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Evaluation of the Sensititre Yeast One microdilution method for susceptibility testing of *Candida* species to anidulafungin, caspofungin, and micafungin

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ABSTRACT

Introduction. Echinocandins represent a new antifungal group with potent activity against *Candida* species. The purpose of our study was to evaluate the utility of the Sensititre Yeast One method to determine the in vitro activity of anidulafungin, micafungin, and caspofungin against *Candida* species isolated from clinical specimens.

Methods. A total of 131 *Candida* strains were tested using Sensititre Yeast One colorimetric microdilution method. They belonged to the following species: 42 *C. albicans*, 36 *C. glabrata*, 21 *C. parapsilosis*, 12 *C. tropicalis*, 10 *C. krusei*, 3 *C. guilliermondii*, 2 *C. famata*, 3 *C. kefyr*, 1 *C. lusitaniae*, 1 *C. zeylanoides*, and 1 *C. lipolytica*. For being considered susceptible the strains had to be inhibited by concentrations ≤2 mg/L of anidulafungin, caspofungin or micafungin.

Results. The 80.1% of the strains tested were inhibited by concentrations ≤0.25 mg/L of anidulafungin and micafungin. The activity of caspofungin was slightly lower (78.6% of strains inhibited by concentrations ≤0.25 mg/L). The 96.9% of strains turned out susceptible to concentrations ≤2 mg/L against the three echinocandins. Two strains of *C. parapsilosis* (9.5%), one of *C. guilliermondii*, and two of *C.* famata showed non-susceptible to one or more echinocandins.

Conclusions. In our series, anidulafungin, micafungin, and caspofungin were effective against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. lusitaniae* and *C. lipolytica*. The 96.9% of strains were susceptible to all three echinocandins. Thus, echinocandins are proved to exhibit excellent activity to the *Candida* species most frequently involved in human infections, except *Candida parapsilosis*.

Key words: anidulafungin, micafungin, caspofungin, *Candida*, yeasts.

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Evaluación del método de microdilución Sensititre Yeast One para determinar la sensibilidad de especies de *Candida* frente a anidulafungina, caspofungina y micafungina

RESUMEN

Introducción. Las equinocandinas representan un nuevo grupo de antifúngicos con gran actividad frente a especies de *Candida*. El propósito de este estudio fue evaluar el método Sensititre Yeast One para determinar la actividad *in vitro* de anidulafungina, micafungina y caspofungina frente a especies de *Candida* aisladas de muestras clínicas.

Métodos. Un total de 131 cepas de *Candida* identificadas como: 42 *C. albicans*, 36 *C. glabrata*, 21 *C. parapsilosis*, 12 *C. tropicalis*, 10 *C. krusei*, 3 *C. guilliermondii*, 2 *C. famata*, 3 *C. kefyr*, 1 *C. lusitaniae*, 1 *C. zeylanoides* y 1 *C. lipolytica*, fueron ensayadas mediante el método colorimétrico de microdilución Sensititre Yeast One. Se consideraron sensibles las cepas inhibidas por concentraciones ≤2 mg/L de anidulafungina, caspofungina o micafungina.

Resultados. El 80,1% de las cepas fueron inhibidas por concentraciones ≤0,25 mg/L de anidulafungina y micafungina. La actividad de caspofungina fue ligeramente inferior (78,6% de las cepas inhibidas por concentraciones ≤0,25 mg/L). El 96,9% de las cepas resultaron sensibles frente a las tres equinocandinas. Dos cepas de *C. parapsilosis* (9,5%), 1 de *C. guilliermondii* y 2 de *C. famata* no mostraron sensibilidad a una o más equinocandinas.

Conclusiones. En nuestra serie, anidulafungina, micafungina y caspofungina fueron efectivas frente a *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. lusitaniae* y *C. lipolytica*. El 96,9% de las cepas fueron sensibles a las tres equinocandinas. Se puede afirmar que las equinocandinas tienen una excelente actividad frente a las especies de *Candida* más frecuentes en infecciones humanas, excepto *Candida parapsilosis*.

Palabras clave: anidulafungina, micafungina, caspofungina, *Candida*, levaduras.

INTRODUCTION

Yeasts are a common cause of infection in humans. Echinocandins represent a new antifungal group with potent activity against *Candida* species. They are semi-synthetic, high-molecular lipoproteins, with variations in their N-linked acyl lipid side chain. Selective antifungal activity of echinocandins is due to the inhibition of the beta-1,3-D-glucan synthase enzyme, an essential component for the formation of the fungal cell wall. The spectrum activity of echinocandins includes fungicide action against *Candida* and fungistatic against *Aspergillus*, the two main etiological agents causing invasive fungal infections[1-4]. However, echinocandins are not active against *Cryptococcus*, *Trichosporon*, *Fusarium*, *Zygomycetes* and other filamentous fungi[5,6].

