Jundishapur J Microbiol. 2016 May; 9(5):e36638. doi: 10.5812/jjm.36638.

Published online 2016 April 23. **Research Article**

In Vitro Activities of Six Antifungal Drugs Against *Candida glabrata* Isolates: An Emerging Pathogen

Nasrin Amirrajab,[1,2] Hamid Badali,[3] Mojtaba Didehdar,[4] Mohammad Hosein Afsarian,[5] Rasoul Mohammadi,[6] Nazanin Lotfi,[3] and Tahereh Shokohi[3,*]

[1]Department of Laboratory Sciences, School of Paramedicine and Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
[2]Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, IR Iran
[3]Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, IR Iran
[4]Department of Medical Mycology and Parasitology, Faculty of Medicine, Arak University of Medical Sciences, Arak, IR Iran
[5]Department of Microbiology, Fasa University of Medical Sciences, Fasa, IR Iran
[6]Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, IR Iran

[*]*Corresponding author*: Tahereh Shokohi, Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, IR Iran. Tel: +98-1133543081-3, Fax: +98-1133543248, E-mail: Shokohi.tahereh@gmail.com

Received 2016 January 29; **Revised** 2016 March 11; **Accepted** 2016 March 26.

Abstract

Background: *Candida glabrata* is a pathogenic yeast with several unique biological features and associated with an increased incidence rate of candidiasis. It exhibits a great degree of variation in its pathogenicity and antifungal susceptibility.
Objectives: The aim of the present study was to evaluate the in vitro antifungal susceptibilities of the following six antifungal drugs against clinical *C. glabrata* strains: amphotericin B (AmB), ketoconazole (KTZ), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ), and caspofungin (CASP).
Materials and Methods: Forty clinical *C. glabrata* strains were investigated using DNA sequencing. The in vitro antifungal susceptibility was determined as described in clinical laboratory standard institute (CLSI) documents (M27-A3 and M27-S4).
Results: The sequence analysis of the isolate confirmed as *C. glabrata* and deposited on NCBI GenBank under the accession number no. KT763084-KT763123. The geometric mean MICs against all the tested strains were as follows, in increasing order: CASP (0.17 g/mL), VCZ (0.67 g/mL), AmB (1.1 g/mL), ITZ (1.82 g/mL), KTZ (1.85 g/mL), and FCZ (6.7 g/mL). The resistance rates of the isolates to CASP, FCZ, ITZ, VZ, KTZ, and AmB were 5%, 10%, 72.5%, 37.5%, 47.5%, and 27.5%, respectively.
Conclusions: These findings confirm that CASP, compared to the other antifungals, is the potent agent for treating candidiasis caused by *C. glabrata*. However, the clinical efficacy of these novel antifungals remains to be determined.

Keywords: In Vitro Antifungal Susceptibility, Broth Microdilution, *Candida glabrata*

1. Background

Among pathogenic fungi, *Candida* species are most common cause of candidemia infections, with high mortality rates, and the incidence of candidemia infections has significantly increased in recent decades (1, 2). Although *C. albicans* is the most frequently detected fungal species in this type of infection, non-*C. albicans* species (*C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*) have been increasingly detected, particularly in high-risk populations. *C. glabrata*, the most prominent of these species (3), shows great variation in pathogenicity and antifungal susceptibility (4). It exerts its most important impact on human health via bloodstream *Candida* infections, particularly in healthy individuals and those treated in the ICU and BICU (5-7). Systemic infections due to *C. glabrata* lead to high mortality, owing to treatment failure (8). *Candida glabrata* reduces antifungal susceptibility to all azole drugs, independently of the presence of acquired resistance.

The prevalence of non-*C. albicans* species is critical because different species have different levels of resistance, either via intrinsic or acquired resistance mechanisms (9). A genetic analysis revealed fluconazole (FCZ) resistance mechanisms in 8% of *C. glabrata* isolates and 0.3% of *C. albicans* isolates (2). The resistance of *C. glabrata* to several antifungal drugs, limiting its medical efficacy (5, 8, 10). The cause of the increased prevalence of *C. glabrata* is unclear, but it could plausibly be related to the low intrinsic susceptibility of *C. glabrata* to azole drugs compared with that of most other *Candida* species (5, 11, 12). The long-term use of azoles has resulted in the development of Multidrug resistance (MDR), which is an important healthcare issue worldwide and poses a significant obstacle to antifungal therapy (13). Limited data are available on the susceptibility profiles of currently available antifungal agents, as only a small number of strains have been tested in most studies.

A rapid, reproducible method of determining the drug susceptibility of *C. glabrata* is needed to select the most appropriate and effective antifungal agent (4).

