Original Article

Susceptibility pattern of *Candida albicans* isolated from Iranian patients to antifungal agents

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Abstract

Background and Purpose: Candidiasis is a major fungal infection, and *Candida albicans* is the major cause of infections in humans. The Clinical and Laboratory Standards Institute (CLSI) developed new breakpoints for antifungal agents against *C. albicans*. In this multi-center study, we aimed to determine the drug susceptibility profile of *C. albicans*, isolated from Iranian population according to new species-specific CLSI.

Materials and Methods: Clinical samples were cultured on Sabouraud dextrose agar and were incubated at room temperature for seven days. The isolates were transferred to Professor Alborzi Clinical Microbiology Research Center, Shiraz, Iran. *C. albicans* were identified by using API 20C AUX system. Broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of amphotericin B, caspofungin, voriconazole, fluconazole, posaconazole, itraconazole, and ketoconazole, based on CLSI document M27-S4 and new breakpoints for some azoles and caspofungin.

Results: Overall, 397 *C. albicans* were isolated from patients admitted to ten university hospitals in Iran. The MIC90 of the isolates to amphotericin B, caspofungin, voriconazole, fluconazole, posaconazole, itraconazole, and ketoconazole were 0.125, 0.125, 0.125, 1, 0.064, 0.5, and 0.125 µg/ml, and rates of resistance were 0.5%, 0.3%, 3.8%, 2.8%, and 2.5% for amphotericin B, caspofungin, voriconazole, fluconazole, and itraconazole, respectively.

Conclusion: According to our data, fluconazole is the drug of choice for management of patients at risk for systemic candidiasis throughout the region, since it is cost-effective with low side effects.

Keywords: *Candida albicans*, Candidiasis, Minimum inhibitory concentration, Amphotericin B

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Introduction

Candida albicans is a yeast fungus that is normally present on the skin and mucous membranes such as oral cavity, vagina, and rectum. It can travel through the blood stream and cause infection in any part of the body. *C. albicans* is the major cause of infection in humans [1-5]; it is also an important part of the normal microbial flora in the oral cavity, gastrointestinal tract, and vagina in healthy humans. Mediate adhesion, biofilm formation, invasion into host cells, yeast-to-hypha transition (phenotypic switching), secretion of hydrolases, contact sensing, and thigmotropism are the pathogenic potentials of *C. albicans* [6].

Several factors increase the incidence rate of systemic candidiasis in colonized patients such as weakened immune system, mucosal and cutaneous barrier disruption, neutrophil dysfunction (quantitative or qualitative), metabolic disorders, and advanced age [2, 7].

Recently, resistance to common antifungals has been reported in different *Candida* species [8-11]. In addition, fungal strains isolated from immunocompromised patients have higher resistance to antifungals because of using

antifungals as prophylaxis [11].

The Clinical and Laboratory Standards Institute (CLSI) developed new breakpoints for antifungal agents against *C. albicans*. Therefore, the new susceptibility pattern of the CLSI breakpoints may be a sensitive tool for management of systemic candidiasis in immunocompromised patients [12, 13]. The present study aimed to determine the drug susceptibility profile of *C. albicans,* according to new species-specific CLSI in a multicenter study in Iran.

Materials and Methods

This study was approved by the Ethics Committee of Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. A total of 397 *C. albicans* were isolated from ten university hospitals (Kerman, Shiraz, Yasouj, Mashhad, Isfehan, Urmia, Tehran, Sanandaj, Ahvaz, and Sari) in Iran. Clinical samples such as blood, urine, bronchoalveolar lavage (BAL), and sputum were cultured on Sabouraud dextrose agar (Merck, Germany) and were incubated at room temperature for seven days.

The isolates were transferred to Professor Alborzi Clinical Microbiology Research Center and were re-cultured twice to confirm purity. *C. albicans* was confirmed by using API 20C AUX system (BioMe'rieux, France), according to the manufacturer's instructions.

Broth microdilution method was applied to determine the minimum inhibitory concentrations (MICs) of amphotericin B, caspofungin, voriconazole, fluconazole, posaconazole, itraconazole, and ketoconazole, based on the CLSI document M27-A3 and CLSI M27-S4 [12]. All the antifungals were purchased from Sigma Aldrich, Germany.

