eISSN: 2645-7687. Journal of Clinical Care and Skills. 2021;2(4):195-199.

in vitro Activity of Seven Antifungals against Different Clinical Candida Species

Journal of Clinical Care and Skills

ARTICLE INFO

Article Type
Original Research

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How to cite this article
Gharaghani M, Rezaei-Matehkolaei R, Shokoohi Gh, Taghavi J, Nouripour-Sisakht S. *in vitro* Activity of Seven Antifungals against Different Clinical Candida Species. Journal of Clinical Care and Skills. 2021;2(4): 195-199.

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Article History
Received: November 24, 2021
Accepted: December 25, 2021
ePublished: January 19, 2022

ABSTRACT

Aims This study aimed to determine the antifungal susceptibility profile of *Candida* spp. with different sources.

Methods This descriptive study was conducted in the Department of Medical Mycology of Yasuj, Iran, from 2018 to 2019. Seventy-six Candida isolates recovered from different samples were used for antifungal profiles. *Candida* spp. isolates were identified at the species level based on molecular methods. The antifungal susceptibility tests with fluconazole, voriconazole, itraconazole, clotrimazole, amphotericin B, nystatin, and caspofungin were assessed according to the Clinical and Laboratory Standards Institute broth microdilution method.

Findings Our study showed that all isolates species were sensitive to antifungal drugs except for 3 (3.9%) isolates resistant to fluconazole. Among the *In vitro* activity of triazoles against all isolates tested, voriconazole had the lowest minimum inhibitory concentration ranges of 0.5-0.015µg/ml. According to the minimum inhibitory concentration of 50%, amphotericin B (0.25µg/ml) and nystatin (0.0625µg/ml) was the most active polyenes against other Candida species.

Conclusion Generally, the clinical correlation between the minimum inhibitory concentration value of different antifungals and different *Candida* spp. isolates are necessary because the resistance profile of Candida spp. is varied and dependent on the different variables.

Keywords Antifungal Agents; Candida; Candidiasis; *in vitro*

CITATION LINKS

[1] Candida and candidiasis [2] An approach to etiology, diagnosis and management of different types of candidiasis [3] Invasive candidiasis [4] Genotypic diversity and antifungal susceptibility pattern of Candida albicans species isolated from hospitalized paediatric patients with urinary tract infection in Iran [5] Molecular characterization and antifungal susceptibility of Candida species isolated from vulvovaginitis in Jahrom city, south of Iran [6] Non-albicans Candida infection: An emerging threat [7] Luliconazole for the treatment of fungal infections: An evidence-based review [8] Luliconazole, a highly effective imidazole, against Fusarium species complexes [9] Resistance to antifungal agents: Mechanisms and clinical impact [10] Reduced fluconazole susceptibility of Candida albicans isolates in women with recurrent vulvovaginal candidiasis: Effects of long-term fluconazole therapy [11] Emergence of resistance of Candida albicans to clotrimazole in human immunodeficiency virus-infected children: In vitro and clinical correlations [12] Antifungal susceptibility profile of Candida albicans isolated from vulvovaginal candidiasis in Xinjiang province of China [13] Identification of Candida species isolated from vulvovaginal candidiasis patients by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in Yasuj southwestern Iran [14] Clinical and microbial epidemiology of otomycosis in the city of Yasuj, southwest Iran, revealing Aspergillus tubingensis as the dominant causative agent [15] Epidemiological Cutoff Values for Antifungal Susceptibility Testing [16] M27: Reference method for broth dilution antifungal susceptibility testing of yeasts [17] Antifungal susceptibility of 1000 Candida bloodstream isolates to 5 antifungal drugs: Results of a multicenter study conducted in Sao Paulo, Brazil, 1995-2003 [18] Antifungal susceptibility testing of Candida isolates from the Candida surveillance study [19] Species distribution and in vitro antifungal susceptibility of vulvovaginal Candida isolates in China [20] Vulvovaginal candidiasis: Species distribution of Candida and their antifungal susceptibility pattern [21] Antifungal susceptibilities of Candida species causing vulvovaginitis and epidemiology of recurrent cases [22] Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals

