Enferm Infecc Microbiol Clin. 2011;**29(Supl 2)**:3-9

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

In vitro activity of echinocandins against non-*Candida albicans:* Is echinocandin antifungal activity the same?

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Keywords:
Echinocandins
Echinocandins activity non-*Candida albicans*
Echinocandin ECVs for non-*Candida albicans*
Echinocandin CBPs or non-*Candida albicans*

ABSTRACT

The echinocandins anidulafungin, caspofungin, and micafungin have a broad and similar spectrum of in vitro and in vivo activity against most *Candida* spp. Minimal inhibitory concentrations (MICs) for *Candida* spp. are usually below 1 µg/mL for most isolates. The exceptions are *Candida parapsilosis* and *C. guilliermondii*. Species-specific clinical breakpoints (CBPs) and epidemiologic cutoff values (ECVs) have been proposed by the Clinical and Laboratory Standards Institute (CLSI) for the eight most common *Candida* spp. versus each echinocandin; these values are useful to detect in vitro antifungal resistance (CBPs) and to identify isolates harboring *fks* mutations or having reduced susceptibility (ECVs). This paper presents a review of the literature (2006-2010) regarding the in vitro activity similarities or differences among the three echinocandins against *Candida* spp.; different parameters or measurements of in vitro potency were evaluated. The focus of the review is the non-*Candida albicans* species.

Actividad in vitro de las equinocandinas frente a *Candida* no *albicans:* ¿todas las equinocandinas son iguales?

Palabras clave:
Equinocandinas
Equinocandinas y *Candida no albicans*
PCE equinocandinas y *Candida no albicans*
PCC equinocandinas y *Candida no albicans*

RESUMEN

Las 3 candinas, anidulafungina, caspofungina y micafungina, comparten el mismo amplio espectro de acción y actividad, tanto in vitro como in vivo, sobre *Candida* spp. Las concentraciones mínimas inhibitorias (CMI) para la mayoría de los aislados de *Candida* spp. son generalmente inferiores a 1 µg/mL, excepto para *C. parapsilosis* y *C. guilliermondii*. El Clinical and Laboratory Standards Institute (CLSI) ha propuesto recientemente puntos de corte clínicos (PCC) y puntos de corte epidemiológicos (PCE) a cada candina para las 8 especies más comunes de *Candida*. Estos valores son útiles para detectar resistencias (PCC) e identificar aislados con mutaciones en el gen *fks* o con sensibilidad reducida (PCE). En este trabajo se revisa la bibliografía (2006-2010) de la actividad in vitro de las equinocandinas frente a *Candida* no *albicans* analizando diferentes parámetros de actividad in vitro para evaluar las diferencias entre ellas.

Introduction

The incidence and prevalence of invasive fungal infections is a major health problem, especially in the large population of immunocompromised patients and/or those with serious underlying diseases[1,2]. The most common fungal pathogens are the species of *Candida* and *Aspergillus*. The mortality rate associated with invasive candidiasis is substantial, the attributed mortality rate for candidemia is as high as 47% depending on the patient population age[2]. In recent years, three echinocandins, anidulafungin (Pfizer), caspofungin (Merck) and micafungin (Astellas) have been licensed for intravenous treatment and prevention of *Candida* and other infections, especially for patients with recent azole exposure[2-5].

The echinocandins represent an important therapeutic advance, because their mode of action is independent from that of other agents. The three echinocandins have a broad and similar spectrum of in vitro activity against most *Candida* spp. and *Aspergillus* spp.as well as in vitro and in vivo activity against triazole-resistant or less susceptible species such as *C. krusei* and *C. glabrata*[6-17]. Minimal inhibitory concentrations (MICs) for *Candida* spp. or minimal effective

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concentrations (MECs) for *Aspergillus* spp. are usually below 1 µg/mL for most isolates[6,8]. The exceptions are *Candida parapsilosis* and *C. guilliermondii*, but these results do not appear to influence the response to therapy[11-17]. We have reviewed the literature of the last five years (2006-2010) regarding the similarities or differences of the in vitro activity of the three echinocandins against non-*Candida albicans* species to answer the question, is there a difference in their in vitro activity against these less common *Candida* spp.? To answer that query the following in vitro data were compared: *a)* echinocandin MIC results by the Clinical and Laboratory Standards Institute (CLSI) method for azole-susceptible and-resistant *Candida* sp. isolates; *b)* CLSI ECVs and CBPs; *c)* CLSI MIC results for echinocandin-resistant isolates or those harboring mechanisms of resistance; *d)* IC_{50} results; *e)* fungicidal activity by killing curve assays. The focus will be the data for non-*Candida albicans*.

New definitions: ECVs and CBPs

The drug-susceptible wild-type (WT) is the population of isolates that do not exhibit acquired or mutational resistance to the drug being evaluated, in contrast to the drug-resistant non-WT isolates that harbor one or more resistant markers[18]. The highest WT MIC is the ECV. On the other hand, CBPs categorize the isolate as either treatable (susceptible) or non-treatable (resistant). An organism with an MIC above the drug ECV shows reduced susceptibility as compared to the WT population, but it may respond to treatment with the drug being evaluated if this MIC is lower than the CBP[18].

