



Antifungal Susceptibility of *Candida albicans* Isolates at a Tertiary Care Hospital in Bulgaria

Hristina Yotova Hitkova^{1,*}, Diana Simeonova Georgieva¹, Preslava Mihailova Hristova¹ and Mariya Petrova Sredkova¹

¹Department of Microbiology, Virology and Medical Genetics, Faculty of Pharmacy, Medical University, Pleven, Bulgaria

*Corresponding author: Department of Microbiology, Virology and Medical Genetics, Faculty of Pharmacy, Medical University, Pleven, Bulgaria. Tel: +359-899116021, Email: hitkova@abv.bg

Received 2019 April 08; Revised 2019 June 22; Accepted 2019 June 24.

Abstract

Background: The precise identification of yeasts and their antifungal sensitivity is a key factor in the choice of a suitable drug for treatment and prevention of fungal infections.

Objectives: The aim of the present study was to determine in vitro susceptibility of clinical *Candida albicans* isolates to nine antifungal agents.

Methods: A total of 61 *C. albicans* isolates were tested. Antifungal susceptibility was evaluated by determination of minimum inhibitory concentrations (MIC).

Results: Fifty *C. albicans* isolates were susceptible to nine antifungal agents. The remaining 11 yeasts were resistant to one or more antifungals. All *C. albicans* were susceptible to amphotericin B with MICs 0.25 to 1 $\mu\text{g}/\text{mL}$ and exhibited sensitivity to 5-fluorocytosine with MIC₉₀ of 0.125 $\mu\text{g}/\text{mL}$. The MIC₅₀ and MIC₉₀ of fluconazole were 0.5 and 32 $\mu\text{g}/\text{mL}$, whereas the MIC₅₀ and MIC₉₀ of voriconazole were considerably lower - 0.0078 and 2 $\mu\text{g}/\text{mL}$. The isolates showed susceptibility to echinocandins with MIC₉₀ of micafungin, anidulafungin and caspofungin of 0.015, 0.031, and 0.125 $\mu\text{g}/\text{mL}$, respectively. Of particular interest was detection of seven *C. albicans* isolates, which expressed a high-level resistance to all azoles and one of them was also resistant to echinocandins.

Conclusions: In conclusion, detection of resistance in *C. albicans*, which is a species typically susceptible to antifungals, is of great importance for clinical practice.

Keywords: *Candida albicans*, Antifungal Agents, Resistance

1. Background

Microorganisms of genus *Candida* belong to the normal microbial flora of the oral cavity, gastrointestinal tract, and vagina. Although they normally colonize the human mucosal surfaces, members of the genus could cause a wide range of infections, characterized by different clinical manifestations and severity (1-4). For the needs of the clinical practice, differentiation of *Candida albicans* and non-*albicans* species has been accepted. Nowadays, a shift towards non-*albicans* species is observed, yet *C. albicans* is the most prevalent species (5-11). According to some current publications, the ratio of isolation of this species among the other *Candida* spp. is about 50% (5, 6, 9). *Candida albicans* is still a leading causative agent of candidemias and it is responsible for over 50% of them (12). Therefore, the correct identification and sensitivity testing of *C. albicans* are key points in the choice of an appropriate drug for prophylaxis and treatment of infections, caused by this species.

Azoles are the most commonly used antifungals and they act by inhibiting the cytochrome P-450-dependent enzyme lanosterol 14- α -demethylase. This enzyme is involved in the biosynthesis of the fungal-specific membrane sterol ergosterol. Fluconazole, a member of the triazole group, is the most commonly used agent for treatment of *Candida* infections. It has been shown that *C. albicans* could develop a high-level resistance to azoles, which is associated with the type of infection and previous fluconazole application (10). Echinocandins are a relatively new class of antifungal agents and their mechanism of action is based on inhibiting the β -(1,3)-glucan synthetase enzyme. These antifungals possess fungicidal activity against azole-susceptible and azole-resistant *Candida* isolates (13, 14). Resistance to caspofungin was first reported in 2005 among *Saccharomyces cerevisiae*, *C. albicans*, and *C. krusei* strains (15). Generally, echinocandin resistance in *Candida* spp. still remains low; less than 3% (16).

According to the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the antifungal susceptibility of *Candida* spp. should be determined by broth microdilution method, yet disk-diffusion assay and agar-based E-test are also used (5, 6, 8, 17, 18). Recently, CHROMagar *Candida* was used for isolation and presumptive identification of the main yeast species, yet precise identification and susceptibility testing have to be performed in clinically important fungi.