Resistance to echinocandins is uncommon and has little clinical relevance. Acquired resistance is associated with mutations in two "hotspot" regions of Fks1 or Fks2, the probable beta-1,3-D-glucan synthase[7]. All three available echinocandins (anidulafungin, micafungin, and caspofungin), provide excellent clinical efficacy besides low toxicity for the treatment of serious infections by *Candida* species[8-10].

Up to now, there are few studies about the in vitro activity of echinocandins in yeasts[1,3,11-24].There exist two reference methods to analyse the susceptibility of yeasts to echinocandins: broth microdilution method M27-A3, designed by the Clinical Laboratory Standards Institute (CLSI), and the one proposed by the European Committee for Antimicrobial Susceptibility Testing (EUCAST)[25-27]. Both techniques produce comparable results. Among the commercially available methods, only the E-test and Sensititre Yeast One colorimetric broth microdilution are able to determine the susceptibility to echinocandins[16,21,23,24]. This last one has demonstrated an excellent correlation with the reference method from the CLSI.

The purpose of the present study was to assess the performance of the Sensititre Yeast One colorimetric microdilution method to determine the in vitro activity of anidulafungin, micafungin and caspofungin against clinical isolates of *Candida* species.

MATERIALS AND METHODS

We tested a total of 131 strains of *Candida*, belonging to the following species: 42 *C. albicans*, 36 *C. glabrata*, 21 *C. parapsilosis*, 12 *C. tropicalis*, 10 *C. krusei*, 3 *C. guilliermondii*, 2 *C. famata*, 3 *C. kefyr*, 1 *C. lusitaniae*, 1 *C. zeylanoides*, and 1 *C. lipolytica*. The strains were identified according to the assimilation profile of carbon compounds by the commercial system ID 32C (bioMérieux, France).

The Sensititre Yeast One panels (Trek Diagnostic Systems, UK) contained serial twofold dilutions of anidulafungin (0.015 to 16 mg/L), micafungin (0.008 to 16 mg/L), and caspofungin (0.008 to 16 mg/L). On the day of the test, working yeast suspensions were prepared to a final turbidity of 0.5 McFarland standards. Twenty microliters of each suspension was then diluted with 11 ml of Yeast One inoculum broth. The dry Sensititre Yeast One panels were rehydrated with the working yeast suspension using an appropriate multichannel pipetting device to dispense 100 µl into each well. Panels were then incubated at 35°C for 24 to 48 h. Quality control was ensured by testing the CLSI-recommended strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258. Minimum inhibitory concentrations (MICs) endpoints were read after 24 h of incubation. Yeast One endpoints were determined to be the lowest concentration at which the colour in a well changed from red (positive, indicating growth) to blue (negative, indicating no growth)[13]. All strains inhibited by concentrations ≤2 mg/L of anidulafungin, caspofungin or micafungin were considered susceptible, and those inhibited by concentrations >2 mg/L not susceptible. We determined the MICs for both growth inhibition of 50% and 90% of the strains, for all strains, at the recommended endpoints and time intervals.

RESULTS

Table 1 summarizes the in vitro activity of anidulafungin, micafungin and caspofungin against 131 strains of *Candida* species tested with the Sensititre Yeast One broth microdilution method. Table 2 shows the cumulative percentages of susceptibility.

Anidulafungin, micafungin and caspofungin proved to have good activity against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. lusitaniae* and *C. lipolytica*. The 80.1% of all strains tested were inhibited by concentrations ≤0.25 mg/L of anidulafungin and micafungin. The activity of caspofungin was slightly lower (78.6% of strains inhibited by concentrations ≤0.25 mg/L). A total of 127 strains (96.9%) were susceptible to ≤2 mg/L against all three echinocandins. Minimum inhibitory concentrations (MICs) were higher for *C. parapsilosis*: 47.6% strains with MIC ≥2 mg/L to anidulafungin and micafungin, 14.3% with the same MIC to caspofungin. One strain of *C. guilliermondii* also throwed up the same MIC against the three echinocandins, as well as one strain of *C. famata* against micafungin and caspofungin, and that of *C. zeylanoides* against caspofungin. Two strains of *C. parapsilosis* (9.5%), one of *C. guilliermondii*, and two of *C. famata* proved to be non-susceptible to one or more echinocandins.

DISCUSSION

Antifungal susceptibility testing results of clinically significant *Candida* species are of interest for empiric and prophylactic therapies. The Sensititre Yeast One colorimetric broth microdilution method appears to be comparable to the CLSI reference method for testing the susceptibility of *Candida* spp. to the echinocandins anidulafungin, caspofungin, and micafungin[16,21].

