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SUSCEPTIBILITY

Resistance to Echinocandins in *Candida* Can Be Detected by Performing the Etest Directly on Blood Culture Samples

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ABSTRACT We examined the rapid evaluation of susceptibility to echinocandins in *Candida* spp. using the Etest performed directly on positive blood cultures and anidulafungin-containing agar plates. We prospectively collected 80 positive blood cultures (Bactec-FX system, Becton-Dickinson, Cockeysville, MD, USA) with echinocandin-susceptible *Candida* spp. ($n = 60$) and echinocandin-intermediate *Candida parapsilosis* ($n = 20$) from patients with candidemia. Additionally, blood culture bottles of nonfungemic/bacteremic patients were spiked with 35 echinocandin-resistant *Candida* species isolates. A total of 2 to 4 drops of medium from each bottle were stroked directly onto both RPMI 1640 agar plates with micafungin and anidulafungin Etest strips (ET_{DIR}) and Sabouraud agar plates containing 2 mg/liter of anidulafungin. The isolates were tested according to the EUCAST method and Etest standard (ET_{SD}). Essential and categorical agreement between the methods was calculated. The essential agreement and categorical agreement between the EUCAST method and ET_{DIR} and ET_{SD} were both >97.4%. The essential agreement between ET_{DIR} and the EUCAST method for both echinocandins was >97%. The categorical agreement between the *FKS* sequence and ET_{DIR} was 97.4%. The ET_{DIR} MICs of anidulafungin and micafungin (≥0.19 mg/liter and ≥0.064 mg/liter, respectively) effectively separated all susceptible *FKS* wild-type isolates from the resistant *FKS* mutant isolates. The categorical agreement (62.6%) between the EUCAST method and growth on anidulafungin-containing plates was poor, with the best agreement observed for *Candida glabrata* (94.2%). When performed directly on positive blood cultures from patients with candidemia, the Etest with micafungin and anidulafungin is a reliable procedure for the rapid testing of susceptibility to echinocandins in *Candida* species isolates.

KEYWORDS *Candida* spp., EUCAST procedure, Etest, echinocandins

The incidence of invasive fungal infections has increased in many institutions, and mortality rates soar when an appropriate antifungal treatment is delayed (1–4). Echinocandins are recommended as the first-line treatment for invasive candidiasis (5, 6). Although the rates of resistance to echinocandins remain low (1, 7–10), recent publications are alerting physicians to an increased rate of resistance in some geographic areas (11, 12). Resistance is associated with a poor prognosis in patients with candidemia treated with echinocandins; consequently, detection may help to optimize antifungal treatment (11, 13). Echinocandin resistance can be detected in the clinical microbiology laboratory using broth microdilution methods, such as EUCAST and CLSI reference methods, or commercial methods, such as with Sensititre YeastOne, Vitek,

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TABLE 1 Distribution of MICs and geometric mean MICs of micafungin and anidulafungin obtained using EUCAST, ET_{SD}, and ET_{DIR} methods

Drug	Species (no. of isolates)	Method	MIC (mg/liter)[a]										
			GM[b]	0.015	0.032	0.064	0.125	0.25	0.5	1	2	4	≥8
Micafungin	*C. albicans* (21)	EUCAST	0.018	20						**1**			
		ET_{DIR}	0.021	20									**1**
		ET_{SD}	0.021	20									**1**
	C. parapsilosis (20)	EUCAST	0.841					1	7	8	4		
		ET_{DIR}	1.149						2	12	6		
		ET_{SD}	1						4	12	4		
	C. tropicalis (23)	EUCAST	0.033	9	11			**2**			**1**		
		ET_{DIR}	0.039	6	14			**1**	**1**			**1**	
		ET_{SD}	0.042	3	17			**1**	**1**		**1**		
	C. glabrata (51)	EUCAST	0.192	20		**6**		**1**	**1**	**5**	**8**	**10**	
		ET_{DIR}	0.224	19	1	**1**	**4**	**2**	**1**	**6**	**8**	**5**	**4**
		ET_{SD}	0.178	20		**1**	**5**	**1**	**5**	**5**	**10**	**3**	**1**
Anidulafungin	*C. albicans* (21)	EUCAST	0.016	18	2			**1**					
		ET_{DIR}	0.021	20									**1**
		ET_{SD}	0.021	20									**1**
	C. parapsilosis (20)	EUCAST	1.866						1	4	11	4	
		ET_{DIR}	2.928							1	7	12	
		ET_{SD}	3.031							1	6	13	
	C. tropicalis (23)	EUCAST	0.023	18		4				**1**			
		ET_{DIR}	0.038	7	12	1		**1**	**1**		**1**		
		ET_{SD}	0.029	15	5			**1**	**1**		**1**		
	C. glabrata (51)	EUCAST	0.258	8	12			**4**	**4**	**6**	**13**	**4**	
		ET_{DIR}	0.311	16	4			**1**	**5**	**2**	**10**	**12**	**1**
		ET_{SD}	0.239	20				**3**	**5**	**5**	**12**	**4**	**2**