Standard methodology to test echinocandins

Before 2004, echinocandin MICs for *Candida* spp. were obtained using different testing conditions, because the standard methodology at the time was for testing azoles, flucytosine and amphotericin B. Further, the in vitro data available was mostly for caspofungin and anidulafungin versus *C. albicans* and *Aspergillus fumigatus*. For example, according to a 2003 review of the literature, the amount of published caspofungin in vitro data for *C. albican*s was ~ 50 to 75% higher than that for anidulafungin and micafungin, respectively; similar numbers were reported for non-*Candida albicans*[8]. For that reason, head-to-head comparisons of the in vitro activity of the three echinocandins were rarely found in the literature[10]. In addition, different incubation times (24 and 48 h) and different criteria of MIC determination (50 to 100% growth inhibition) were used due to the trailing growth observed with some isolates[19]. Therefore, it was difficult to compare the in vitro activities of these agents. It was not until 2004, based on data from a collaborative study[20], that standard guidelines were identified to test the susceptibilities of *Candida* spp. and *Aspergillus* spp. to caspofungin. These guidelines became the standard parameters for the antifungal susceptibility testing and vitro resistance detection of *Candida* spp. versus the three echinocandins. The revised versions of the CLSI documents published between 2008 and 2009 describe these parameters for broth microdilution (CLSI M27-A3 and M27-S3 documents) and disk diffusion (CLSI M44-A2 and M44-S3 documents) methods[21-24]. The development of standardized methodology for the echinocandins made possible the study of resistance mechanisms and the development of CBPs, and more recently the definition of ECVs for the echinocandins and other antifungal agents[6,25].

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not developed standardized methodology or established CBPs for echinocandins. However, a recent EUCAST study has indicated that it is possible to differentiate between anidulafungin susceptible WT isolates from the *fks* hot spot mutants by using a cutoff endpoint that was two dilutions higher than the MIC_{50}[26]. EUCAST will be proposing anidulafungin CBPs for *Candida* spp. in the near future (Rodríguez-Tudela, personal communication).

Echinocandin MIC results for azole-susceptible and-resistant isolates

To compare the in vitro antifungal activities of the three echinocandins, we selected articles that reported MICs obtained by the CLSI standard guidelines for testing these agents (24 h incubation time and 50 % or more growth inhibition)[6,9,27]. However, for the comparison depicted in Table 1, we have also included the aggregated MIC results from 60 studies that were obtained by CLSI standard parameters for other antifungal agents prior to 2004[8]. In addition to MIC ranges, we have listed two important and recently reported in vitro susceptibility values: modal MICs (most frequent MIC value for each WT MIC distribution) and ECVs[6,25]. As expected, since some early results were obtained using 100% growth inhibition, MIC ranges were wider in the set of aggregated results (left MIC column of Table 1) than in the other sets (right MIC column of Table 1). MICs for *C. parapsilosis* and *C. guilliermondii* were the highest values for the three echinocandins in both sets of data. There are species-dependent variations in MIC results of the three echinocandins, where anidulafungin modal MICs (most frequent endpoints) were higher for four species (*C. glabrata*, *C. guilliermondii*, *C. lusitaniae* and *C. parapsilosis*) and lower for one species (*C. krusei*). On the other hand, micafungin modal MICs were lower for three species (*C. albicans, C. glabrata* and *C. tropicalis*) and caspofungin for one species (*C. kefyr*). However, these differences were within one to two dilutions, which is the expected MIC variability for QC isolates and it is commonly observed in studies designed to identify testing parameters. MIC ranges were also within one to two dilutions for most species, but caspofungin MIC ranges were wider for *C. tropicalis* and *C. dubliniensis*. The echinocandin modal MICs were similar for *C. tropicalis*, but although ECVs are not available for *C. dubliniensis*[27], the caspofungin modal MIC was four dilutions higher than those of the other two agents (Table 1); this difference could indicate their superior in vitro activity. Yet, the overall correlation of MICs of the three echinocandins has been high (comparisons of MICs of anidulafungin and micafungin to those of caspofungin as well as versus one another: R, 0.85, 0.84 and 0.89, respectively, and 92% to 97% agreement)[25].

The question is, is there a clinical advantage for these small differences? The clinical advantage for the better activity (lower MICs) of either micafungin or anidulafungin versus *C. dubliniensis* is yet to be determined. Most clinical data are for *C. albicans* and they are scarce for the other species, especially for *C. dubliniensis*[28]. Even the recent study regarding the efficacy of caspofungin for non-*Candida albicans* did not include patients infected with *C. dubliniensis*[15]. The echinocandin efficacy in the different clinical trials (according to MICs) has been similar and species dependent: 88% to 92% for *C. glabrata*, 71% to 90% for *C. tropicalis* (highest efficacy percentage with anidulafungin), 75% to 88% for *C. parapsilosis* (lowest efficacy percentage with caspofungin) and 50% to 77% for *C. krusei* (lowest efficacy percentage with anidulafungin). There were only 6 to 13 MICs of the latter species in the analysis, the number of MICs for the other species was below 100, and the majority of MICs were susceptible (e.g., $\leq$ 0.25 µg/mL for the most susceptible species and $\leq$ 2 µg/mL for *C. parapsilosis*)[25]. Recently, micafungin breakthrough has been reported when micafungin MICs for some of the isolates were below corresponding ECVs and/or CBPs (WT isolates) for 6 of 7 *C. parapsilosis*, 1 of 6 *C. glabrata*, 1 of 3 *C. tropicalis* and 1 *C. krusei*; no *fks* mutations were observed for these susceptible *C. glabrata* and *C. tropicalis* isolates, and the mutation for the *C. krusei* isolate was outside the "hotspot" region. However, MICs for the other eight isolates could be considered resistant by the newly adjusted CBPs for echinocandins; they harbored gene mutations[28]. All these results underline the influence of both clinical and in vitro resistance in treatment failure.