2. Objectives

The aim of the present study was to determine in vitro susceptibility of *C. albicans* isolates to nine antifungal agents.

3. Methods

3.1. Yeast Isolates

The sample of this study was a group of 61 *C. albicans* isolates collected from various clinical materials of overall 61 patients admitted to University Hospital; Dr. G. Stranski-Pleven, Bulgaria between September, 2016 and December, 2017. This hospital is a 920-bed tertiary-care facility, localized in the central part of Northern Bulgaria. The distribution of the isolates according to the patient specimens was as follows: Urines (15), lower respiratory tract samples (15), throat swabs and oral cavity samples (10), wound aspirates (8), vaginal swabs (6), feces (5), and blood cultures (2).

3.2. Species Identification

Yeast isolates were preliminary identified on the basis of typical green colored colonies on CHROMagar *Candida* (BD, UK). The definitive identification up to species level was performed by MIKROLATEST CANDIDATEST 21 (Erba Lachema, CZ) and VITEK 2 compact system (BioMerieux, France), using YST ID cards. Additional tests, such as germ-tube test and chlamyospore formation were used if necessary.

3.3. Antifungal Susceptibility Testing

Minimum inhibitory concentrations (MICs) of Amphotericin B (APH), 5-Fluorocytosine (FCY), Fluconazole (FCA), Voriconazole (VOR), Posaconazole (POS), Itraconazole (ITR), Micafungin (MIF), Anidulafungin (ANF), and Caspofungin (CAS) were tested with Micronaut-AM (MERLIN Diagnostika GmbH, Germany). The prepared yeast suspensions were adjusted to 0.5 McFarland and 100 μ L were inoculated to each well of the microplates. The results were read after 48 hours of incubation at 35°C. The MICs

were interpreted according to EUCAST, 2018 (19) with breakpoints in μ g/mL as follows: APH susceptibility ($S \leq 1$ and resistance ($R > 1$), FCA $S \leq 2$ and $R > 4$, VOR $S \leq 0.064$ and $R > 0.25$, POS and ITR $S \leq 0.064$ and $R > 0.064$, ANF $S \leq 0.032$ and $R > 0.032$, MIF $S \leq 0.016$ and $R > 0.016$. The interpretation of the results for FCY was performed according to the manufacturer's instruction: $S \leq 4$ and $R > 32 \mu$ g/mL. The EUCAST breakpoints for CAS have not yet been established and it is recommended that isolates that are susceptible to ANF as well as to MIF should be considered susceptible to CAS. For each drug, MIC₅₀ and MIC₉₀ were calculated.

3.4. Multidrug Resistance

Multidrug resistance was defined as simultaneous resistance to ≥ 1 agent in two or more classes of antifungal agents (1).

4. Results

From a total of 61 *C. albicans* isolates, 50 (81.97%) were susceptible to all tested antifungal agents. The remaining 11 yeasts were resistant to one or more antifungals: two resistant to fluconazole, two resistant to fluconazole and itraconazole, six resistant to all azoles, and one resistant to azoles and echinocandins.

The results of susceptibility testing to nine antifungal agents are presented in Table 1 as MIC ranges, MIC₅₀, MIC₉₀, and resistance rates. As shown, the MICs ranges of azoles were very wide: from < 0.002 to $>128 \mu$ g/mL. The MIC₅₀ and MIC₉₀ for fluconazole were 0.5 and 32 μ g/mL, whereas the MIC₅₀ and MIC₉₀ for voriconazole were considerably lower, 0.0078 and 2 μ g/mL. The resistance rates of azoles varied from 11.47% for voriconazole and posaconazole to 18.03% for fluconazole. The data about echinocandins showed susceptibility to micafungin, anidulafungin, and caspofungin with MIC₉₀s of 0.015, 0.031, and 0.125 μ g/mL, respectively. The tested isolates exhibited sensitivity to 5-fluorocytosine with MIC₉₀ of 0.125 μ g/mL. All *C. albicans* were susceptible to amphotericin B with MICs 0.25 to 1 μ g/mL.

Seven *C. albicans* isolates expressed a high-level resistance to all azoles (Table 2). Surprisingly, one of them was also highly resistant to echinocandins with anidulafungin and caspofungin MICs $> 8 \mu$ g/mL and micafungin MIC - 2 μ g/mL. This multidrug resistant strain (No. 154/17) was recovered from a pleural aspirate of a surgical patient. On the whole, resistant yeasts originated from patients at intensive care units (ICU) and surgical wards.