*C. albican*s isolates were re-cultured on Sabouraud dextrose agar (Merck, Germany) at 35ºC for 24 hours and diluted with sterile distilled water to concentrations of 1×10^6 to 5×10^6 cells/mL, based on 0.5 McFarland standard. RPMI-1640 (Sigma-Aldrich, Germany) culture medium was buffered with 3-Morpholinopropanesulfonic acid (Sigma-Aldrich, Germany), adjusted to pH 7.0 and supplemented with 2% glucose. Serial dilutions (64 to 0.125 µg/ml for fluconazole and 16 to 0.032 µg/ml for other drugs) of each antifungal agent in RPMI were prepared in 96-well microplate (Jetbiofil, China) and were mixed with 100 µl of yeast suspension.

The microplates were incubated at 35ºC for 24 and 48 hours. Positive and negative controls (a well without antifungal and a well without yeast suspension) were added to each examination set. A standard strain of *C. parapsilosis* ATCC 22019 was used as a control in the tests, the results of which were in the expected CLSI range. The amount of growth in each well was reported visually and compared with the amount in the growth control wells (with no antifungal agents).

The MIC of amphotericin B was described as the lowest concentration of the drug that could stop any visible yeast growth, compared to positive controls. The MICs for the azole family (fluconazole, voriconazole, posaconazole, itraconazole, and ketoconazole) and caspofungin were described as the lowest concentration of the drug that could reduce 50% of fungal growth [9, 14]. Isolated *C. albicans* strains were categorized as sensitive, intermediate, and resistant, according to CLSI M27-S4 (new CLSI breakpoint; Table 1).

Data were collected and entered into WHONET, version 5.6. WHONET is a free, simple software developed for the analysis of microbiological and clinical data, with special focus on antimicrobial susceptibility test results.

Results

Among the 397 isolated *C. albicans*, total rates of resistance for amphotericin B, caspofungin, voriconazole, fluconazole, and itraconazole were 0.5%, 0.3%, 3.8%, 2.8%, and 2.5%, respectively. Since there are no new CLSI breakpoints for posaconazole and ketoconazole, their MIC50 and MIC90 were reported in this study. For posaconazole, MIC50 and MIC90 were 0.016 and 0.064 µg/ml, respectively, while for ketoconazole, they were 0.016, 0.125 µg/ml, respectively.

Resistance to amphotericin B was mostly observed in Ahvaz, whereas resistance to

Table 1. Antifungal breakpoints used in this study, according to Clinical and Laboratory Standards Institute M27-S4 and new breakpoints

	Susceptible	Susceptible dose-dependent	Resistant
Amphotericin B	≤1 µg/ml	-	≥ 1µg/ml
Caspofungin	≤0.25 µg/ml	0.25-0.5	≥1µg/ml
Voriconazole	≤0.12 µg/ml	0.25-0.5	≥ 1 µg/ml
Fluconazole	≤2	4	≥8
Posaconazole*	-	-	-
Itraconazole	≤0.12	0.25-0.5	≥1
Ketoconazole*	-	-	-

* No clinical breakpoints for these drugs

Table 2. Antifungal susceptibility testing of 397*Candida albicans* isolated from ten cities in Iran