[a]The MICs obtained by the ET_{SD} and ET_{DIR} were increased to the concentration of the next 2-fold dilution matching the micafungin dilution scale used for the EUCAST procedure. Numbers in bold indicate resistant isolates.
[b]GM, geometric mean.

disk diffusion, or plastic gradient strips. Alternatively, mutations in *FKS1* and *FKS2* (*Candida glabrata* only) can be detected using molecular techniques (12, 14, 15).

Phenotypic procedures for *Candida* antifungal susceptibility testing require pure-cultured isolates and are hindered by a slow turnaround time (48 to 72 h from the diagnosis of candidemia). We previously reported that the Etest performed directly on positive blood samples for yeasts was comparable to the standard CLSI and EUCAST approaches for the detection of both wild-type and azole-resistant *Candida* species isolates within 24 h of the diagnosis (2, 16). Unfortunately, given the lack of resistant isolates, we were unable to study the role of the procedure in the detection of the resistance of *Candida* to echinocandins.

On the basis of a set of echinocandin-resistant *Candida* isolates, we assessed the role of the Etest performed directly on artificially spiked blood cultures for the detection of resistance to echinocandins. Anidulafungin-containing agar plates were also tested for the screening of resistant isolates.

(This study was partially presented at the 28th European Congress of Clinical Microbiology and Infectious Diseases in Madrid, Spain, 2018 [17].)

RESULTS

The antifungal activities of micafungin and anidulafungin determined by EUCAST, Etest strips (ET_{DIR}), and Etest standard (ET_{SD}) procedures against the 115 isolates are shown in Table 1. The geometric mean (GM) MICs obtained by the EUCAST method and the Etest (ET_{DIR} and ET_{SD}) were similar ($P > 0.05$).

The essential agreement between ET_{DIR} and ET_{SD} of micafungin and anidulafungin was 98.3% and 100%, respectively, with few exceptions (Table 2). The essential agreement between ET_{DIR} and the EUCAST method was >97% for both micafungin and anidulafungin and 100% for micafungin against *Candida parapsilosis* and *Candida tropicalis* and for anidulafungin against *C. glabrata*.

The categorical agreement between ET_{DIR} and ET_{SD} was 100% for all species and

TABLE 2 Essential agreement and categorical agreement between the methods for micafungin and anidulafungin

Species	Agreement (% of isolates)							
	Essential[a]				Categorical			
	ET_{SD} vs ET_{DIR}[b]		EUCAST vs ET_{SD}/ET_{DIR}		EUCAST vs ET_{DIR}		*FKS* sequence vs ET_{DIR}/EUCAST	
	MYC[c]	AND[d]	MYC	AND	MYC	AND	MYC	AND
C. albicans	100	100	95.2/95.2	95.2/95.2	100	100	100	100
C. parapsilosis	100	95	100/100	90/95	100	100	100	100
C. tropicalis	100	100	100/100	95.6/95.6	100	91.3	100	100
C. glabrata	96.1	100	98/98.1	100/100	100	100	94.2	94.2
Overall	98.3	100	98.2/98.2	97.5/97.4	100	98.3	97.4	97.4

[a]Percentages of isolates in which the antifungal MIC differed ±2-log dilutions over the methods.
[b]ET_{SD}, Etest standard; ET_{DIR}, Etest direct.
[c]MYC, micafungin.
[d]AND, anidulafungin.