Table 2 depicts a similar comparison of MIC data as that in table 1, but this time the comparison is for echinocandin MICs obtained for fluconazole-resistant isolates of three *Candida* spp.[9,25]. Micafungin

Table 1
Comparative in vitro activity (MICs) of three echinocandin antifungal agents against isolates of *Candida* species

Species	No. tested	Agent[a]	MIC range[b]	No. tested	MIC range[c]	Modal[d] MIC	ECV[e]
C. albicans	2,394	AND	< 0.015-> 8	4,283	< 0.015-1	0.03	0.12
	4,265	CAS	< 0.015-> 8	4,283	< 0.015-0.5	0.03	0.12
	966	MCF	< 0.015-0.5	4,283	< 0.015-0.5	0.015	0.03
C. dubliniensis	92	AND	0.12-8	20	≤ 0.03	≤ 0.03	NA
	177	CAS	0.015-1	20	0.25-0.5	0.5	NA
	40	MCF	0.06-1	20	≤ 0.03	≤ 0.03	NA
C. famata	11	AND	0.015-> 8	NA	NA	NA	NA
	13	CAS	0.06-> 8	NA	NA	NA	NA
	1	MCF	NA	NA	NA	NA	NA
C. glabrata	993	AND	< 0.015-8	1,236	0.015-4	0.06	0.25
	1,289	CAS	< 0.015-> 8	1,236	0.015-8	0.03	0.12
	1,072	MCF	≤ 0.015-> 8	1,236	0.015-2	0.015	0.03
C. guilliermondii	27	AND	0.06-4	88	0.06-4	2	16
	158	CAS	0.12-> 8	88	0.03-> 8	0.5	4
	24	MCF	0.03-2	88	0.015-> 8	0.5	4
C. kefyr	21	AND	0.03-0.5	61	0.015-0.12	0.06	0.25
	27	CAS	0.06-1	61	< 0.015-0.03	0.015	0.03
	4	MCF	0.06-0.5	61	0.015-0.06	0.06	0.12
C. krusei	207	AND	< 0.015-8	270	0.015-0.5	0.03	0.12
	221	CAS	0.12-> 4	270	0.015-1	0.06	0.25
	82	MCF	0.06-4	270	0.015-0.25	0.06	0.12
C. lusitaniae	81	AND	0.03-8	99	0.06-1	0.5	2
	114	CAS	0.12-4	99	0.03-1	0.25	0.5
	23	MCF	0.03-0.06	99	< 0.01-1	0.12	0.5
C. parapsilosis	231	AND	0.015-> 8	1,238	0.015-4	2	4
	103	CAS	0.03-≥ 8	1,238	0.015-4	0.5	1
	439	MCF	0.03-≥ 8	1,238	0.015-2	0.5	4
C. tropicalis	548	AND	0.03-> 8	996	< 0.015-2	0.03	0.12
	811	CAS	0.015-> 8	996	< 0.015-> 8	0.03	0.12
	364	MCF	< 0.015-> 8	996	< 0.015-1	0.015	0.12

[a]AND: anidulafungin; CAS: caspofungin; MFC: micafungin.
[b]MICs determined in RPMI broth at different incubation times and MIC determination criteria.
[c]MICs determined according to testing guidelines for echinocandins and *Candida* spp.: RPMI broth, 24 h incubation, prominent inhibition endpoint (≥ 50% inhibition)[21,22]; MICs for bloodstream isolates.
[d]Modal MIC, more frequent MIC.
[e]ECV, epidemiological cutoff value.
New *C. dubliniensis* data summarized from reference 27; other data from references 6, 8, and 25.

$MICs_{90}$ were the lowest for these isolates (superior in vitro activity), but again the differences were mostly within one to two dilutions. The micafungin MIC_{90}s for azole-resistant isolates of *C. glabrata* (MIC_{90}s 0.015 μg/mL) suggests that this agent had better in vitro activity than the other two agents, especially when compared to that of anidulafungin (MIC_{90}s 0.12 μg/mL). However, both values are below their respective ECVs or WT endpoints (Tables 1 and 2).