2. Objectives

The present investigation evaluated the in vitro antifungal susceptibilities of six antifungal agents to 40 clinical *C. glabrata* strains: amphotericin B (AmB), ketoconazole (KTZ), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ), and caspofungin (CASP).

3. Materials and Methods

3.1. Fungal Isolates

From 2008 to 2011, 40 (3.8%) strains of *C. glabrata* in 1055 yeast isolates were recovered from patients with candidiasis. All the patient data, including gender, age, host status, and source of the isolates, are shown in Table 1. The patients were between 4 and 84 years, with a mean age of 44.65 $\pm$ 17.39 years. *C. glabrata* was the most common species recovered from the clinical samples: bronchoalveolar lavage (n = 14, 35%), blood (n = 2, 5%), urine (n = 5, 12.5%), vagina (n = 10, 25%), sputum (n = 4, 10%), wound (n = 3, 7.5%), abdominal drain (n = 1, 2.5%), and tracheal aspirate (n = 1, 2.5%). The most frequent underlying disease was respiratory failure, followed by diabetes, malignancy, septicemia, and vaginal candidiasis. The isolates were deposited at reference culture collections in the invasive fungi research center (IFRC; Sari, Iran).

To differentiate the *Candida* species, stock cultures were initially grown on malt extract agar, supplemented with chloramphenicol (MEA; Difco Laboratories, Detroit, MI, USA) and CHROMagar *Candida* (BD Biosciences Mississanga, Ontario, Canada) at 24°C in the dark. All the strains were preliminarily identified at the species level, based on microscopic and macroscopic characteristics (i.e., germ tube formation, chlamydospores production, and temperature growth analysis at elevated temperatures of 42 - 45°C), and carbohydrate assimilation. The strains were subsequently confirmed by PCR assays and sequencing, using the following primers: 5'-GTC AAA TGC CAC AAC AAC AAC CT-3' and 5'-AGC ACT TCA GCA GCG TCT TCA G-3' (14). Briefly, the amplification was performed with a cycle of 7 minutes at 94°C for primary denaturation, followed by 30 cycles at 94°C (60 seconds), 55°C (60 seconds), and 74°C (60 seconds), and a final extension step at 74°C for 10 minutes (14). PCR amplicons were purified with Sephadex G-50 fine (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and sequencing was conducted using an ABI 3730XL automatic sequencer (Applied Biosystems, Foster city, CA, USA). The sequence data obtained in this study were adjusted using the SeqMan of Lasergene software (DNAStar Inc., Madison, Wisconsin, USA) and compared with those in the GenBank database (http://blast.ncbi.nlm.nih.gov) and local database of the CBS-KNAW fungal biodiversity centre, Utrecht, Netherlands. Prior to use, the identified strains were stored at -70°C in a Tryptone soya broth medium (Oxoid, CM0129) containing 2% glucose, 2% peptone, and 20% glycerol. This research was approved by the ethics committee (Ethical no. 92-3-8) of Mazandaran University of Medical Sciences, Sari, Iran.

3.2. Antifungal Susceptibility Testing

In vitro antifungal susceptibility testing was determined according to the recommendations of the clinical and laboratory standards institute (CLSI) M27-A3 and M27-S4 documents) (15, 16). AmB (Sigma-Aldrich, USA), FCZ (Pfizer Central Research, UK), KTZ (Sigma-Aldrich), ITZ (Sigma-Aldrich), VCZ (Pfizer Central Research, UK), and CASP (Merck, Whitehouse Station, NJ, USA) were obtained as reagent-grade powders from the respective manufacturers for preparation of CLSI microdilution trays. The antifungal agents were diluted in standard RPMI-1640 medium with L-glutamine without bicarbonate (Sigma Chemical Co.), buffered to pH 7.0 with 0.165 M-morpholinepropanesulfonic acid (Sigma) was used to yield twice as strong concentrations, which were dispensed into 96-well microdilution trays, with a final concentration of 0.016 - 16 μg/mL for AmB, KTZ, ITZ, and VCZ; 0.063 - 64 μg/mL for FCZ; and 0.008 - 8 μL/mL for CASP.