echinocandins. The categorical agreement between ET_{DIR} and the EUCAST method was 100% for micafungin and 98.3% for anidulafungin, where misclassifications were found in two *C. tropicalis* isolates (8.7% of major errors in *C. tropicalis*) in which the EUCAST method indicated resistance to micafungin but susceptibility to anidulafungin, whereas ET_{DIR} (and ET_{SD}) indicated resistance to both drugs (Table 3 and Table 2). The isolates harbored *FKS1* HS1 mutations (F641L and R647G) (Fig. 1). The categorical agreement between ET_{DIR} and the *FKS* sequence was 97.4% for both echinocandins (Table 2); the agreement was 100% for all species, with the exception of *C. glabrata* (94.2%), because of three isolates in which ET_{DIR} (and the EUCAST method) for both echinocandins indicated resistance but the *FKS1* and *FKS2* sequences were the wild types (overall 2.6% of major errors). Figure 2 shows the distributions of the micafungin and anidulafungin MICs obtained by ET_{DIR} and the EUCAST method (*C. parapsilosis* was excluded). An ET_{DIR} MIC of anidulafungin of ≥0.19 mg/liter and/or an MIC of micafungin of ≥0.064 mg/liter against *Candida albicans*, *C. tropicalis*, and *C. glabrata* effectively separated the phenotypically resistant isolates/*FKS* mutants from the susceptible isolates/*FKS* wild types (100% categorical agreement with combined gold standards for both agents).

Overall, the categorical agreement between the EUCAST method and growth on anidulafungin-containing plates was 62.6%. Major errors were found in all *C. albicans* and *C. tropicalis* echinocandin-susceptible isolates (the EUCAST method indicated susceptibility, but growth was visible on the plates [34.8%]). All *C. parapsilosis* isolates were able to grow on the plates. The best agreement was observed for *C. glabrata* (94.2%), with 100% of susceptible isolates not growing on the plates and 90.3% of the resistant isolates growing on the plates. Very major errors (the EUCAST method indicated resistance but there was no visible growth on the plates [2.6%]) were found in the three echinocandin-resistant *FKS* wild-type *C. glabrata* isolates.

DISCUSSION

Our study shows that performing the ET_{DIR} directly on positive blood cultures can speed up echinocandin susceptibility testing within 24 h of the detection of *Candida*. The results obtained by this rapid, easy, and inexpensive procedure mirrored those obtained by the EUCAST method and ET_{SD} and those obtained with the *FKS* gene sequence.

Current Infectious Diseases Society of America (IDSA) guidelines for the treatment of patients with candidemia recommend echinocandin susceptibility testing on isolates causing fungemia, particularly for patients previously exposed to echinocandins or infected by *C. glabrata* (5). Microdilution methods are preferred, although they require pure-cultured isolates, they are time consuming, and the results are not available until 48 to 72 h after diagnosis. Given that a delay in starting an appropriate antifungal treatment invariably leads to a poorer prognosis, the results of antifungal susceptibility must be anticipated where possible.

We previously showed that ET_{DIR} performed directly on positive blood cultures

TABLE 3 Micafungin and anidulafungin MICs against the 35 echinocandin-resistant *Candida* species isolates obtained using the EUCAST, ET_{SD}, and ET_{DIR} procedures