ECVs and CBPs for *Candida* spp. and echinocandins

As for any antimicrobial agent, detection of echinocandin in vitro resistance is an important factor in the treatment of invasive infections for which these agents have been licensed, mostly for *Candida* infections. For in vitro results to be meaningful, CBPs or ECVs should be available and should have been established using data obtained by standardized methods. In 2008, the CLSI established a susceptible CBP (≤ 2 μg/mL) for echinocandins and all *Candida* spp.[21,22]. However, using *fks1* mutant strains, Garcia-Effron et al. demonstrated that although caspofungin MICs > 2 μg/mL captured almost 100% of mutant strains, the MICs that captured 95% of these mutants were lower (> 0.5 μg/mL for *C. albicans* and > 0.25 μg/mL for *C. glabrata*) for both anidulafungin and micafungin[29,30]. Because of that, Pfaller el al. defined WT MIC distributions for a large number of isolates and proposed species-specific ECVs for each echinocandin and eight *Candida* spp.[6]. Most of these species-specific ECVs are ≤ 0.25 μg/mL; the exceptions were the ECVs for *C. guilliermondii* (ECVs, 4-16 μg/mL), *C. lusitaniae* (ECVs, 0.5-2 μg/mL) and *C. parapsilosis* (ECVs, 1-4 μg/mL) (Tables 1 and 3). The lower ECVs indicate that while micafungin MICs of ≤ 0.03 μg/mL for *C. glabrata* encompassed 98% of the isolates, similar percentages of isolates are encompassed by caspofungin MICs of 0.12 μg/mL or anidulafungin MICs of 0.25 μg/mL. Therefore, these different values belong to the WT or susceptible population of each echinocandin or are essentially the same.

Table 2
Comparative in vitro activity (MICs) of three echinocandin antifungal agents against fluconazole-resistant isolates of Candida species[a,b]

		(μg/mL)[c] of MIC_{90}		
Species	**No. tested**	**AND**	**CAS**	**MFC**
C. albicans	41	0.06	0.06	0.03
C. glabrata	110	0.12	0.06	0.015
C. krusei	146	0.12	0.25	0.06
All *Candida*	315	0.12	0.25	0.06

[a]MICs determined according to CLSI testing guidelines for echinocandins and Candida spp.: RPMI broth, 24 h incubation, prominent inhibition endpoint (≥ 50% inhibition)[21,22].
[b]AND: anidulafungin; CAS: caspofungin; MFC: micafungin.
[c]MIC_{90}: MIC encompassing 90% of isolates tested.
MICs in μg/mL.
Adapted from references 9 and 25.

More recently, the original susceptible echinocandin CBP has been adjusted to species-specific for six *Candida* spp.[25]. As expected, adjusted echinocandin CBPs are also higher for *C. parapsilosis* and *C. guilliermondii* (susceptible, ≤ 2 μg/mL; resistant, ≥ 8 μg/mL) than for the other more common species (susceptible, ≤ 0.25 μg/mL; resistant, ≥ 1 μg/mL) and even lower for *C. glabrata* (susceptible, ≤ 0.12 μg/mL; resistant, ≥ 0.5 μg/mL). These species-specific CBPs would better recognize non-treatable or resistant isolates.

Table 3
Echinocandin MIC results and other properties for clinical isolates of *Candida* sp. harboring fks 1 and fks2 resistant mutations[a]

		MICs (mg/L) of:[d]			Glucan Synthesis IC_{50} (ng/mL) of:[e]		
Isolate (No.)[b]	**Fks change[c]**	**AND**	**CAS**	**MCF**	**AND**	**CAS**	**MCF**
C. albicans (6)	None	0.03-0.12 (0.12)	0.25-0.5 (0.12)	0.03-0.06 (0.03)	0.89-7.96	0.5-3.88	10.2-58.2
C. albicans (14)	S645F, S64Y, F641S, S645P	1-2 (≥ 1)	4-8 (≥ 1)	1-4 (≥ 1)	989-2,739	245.4-2,705	1,085-2,533
C. glabrata (6)	None	0.06-0.12 (0.25)	0.06-0.25 (0.12)	0.06-0.12 (0.03)	1.25-1.91	1.79-3.11	0.56-1.29
C. glabrata (27)	F659V, F659S, F625S, W1375L, S663P, S629P, D632G, D632E, others	0.12, 0.25, 0.5-> 2 (≥ 0,5)	0.25, ≥ 2 (≥ 0,5)	0.06, 0.12, 0.25-> 2 (≥ 0.5)	86.6-4,003.5	115-5,245.5	88.7-2,569
C. krusei (2)	None	0.25 (0.12)	0.25 (0.25)	0.25 (0.12)	NA	NA	NA
C. krusei (3)	L658W, L701M, Phe655Cys, H675HQ[f]	0.25,4 (≥ 1)	≥ 1-8 (≥ 1)	0.25,4 (≥ 1)	NA	NA	NA
C. tropicalis (4)	None	0.06-0.12 (0.12)	0.12-0.5 (0.12)	0.06 (0.12)	2.689-4.66	2.33-7.562	6.168-7.545
C. tropicalis (9)	F641S, F26S, L644W, T227C, Phe-Leu, Ser-Pro,S80S	0.5-2 (≥ 1)	1-4 (≥ 1)	0.5, 0.5, 0.5-≥ 1 (≥ 1)	31.62-407	76.88-369.6	75.66-289.8
C. parapsilosis (9)	P660[g]	≤ 2.2-8 (≥ 8)	0.5, 0.5, 1.4-2.2 (≥ 8)	≥ 2.2-8 (≥ 8)	110-442	12.53-79.19	245-493.63
C. orthopsilosis (2)	P660A	0.79-1.3 (NA)	0.79-1 (NA)	0.63-1.3 (NA)	141.0-163.17	58.16-75.26	77.52-152.2
C. metapsilosis (2)	P660A	0.63-0.79 (NA)	0.79-1(NA)	1.59 (NA)	126.4-133.3	25.15-40.28	70.32-13.73