Introduction

Candidiasis includes the different forms of opportunistic yeast infection. Clinical symptoms of this infection can be varied and dependent on the site of infection [1]. Candida infections can occur in the superficial, mucocutaneous, and systemic forms such as otomycosis, vaginitis, and candiduria. This infection can lead to a life-threatening disease, especially in immunocompromised patients [2]. An untreated Candida infection can affect other organs and lead to a systemic infection. The long-term prognosis with systemic candidiasis depends on the severity and location of the Candida infection, the general health of the infected person, and the timing of diagnosis and treatment [3].

Different species of the Candida genus are introduced as the causative agents of candidiasis. Among these species, *Candida albicans* accounted for the main causative agent of this infection [4, 5]. However, the prevalence of non-albicans species in recent years increased (including *C. glabrata*, *C. krusei*, *C. tropicalis*, and C. *parapsilosis*) [6-8].

There are different classes of antifungal drugs for the treatment of candidiasis; drug resistance has been a problem in recent years [9]. The broad-spectrum use of antifungal drugs, prophylaxis in immunocompromised and hospitalized patients has increased resistance to antifungal drugs. For instance, reduced fluconazole (FLO) susceptibility has been reported in a patient with recurrent vulvovaginitis who received long-term fluconazole therapy [10]. Besides, in another study, resistance to clotrimazole (CLO) was reported among *C. albicans* isolates [11]. Reduced susceptibility of non-albicans *Candida* spp. to antifungal agents has been reported [10]. Hence, to find a way to prevent and control such infections, it is important to select as early as possible the antifungal treatment of choice and understand the resistance profile of causative agents to various antifungals [12].

Given the reduction and the possible differences in drug susceptibility in *Candida* spp. isolates, this study aimed to perform antifungal susceptibility profiles of *Candida* spp. isolated from different sources.

Material and Methods

This descriptive study was conducted in the Department of Medical Mycology of Yasuj, Iran, from 2018 to 2019.

Yeast isolation and identification: *Candida* spp. were collected and identified based on the PCR-RFLP method during the previous study [13, 14]. These species with clinical origin were isolated in different forms of candidiasis included vaginitis (n=55), candiduria (n=12), and otomycosis (n=9). All *Candida* spp. isolates were stored in distilled water at 4°C. For the preparation of isolates, all Candida species isolates were subcultured on Sabouraud Dextrose Agar (Merck, Germany) plates and incubated at 37°C.

Antifungal assay: Seventy-six *Candida* spp. were tested for antifungal susceptibility against amphotericin B (AMB), nystatin (NYS), caspofungin (CAS), fluconazole, voriconazole (VRC), itraconazole (ITR), and clotrimazole (Sigma, St. Louis, MO, USA). For this purpose, all *Candida* spp. isolates included 37 *Candida albicans*, 18 *C. glabrata*, 11 *C. parapsilosis*, 7 *C. krusei*, 1 *C. tropicalis*, 1 C. *kefyr*, and 1 *C. femata* were cultured in Sabouraud Dextrose Agar, incubated at 37°C for 24h. Then, all fresh colonies were dissolved in distilled water and adjusted to 0.5 McFarland according to the Clinical and Laboratory Standards Institute M27-S4 document. The standard suspension of yeast cells was diluted 1:1000 in RPMI 1640. Stock solutions of all drugs were prepared in dimethyl sulfoxide solution (DMSO, Fluka, Germany). All stock solutions were preserved at -4°C until used. The serial dilution from 0.031 to 16µg/ml for amphotericin B, nystatin and clotrimazole, 0.015 to 8µg/ml for caspofungin, itraconazole, and voriconazole 0.062 to 32µg/ml for fluconazole was prepared and dissolved in RPMI 1640 medium. Spectrophotometrically, adjusting homogeneous suspensions was done at the wavelength of 530nm to optical densities of 75-77% transmission. In the next step, 100µL of yeast suspension with 100µL of each antifungal serial dilution was added to 96-microtiter plates. All microplates were incubated at 35°C for 24-48h. Finally, minimum inhibitory concentration (MIC), MIC Geometric (MIC GM), and the MIC that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were determined for each antifungal. For interpreting MIC values used Clinical and Laboratory Standards Institute breakpoints and epidemiological cut-off value (ECV) [15, 16]. *Candida parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used as quality control strains.