Species	*FKS* mutation	MIC (mg/liter)[a]					
		EUCAST		ET_{DIR}[b]		ET_{SD}[c]	
		MYC[d]	AND[e]	MYC	AND	MYC	AND
C. albicans	F641S (*FKS1* HS1)	1	0.25	>32	>32	>32	>32
C. tropicalis[f]	R647G (*FKS1* HS1)	0.25	0.064	0.38	0.19	0.38	0.19
C. tropicalis	S645F (*FKS1* HS1)	2	1	3	2	2	2
C. tropicalis[f]	F641L (*FKS1* HS1)	0.25	0.064	0.19	0.38	0.19	0.38
C. glabrata	Δ659 (*FKS2* HS1)	1	0.5	0.25	0.25	0.25	0.25
C. glabrata	Δ659 (*FKS2* HS1)	0.064	1	1.5	2	1.5	2
C. glabrata	Δ659 (*FKS2* HS1)	4	4	>32	>32	>32	>32
C. glabrata	Δ659 (*FKS2* HS1)	2	1	1	1.5	0.5	1
C. glabrata	Δ659 (*FKS2* HS1)	4	2	2	4	1.5	3
C. glabrata	Δ659 (*FKS2* HS1)	2	1	1	3	0.75	1
C. glabrata	Δ659 (*FKS2* HS1)	4	4	1.5	2	2	4
C. glabrata	Δ659 (*FKS2* HS1)	4	2	2	4	2	2
C. glabrata	Δ659 (*FKS2* HS1)	4	2	1	3	1	1
C. glabrata	Δ659 (*FKS2* HS1)	1	2	1.5	3	1	2
C. glabrata	Δ659 (*FKS2* HS1)	1	2	1.5	2	1.5	2
C. glabrata	Δ659 (*FKS2* HS1)	1	1	0.5	1.5	0.5	1
C. glabrata	Δ659 (*FKS2* HS1)	4	2	1	3	2	2
C. glabrata	Δ659 (*FKS2* HS1)	2	4	3	4	3	4
C. glabrata	S663P (*FKS2* HS1)	2	1	3	2	2	2
C. glabrata	S663P (*FKS2* HS1)	2	2	1	2	4	8
C. glabrata	S663P (*FKS2* HS1)	4	4	6	3	1	2
C. glabrata	S663P (*FKS2* HS1)	4	2	4	4	2	2
C. glabrata	S663P (*FKS2* HS1)	4	2	4	4	1	2
C. glabrata	S663P (*FKS2* HS1)	2	1	3	1.5	2	3
C. glabrata	S663P (*FKS2* HS1)	2	2	6	4	0.5	1
C. glabrata	W715L (*FKS2*)	2	2	2	4	2	2
C. glabrata	W715L (*FKS2*)	0.5	0.5	2	2	0.38	0.5
C. glabrata	W715L (*FKS2*)	4	2	8	1	4	2
C. glabrata	D666N (*FKS2* HS1)	0.064	0.25	0.125	0.5	0.125	0.5
C. glabrata	D666N (*FKS2* HS1)	0.064	0.5	0.064	0.38	0.094	0.38
C. glabrata	S663Y (*FKS2* HS1)	1	2	1	2	0.5	1.5
C. glabrata	E655A (*FKS2*)	0.25	0.5	0.125	1	0.125	0.38
C. glabrata[g]	Wild type	0.06	0.25	0.125	0.5	0.06	0.25
C. glabrata[g]	Wild type	0.06	0.25	0.19	0.38	0.125	0.38
C. glabrata[g]	Wild type	0.06	0.25	0.125	0.5	0.125	0.25

[a]EUCAST breakpoints used to classify the isolates as resistant: *C. albicans* (micafungin, >0.016; anidulafungin, >0.032); *C. glabrata* (micafungin, >0.032; anidulafungin, >0.064); *C. tropicalis* (micafungin [based on ECOFF], >0.06; anidulafungin, >0.032) (34, 37).
[b]ET_{DIR}, Etest direct.
[c]ET_{SD}, Etest standard.
[d]MYC, micafungin.
[e]AND, anidulafungin.
[f]*C. tropicalis* isolates showing resistance to micafungin but susceptibility to anidulafungin by the EUCAST method; both Etest procedures showed resistance to micafungin and anidulafungin.
[g]*C. glabrata* isolates showing phenotypic resistance to anidulafungin and micafungin but wild-type *FKS1* and *FKS2* genes.

showed good agreement with the CLSI M27-A3 procedure (2). The study proved useful for ruling out false resistance, which can facilitate antifungal de-escalation (for example, switching from echinocandins to fluconazole). As for the ability of ET_{DIR} to detect resistance, we demonstrated that it was reliable for caspofungin-resistant basidiomycete yeast or fluconazole-resistant non-*albicans Candida*. Unfortunately, we did not test fluconazole-resistant *C. albicans* isolates or echinocandin-resistant *Candida* species isolates. Our subsequent study demonstrated that ET_{DIR} was able to detect fluconazole-resistant *C. albicans* isolates (16). We conducted the present study with well-characterized echinocandin-resistant *Candida* species to complete the testing. We studied the agreement between ET_{DIR} and the EUCAST reference method for micafungin and anidulafungin. Caspofungin, the agent tested in our first paper (2), was not tested here owing to interlaboratory variability (18). For that reason, EUCAST does not provide caspofungin breakpoints.

