata*.

5. Discussion

Fungal UTI has become an important nosocomial infection over the past decades among hospitalized patients. In addition, the extensive use of antifungals in hospitals may be a risk of emergence of resistant fungal strains (17, 18). For example, fluconazole is an important antifungal drug that is usually used to treat systemic fungal infections caused by *Candida* species. In addition, prophylaxis against systemic fungal diseases is also more prevalent by fluconazole.

The susceptibility of *Candida* species to frequently used antifungal drugs has various degrees. It has been reported that non-albicans species, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. lusitaniae* have had higher resistance rates against fluconazole than *C. albicans* (19).

C. krusei is one of the rare isolates of candiduria that is basically resistant to fluconazole (20), however several reports have different results. It is important to note that in the present study *C. krusei* was dose dependent to amphotericine B, nystatin, and ketoconazole and sensitive to econazole. In addition, this isolate was quite resistant to both itraconazole and fluconazole antifungal drugs. Ozcelik *et al.* (18) have reported that this isolate is quite sensitive to amphotericine B, in contrast, Pfaller *et al.* (16) showed that *C. krusei* is resistant to amphotericine B. In addition, Cheng *et al.* (13) showed that several strains of *C. krusei* isolated from candidemia were resistant to amphotericine B. In a study conducted by Yang *et al.* (14) 70% of *C. krusei* isolates ,collected from different hospitals of several regions of Taiwan, were resistant to fluconazole. They concluded that different resistance rates to fluconazole associated with different conditions in hospitals of each region.

The resistance rate of *C. glabrata* to fluconazole has gradually increased during last decades (19). Manzano-Gayosso *et al.* (4) study revealed that itraconazole, amphotericine B, and ketoconazole had less antifungal activity against *C. glabrata* isolates. In a study conducted by Laverdiere *et al.* (17), 4% of the *Candida* species isolated from different parts of ICUs patients were resistant to fluconazole and/or itraconazole. They believed that extensive use of antifungals in hospitals may be a risk of emergence of resistant fungal strains.

It is suggested by the current study that controlled surveys must be undertaken to optimize antifungal therapy based on characteristics of *Candida* strains. The current study indicated that 7.5% of *C. glabrata* isolates were resistant to fluconazole. It should be considered when *C. glabrata* is commonly isolated , fluconazole is a frequent choice for treatment and prevention of fungal diseases. The highest fluconazole sensitivity rates were recognized among *C. albicans* with 9.7%, while none of the isolates of *C. tropicalis*, *C. krusei* and *Geotrichum* spp. were susceptible.

There was no econazole resistance identified in the current study, and higher econazole sensitivity was found in *C. albicans* in 58.1% of isolates. This result strongly indicates that econazole is very effective against *C. albicans.*

It is concluded that *Candida* species isolated from candiduria in hospitalized patients have excellent *in vitro* activities against econazole. Other suitable antifungal drugs were itraconazole, nystatin and amphotericine. Whereas, resistance against ketoconazole (26.9%) and especially fluconazole (48.4%) was significant.

Acknowledgments

This work was supported with grant by the Ahvaz Jundishapur University of Medical Sciences, Iran (No. U88047). In addition this article has been extracted from an MD thesis (Maryam Beheshti Fard).

Financial Disclosure

The authors state no conflict of interest.

Funding/Support

None declared.

Authors' Contribution

None declared.