Location	Antibiotic name	%R	%I	%S	%R 95% CI	MIC50* µg/ml	MIC90 µg/ml	Geom.mean	MIC range µg/ml
Kerman (37)	Amphotericin B	0	0	100	0.0-11.7	0.032	0.25	0.036	0.016-0.25
	Caspofungin	0	0	100	0.0-11.7	0.016	0.125	0.026	0.016-0.125
	Voriconazole	0	8.1	91.9	0.0-11.7	0.016	0.064	0.025	0.016-1
	Fluconazole	0	2.7	97.3	0.0-11.7	0.125	1	0.183	0.016-4
	Posaconazole	0	0	0	0.0-11.7	0.016	0.064	0.028	0.016-16
	Itraconazole	2.7	29.7	67.6	0.1-15.8	0.032	0.25	0.045	0.016-2
	Ketoconazole	0	0	0	0.0-11.7	0.016	0.064	0.025	0.016-1
Shiraz (80)	Amphotericin B	0	0	100	0.0-5.7	0.032	0.25	0.057	0.025-0.5
	Caspofungin	1.2	7.5	91.2	0.1-7.6	0.125	0.25	0.126	0.032-64
	Voriconazole	12.5	0	87.5	6.5-22.2	0.032	16	0.075	0.032-16
	Fluconazole	13.8	11.2	75	7.4-23.8	0.5	64	0.971	0.125-64
	Posaconazole	0	0	0	0.0-5.7	0.032	16	0.067	0.032-16
	Itraconazole	6.2	17.5	76.2	2.3-14.6	0.032	0.125	0.068	0.016-16
	Ketoconazole	0	0	0	0.0-5.7	0.032	0.25	0.066	0.032-16
Yasouj (18)	Amphotericin B	0	0	100	0.0-21.9	0.016	0.064	0.023	0.016-0.125
	Caspofungin	0	0	100	0.0-21.9	0.016	0.25	0.036	0.016-0.25
	Voriconazole	0	16.7	83.3	0.0-21.9	0.016	1	0.032	0.016-1
	Fluconazole	0	0	100	0.0-21.9	0.125	0.5	0.142	0.064-2
	Posaconazole	0	0	0	0.0-21.9	0.016	0.125	0.021	0.016-0.25
	Itraconazole	0	66.7	33.3	0.0-21.9	0.125	0.5	0.112	0.016-1
	Ketoconazole	0	0	0	0.0-21.9	0.016	0.125	0.024	0.016-0.5
Mashhad (67)	Amphotericin B	0	0	100	0.0-6.8	0.016	0.125	0.031	0.016-0.125
	Caspofungin	0	0	100	0.0-6.8	0.064	0.125	0.061	0.016-0.25
	Voriconazole	0	1.5	98.5	0.0-6.8	0.016	0.032	0.022	0.016-1
	Fluconazole	0	1.5	98.5	0.0-6.8	0.125	0.25	0.13	0.032-4
	Posaconazole	0	0	0	0.0-6.8	0.016	0.032	0.022	0.01-0.125
	Itraconazole	4.5	25.4	70.1	1.2-13.4	0.032	1	0.073	0.016-125
	Ketoconazole	0	0	0	0.0-6.8	0.016	0.032	0.022	0.016-0.25
Isfahan (37)	Amphotericin B	0	0	100	0.0-11.7	0.064	0.125	0.052	0.016-0.125
	Caspofungin	0	2.7	97.3	0.0-11.7	0.016	0.032	0.021	0.016-1
	Voriconazole	2.7	35.1	62.2	0.1-15.8	0.032	1	0.081	0.016-2
	Fluconazole	0	0	100	0.0-11.7	0.125	2	0.221	0.064-2
	Posaconazole	0	0	0	0.0-11.7	0.032	1	0.058	0.016-1
	Itraconazole	2.7	67.6	29.7	0.1-15.8	0.125	1	0.173	0.016-2
	Ketoconazole	0	0	0	0.0-11.7	0.125	0.5	0.069	0.016-1
Urmia (19)	Amphotericin B	0	0	100	0.0-20.9	0.032	0.064	0.037	0.016-0.125
	Caspofungin	0	0	100	0.0-20.9	0.016	0.032	0.018	0.016-0.064
	Voriconazole	0	5.3	94.7	0.0-20.9	0.016	0.064	0.021	0.016-0.5
	Fluconazole	0	0	100	0.0-20.9	0.25	0.5	0.242	0.064-2
	Posaconazole	0	0	0	0.0-20.9	0.016	0.064	0.02	0.01-0.125
	Itraconazole	0	5.3	94.7	0.0-20.9	0.016	0.032	0.02	0.016-0.125
	Ketoconazole	0	0	0	0.0-20.9	0.016	0.016	0.017	0.016-0.064

Continue Table 2. Antifungal susceptibility testing of 397 *Candida albicans* isolated from ten cities in Iran