The plates were stored at -70°C until used. Homogeneous conidial suspensions were measured spectrophotometrically at wavelengths of 530 nm to a percent transmission in the range of 75 - 77. The final densities of the stock inoculum suspensions of the tested isolates ranged from 2.5 to 5 $\times 10^3$ colony-forming units/mL, as determined by quantitative colony counts on Sabouraud glucose agar (Difco). They were incubated at 35°C and examined visually after 24 and 48 hours to determine the MIC values. The MIC endpoints were determined using a reading mirror and were defined as the lowest concentration of the drug that prevented recognizable growth (i.e., exerted 100% inhibition for AmB) or significant (50%) growth diminution levels (all other agents) compared with the growth of a drug-free control. *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) strains were chosen as quality controls. Analysis of these strains was performed with every new batch of MIC determinations. The MIC range, geometric mean, MIC50, and MIC90 are provided for all the isolates. All the tests were performed in duplicate, and differences among mean values were determined by a Student's t-test. The statistical analyses were conducted using the statistical SPSS package

Table 1. Variation in the MIC Profiles of the Antifungal Agents by Gender, Age, Host Status, and Source of the Isolates[a]

C. glabrata Collection no.	Sex/Age	Host Status	Source	GenBank Accession No.	AmB	FCZ	KTZ	ITZ	VCZ	CASP
C. glabrata ((IFRC 1451)	M/44	Respiratory failure	BAL	KT763084	1	2	1	0.5	0.25	0.063
C. glabrata (IFRC 1452)	M/37	ND	BAL	KT763085	1	4	0.5	4	0.5	0.125
C. glabrata (IFRC 1453)	F/39	Immunocompetent	Vagina	KT763086	1	4	8	2	0.5	0.25
C. glabrata (IFRC 1454)	M/56	ND	Sputum	KT763087	1	64	8	16	4	0.25
C. glabrata (IFRC 1455)	M/40	Respiratory failure	Wound	KT763088	2	4	8	16	0.25	0.25
C. glabrata (IFRC 1456)	F/31	ND	Sputum	KT763089	1	4	8	16	0.5	0.25
C. glabrata (IFRC 1457)	M/54	ND	Urine culture	KT763090	1	8	2	2	0.5	0.25
C. glabrata (IFRC 1458)	F/4	Burn	Urine culture	KT763091	1	4	16	8	0.5	1.0
C. glabrata (IFRC 1459)	F/69	Respiratory failure-burn	Wound	KT763092	0.25	4	0.25	0.5	0.125	0.125
C. glabrata (IFRC 1460)	M/27	Burn	Urine culture	KT763093	1	4	1	0.5	0.5	0.25
C. glabrata (IFRC 1461)	M/27	HCV-burn	Wound	KT763094	1	8	4	4	2	0.25
C. glabrata (IFRC 1462)	M/27	Burn	Sputum	KT763095	1	8	1	2	0.5	0.063
C. glabrata (IFRC 1463)	M/48	Leukemia	BAL	KT763096	2	16	16	8	16	0.25
C. glabrata (IFRC 1464)	F/29	Immunocompetent	Vagina	KT763097	2	8	4	2	2	0.125
C. glabrata (IFRC 1465)	F/33	ND	BAL	KT763098	1	16	4	4	1	0.125
C. glabrata (IFRC 1466)	M/58	Respiratory failure	BAL	KT763099	0.5	4	0.25	0.5	0.125	0.25
C. glabrata (IFRC 1467)	M/81	Respiratory failure	BAL	KT763100	1	4	1	2	0.25	0.25
C. glabrata(IFRC 1468)	M/55	Solid organ recipient	BAL	KT763101	2	64	8	4	8	0.25
C. glabrata (IFRC 1469)	M/28	Respiratory failure	BAL	KT763102	1	4	0.5	0.5	0.25	0.125
C. glabrata (IFRC 1470)	F/24	Immunocompetent	Vagina	KT763103	0.5	16	2	0.5	0.5	0.25
C. glabrata (IFRC 1471)	M/47	Solid organ recipient	BAL	KT763104	1	8	2	0.5	0.5	0.125
C. glabrata (IFRC 1472)	M/67	Respiratory failure	BAL	KT763105	1	4	2	2	0.5	0.25
C. glabrata (IFRC 1473)	M/63	ND	Blood	KT763106	1	4	1	0.5	0.25	0.125
C. glabrata (IFRC 1474)	M/65	Immunocompetent	BAL	KT763107	2	4	0.5	2	0.25	0.063
C. glabrata (IFRC 1475)	M/44	Respiratory failure	Tracheal aspirate	KT763108	2	16	2	2	1	0.125
C. glabrata (IFRC 1476)	M/48	Bone marrow recipient	BAL	KT763109	2	8	16	8	8	0.125
C. glabrata (IFRC 1477)	M/84	ND	Urine culture	KT763110	1	8	2	0.5	0.5	0.25
C. glabrata (IFRC 1478)	F/41	Immunocompetent	Vagina	KT763111	2	64	16	8	16	0.25
C. glabrata (IFRC 1479)	M/54	ND	Abdominal drain	KT763112	1	16	4	2	4	0.063
C. glabrata (IFRC 1480)	F/33	Immunocompetent	Vagina	KT763113	1	16	4	4	8	0.063
C. glabrata (IFRC 1481)	M/22	Respiratory failure	Sputum	KT763114	1	8	4	4	16	0.25
C. glabrata (IFRC 1482)	F/35	Immunocompetent	Vagina	KT763115	2	8	8	8	16	0.063
C. glabrata (IFRC 1483)	M/57	Solid organ recipient	Urine culture	KT763116	0.5	16	1	2	0.25	0.25
C. glabrata (IFRC 1484)	F/36	Immunocompetent	Vagina	KT763117	1	4	0.5	2	0.125	0.125
C. glabrata (IFRC 1485)	F/35	Immunocompetent	Vagina	KT763118	2	8	0.5	0.5	0.25	0.063
C. glabrata(IFRC 1486)	M/37	Respiratory failure	BAL	KT763119	1	16	4	2	8	0.125
C. glabrata (IFRC 1487)	M/79	Cancer	BAL	KT763120	1	8	0.5	2	0.125	0.25
C. glabrata (IFRC 1488)	M/50	ND	Blood	KT763121	1	4	8	4	2	0.25
C. glabrata (IFRC 1489)	F/32	Immunocompetent	Vagina	KT763122	1	8	0.5	0.5	0.25	0.5
C. glabrata (IFRC 1490)	F/46	Diabetes	Vagina	KT763123	2	64	4	2	0.5	0.25