5. Discussion

The current study presents information about antifungal susceptibility profiles of 61 *C. albicans* isolates, obtained

Table 1. Susceptibility of *Candida albicans* Isolates to Antifungal Agents: MIC Ranges, MIC₅₀, MIC₉₀ and Resistance Rates

Antifungal Agents	<i>Candida albicans</i> (N = 61)			Resistance Rate, %
	MIC Ranges, $\mu\text{g/mL}$	MIC ₅₀ , $\mu\text{g/mL}$	MIC ₉₀ , $\mu\text{g/mL}$	
Fluconazole	< 0.002 - >128	0.5	32	18.03
Itraconazole	< 0.031 - >4	0.031	4	14.75
Posaconazole	< 0.0078 - > 8	0.0078	8	11.47
Voriconazole	< 0.0078 - > 8	0.0078	2	11.47
Anidulafungin	< 0.002 - > 8	0.031	0.031	1.64
Caspofungin	< 0.0625 - > 8	0.125	0.125	1.64
Micafungin	< 0.015 - 2	0.015	0.015	1.64
5-fluorocytosine	< 0.0625 - 8	0.0625	0.125	0
Amphotericin B	0.25 - 1	0.5	1	0

Table 2. Antifungal Resistance Patterns of *Candida albicans* Isolates (N = 7)

Isolate ^a	Sample	Ward	Antifungal Agents (MIC in $\mu\text{g/mL}$ - Interpretation)						
			FCA	ITR	POS	VOR	ANF	CAS	MIF
60/16	Blood culture	Cardio	> 128-R	> 4-R	> 8-R	> 8-R	0.031-S	0.125-S	0.015-S
92/16	Vaginal swab	Obstetric	> 128-R	4-R	8-R	1-R	0.002-S	0.0625-S	0.015-S
186/17	wound aspirate	Surgery	> 128-R	4-R	8-R	> 8-R	0.031-S	0.125-S	0.015-S
220/17	Oral cavity	ICU	32-R	4-R	8-R	2-R	0.015-S	0.0625-S	0.015-S
225/17	BAL	ICU	> 128-R	4-R	8-R	8-R	0.015-S	0.0625-S	0.015-S
229/17	Oral cavity	ICU	32-R	4-R	8-R	2-R	0.015-S	0.125-S	0.015-S
154/17	Pleural aspirate	Surgery	> 128-R	4-R	8-R	> 8-R	> 8-R	> 8-R	2-R

^aThe isolates are presented as Lab No./year of isolation.

from different clinical specimens. Of particular interest were the results for azoles, which were characterized with wide MIC ranges and large distinctions in the values of MIC₅₀ and MIC₉₀. However, MIC₅₀s were within the S breakpoints, MIC₉₀s were significantly greater than the R breakpoints - 32, 8, 4 and 2 $\mu\text{g/mL}$ of fluconazole, posaconazole, itraconazole, and voriconazole, respectively. This is due to detection of a few isolates with a high-level resistance to all azoles. Previously published data in Bulgaria indicated fluconazole MIC₉₀ of 8 mg/L (20). This value was observed in testing of 106 clinical *C. albicans* isolates collected between 2009 and 2013. In a study covered by 35 countries in the period 1997 to 2001, the fluconazole MIC₅₀s for *C. albicans* were less than 8 $\mu\text{g/mL}$, yet five countries had populations with MIC₉₀s more than 8 $\mu\text{g/mL}$ (8).

The published data about fluconazole resistance in *C. albicans* varied widely. Different susceptibilities were found according to specimen types and wards (8, 11). The discrepancies in MIC breakpoints according to the CLSI or EUCAST also influence resistance rates. Therefore, the interpretative criteria are important in comparing the results.

On the basis of microbiological and clinical parameters clinical breakpoints (CBPs) have been established for a specific drug and a definitive fungal species. Nowadays, there is a tendency for harmonization of CBPs recommended by the CLSI and EUCAST (21). Using the CLSI agar diffusion assay, Azevedo et al. (5) detected 98% susceptibility in evaluation of 2393 strains, collected from 1999 to 2003. Ying et al. (11) determined 4.3% resistance rate (MICs > 64 $\mu\text{g/mL}$) among 2170 isolates, obtained between 2005 and 2009, yet the authors observed that yeasts from different specimens and clinical services had various degrees of antifungal susceptibility.