[a] MICs determined according to CLSI testing guidelines for echinocandins and *Candida* spp.: RPMI broth, 24 h incubation, prominent inhibition endpoint (≥ 50% inhibition) (21,22).
[b] No., number of control (WT) and mutant isolates of each species included in the different studies.
[c] Amino acid substitutions for the mutant isolates; None, control isolate or WT strains, no harboring mutations.
[d] AND (anidulafungin) CAS (caspofungin) MFC (micafungin) MIC ranges for WT and mutant isolates.
ECVs are within parentheses next to MICs for WT isolates and CBPs are within parentheses next to MICs for mutants.
[e] Glucan synthase 50% inhibition profile ranges for WT and mutant isolates from references 28,29,38,39.
[f] Mutation, outside the "hotspot"region.
[g] Naturally occurring amino acid change.
MICs in µg/mL.
Adapted from references 28-30,32-40.

MICs for echinocandin resistant (harboring *fks* mutations) isolates

The principal target of activity is the protein Fksp encoded by three *fks* genes; drug binding with this target leads (susceptible isolates) to fungal cell glucan depletion due to the inhibition of β-1,3-$_{D}$-glucan synthase. Echinocandin resistance for *C. albicans* clinical isolates has been associated with high MICs (as compared to those for WT isolates), mutations conferring amino acid substitutions in the *fks1* and *fks2* genes (two "hotspot" regions) of the β-1-3,-D-glucan synthase complex, therapeutic failure (secondary resistance) and/or breakthrough infections (mostly caspofungin related)[29,31,32]. Similar mutations (e.g., Fks1 and/or Fks2 residues) have been documented in *C. glabrata*, *C. krusei*, *C. tropicalis*, the *C. parapsilosis* group, including *C. orthopsilosis* and *C. metapsilosis*) and outside the "hotspot" regions for *C. krusei* (Table 3) and *C. dubliniensis*[28,30,32-40]. Kinetic inhibition studies have demonstrated that the sensitivity of the glucan synthase was also reduced due to substitution at various amino acid positions and that this reduction was not observed in control or WT isolates. However, *fks1* or *fks2* mutations remain uncommon regardless of the MIC endpoint; the following incidences have been recently reported: in 1 of 32 *C. albicans*, 4 of 32 *C. glabrata*, 2 of 12 *C. tropicalis*, and none in *C. krusei*, *C. parapsilosis* and *C. guilliermondii* isolates[36].

Table 3 summarizes MICs of the three echinocandins for non-*Candida albicans* isolates for which mutations have been described; we also listed a set of *C. albicans* as a comparison. These isolates were recovered from patients receiving anidulafungin (one patient), caspofungin and micafungin therapy and/or after therapeutic failure. As expected, most caspofungin MICs were higher than micafungin and anidulafungin MICs. However, if we examine these in vitro results according to recently defined echinocandin ECVs and CBPs, most mutant isolates can be considered either non-WT or resistant, because MICs are above corresponding ECVs and/or the susceptible CBP of ≤ 0.12 to ≤ 0.25 µg/mL for *C. glabrata*, *C. albicans*, *C. krusei* and *C. tropicalis*[6,25]. The MICs below the CBP for mutant isolates suggest the superior activity of the particular echinocandin (s) because there is no cross-resistance of this agent with either of the other two. Although cross-resistance was evident among the three agents for most of the isolates harboring *fks* mutations, there was no cross-resistance between the following drug/species combinations: *a)* anidulafungin and micafungin with caspofungin for 2 to 3 of the 27 isolates of *C. glabrata*, 1 of 3 isolates of *C. krusei* (mutation outside the "hotspot" regions) and 1 of the 9 isolates of *C. tropicalis* with *fks* mutations; *b)* micafungin with the other two agents for another 2 of the 9 isolates of the latter species (Table 3). However, with the exception of two anidulafungin MICs for *C. glabrata* (MICs of 0.12 and 0.25 µg/mL), the mutant isolates were identified by the ECVs as non-WT isolates (91% mutants identified by anidulafungin ECVs and 100% by ECVs of the other two agents) or having decreased echinocandin susceptibility. Another consideration, and as discussed below, is that in the presence of serum, some of these lower MICs have shifted or increased and the potency differences among the echinocandins was reduced[30,35]. All these factors obfuscate comparisons of echinocandin in vitro activity. It is interesting that 6 of the 36 MICs for WT isolates (not harboring *fks* mutations) of *C. glabrata*, *C. krusei* and *C. tropicalis* were above the corresponding ECV; these results could be considered major errors (false resistance errors).