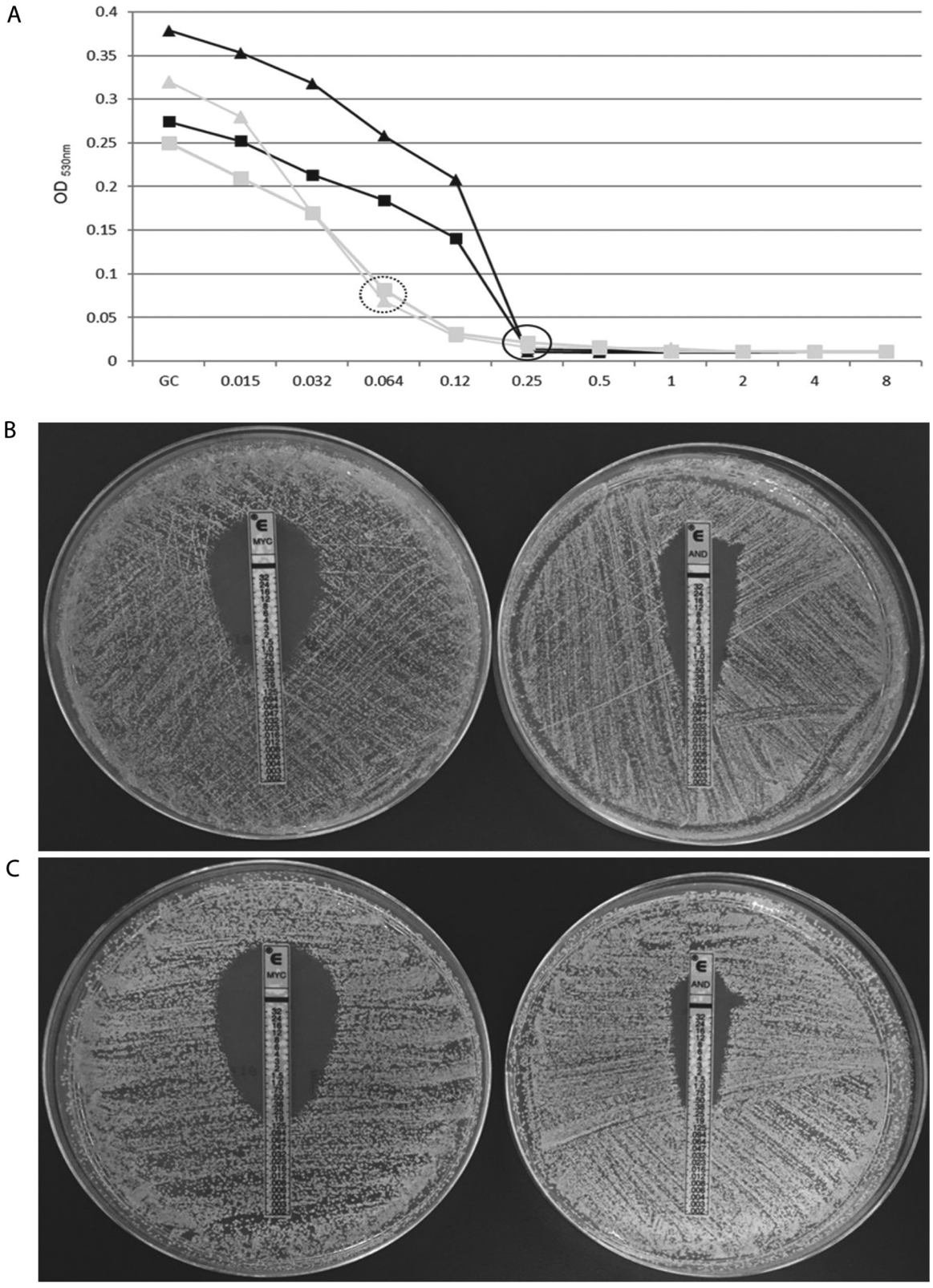

FIG 1 (A) Echinocandin growth inhibition curves obtained by the EUCAST method indicating resistance to micafungin (black) but susceptibility to anidulafungin (grey) for *C. tropicalis* isolates with R647G (triangles) or F641L (squares) mutations. Black circle indicates micafungin MIC; dotted circle indicates anidulafungin MIC; GC, growth control. ET_{DIR} of two *C. tropicalis* isolates with R647G (B) and F641L (C) mutations indicating resistance to both echinocandins. MYC, micafungin; AND, anidulafungin.