Many publications have also revealed high activity of fluconazole against *C. albicans* (12, 22-28). On the other hand, Zarei Mahmoudabadi et al. (18) reported only 15.5% sensitivity in testing of 58 urine strains. Kaur et al. (29) determined that among 33 isolates, 12 (36.36%) were fluconazole resistant with MICs > 64 $\mu\text{g/mL}$. Similar results were presented by Turkish authors; 34% resistance among 201 strains with MICs > 256 $\mu\text{g/mL}$ (17). In contrast, Yuksekkaya et al. (30) reported that 56 urine isolates were flu-

conazole susceptible with MIC ranges, MIC₅₀ and MIC₉₀ as 0.25 to 4, 0.25 and 0.5 µg/mL, respectively. Recently, the resistance rates in *C. albicans* have been higher than those previously observed because of establishment of new MIC breakpoints (31).

The current researchers observed that resistance rates of voriconazole and posaconazole (11.47% for both) were lower than that of fluconazole (18.03%). The MIC₅₀ and MIC₉₀ of voriconazole were considerably lower than those obtained for fluconazole. In general, the researchers determined that voriconazole exhibited better activity among the other azoles, yet the MIC₉₀ value was significantly higher than that previously reported in Bulgaria - 0.047 mg/L (20). According to Pfaller et al. (25), voriconazole demonstrates 16- to 32-fold greater activity than fluconazole against yeast species. Similar results were found in other publications (5, 8, 32). The mechanisms of fluconazole resistance in *C. albicans* have been most extensively studied, whereas those against the other azoles have not yet been clearly defined (10). Many genetic determinants have been investigated, such as point mutations in *ERG11*, increased expression of *ERG11* due to mutations in transcriptional regulator *UPS2*, overexpression of drug efflux pumps *Mdr1p* and *Cdr1p/Cdr2p*, inactivation of *ERG3*, aneuploidy and other chromosomal abnormalities (1, 10, 12). High-level fluconazole resistance rarely refers to a single mechanism, thus multiple molecular processes may be involved (12).

In the current study, six isolates (9.84%) were highly resistant to all tested azoles. The current results are similar to those reported by Yenisehirli et al. (17). The authors determined that 29 (14%) from 201 *C. albicans* isolates were simultaneously resistant to ketoconazole, fluconazole, itraconazole, posaconazole, and voriconazole. Although antifungal resistance usually involves single drugs of the classes, once established, resistance mechanisms could be shared by drugs (33). Larger studies have documented that *C. albicans* isolates with elevated MICs of fluconazole were generally less susceptible to other triazoles, indicating development of resistance to several drugs of the same class (6, 25, 34). Cross-resistance of all azoles was explained by the expression of the ATP-Binding Cassette (ABC) transporters (i.e., *CDR1* or *CgCDR1*) (35).

The current researchers detected a high-level resistance of azoles and echinocandins in one *C. albicans* isolate (1.64%). Echinocandin resistance associated with cross-resistance of azoles was reported in *C. glabrata* (36). The current results are in concordance with the data for a low rate of echinocandin resistance in *C. albicans* (22, 37, 38). Shokohi et al. (39) detected two (4.6%) caspofungin resistant strains among 44 isolates. However, Taghipour et al. (9) reported that from a total of 43 *C. albicans*, 15 were resis-

tant to caspofungin, 16 to itraconazole, and 30 to amphotericin B. In a Bulgarian multicenter study, all 204 yeast isolates were susceptible to anidulafungin (20). Echinocandin resistance is uncommon among *C. albicans* and its genetic mechanism involves mutations in two highly conserved “hot spot” regions of the target gene *FKS1*, thus molecular testing for *FKS* mutant strains is recommended (36). The *FKS1* mutations, mainly F641S/L and S645F/P/Y, confer cross-resistance among the drugs of the class (40).

In the current study, all tested yeasts were sensitive to amphotericin B. These results could be explained with the fact that this drug is rarely used in Bulgaria. The current data are similar to those reported worldwide (17, 22, 24, 30, 32, 41). In testing 625 invasive *C. albicans* isolates, Zhang et al. (28) determined only seven (1.1%) amphotericin B resistant isolates, whereas Iranian authors observed resistance rates from about 7% (6), 11% (39) to 30% (42).

5.1. Conclusions

Although about 80% of *C. albicans* were susceptible to nine antifungal agents, a few isolates showed a high-level resistance to all azoles and one isolate was also resistant to echinocandins. Detection of resistance in *C. albicans*, which is a species typically susceptible to antifungals, determines the need for further monitoring of antifungal sensitivity of clinical yeast isolates in the hospital under study.