Abbreviations: AmB, amphotericin B; FCZ, fluconazole; KTZ, ketoconazole; ITZ, itraconazole; VRZ, voriconazole; CASP, caspofungin; BAL, broncho Alveolar Lavage; HCV, hepatitis C virus; IFRC; invasive Fungi tesearch center.
[a] The MIC values are expressed in μg/mL.

(version 7.0). A P value of < 0.05 was considered statistically significant.

4. Results

All 40 isolates were classified as *C. glabrata* based on DNA sequencing. The DNA sequences showed > 99% sequence similarity to the available *C. glabrata* type isolate in the GenBank database. The sequences of all isolates were submitted to the NCBI GenBank and assigned under the accession nos.KT763084-KT763123 (Table 1). The in vitro antifungal susceptibility of the six antifungal drugs to *C. glabrata* isolates is summarized in Table 2, showing the geometric mean MICs, MIC ranges, MIC50s, and MIC90s.

The MIC results of all the *C. glabrata* isolates revealed that they were highly susceptible to CASP but not to the azole agents. FCA showed the widest range and highest MICs (2 - 64 μg/mL), followed by VCZ, KTZ, ITZ, and CASP (0.125 - 16, 0.25 - 16, 0.5 - 16, and 0.063 - 1 μg/mL, respectively). The GM MICs against all the tested strains were as follows, in increasing order: CASP (0.17 μg/mL), VCZ (0.67 μg/mL), AmB (1.1 μg/mL), ITZ (1.82 μg/mL), KTZ (1.85 μg/mL), and FCZ (6.7 μg/mL). The results showed that, in terms of the MIC50 and MIC90, CASP (both 0.25 μg/mL) was more active than FCZ (8 and 16 μg/mL, respectively). For all strains of *C. glabrata*, the AmB MIC was 2-log2-dilution steps and 1-log2-dilution steps less active than CASP. The MICs of 30 (75%) strains of *C. glabrata* against five of the antifungal drugs were high, and the MICs of 10 (25%) strains to all the tested drugs were low. Only 4 (10%) *C. glabrata* strains were FCZ resistant. The remaining strains (n = 36, 90%) were susceptible dose-dependent (SDD), with an FCZ value of $\leq$ 32 μg/mL. Eleven (27.5%) strains were SDD to ITZ, and an additional 29 (72.5%) strains were resistant to ITZ.

5. Discussion

An increasing incidence of candidiasis caused by *C. glabrata* was reported during the 1990s. *C. albicans* was the most predominant agent of bloodstream infections, followed by *C. glabrata* in north America, while *C. glabrata* was the third one in Europe. In Latin America, *C. parapsilosis* was followed by *C. albicans* and *C. glabrata* was the fourth (17). By contrast, the prevalence of *C. glabrata* infections was reported to be low in Iran (18).

Owing to the increasing frequency of *C. glabrata* isolates in clinical specimens and the association of this species with resistance to antifungal drugs (i.e., azoles and echinocandins), *C. glabrata* drug resistance patterns have a disproportionate impact on treatment strategies and surveillance programs (9, 19). Timely targeted administration of antifungal agents in patients with invasive candidiasis requires reliable, up-to-date epidemiological data on both the distribution and susceptibility of the species. Such data are important not only in health centers where antifungal susceptibility testing is not routinely performed, but also in evaluations of invasive yeast infections (17). The intrinsically low susceptibility of *C. glabrata*, an emerging opportunistic fungal pathogen, to azole antifungals has made its treatment challenging, and infection is accompanied by frequent relapse and failure (20).