Tehran (25)	Amphotericin B	0	0	100	0.0-16.6	0.016	0.064	0.026	0.016-0.125
	Caspofungin	0	0	100	0.0-16.6	0.016	0.016	0.016	0.016-0.016
	Voriconazole	0	8	92	0.0-16.6	0.016	0.064	0.025	0.016-1
	Fluconazole	0	0	100	0.0-16.6	0.064	1	0.11	0.064-2
	Posaconazole	0	0	0	0.0-16.6	0.016	0.016	0.017	0.016-0.064
	Itraconazole	0	92	8	0.0-16.6	0.5	1	0.369	0.064-1
	Ketoconazole	0	0	0	0.0-16.6	0.016	0.064	0.02	0.01-0.125
Sanandaj (63)	Amphotericin B	1.6	0	98.4	0.1-9.7	0.032	0.064	0.031	0.016-32
	Caspofungin	0	0	100	0.0-7.2	0.016	0.032	0.018	0.016-0.125
	Voriconazole	0	0	100	0.0-7.2	0.016	0.016	0.016	0.016-0.032
	Fluconazole	0	0	100	0.0-7.2	0.064	0.064	0.07	0.064-1
	Posaconazole	0	0	0	0.0-7.2	0.016	0.032	0.018	0.016-0.125
	Itraconazole	0	9.5	90.5	0.0-7.2	0.016	0.064	0.023	0.016-0.25
	Ketoconazole	0	0	0	0.0-7.2	0.016	0.016	0.017	0.016-0.25
Ahvaz (25)	Amphotericin B	4	0	96	0.2-22.3	0.032	0.064	0.037	0.016-16
	Caspofungin	0	0	100	0.0-16.6	0.016	0.016	0.018	0.016-0.25
	Voriconazole	8	8	84	1.4-27.5	0.016	1	0.038	0.016-2
	Fluconazole	0	0	100	0.0-16.6	0.125	2	0.225	0.064-2
	Posaconazole	0	0	0	0.0-16.6	0.016	0.032	0.018	0.016-0.064
	Itraconazole	0	44	56	0.0-16.6	0.064	0.25	0.077	0.016-0.5
	Ketoconazole	0	0	0	0.0-16.6	0.016	0.064	0.026	0.016-0.125
Sari (26)	Amphotericin B	0	0	100	0.0-16.0	0.032	0.064	0.037	0.016-0.064
	Caspofungin	0	0	100	0.0-16.0	0.016	0.016	0.016	0.016-0.032
	Voriconazole	7.7	3.8	88.5	1.3-26.6	0.016	0.125	0.026	0.016-2
	Fluconazole	0	0	100	0.0-16.0	0.125	0.5	0.141	0.064-2
	Posaconazole	0	0	0	0.0-16.0	0.016	0.032	0.021	0.016-1
	Itraconazole	0	100	0	0.0-16.0	0.5	1	0.363	0.125-1
	Ketoconazole	0	0	0	0.0-16.0	0.016	0.032	0.022	0.016-1
Total (397)	Amphotericin B	0.5	0	99.5	0.1-2.0	0.032	0.125	0.037	0.016-32
	Caspofungin	0.3	1.8	98	0-1.7	0.016	0.125	0.035	0.016-64
	Voriconazole	3.8	6.5	89.7	2.2-6.3	0.016	0.125	0.033	0.016-16
	Fluconazole	2.8	2.8	94.5	1.5-5.1	0.125	1	0.204	0.016-64
	Posaconazole	0	0	0	0.0-1.2	0.016	0.064	0.029	0.01-16
	Itraconazole	2.5	36.8	60.7	1.3-4.7	0.064	0.5	0.073	0.016-125
	Ketoconazole	0	0	0	0.0-1.2	0.016	0.125	0.029	0.01-16

*Minimum inhibitory concentration

caspofungin, itraconazole, voriconazole, and fluconazole was most commonly noted in Shiraz. The lowest MIC90 of all the isolates (0.064 µg/ml) pertained to posaconazole (Table 2).

Discussion

C. albicans is reported to be the most prevalent *Candida spp*. isolated from different populations , with the rates of 17.5%, 48%, 55% , 56.8%, and 82.2% in Shiraz, Mazandaran, Tehran, and Kerman, respectively [3, 8, 15-17], indicating the important role of this species in fungal infections.

Amphotericin B, which belongs to the polyene class of antifungal agents, is an effective treatment for some fungal infections. This drug is available in the form of complexes with sodium deoxycholate,

cholesteryl sulfate complex, lipid complex, and liposomal formulation. Amphotericin B binds with ergosterol, forming a transmembrane channel that causes monovalent ion leakage (K^+, Na^+, H^+, and Cl^-), leading to fungal cell death [18].