Footnotes

Authors' Contribution: Study concept and design: Hristina Yotova Hitkova. Acquisition of data: Diana Simeonova Georgieva and Preslava Mihailova Hristova. Analysis and interpretation of data: Hristina Yotova Hitkova and Diana Simeonova Georgieva. Drafting of the manuscript: Hristina Yotova Hitkova. Critical revision of the manuscript for important intellectual content: Mariya Petrova Sredkova.

Conflict of Interests: The authors declare that there was no conflict of interest in this study.

Financial Disclosure: Authors declare that they had no financial interests related to the material in the manuscript.

Funding/Support: This study was carried out with financial support of Medical University - Pleven through University Grants Commission (Project No. 16/2017).

References

1. Arendrup MC, Patterson TF. Multidrug-resistant Candida: Epidemiology, molecular mechanisms, and treatment. *J Infect Dis.* 2017;**216**(suppl_3):S445-51. doi: [10.1093/infdis/jix131](https://doi.org/10.1093/infdis/jix131). [PubMed: 28911043].

2. Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol*. 2013;**62**(Pt 1):10–24. doi: [10.1099/jmm.0.045054-0](https://doi.org/10.1099/jmm.0.045054-0). [PubMed: [23180477](https://pubmed.ncbi.nlm.nih.gov/23180477/)].
3. Singh G, Raksha AU. Candidal infection: Epidemiology, pathogenesis and recent advances for diagnosis. *Bul Pharm Med Sci*. 2013;**1**(1).
4. Yapar N. Epidemiology and risk factors for invasive candidiasis. *Ther Clin Risk Manag*. 2014;**10**:95–105. doi: [10.2147/TCRM.S40160](https://doi.org/10.2147/TCRM.S40160). [PubMed: [24611015](https://pubmed.ncbi.nlm.nih.gov/24611015/)]. [PubMed Central: [PMC3928396](https://pubmed.ncbi.nlm.nih.gov/PMC3928396/)].
5. Azevedo AC, Bizerra FC, da Matta DA, de Almeida LP, Rosas R, Colombo AL. In vitro susceptibility of a large collection of Candida Strains against fluconazole and voriconazole by using the CLSI disk diffusion assay. *Mycopathologia*. 2011;**171**(6):411–6. doi: [10.1007/s11046-010-9387-1](https://doi.org/10.1007/s11046-010-9387-1). [PubMed: [21181497](https://pubmed.ncbi.nlm.nih.gov/21181497/)].
6. Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J Microbiol*. 2011;**3**(4):183–8. [PubMed: [22530086](https://pubmed.ncbi.nlm.nih.gov/22530086/)]. [PubMed Central: [PMC3330181](https://pubmed.ncbi.nlm.nih.gov/PMC3330181/)].
7. Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida infection: An emerging threat. *Interdiscip Perspect Infect Dis*. 2014;**2014**:615958. doi: [10.1155/2014/615958](https://doi.org/10.1155/2014/615958). [PubMed: [25404942](https://pubmed.ncbi.nlm.nih.gov/25404942/)]. [PubMed Central: [PMC4227454](https://pubmed.ncbi.nlm.nih.gov/PMC4227454/)].
8. Hazen KC, Baron EJ, Colombo AL, Girmenia C, Sanchez-Sousa A, del Palacio A, et al. Comparison of the susceptibilities of Candida spp. to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. *J Clin Microbiol*. 2003;**41**(12):5623–32. doi: [10.1128/jcm.41.12.5623-5632.2003](https://doi.org/10.1128/jcm.41.12.5623-5632.2003). [PubMed: [14662952](https://pubmed.ncbi.nlm.nih.gov/14662952/)]. [PubMed Central: [PMC309015](https://pubmed.ncbi.nlm.nih.gov/PMC309015/)].
9. Taghipour S, Rezaei-Matehkolaei A, Zarei Mahmoudabadi A. Antifungal susceptibility profiles of Candida species isolated from Ahvaz Jundishapur Educational Hospitals. *Jundishapur J Microbiol*. 2018;**11**(11). e78851. doi: [10.5812/jjm.78851](https://doi.org/10.5812/jjm.78851).
10. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans candida species. *Front Microbiol*. 2016;**7**:2173. doi: [10.3389/fmicb.2016.02173](https://doi.org/10.3389/fmicb.2016.02173). [PubMed: [28127295](https://pubmed.ncbi.nlm.nih.gov/28127295/)]. [PubMed Central: [PMC5226953](https://pubmed.ncbi.nlm.nih.gov/PMC5226953/)].