In the present study, the results of the antifungal susceptibility tests of FCZ and ITZ were comparable to those reported in different continents, including the U.S., Canada, Europe, and Asia, with slight differences (21-23). The MIC ranges for AmB were 0.25 - 2 μg/mL for all strains of *C. glabrata*, with a GM MIC of 1.1 μg/mL. In addition, 11 of the 40 (27.5%) strains assayed with AmB were resistant to this antifungal agent, with the level of resistance higher than that reported in previous studies in Iran (24-28). The main problems in the treatment of *C. glabrata* include resistance to numerous azoles (29). The findings indicate that the decreased susceptibility of *Candida* to azole agents may contribute to the increased proportion of infections caused by these species (5, 30).

In the present study, the highest resistance rate (72.5%) was to ITZ, both consistent (2, 21, 22) and inconsistent (24, 27) with the findings of previous studies. The findings on resistance to ITZ differ slightly from those reported in the U.S., Canada, Europe, and Latin America (2, 21, 22). In the latter regions, *C. glabrata* was reported to be most resistant to ITZ, followed by *C. krusei* and *C. tropicalis*. The resistance to KTZ (47.5%) in the current study was similar to the rate of 50% reported by Razzaghi-Abyaneh et al. (18) but higher than that reported in other studies (25-27). The resistance rate to FCZ (10%) was somewhat similar (6%) to the findings of Shokohi et al. (24). Compared with previous research, the resistance rate to FCZ (10%) in the current study was relatively low. As noted elsewhere, the prevalence of FCZ resistance among *C. glabrata* isolates varies, depending on the country and region (31). In the present study, the percentage of SDD isolates was notably high (90%), with a rapid decline in the rate of FCZ susceptibility. It seems that a higher dose of FCZ is required as a drug of choice for invasive *C. glabrata* infections (32).

In the present study, the VCZ resistance rate (37.5%) was consistent with that of a recent report (38%) (18). In other studies, *C. glabrata* strains were reported to be susceptible to VCZ (21, 22, 26). Data from health care centers in the U.S. and Denmark provide more information about MDR in *C. glabrata* strains. One potential explanation for the emergence of MDR in *C. glabrata* is the haploid nature of the organism, which makes it particularly successful in acquiring and expressing resistant mutations under prolonged drug pressure (10, 33, 34). The observations herein support the potential emergence of MDR isolates of *C. glabrata*, with 70% cross-resistance to azole classes. Cross-resistance between the new and azoles, such as FCZ and VCZ, is a cause for concern (17, 35).

AmB is becoming less effective against azole-resistant *C. glabrata* and *C. krusei*. Experts therefore recommend using higher-than-normal doses. As higher doses of polyenes increase the risk of nephrotoxicity, and VCZ may be ineffective in treating certain strains of *C. glabrata*, there is consensus about the administration of echinocandins (CASP, anidulafungin, and micafungin) as the drugs of choice for azole-resistant *C. glabrata* and *C. krusei* infections (36). Echinocandins are the preferred therapy for patients with

Table 2. In Vitro Susceptibilities of 40 Clinical Isolates of *Candida glabrata* to Six Antifungal Agents

Isolate	Antifungal Agent	No. (Cumulative %) of Isolates Inhibited at MIC, μg/mL														MIC Range, μg/mL	MIC 50, μg/mL	MIC 90, μg/mL	G Mean, μg/mL
		0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64				
C. glabrata (n = 40)																			
	AmB						1 (2.5)	3 (10)	25 (72.5)	11 (100.0)						0.25 - 2	1	2	1.109569
	FCZ									1 (2.5)	15 (40)	12 (70)	8 (90)	0 (90)	4 (100)	2 - 64	8	16	6.7271713
	KTZ						2 (5)	7 (22.5)	6 37.5)	6 (52.5)	8 (72.5)	7 (90)	4 (100)			0.25 - 16	2	8	1.851749
	ITZ							11 (2.75)	0 (2.75)	14 (62.5)	7 (80)	5 (92.5)	3 (100)			0.5 - 16	2	8	1.821169
	VCZ					4 (10)	9 (32.5)	12 (62.5)	2 (67.5)	3 (75)	2 (80)	4 (90)	4 (100)			0.125 - 16	0.5	8	0.67295
	CASP				7 (17.5)	11 (45)	20 (95)	1 (97.5)	1 (100)							0.063 - 1	0.25	0. 25	0.168

Abbreviations: AmB, amphotericin B; FCZ, fluconazole; KTZ, ketoconazole; ITZ, itraconazole; VCZ voriconazole, CASP, caspofungin; IFRC, invasive fungi research center.

renal failure, when polyenes cannot not be administered (37).