Findings

The antifungal susceptibility profiles of seven antifungals are shown in Table 1. As shown, the lowest range of MIC values was detected to voriconazole (0.015-0.5µg/ml), whereas the highest range was attributed to fluconazole (0.031-16µg/ml). Also, 3.9% of *Candida* spp. were resistant to fluconazole. Among non-albicans species, *C. glabrata* had the highest MIC range (0.0625-16µg/ml) for fluconazole. 100% of *Candida* spp. isolates had the MIC values lower than the defined ECV for voriconazole, itraconazole, and amphotericin B. There are no defined breakpoints or ECV for nystatin and clotrimazole for *Candida spp.*; the MIC ranges for these antifungals were relatively low. The 90% of *Candida* spp. isolates were inhibited at 0.5µg/ml of clotrimazole and nystatin.

Table 1) Geometric mean of MIC, MIC ranges, MIC_{50}, and MIC_{90} values obtained by testing the susceptibility of 76 *Candida* spp. isolates obtained from vulvovaginal candidiasis, urine culture, and otomycosis patients against seven antifungal agents

Origin	Antifungals	MIC Distribution (µg/ml)											MIC GM (µg/ml)	Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	R (%)	ECV%
		16	8	4	2	1	0.5	0.25	0.12	0.062	0.031	0.015						
***Candida albicans* (n:37)**																		
Vaginitis	FLO			1	4	2	6	6	3	15			0.7	0.062-4	0.125	2		
	CLO				1	4	3	1	3	18	7		0.59	0.031-2	0.062	1		
	ITR					2	6	7	12	1		9	0.6	0.015-1	0.125	0.5		
	VRC						5	6	5	2	7	12	0.5	0.015-0.5	0.032	0.5		
	CAS						15	8	5	1	1	7	0.6	0.015-0.5	0.25	0.5		
	AMB					4	4	15	4	1	9		0.6	0.031-1	0.5	0.7		100
	NYS				5	1	5		6	14	6			0.031-2				
***Candida glabrata* (n:18)**																		
Vaginitis	FLO	2		2	5	3	1	2		1	2		2	0.0625-16	1	4	2 (11.1)	
	CLO					1	1	1		4	5	6	0.13	0.031-2	0.0625	0.65		
	ITR					2	2	3	1	1	3	6	0.14	0.031-2	0.18	1.3		100
	VRC							8	1	4		5	0.14	0.031-.5	0.18	0.5		100
	CAS							2	8	3		5	0.11	0.031-.5	0.25	0.32		
	AMB				1	2	4	4		2	2	3	0.5	0.031-4	0.5	2		100
	NYS									6	8	4		0.031-0.125				
***Candida parapsilosiss* (n:11)**																		
Otomycosis	FLO							1	1	9			0.25	0.0625-0.25	0.03	0.125		
	CLO				1	1		1	1	7			0.13	0.0625-2	0.625	1		
	ITR					2	5	1		3			0.2	0.0625- 1	0.5	1		
	VRC						1			10			0.02	0.0625-0.5	0.015	0.062		
	CAS					1		6	1	3			0.18	**0.0625-1**	0.25	0.25		
	AMB				3	1	3	1		3			0.3	0.0625-2	0.5	2		100
	NYS						2			9				0.0625-0.5				
***Candida kruzei* (n: 7)**																		
Vaginitis	FLO		1			1	4				1			**0.031-8**			1 (14.28)	
	CLO							1		1		5		**0.015-0.25**				
	ITR						2	1		2	2			0.031-0.5				100
	VRC									1	1	5		**0.015-0.062**				
	CAS						1			1	1	4		**0.015-0.5**				
	AMB						1	1		1	4			0.031-0.5				100
	NYS									2	5			0.031-0.062				
***Candida trpoicalis* (n:1)**																		
Vaginitis	FLO				1													
	CLO									1								
	ITR							1										
	VRC									1								
	CAS									1								
	AMB							1										
	NYS							1										
***Candida famata* (n:1)**																		
Urine culture	FLO						1											
	CLO									1								
	ITR									1								
	VRC									1								
	CAS									1								
	AMB									1								
	NYS								1									
***Candida kefyr* (n:1)**																		
Vaginitis	FLO							1										
	CLO							1										
	ITR					1												
	VRC									1								
	CAS									1								
	AMB				1													
	NYS									1								