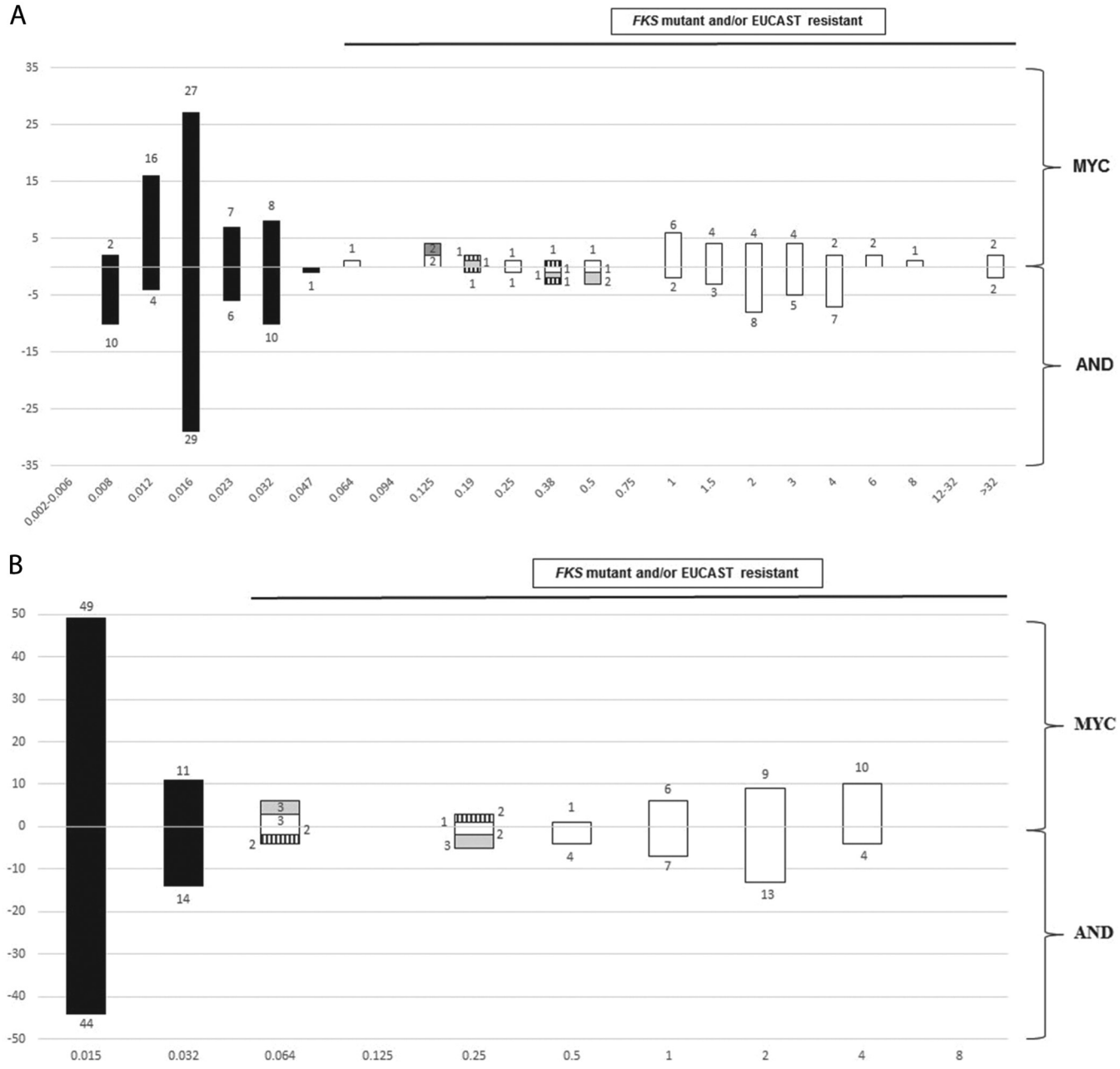

FIG 2 Distribution of the micafungin (MYC) and anidulafungin (AND) MICs obtained by ET_{DIR} (A) and the EUCAST method (B) against 95 isolates (*C. parapsilosis* isolates were excluded). Black bars, *FKS* wild-type isolates classified as susceptible by the EUCAST method; grey bars, *FKS* wild-type *C. glabrata* isolates classified as resistant by the EUCAST method; hatched bars, *FKS* mutant *C. tropicalis* isolates classified as anidulafungin susceptible and micafungin resistant by the EUCAST method; white bars, *FKS* mutant isolates classified as resistant by the EUCAST method.

We found very high essential and categorical agreement between the ET_{DIR}, ET_{SD}, and EUCAST methods for echinocandin susceptibility testing against *Candida* spp. No very major errors (false susceptibility) were detected for any of the isolates tested. However, a few major errors were found for anidulafungin in two *C. tropicalis* isolates with *FKS1* mutations that were classified as anidulafungin-susceptible and micafungin-resistant by the EUCAST method and as resistant by ET_{DIR}. Previous studies reported *C. albicans* and *Candida kefyr* isolates with the *FKS1* mutations R647G and P649H and the above-mentioned phenotype using CLSI methods (19, 20), thus suggesting that the amino acids R647 and P649 are key for glucan synthase inhibition by micafungin. Another study reported a *C. tropicalis* isolate harboring the F641L *FKS1* mutation with

dose-dependent susceptibilities to both micafungin and anidulafungin, again, by using CLSI methods (21). Since EUCAST does not yet have breakpoints for micafungin against *C. tropicalis*, our isolates had to be classified using epidemiologic cutoff values (ECOFFs). However, our and other previously reported observations for *C. glabrata* (22–25) suggest that the presence of *FKS* mutations itself is not sufficient to predict the pattern of resistance to anidulafungin or micafungin. Therefore, we decided to test anidulafungin and micafungin in parallel. On the basis of our results, micafungin may be a good surrogate marker of echinocandin resistance in *C. albicans* and *C. tropicalis* isolates.