A valuable finding of the present study was that 95% of the isolates were susceptible to CASP, with an MIC range of 0.063 - 1 μg/mL. These results are consistent with those of the Global ARTEMIS and national SENTRY antifungal surveillance studies (17, 38). According to previous research, antifungals with low MICs and high activity against *C. glabrata* can be administered to high-risk patients (39). The results of the present study demonstrated that CASP was the most effective agent, with the lowest MIC50 (0.25 μg/mL) and MIC90 (0.25 μg/mL) and geometric mean MIC (0.168 μg/mL) values against all *C. glabrata* strains. These results are somewhat consistent with data published in other studies (24, 26, 28). The emergence of high multiresistant *C. glabrata* is a concern, given the fact that neither azoles nor AmB are an optimal treatment for *C. glabrata* infections (35, 40). This constitutes a strong warning to use fewer effective antifungal agents in our hospitals and with greater caution, in order to relieve pressure on sensitive *Candida* strains (41). Caution is thus recommended with CASP therapy for *C. glabrata* infections when azole resistance is predicted (42). The resistance of *C. glabrata* clinical isolates to both azoles and echinocandins has emerged over time. This is problematic, owing to its treatment limitations (7, 10).

In conclusion, CASP exhibited potent activity against *C. glabrata* clinical isolates and showed the least evidence of emerging resistance. The relevance of these in vitro findings to the treatment of *C. glabrata* infections in clinical practice remains to be determined.

Acknowledgments

This study was financially supported by a grant number 92-2 from Mazandaran University of Medical Sciences, Sari, Iran and Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, which we gratefully acknowledge. We also would like to thank Iman Haghani for technical assistance.

Footnotes

Authors' Contribution: Study concept and design: Tahereh Shokohi; acquisition of data: Nasrin Amirrajab, Mojtaba Didehdar, Mohammad Hosein Afsarian, Rasoul Mohammadi, and Nazanin Lotfi; analysis and interpretation of data: Tahereh Shokohi, Nasrin Amirrajab, and Hamid Badali; drafting of the manuscript: Tahereh Shokohi, Nasrin Amirrajab, and Hamid Badali; critical revision of the manuscript: Tahereh Shokohi, and Hamid Badali; study supervision: Tahereh shokohi.

Funding/Support: This research was financially supported by a grant from Mazandaran University of Medical Sciences and Ahvaz Jundishapur University of Medical Sciences.

References

1. Pfaller MA. The epidemiology of invasive candidiasis. Washington: ASM Press; 2012.
2. Yazdanparast SA, Khodavaisy S, Fakhim H, Shokohi T, Haghani T, Nabili M, et al. Molecular characterization of highly susceptible Candida africana from Vulvovaginal Candidiasis. *Mycopathologia*. 2015;**180**(5-6):137–23. doi: 10.1080/13693780802144339. [PubMed: 18651308].

3. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev.* 2012;**36**(2):288–305. doi: 10.1111/j.1574-6976.2011.00278.x. [PubMed: 21569057].
4. Glockner A, Cornely OA. Candida glabrata–unique features and challenges in the clinical management of invasive infections. *Mycoses.* 2015;**58**(8):445–50. doi: 10.1111/myc.12348. [PubMed: 26207423].
5. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, et al. Species identification and antifungal susceptibility testing of Candida bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. *J Clin Microbiol.* 2012;**50**(11):3435–42. doi: 10.1128/JCM.01283-12. [PubMed: 22875889].
6. Lotfi N, Shokohi T, Nouranibaladezaei SZ, Nasrollahi Omran A, Kondori N. High recovery rate of non-albicans Candida species isolated from burn patients with candidemia in Iran. *Jundishapour J Microbiol.* 2015;**8**(10):e22929. doi: 10.5812/jjm.22929.
7. Fesharaki SH, Haghani I, Mousavi B, Kargar ML, Boroumand M, Anvari MS, et al. Endocarditis due to a co-infection of Candida albicans and Candida tropicalis in a drug abuser. *J Med Microbio.* 2013;**62**(pt11):1763–7. doi: 10.1128/AAC.03255-14. [PubMed: 24890592].
8. Pfaller MA, Messer SA, Boyken L, Hollis RJ, Rice C, Tendolkar S, et al. In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of Candida spp. and Cryptococcus neoformans collected during 2001 and 2002 in the ARTEMIS global antifungal surveillance program. *Diagn Microbiol Infect Dis.* 2004;**48**(3):201–5. doi: 10.1016/j.diagmicrobio.2003.09.008. [PubMed: 15023430].
9. Ben-Ami R, Hilerowicz Y, Novikov A, Giladi M. The impact of new epidemiological cutoff values on Candida glabrata resistance rates and concordance between testing methods. *Diagn Microbiol Infect Dis.* 2014;**79**(2):209–13. doi: 10.1016/j.diagmicrobio.2014.02.008. [PubMed: 24657169].
10. Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of Candida glabrata. *J Clin Microbiol.* 2012;**50**(4):1199–203. doi: 10.1128/JCM.06112-11. [PubMed: 22278842].
11. Tscherner M, Schwarzmüller T, Kuchler K. Pathogenesis and antifungal drug resistance of the human fungal pathogen Candida glabrata. *Pharmaceuticals.* 2011;**4**(1):169–86. doi: 10.3390/ph4010169.
12. Lyon GM, Karatela S, Sunay S, Adiri Y, Candida Surveillance Study I. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. *J Clin Microbiol.* 2010;**48**(4):1270–5. doi: 10.1128/JCM.02363-09. [PubMed: 20129963].
13. Prasad R, Kapoor K. Multidrug Resistance in Yeast Candida. *Int Rev Cytol.* 2005;**242**:215–48. doi: 10.1016/s0074-7696(04)42005-1.
14. Afsarian MH, Badali H, Boekhout T, Shokohi T, Katiraee F. Multilocus sequence typing of Candida albicans isolates from Burn Intensive Care Unit (BICU) in Iran. *J Med Microbiol.* 2015;**64**:248–53. [PubMed: 14662965].
15. Clinical and Laboratory Standards Institute. . Reference method for broth dilution antifungal susceptibility testing of yeasts: 3rd informational supplement. ; 2008.
16. Clinical and Laboratory Standards Institute. . Reference method for broth dilution antifungal susceptibility testing of yeasts: fourth informational supplement. ; 2012.
17. Xiao M, Fan X, Chen SC, Wang H, Sun ZY, Liao K, et al. Antifungal susceptibilities of Candida glabrata species complex, Candida krusei, Candida parapsilosis species complex and Candida tropicalis causing invasive candidiasis in China: 3 year national surveillance. *J Antimicrob Chemother.* 2015;**70**(3):802–10. doi: 10.1093/jac/dku460. [PubMed: 25473027].
18. Razzaghi-Abyaneh M, Sadeghi G, Zeinali E, Alirezaee M, Shams-Ghahfarokhi M, Amani A, et al. Species distribution and antifungal susceptibility of Candida spp. isolated from superficial candidiasis in outpatients in Iran. *J Mycol Med.* 2014;**24**(2):e43–50. doi: 10.1016/j.mycmed.2014.01.004. [PubMed: 24767256].
19. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. *Clin Microbiol Infect.* 2012;**18 Suppl 7**:19–37. doi: 10.1111/1469-0691.12039. [PubMed: 23137135].
20. Ramsay S, Astill N, Shankland G, Winter A. Practical management of recurrent vulvovaginal candidiasis. *Trends Urol Gynaecol Sex Health.* 2009;**14**(6):18–22. doi: 10.1002/tre.127.
21. Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, et al. International surveillance of bloodstream infections due to Candida species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *J Clin Microbiol.* 2001;**39**(9):3254–9. [PubMed: 11526159].
22. Pfaller MA, Diekema DJ, Jones RN, Messer SA, Hollis RJ, Sentry Participants Group . Trends in antifungal susceptibility of Candida spp. isolated from pediatric and adult patients with bloodstream infections: SENTRY Antimicrobial Surveillance Program, 1997 to 2000. *J Clin Microbiol.* 2002;**40**(3):852–6. [PubMed: 11880404].