Discussion

Candidiasis is one of the most opportunistic fungal infections, especially among immunocompromised patients. The increased incidence of drug resistance, especially in hospitalized patients, causes an important public issue for opportunistic infection management [9]. We investigated the *in vitro* activity of 76 *Candida* spp. isolates obtained from different sources. In the present study, voriconazole had the lowest MIC ranges among *Candida* spp.. Generally, the resistance of *Candida* spp. to voriconazole has been varied in different studies. For instance, this value was 0.2% reported by Da Matta *et al.* up to 1.1% in Lyon *et al.,* studies [17, 18]. However, similar to our finding, Gharaghani *et al.* reported that 100% of urinary *C. albicans* isolates were sensitive to voriconazole [4].

Fluconazole is the main treatment choice in different forms of candidiasis, especially in vaginitis. In the present study, 11.1% of *C. glabrata* isolated from two patients with vaginitis were resistant to fluconazole. Wang *et al.,* reported that 73.3% of vulvovaginal *C. glabrata* isolates were resistant to fluconazole [19]. However, this rate was 8.3% in Yan *et al.,* study [12]. It

seems that the history of fluconazole therapy, the origin of isolates, and the sample are also involved in these differences. Our results indicated that triazole drugs showed well *in vitro* activity against all *C. albicans* isolates. So that, 90% of isolates were inhibited by within 2µg/ml of fluconazole and 0.5µg/ml of voriconazole and itraconazole. This data was in agreement with other studies [4, 20].

Polyene drugs, especially amphotericin B frequently used in systemic forms of candidiasis. In our study, 100% of *C. glabrata* isolates were sensitive to amphotericin B with MIC range 2-0.015µg/ml. Also, nystatin had a low MIC_{50} value (0.0625µg/ml) among *C. glabrata* species. These results confirmed that the polyenes have broad-spectrum activity against pathogenic yeasts. However, Richter *et al.* reported the high MIC ranges (2-4µg/ml) for nystatin against *C. glabrata* isolates [21]. This range was 2- 16µg/ml for nystatin against oral *C. glabrata* isolates in Blignaut *et al.'s* study [22]. Although the source of the isolates of these studies is the same as ours, the difference may be due to the history of use of nystatin in patients.

Conclusion

The resistance profile of *Candida* spp. isolates are varied and dependent on the different variables. As demonstrated, resistance to fluconazole, the most frequent antifungals used in different forms of candidiasis, could suggest the need for regular investigations into antifungal resistance in medical centers to manage more efficiently candidiasis.

Acknowledgments: The authors would like to thank the University Grant Commission.

Ethical Permissions: This study was approved by the ethics committee of Yasuj University of Medical Sciences (IR.YUMS.REC.1397.110).

Conflicts of Interests: This study is part of the M.D thesis conducted in the department of medical mycology of Yasuj, Iran.

Authors' Contribution: Gharaghani M (First Author), Main researcher/Introduction writer/Statistical Analyst (25%); Rezaei Matehkolaei A (Second Author), Assistant researcher/Discussion writer/Statistical analyst (20%), Shokoohi Gh (Third Author), Assistant Researcher/Methodologist/Discussion writer (15%); Taghavi J (Fourth Author), Assistant Researcher/Introduction Writer (15%); Nouripour-Sisakht S (Fifth Author), Main Researcher (25%)

Funding/Support: This study was supported financially by the Deputy of Research of Yasuj University of Medical Sciences (Grant No: 1397.10).

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