Table 1 **In vitro susceptibily of 131 strains of *Candida* species to anidulafungin, micafungin, and caspofungin (Sensititre Yeast One method)**

Candida species	Number of isolates	Antifungal agents	MIC (mg/L) Range	50%	90%
C. albicans	42	Anidulafungin	≤0.015 - 1	0.03	0.125
		Micafungin	≤0.015 - 0.5	≤0.015	0.03
		Caspofungin	0.03 - 0.25	0.06	0.125
C. glabrata	36	Anidulafungin	≤0.015 - 0.125	0.03	0.125
		Micafungin	≤0.015 - 0.25	≤0.015	0.03
		Caspofungin	≤0.015 - 0.25	0.06	0.125
C.parapsilosis	21	Anidulafungin	0.25 - 4	1	2
		Micafungin	0.06 - 4	1	2
		Caspofungin	0.125 - 2	1	2
C. tropicalis	12	Anidulafungin	≤0.015 - 0.25	0.03	0.06
		Micafungin	≤0.015 - 0.03	0.03	0.03
		Caspofungin	0.03 - 0.5	0.03	0.03
C. krusei	10	Anidulafungin	0.03 - 0.06	0.06	0.06
		Micafungin	0.25 - 0.5	0.25	0.25
		Caspofungin	0.25 - 0.5	0.25	0.5
Candida spp.*	10	Anidulafungin	0.015 - 4	0.25	1
		Micafungin	0.03 - 4	0.25	2
		Caspofungin	0.03 - ≥8	1	4

* *C. guilliermondii* (3), *C. famata* (2), *C. kefyr* (2), *C. lusitaniae* (1), *C. zeilanoydes* (1), *C. lipolytica* (1).

Our data confirm the antifungal susceptibility patterns among *Candida* species, and highlight the need to perform antifungal susceptibility testing in clinically relevant species. While anidulafungin MICs are similar to those of micafungin, caspofungin MICs are higher[11,19,24,28]. Our results are comparable with those obtained by other studies. Between 95 and 99% of clinical isolates of *Candida* are susceptible[1-3,11,14,15,19,20,22,24]. In our series, 96.9% of the strains were susceptible to all three echinocandins tested.

As proposed by some authors, we classified *Candida* species into two groups in terms of their in vitro susceptibility to echinocandins. There was a group of very susceptible species (MIC_{90} between 0.015 and 0.25 mg/L) such as *C. albicans, C. dubliniensis, C. glabrata, C. tropicalis, C. krusei* and *C. kefyr*, as well as another of less susceptible species (MIC_{90} between 0.25 and 2 mg/L) like *C. parapsilosis, C. guilliermondii* and *C. famata*[11,15,19]. The highest MICs were for *Candida parapsilosis*[1,22,24,28]. The different susceptibility of *C. parapsilosis* strains could be due to the existence of two recently described species included in this one, *C. orthopsilosis* and *C. metapsilosis*, which produce more susceptibility to echinocandins[24,29].

We can affirm that echinocandins exhibit excellent activity against the *Candida* species most frequently involved in human infections, except for *Candida parapsilosis*. About 80% of the predominant species implicated in human infections are inhibited by concentrations ≤0.25 mg/L. Echinocandins are also active against occasionally pathogenic *Candida* species with lower susceptibility to antifungal agents, and species usually resistant to azoles[4,11,12,17-19]. Likewise, echinocandins constitute a good therapeutic option, particularly in candidemia and invasive candidosis in non-neutropenic patients.

Table 2 **Cumulative percentages of susceptibility to anidulafungin, micafungin, and caspofungin of 131 *Candida* strains (Sensititre Yeast One method).**

Candida species	Antifungal agent	Cumulative percentages of susceptibility (MIC mg/L)									
		≤0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	≥8
C. albicans (42)	Anidulafungin	42.9	69.0	76.2	97.6	-	-	100			
	Micafungin	66.7	95.2	97.6	-	-	100				
	Caspofungin		47.6	78.6	97.6	100					
C. glabrata (36)	Anidulafungin	33.3	63.9	83.3	100						
	Micafungin	66.1	91.7	97.2	-	100					
	Caspofungin	2.8	16.7	75.0	97.2	100					
C. parapsilosis (21)	Anidulafungin				4.8	-	-	52.4	90.5	100	
	Micafungin			4.8	-	-	19.0	52.4	95.2	100	
	Caspofungin				4.8	9.5	42.6	85.7	100		
C. tropicalis (12)	Anidulafungin	41.7	83.3	91.7	-	100					
	Micafungin	25.0	100								
	Caspofungin		91.7	-	-	-	100				
C. krusei (10)	Anidulafungin		30.0	100							
	Micafungin				30.0	100					
	Caspofungin					80.0	100				
Candida spp.* (10)	Anidulafungin	10.0	-	-	40.0	50.0	-	90.0	-	100	
	Micafungin		10.0	20.0	40.0	50.0	-	70.0	90.0	100	
	Caspofungin		20.0	-	-	40.0	-	60.0	80.0	90.0	100

* *C. guilliermondii* (3), *C. famata* (2), *C. kefyr* (2), *C. lusitaniae* (1), *C. zeilanoydes* (1), *C. lipolytica* (1).

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