Most echinocandin MICs were below the ECV of 4 µg/mL (anidulafungin and micafungin) and of 1 µg/mL (caspofungin) for *C. parapsilosis* or WT values[6]. These results could be considered very major errors (false susceptibility errors). However, the highest micafungin MICs (4-8 µg/mL) were reported for five micafungin breakthrough isolates[28] and the caspofungin MICs for isolates recovered from patients on caspofungin treatment or prophylaxis[40]. Only the naturally occurring Pro to Ala substitution has been associated with the echinocandin MICs for *C. parapsilosis* summarized in table 3[28,40]. Usually, echinocandin MICs for *C. orthopsilosis* and *C. metapsilosis* have been lower than those for *C. parapsilosis*[40-42], but gene mutations have been investigated in only one of those studies[40] and species-specific ECVs are not available for these two species. More research is needed to really understand the impact of these results on treatment outcome.

We also have compared IC_{50} (50 % inhibitory concentration of the glucan synthase complex [GS]) results of the three echinocandins for isolates harboring *fks1* or *fks2* mutations (Table 3). Although, most

IC_{50} data have been obtained by the same group of investigators, it is evident that the mutant isolates yielded higher IC_{50} values than the control or WT isolates for each echinocandin[29,30,39,40]; these increases were equivalent to a decreased echinocandin in vitro sensitivity. The IC_{50} data of the three echinocandins were similarly variable among the species with a few exceptions: lower IC_{50} ranges for micafungin versus *C. glabrata* and *C. tropicalis* and for caspofungin versus *C. parapsilosis*. As expected, the lower IC_{50} values were reflected in lower MICs and better sensitivity of the enzyme to the agent.

Serum effect on echinocandin antifungal activity

Echinocandins are extensively bound to serum (> 95%) and several investigators have examined the influence of serum in drug protein binding and in vitro activity[29,30,35,40,43]. Using in vitro growth assays, it has been demonstrated that protein binding shifted the antifungal activity of echinocandins against *Aspergillus* spp. and several *Candida* spp., resulting in nearly equivalent MICs or MECs[43]. The effect was less pronounced with caspofungin (2 to 8 fold MIC increase) than with anidulafungin (8 to 128 fold MIC increase) and micafungin (16 to 128 fold MIC increase). Therefore, the initial differences between higher caspofungin MICs and those of the two other agents (without serum) were less evident when MICs were obtained in medium containing 50% serum. These results suggested that serum was affecting the echinocandins directly by lowering their ability to inhibit the target enzyme as demonstrated by the increase of MIC and IC_{50} values. Wiederhold et al. provided similar evidence for *C. glabrata*, the apparent greater in vitro activity of anidulafungin versus caspofungin against this species was also diminished in the presence of serum[35]. More importantly, in the immunosuppressed animal model of invasive candidiasis, both echinocandins were similarly effective in reducing the kidney fungal burden.

Ultrastructural damage

On the other hand, anidulafungin was superior to caspofungin against *C. parapsilosis* in another study[44]. Although both echinocandins (0.25 mg/mL doses) produced similar ultrastructual damage to a "susceptible" caspofungin isolate (MIC 1 µg/mL), a higher dose of caspofungin (16 mg/mL) than of anidulafungin (2 mg/mL) was needed to produce the same damage to a non-susceptible caspofungin isolate (MIC 8 µg/mL). Based on the new CBP (≥ 8 µg/mL) and ECV (4 µg/mL) for *C. parapsilosis* and anidulafungin, these isolates were WT (MIC range 0.03 to 2 µg/mL) and therefore more likely to respond to anidulafungin. In contrast to Garcia et al.'s results[40], amino acid changes were not found in any of these isolates despite the high caspofungin and micafungin MICs for some of their isolates. Mutations or elevated MICs are not the best predictors of treatment outcome. Switching to another echinocandin could not increase treatment efficacy because the in vivo response to echinocandin treatment has been similar as discussed above. Although the response to therapy for *C. parapsilosis* has been comparable to that for other species, the eradication rate for isolates with the highest CLSI anidulafungin MICs (range 0.03 to 2 µg/mL) has been lower than that for other *Candida* spp. in invasive candidiasis[13,16]. However, only a few (9 to 10%) isolates of *C. parapsilosis* and none of *C. guilliermondii* were included in anidulafungin clinical trials; the majority of the patients, as for the other two echinocandin trials, were infected with *C. albicans* and *C. glabrata*.

Echinocandin pharmacodynamic parameters for *Candida* spp.

Standard dosing for each echinocandin gives similar free drug 24 h AUCs (about 112–126 mg.h/L) and an AUC/MIC target of about 20 has been associated with drug exposure and efficacy for *Candida* spp[25,45]. However, recently in candidiasis animal models infected with *C. albicans, C. glabrata*, and *C. parapsilosis*, more of each echinocandin has been required (on a mg/kg basis) to treat *C. parapsilosis*, and caspofungin required less drug on a mg/kg basis for efficacy than the other two echinocandins[45]. These results underline the need for species-specific data and that the effectiveness of each echinocandin can be species-specific.