11. Ying Y, Zhang J, Huang SB, Liu FD, Liu JH, Zhang J, et al. Fluconazole susceptibility of 3,056 clinical isolates of Candida species from 2005 to 2009 in a tertiary-care hospital. *Indian J Med Microbiol*. 2015;**33**(3):413–5. doi: [10.4103/0255-0857.158569](https://doi.org/10.4103/0255-0857.158569). [PubMed: [26068346](https://pubmed.ncbi.nlm.nih.gov/26068346/)].
12. Berkow EL, Lockhart SR. Fluconazole resistance in Candida species: A current perspective. *Infect Drug Resist*. 2017;**10**:237–45. doi: [10.2147/IDR.S118892](https://doi.org/10.2147/IDR.S118892). [PubMed: [28814889](https://pubmed.ncbi.nlm.nih.gov/28814889/)]. [PubMed Central: [PMC5546770](https://pubmed.ncbi.nlm.nih.gov/PMC5546770/)].
13. Messer SA, Diekema DJ, Boyken L, Tendolkar S, Hollis RJ, Pfaller MA. Activities of micafungin against 315 invasive clinical isolates of fluconazole-resistant Candida spp. *J Clin Microbiol*. 2006;**44**(2):324–6. doi: [10.1128/JCM.44.2.324-326.2006](https://doi.org/10.1128/JCM.44.2.324-326.2006). [PubMed: [16455878](https://pubmed.ncbi.nlm.nih.gov/16455878/)]. [PubMed Central: [PMC1392702](https://pubmed.ncbi.nlm.nih.gov/PMC1392702/)].
14. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. In vitro susceptibilities of Candida spp. to caspofungin: four years of global surveillance. *J Clin Microbiol*. 2006;**44**(3):760–3. doi: [10.1128/JCM.44.3.760-763.2006](https://doi.org/10.1128/JCM.44.3.760-763.2006). [PubMed: [16517851](https://pubmed.ncbi.nlm.nih.gov/16517851/)]. [PubMed Central: [PMC1393154](https://pubmed.ncbi.nlm.nih.gov/PMC1393154/)].
15. Park S, Kelly R, Kahn JN, Robles J, Hsu MJ, Register E, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical Candida sp. isolates. *Antimicrob Agents Chemother*. 2005;**49**(8):3264–73. doi: [10.1128/AAC.49.8.3264-3273.2005](https://doi.org/10.1128/AAC.49.8.3264-3273.2005). [PubMed: [16048935](https://pubmed.ncbi.nlm.nih.gov/16048935/)]. [PubMed Central: [PMC1196231](https://pubmed.ncbi.nlm.nih.gov/PMC1196231/)].
16. Castanheira M, Messer SA, Rhomberg PR, Pfaller MA. Antifungal susceptibility patterns of a global collection of fungal isolates: Results of the SENTRY Antifungal Surveillance Program (2013). *Diagn Microbiol Infect Dis*. 2016;**85**(2):200–4. doi: [10.1016/j.diagmicrobio.2016.02.009](https://doi.org/10.1016/j.diagmicrobio.2016.02.009). [PubMed: [27061369](https://pubmed.ncbi.nlm.nih.gov/27061369/)].
17. Yenisehirli G, Bulut N, Yenisehirli A, Bulut Y. In vitro susceptibilities of Candida albicans isolates to antifungal agents in Tokat, Turkey. *Jundishapur J Microbiol*. 2015;**8**(9). e28057. doi: [10.5812/jjm.28057](https://doi.org/10.5812/jjm.28057). [PubMed: [26495115](https://pubmed.ncbi.nlm.nih.gov/26495115/)]. [PubMed Central: [PMC4609313](https://pubmed.ncbi.nlm.nih.gov/PMC4609313/)].
18. Zarei Mahmoudabadi A, Zarrin M, Beheshti Fard M. Antifungal susceptibility of Candida species isolated from candiduria. *Jundishapur J Microbiol*. 2012;**6**(1):24–8. doi: [10.5812/jjm.4633](https://doi.org/10.5812/jjm.4633).
19. European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST). *EUCAST definitive document E.DEF 7.3: Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts*. 2018.
20. Ivanova Z, Kantardjiev T, Kouzmanov A, Boyanova L. Antifungal susceptibility of invasive yeast isolates from Bulgarian patients against anidulafungin, voriconazole and fluconazole. *12th National Congress Clin Microbiol Infect*. 2014 Apr 24–26. 2011. p. 73–4.