23. Taj-Aldeen SJ, Kolecka A, Boesten R, Alolaqi A, Almaslamani M, Chandra P, et al. Epidemiology of candidemia in Qatar, the Middle East: performance of MALDI-TOF MS for the identification of Candida species, species distribution, outcome, and susceptibility pattern. *Infection.* 2014;**42**(2):393–404. doi: 10.1007/s15010-013-0570-4. [PubMed: 24352810].
24. Shokohi T, Bandalizadeh Z, Hedayati MT, Mayahi S. In vitro antifungal susceptibility of Candida species isolated from oropharyngeal lesions of patients with cancer to some antifungal agents. *Jundishapur J Microbiol.* 2011;**4**(Supplement 1):S19–26.
25. Badiee P, Alborzi A, Shakiba E, Farshad S, Japoni A. Susceptibility of Candida species isolated from immunocompromised patients to antifungal agents. *East Mediterr Health J.* 2011;**17**(5):425–30. [PubMed: 21796956].
26. Badiee P, Alborzi A, Davarpanah MA, Shakiba E. Distributions and antifungal susceptibility of Candida species from mucosal sites in HIV positive patients. *Arch Iran Med.* 2010;**13**(4):282–7. [PubMed: 20597560].
27. Zomorodian K, Rahimi MJ, Pakshir K, Motamedi M, Ghiasi MR, Rezashah H. Determination of antifungal susceptibility patterns among the clinical isolates of Candida species. *J Glob Infect Dis.* 2011;**3**(4):357–60. doi: 10.4103/0974-777X.91059. [PubMed: 22223999].
28. Katiraee F, Khosravi AR, Khalaj V, Hajiabdolbaghi M, Khaksar AA, Rasoulinejad M. In vitro antifungal susceptibility of oral candida species from Iranian HIV infected patients. *Tehran Univ Med Sci.* 2012;**70**(2).
29. Fidel PJ, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. *Clin Microbiol Rev.* 1999;**12**(1):80–96. [PubMed: 9880475].
30. Pfaller MA, Messer SA, Hollis RJ, Boyken L, Tendolkar S, Kroeger J, et al. Variation in susceptibility of bloodstream isolates of Candida glabrata to fluconazole according to patient age and geographic location in the United States in 2001 to 2007. *J Clin Microbiol.* 2009;**47**(10):3185–90. doi: 10.1128/JCM.00946-09. [PubMed: 19656983].
31. Pfaller MA, Diekema DJ, International Fungal Surveillance Participant G. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of Candida. *Clin Microbiol Infect.* 2004;**10 Suppl 1**:11–23. [PubMed: 14748799].
32. Ruan SY, Chu CC, Hsueh PR. In vitro susceptibilities of invasive isolates of Candida species: rapid increase in rates of fluconazole susceptible-dose dependent Candida glabrata isolates. *Antimicrob Agents Chemother.* 2008;**52**(8):2919–22. doi: 10.1128/AAC.00323-08. [PubMed: 18458136].
33. 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. doi: 10.1086/500939. [PubMed: 16511756].
34. Zimbeck AJ, Iqbal N, Ahlquist AM, Farley MM, Harrison LH, Chiller T, et al. FKS mutations and elevated echinocandin MIC values among Candida glabrata isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother.* 2010;**54**(12):5042–7. doi: 10.1128/AAC.00836-10. [PubMed: 20837754].
35. Alexander BD, Schell WA, Miller JL, Long GD, Perfect JR. Candida glabrata fungemia in transplant patients receiving voriconazole after fluconazole. *Transplantation.* 2005;**80**(6):868–71. [PubMed: 16210978].
36. Spellberg BJ, Filler SG, Edwards JJ. Current treatment strategies for disseminated candidiasis. *Clin Infect Dis.* 2006;**42**(2):244–51. doi: 10.1086/499057. [PubMed: 16355336].
37. Kartsonis N, Killar J, Mixson L, Hoe CM, Sable C, Bartizal K, et al. Caspofungin susceptibility testing of isolates from patients with esophageal candidiasis or invasive candidiasis: relationship of MIC to treatment outcome. *Antimicrob Agents Chemother.* 2005;**49**(9):3616–23. doi: 10.1128/AAC.49.9.3616-3623.2005. [PubMed: 16127030].
38. Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance. *J Clin Microbiol.* 2013;**51**(8):2571–81. doi: 10.1128/JCM.00308-13. [PubMed: 23720791].
39. Mortezaee VKP, Mahmoudi M, Safara M, Shekari H. In vitro Susceptibilities of Iranian Clinical nonalbicans Candida species to Caspofungin and Ketoconazole. *J Basic Appl Sci Res.* 2012;**2**:1608–14.
40. Pappas PG, Kauffman CA, Andes D, Benjamin DJ, Calandra TF, Edwards JJ, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2009;**48**(5):503–35. doi: 10.1086/596757. [PubMed: 19191635].
41. Panackal AA, Gribskov JL, Staab JF, Kirby KA, Rinaldi M, Marr KA. Clinical significance of azole antifungal drug cross-resistance in Candida glabrata. *J Clin Microbiol.* 2006;**44**(5):1740–3. doi: 10.1128/JCM.44.5.1740-1743.2006. [PubMed: 16672401].
42. Pfaller MA, Diekema DJ, Andes D, Arendrup MC, Brown SD, Lockhart SR, et al. Clinical breakpoints for the echinocandins and Candida revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat.* 2011;**14**(3):164–76. doi: 10.1016/j.drup.2011.01.004. [PubMed: 21353623].