References

1. Bukhary ZA. Candiduria: a review of clinical significance and management. *Saudi J Kidney Dis Transpl*. 2008;**19**(3):350-60.
2. Nayman Alpat S, Ozgunes I, Ertem OT, Erben N, Doyuk Kartal E, Tozun M, et al. [Evaluation of risk factors in patients with candiduria]. *Mikrobiyol Bul*. 2011;**45**(2):318-24.
3. 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.
4. Manzano-Gayosso P, Hernandez-Hernandez F, Zavala-Velasquez N, Mendez-Tovar LJ, Naquid-Narvaez JM, Torres-Rodriguez JM, et al. [Candiduria in type 2 diabetes mellitus patients and its clinical significance. Candida spp. antifungal susceptibility]. *Rev Med Inst Mex Seguro Soc*. 2008;**46**(6):603-10.
5. Sellami A, Sellami H, Makni F, Bahloul M, Cheikh-Rouhou F, Bouaziz M, et al. [Candiduria in intensive care unit: significance and value of yeast numeration in urine]. *Ann Fr Anesth Reanim*. 2006;**25**(6):584-8.
6. Weinberger M, Sweet S, Leibovici L, Pitlik SD, Samra Z. Correlation between candiduria and departmental antibiotic use. *J Hosp Infect*. 2003;**53**(3):183-6.
7. Achkar JM, Fries BC. Candida infections of the genitourinary tract. *Clin Microbiol Rev*. 2010;**23**(2):253-73.
8. Nucci M. Candiduria in hospitalized patients: a review. *Braz J Infect Dis*. 2000;**4**(4):168-72.
9. Dalen DM, Zvonar RK, Jessamine PG. An evaluation of the management of asymptomatic catheter-associated bacteriuria and

candiduria at The Ottawa Hospital. *Can J Infect Dis Med Microbiol*. 2005;**16**(3):166-70.
10. Carvalho M, Guimaraes CM, Mayer JR, Jr., Bordignon GP, Queiroz-Telles F. Hospital-associated funguria: analysis of risk factors, clinical presentation and outcome. *Braz J Infect Dis*. 2001;**5**(6):313-8.
11. Fisher JF, Sobel JD, Kauffman CA, Newman CA. Candida urinary tract infections–treatment. *Clin Infect Dis*. 2011;**52 Suppl 6**:S457-66.
12. Yang YL, Cheng MF, Chang YW, Young TG, Chi H, Lee SC, et al. Host factors do not influence the colonization or infection by fluconazole resistant Candida species in hospitalized patients. *J Negat Results Biomed*. 2008;**7**:12.
13. Cheng MF, Yu KW, Tang RB, Fan YH, Yang YL, Hsieh KS, et al. Distribution and antifungal susceptibility of Candida species causing candidemia from 1996 to 1999. *Diagn Microbiol Infect Dis*. 2004;**48**(1):33-7.
14. Yang YL, Cheng HH, Ho YA, Hsiao CF, Lo HJ. Fluconazole resistance rate of Candida species from different regions and hospital types in Taiwan. *J Microbiol Immunol Infect*. 2003;**36**(3):187-91.
15. Pakshir K, Bahaedinie L, Rezaei Z, Sodaifi M, Zomorodian K. In vitro activity of six antifungal drugs against clinically important dermatophytes. *Jundishapur J Microbiol*. 2011;**2**(4):158-63.
16. Pfaller MA, Diekema DJ, Colombo AL, Kibbler C, Ng KP, Gibbs DL, et al. Candida rugosa, an emerging fungal pathogen with resistance to azoles: geographic and temporal trends from the artemis disk antifungal surveillance program. *J Clin Microbiol*. 2006;**44**(10):3578-82.
17. Laverdiere M, Labbe AC, Restieri C, Rotstein C, Heyland D, Madger S, et al. Susceptibility patterns of Candida species recovered from Canadian intensive care units. *J Crit Care*. 2007;**22**(3):245-50.
18. Ozcelik B, Kaynak F, Cesur S, Sipahi B, Sultan N. In vitro activities of voriconazole as a triazole derivative and caspofungin as an echinocandin were compared with those of some antifungal agents against Candida species isolated from clinical specimens. *Jpn J Infect Dis*. 2007;**60**(5):302-4.
19. Al-Abeid HM, Abu-Elteen KH, Elkarmi AZ, Hamad MA. Isolation and characterization of Candida spp. in Jordanian cancer patients: prevalence, pathogenic determinants, and antifungal sensitivity. *Jpn J Infect Dis*. 2004;**57**(6):279-84.
20. Quindos G, Abarca L, Carrillo-Munoz AJ, Arevalo MP, Bornay FJ, Casals JB, et al. Multicenter survey of in vitro antifungal resistance in yeasts of medical importance isolated from Spanish patients. *Rev Iberoam Micol*. 1999;**16**(2):97-100.