21. Sanglard D. Emerging threats in antifungal-resistant fungal pathogens. *Front Med (Lausanne)*. 2016;**3**:11. doi: [10.3389/fmed.2016.00011](https://doi.org/10.3389/fmed.2016.00011). [PubMed: [27014694](https://pubmed.ncbi.nlm.nih.gov/27014694/)]. [PubMed Central: [PMC4791369](https://pubmed.ncbi.nlm.nih.gov/PMC4791369/)].
22. Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, Viscoli C. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. *PLoS One*. 2011;**6**(9). e24198. doi: [10.1371/journal.pone.0024198](https://doi.org/10.1371/journal.pone.0024198). [PubMed: [21935385](https://pubmed.ncbi.nlm.nih.gov/21935385/)]. [PubMed Central: [PMC3174155](https://pubmed.ncbi.nlm.nih.gov/PMC3174155/)].
23. Castanheira M, Deshpande LM, Davis AP, Rhomberg PR, Pfaller MA. Monitoring antifungal resistance in a global collection of invasive yeasts and molds: Application of CLSI epidemiological cutoff values and whole-genome sequencing analysis for detection of azole resistance in Candida albicans. *Antimicrob Agents Chemother*. 2017;**61**(10). doi: [10.1128/AAC.00906-17](https://doi.org/10.1128/AAC.00906-17). [PubMed: [28784671](https://pubmed.ncbi.nlm.nih.gov/28784671/)]. [PubMed Central: [PMC5610521](https://pubmed.ncbi.nlm.nih.gov/PMC5610521/)].
24. Fleck R, Dietz A, Hof H. In vitro susceptibility of Candida species to five antifungal agents in a German university hospital assessed by the reference broth microdilution method and Etest. *J Antimicrob Chemother*. 2007;**59**(4):767–71. doi: [10.1093/jac/dkl555](https://doi.org/10.1093/jac/dkl555). [PubMed: [17293369](https://pubmed.ncbi.nlm.nih.gov/17293369/)].
25. Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, et al. Use of fluconazole as a surrogate marker to predict susceptibility and resistance to voriconazole among 13,338 clinical isolates of Candida spp. tested by clinical and laboratory standards institute-recommended broth microdilution methods. *J Clin Microbiol*. 2007;**45**(1):70–5. doi: [10.1128/JCM.01551-06](https://doi.org/10.1128/JCM.01551-06). [PubMed: [17079501](https://pubmed.ncbi.nlm.nih.gov/17079501/)]. [PubMed Central: [PMC1828974](https://pubmed.ncbi.nlm.nih.gov/PMC1828974/)].
26. Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against Candida spp.: results from a global antifungal surveillance program. *J Clin Microbiol*. 2008;**46**(2):551–9. doi: [10.1128/JCM.01952-07](https://doi.org/10.1128/JCM.01952-07). [PubMed: [18094129](https://pubmed.ncbi.nlm.nih.gov/18094129/)]. [PubMed Central: [PMC2238086](https://pubmed.ncbi.nlm.nih.gov/PMC2238086/)].
27. Badiee P, Choopanzadeh M, Moghadam AG, Nasab AH, Jafarian H, Shamsizadeh A, et al. Antifungal susceptibility patterns of colonized Candida species isolates from immunocompromised pediatric patients in five university hospitals. *Iran J Microbiol*. 2017;**9**(6):363–71. [PubMed: [29487735](https://pubmed.ncbi.nlm.nih.gov/29487735/)]. [PubMed Central: [PMC5825937](https://pubmed.ncbi.nlm.nih.gov/PMC5825937/)].
28. Zhang L, Zhou S, Pan A, Li J, Liu B. Surveillance of antifungal susceptibilities in clinical isolates of Candida species at 36 hospitals in China from 2009 to 2013. *Int J Infect Dis*. 2015;**33**:1–4. doi: [10.1016/j.ijid.2014.12.033](https://doi.org/10.1016/j.ijid.2014.12.033). [PubMed: [25541294](https://pubmed.ncbi.nlm.nih.gov/25541294/)].
29. Kaur R, Dhakad MS, Goyal R, Kumar R. Emergence of non-albicans Candida species and antifungal resistance in intensive care unit patients. *Asia Pacific J Trop Biomed*. 2016;**6**(5):455–60. doi: [10.1016/j.apjtb.2015.12.019](https://doi.org/10.1016/j.apjtb.2015.12.019).
30. Yuksekkaya S, Findik D, Arslan U. [Molecular epidemiology and antifungal susceptibility of Candida species isolated from urine sam-