In the current study, *C. albicans* sensitivity to amphotericin B was 99.5%. The highest resistance rates to this antifungal agent were observed in Sanandaj (1.6%) and Ahvaz (4%). The resistance rates of *C. albicans* to amphotericin B were reported to be 2.6% [8] and 7% [5] in Shiraz and Mazandaran. However, no resistance to amphotericin B was reported in a study by Mohammadi et al. [19]. This discrepancy can be explained with the recurrent use of amphotericin B in some patients.

The mechanism of azole antifungals incorporates blocking the synthesis of ergosterol by inhibiting lanosterol 14α-demethylase. This enzyme has various potentials depending on the drug. For instance, posaconazole is significantly more potent than itraconazole in inhibiting 14-alpha demethylase [14]. Fluconazole and itraconazole are more cost-effective and less toxic drug forms of the azole family with excellent patient tolerance. The total rates of resistance to fluconazole and itraconazole in all the cities were 2.8% with 2.8% intermediate and 2.5% with 36.8% intermediate. The resistance rates against fluconazole were reported to be 4.6% [5], 34.2% [19], 10.5% [3], and 34% [9], while for itraconazole these rates were 7% [5], 43% [19], 21% [9], and 33.7% [3] in Shiraz, Ilam, and Turkey (with the first two rates belonging to Shiraz).

Cross-resistance should be considered for some antifungal agents from the azole family [20]. The existing difference may be due to population or regional studies. Moreover, fluconazole in some immunocompromised patients was used as a prophylaxis, which can explain the differences in resistance rates in some populations.

Voriconazole is another triazole, which is generally administered to treat serious, invasive fungal infections in immunocompromised patients. The resistance rate of voriconazole in the present study was 3.8% (6.5% intermediate), and in other studies, it was reported to be 2.3% [5], 6% [3, 19], and 14% [9].

Posaconazole is a new antifungal agent in Iran, and there is a scarcity of reports on its susceptibility pattern. The new CLSI did not report any breakpoints for this antifungal, but its resistance rate was reported to be 6.7% [19]. In a study by Yenisehirli et al., antifungal susceptibility tests were performed using E-test method and the resistance rate of *C. albicans* isolates to posaconazole was reported 14%. In the present study, resistance rates for all the azoles were high [9]. In the current study, MIC90 of posaconazole was 0.064 μg/ml.

Ketoconazole is usually prescribed for fungal infections of the skin and mucous membranes (as a cream and shampoo), and its oral administration is limited due to risk of hepatic damage. The MIC90 for this agent in the current study was 0.125 μg/ml, indicating effectiveness against *C. albicans*. The resistance rates for this antifungal were reported to be 7% [5], 9.4% [3], 34.9% [19], and 32% [9] in Shiraz, Ilam, and Turkey (with first two rates belonging to Shiraz).

Caspofungin is a lipopeptide antifungal agent and a member of a new class of echinocandin antifungals. It was the inhibitor of fungal (1→3)-β-D-glucan synthesis. In our study, 0.3% of all the *C. albicans* isolates were resistant, 1.8% were intermediate, and 98% were susceptible to this antifungal agent. Shekohi et al. [8] reported no resistance to this agent; whereas, 1.8% were resistant to caspofungin in South of Iran [10]. This drug is an effective, but costly, antifungal agent. In 2014, Haddadi et al. reported high resistance, especially to voriconazole, fluconazole, posaconazole, itraconazole, and ketoconazole in the pediatric patients with neutropenia [11].

The susceptibility pattern of *C. albicans* to antifungal agents is dependent on study population, duration of prophylaxis, and treatment of patients against invasive fungal infections in each region. Susceptibility pattern for antifungal drugs varies with region. Species-specific resistance rate plays an important role in resistance surveillance. Constant susceptibility testing and determination of susceptibility pattern for antifungal agents seems to be necessary. According to our data, fluconazole is the drug of choice since it is cost-effective with low side effects for management of patients at risk for systemic candidiasis throughout the region.

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Author's contribution

P.B. designed the experiments, analyzed the data, and cooperated with writing the manuscript; H.J. performed experiments and assisted with

writing the manuscript; H.B., K.D., A.GM., A.HN., R.M., H.M., MJ.N, A.Sh., and J.S. collected samples. All the authors discussed the results and implications and provided their comments during all the stages.

Conflicts of interest

None declared.

Financial disclosure

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