Table 4
Concentration and time to reach the fungicidal endpoint for each species and agent

Specie (no. strains evaluated)	Concentration and time to reach the fungicidal endpoint with each agent[a]		
	Anidulafungin mg/l (h)	Caspofungin mg/l (h)	Micafungin mg/l (h)
C. krusei (7)	≥ 2 (19-20)	≥ 2 (21-37)	≥ 0.25 (25-30)
C. krusei (2)	–	–	0.25 (5-7)
C. krusei Cas-R[b] (1)	8 (24)	NR	16 (24)
C. metapsilosis (3)	≥ 1 (14-23)	32 (19)	≥ 1 (4-30)
C. orthopsilosis (4)	≥ 4 (14-27)	16 (34-38)	≥ 1 (13-30)
C. parapsilosis (4)	32 (41)	NR	NR
C. guilliermondii (8)	–	NR	NR
C. glabrata (2)	–	–	≥ 0.03 (12-13)
C. glabrata (1)	0.03 (29)	–	–
C. glabrata Cas-R[b] (2)	1-4 (24)	–	–
C. tropicalis (1)	–	–	NR
C. tropicalis (1)	1 (13)	–	–

–: no data; NR: not reached.
[a]Fungicidad end point: 3-log decrease in CFU/mL and 99% Killingrate.
[b]Cas-R, caspofungin resistant isolates.
Adapted from references 41, 47-53.

Echinocandins killing kinetics for non-*Candida albicans* species

The major host defence, the phagocytic killing of neutrophils, monocytes and macrophages, is reduced in immunocompromised patients[46]. Fungicidal activity and a rapid killing activity are important features (or advantages) of antifungal agents for infection eradication, especially in immunocompromised patients. Both parameters are measured by time-killing curves. Only two studies have compared the killing activity of the three agents against *C. krusei, C. metapsilosis, C. orthopsilosis* and *C. parapsilosis*[41,47]. Other investigators have evaluated the fungicidal activity of one or two echinocandins against *C. glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. guilliermondii*[48-54]. Therefore, the more reliable comparative data are those from the two studies that compared the three echinocandins. Table 4 summarizes the concentration of each agent and time required for each species and echinocandin to attain the fungicidal endpoint (99.9% of killing or 3-log decreases) from the different studies; Figure 1 depicts the number of viable cells per millilitre after 24 h with different echinocandin concentrations. The discussion of echinocandin fungicidal activity is based on those endpoints.

In general, the echinocandin killing activity against *C. krusei* begins at the MIC. The fungicidal activity of anidulafungin and caspofungin was achieved with ≥ 2 µg/mL and between 19 to 20 h and 21 to 37 h, respectively against seven susceptible *C. krusei* isolates[47]. A lower micafungin concentration was needed (≥ 0.25 µg/mL) after 25 to 30 h. As expected, a longer time (24 h) and higher anidulafungin (8 µg/mL) and micafungin (16 µg/mL) concentrations were required against a C. *krusei* caspofungin-resistant isolate in the same study. In another study, a shorter time (5 to 7 h) has been reported for micafungin to be fungicidal at concentrations ≥ MIC[48] (Table 4 and Figure 1). So far, micafungin has shown the best killing rate against this species (Figure 1).

Echinocandin killing activity was variable against the *C. parapsilosis* group; none of the echinocandins had fungicidal activity against *C. parapsilosis*[41]. Anidulafungin and micafungin were fungicidal (≥ 1 µg/mL) between 14 to 23 h and 4 to 30 h, respectively, against *C. metapsilosis*, but caspofungin activity was low (19 h with 32 µg/mL and 25 h with 16 µg/mL). Also, low results have been observed for