- ples of patients in intensive care unit]. *Mikrobiyol Bul.* 2011;**45**(1):137-49. Turkish. [PubMed: [21341168](#)].
31. Fothergill AW, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in *Candida* species. *J Clin Microbiol.* 2014;**52**(3):994-7. doi: [10.1128/JCM.03044-13](#). [PubMed: [24403302](#)]. [PubMed Central: [PMC3957792](#)].
 32. Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother.* 2006;**50**(6):2009-15. doi: [10.1128/AAC.00163-06](#). [PubMed: [16723559](#)]. [PubMed Central: [PMC1479149](#)].
 33. White TC, Holleman S, Dy F, Mirels LF, Stevens DA. Resistance mechanisms in clinical isolates of *Candida albicans*. *Antimicrob Agents Chemother.* 2002;**46**(6):1704-13. doi: [10.1128/aac.46.6.1704-1713.2002](#). [PubMed: [12019079](#)]. [PubMed Central: [PMC127245](#)].
 34. Cartledge JD, Midgley J, Gazzard BG. Clinically significant azole cross-resistance in *Candida* isolates from HIV-positive patients with oral candidosis. *AIDS.* 1997;**11**(15):1839-44. doi: [10.1097/00002030-199715000-00008](#). [PubMed: [9412702](#)].
 35. Sanglard D, Coste AT. Activity of isavuconazole and other azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. *Antimicrob Agents Chemother.* 2016;**60**(1):229-38. doi: [10.1128/AAC.02157-15](#). [PubMed: [26482310](#)]. [PubMed Central: [PMC4704203](#)].
 36. Perlin DS. Echinocandin resistance in *Candida*. *Clin Infect Dis.* 2015;**61** Suppl 6:S612-7. doi: [10.1093/cid/civ791](#). [PubMed: [26567278](#)]. [PubMed Central: [PMC4643482](#)].
 37. Castanheira M, Woosley LN, Diekema DJ, Messer SA, Jones RN, Pfaller MA. Low prevalence of *fks1* hot spot 1 mutations in a worldwide collection of *Candida* strains. *Antimicrob Agents Chemother.* 2010;**54**(6):2655-9. doi: [10.1128/AAC.01711-09](#). [PubMed: [20368396](#)]. [PubMed Central: [PMC2876398](#)].
 38. Grossman NT, Chiller TM, Lockhart SR. Epidemiology of echinocandin resistance in *Candida*. *Curr Fungal Infect Rep.* 2014;**8**(4):243-8. doi: [10.1007/s12281-014-0209-7](#). [PubMed: [29780439](#)]. [PubMed Central: [PMC5956517](#)].
 39. Shokohi T, Badali H, Amirrajab N, Ataollahi MR, Kouhpayeh SA, Afsharian MH. In vitro activity of five antifungal agents against *Candida albicans* isolates, Sari, Iran. *Curr Med Mycol.* 2016;**2**(2):34-9. doi: [10.18869/acadpub.cmm.2.2.8](#). [PubMed: [28681018](#)]. [PubMed Central: [PMC5490303](#)].
 40. Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol.* 2011;**6**(4):441-57. doi: [10.2217/fmb.11.19](#). [PubMed: [21526945](#)]. [PubMed Central: [PMC3913534](#)].
 41. Faria-Ramos I, Neves-Maia J, Ricardo E, Santos-Antunes J, Silva AT, Costa-de-Oliveira S, et al. Species distribution and in vitro antifungal susceptibility profiles of yeast isolates from invasive infections during a Portuguese multicenter survey. *Eur J Clin Microbiol Infect Dis.* 2014;**33**(12):2241-7. doi: [10.1007/s10096-014-2194-8](#). [PubMed: [25012821](#)].
 42. Kooshki P, Rezaei-Matehkolaei A, Mahmoudabadi AZ. The patterns of colonization and antifungal susceptibility of *Candida*, isolated from preterm neonates in Khorramabad, South West of Iran. *J Mycol Med.* 2018;**28**(2):340-4. doi: [10.1016/j.mycmed.2018.02.010](#). [PubMed: [29530715](#)].