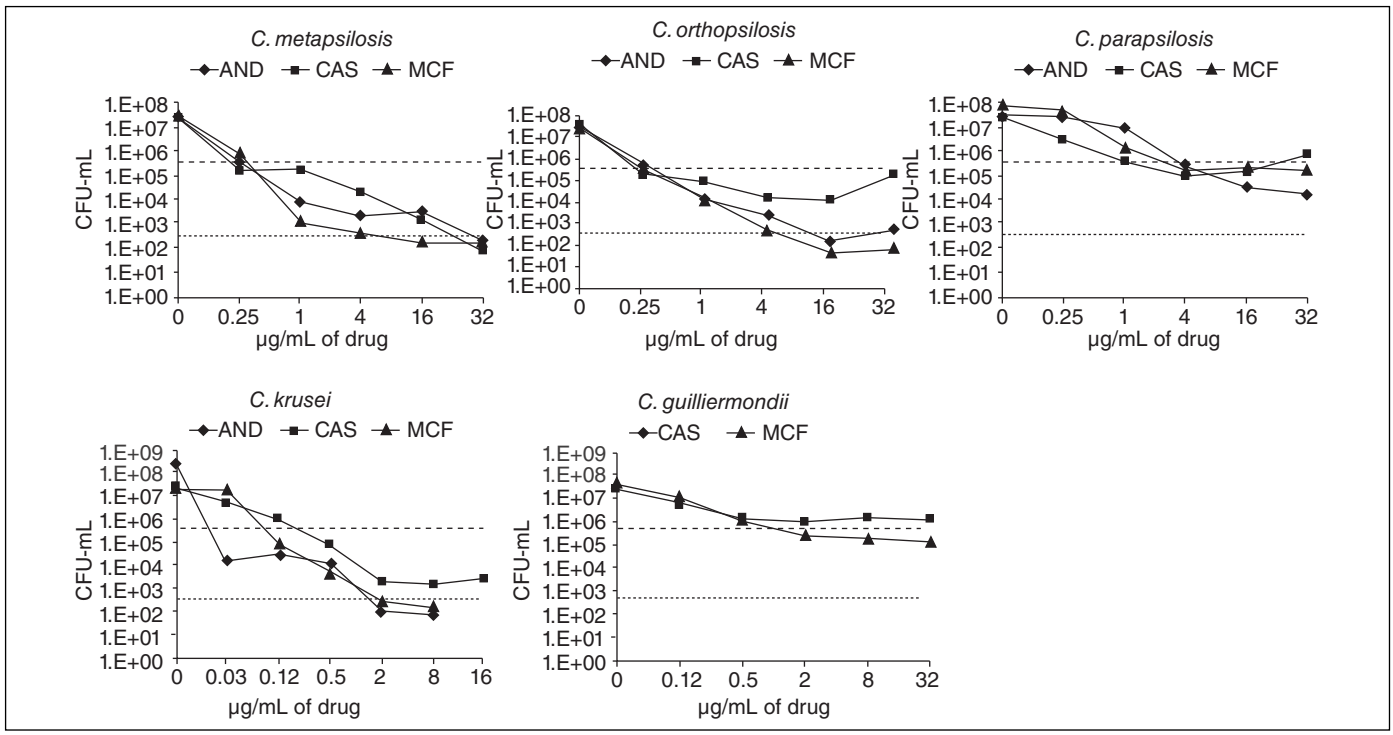

Figure 1. CFU/mL at 24 h with different echinocandin concentrations. Inoculum size: 3-5 × 10^5 CFU/mL (discontinuous line). Above discontinuous line: growth. Below discontinuous line: killing. Doted line: fungicidal endpoint (3-Log decrease in CFU/mL). Adapted from references 41, 47, 48, 50-52.

C. orthopsilosis with anidulafungin (14 to 27 h with 4 µg/mL) and caspofungin (34 and 38 h with 4 and 16 µg/mL), but again micafungin had better activity (≥ 1 µg/mL between 13 to 30 h) (Table 4 and Figure 1). In general, although anidulafungin and micafungin killing rates against the *C. parapsilosis* group are similar and superior to those of caspofungin, the overall best echinocandin fungicidal activity was for *C. metapsilosis*.

Echinocandin results have been obtained in three studies for *C. glabrata*. Ernst et al[48]. reported micafungin killing activity against *C. glabrata* after 12 to 13 h at concentrations between 8 and 16 MICs (0.03-0.06 µg/mL). Cota et al[51]. observed anidulafungin killing activity against 2 of 3 caspofungin-resistant isolates at 24 h with 1 and 4 µg/mL, while this agent's activity was observed at 29 h with 0.03 µg/mL in the third study[52] (Table 4). Unfortunately, caspofungin has not been evaluated for this species and each study only provided data for one of the three echinocandins.

So far neither caspofungin nor micafungin have shown fungicidal activity against *C. guilliermondii* and data are not available for anidulafungin[49,50]. However, caspofungin at a dose of 1 mg/kg/day has proved to be effective (CFU count reduction in kidney) in a murine model of systemic candidemia[49]. By the parameters specified above, no echinocandin killing activity has been observed against *C. tropicalis;* the micafungin maximum killing rate was 90% (1-log decrease) and isolate-dependent in one study[48] and anidulafungin (1 µg/mL) achieved killing activity at 13 h in another study[52]. Similar results were reported by Vargas et al. with caspofungin for six susceptible *C. dubliniensis* isolates[54]. Therefore, it is difficult to compare the potential fungicidal activity for these species until further research is conducted.

Conclusions

Presently, the in vitro activity of the three echinocandins is similar according to MIC results (correlation and agreement percentages are high when echinocandin MICs are compared). The few possible exceptions are: *a)* better in vitro activity of micafungin over anidulafungin for *C. glabrata*, including azole-resistant isolates. But MIC_{90}s of both echinocandins for the latter isolates were below their corresponding ECVs or WT values; that equalized the results; *b)* better activity of anidulafungin and micafungin over caspofungin for a few isolates of *C. dubliniensis* (data from a single study); *c)* no cross-resistance of micafungin and/or anidulafungin with caspofungin and of micafungin with the other two agents for some mutant isolates of *C. glabrata*, *C. krusei* and *C. tropicalis*, but serum appeared to equalize some of those results; *d)* lower IC_{50s} for *C. glabrata* and *C. tropicalis* versus micafungin and for *C. parapsilosis* versus caspofungin; *e)* better anidulafungin than caspofungin activity (ultrastructural damage) for *C. parapsilosis* cells, but the test isolates were caspofungin-resistant yet anidulafungin-susceptible; *f)* more research is needed to identify possible species-dependent differences regarding pharmacokinetic parameters; *g)* superior micafungin fungicidal activity than those of caspofungin and anidulafungin for *C. krusei*, *C. orthopsilosis* and than anidulafungin for *C. metapsilosis* and *C. glabrata* (comparison from different studies for the latter species). Thus far, regardless of potentially different in vitro activity, efficacy (animal and humans) is similar among these agents.

Conflict of interests

The authors declare that they have no conflict of interests.

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