To improve the potential of ET_{DIR} for the detection of both resistant and *FKS* non-wild-type isolates, we calculated the categorical agreement using the *FKS* sequence as the gold standard. The categorical agreement between the *FKS* sequence and ET_{DIR} was very high, thus showing the ability of ET_{DIR} to discriminate between *FKS* mutants and wild types in most cases. However, three major errors were observed in three unusual *C. glabrata* isolates that showed very high anidulafungin and micafungin MICs by the EUCAST method and Etest but wild-type *FKS1* and *FKS2* sequences. We cannot rule out alternative mechanisms of resistance (e.g., efflux pumps), although the ET_{DIR} classified them correctly, in agreement with the EUCAST method, as resistant, suggesting the ability of this procedure to detect wild-type *FKS*/resistant isolates. Future studies on these isolates are required. To separate the *FKS* wild-type/susceptible isolates from *FKS* mutant/resistant isolates, we suggest the following cutoffs for ET_{DIR}: anidulafungin, $\geq$0.19 mg/liter; and/or micafungin, $\geq$0.06 mg/liter.

Recent studies have proved that azole-containing plates are useful when screening for the presence of resistance in *Aspergillus* (26). To determine whether this procedure would be useful for the screening of echinocandin resistance in *Candida*, we decided to use anidulafungin-containing plates at 2 mg/liter on the basis of our previous study, which reports the anidulafungin mutant prevention concentration (27). However, we found poor agreement between this method and the EUCAST method owing to the high percentages of false resistance in *C. albicans* and *C. tropicalis*. A paradoxical effect is common to both species (28, 29). Owing to the lack of echinocandin-resistant *C. parapsilosis* isolates tested and the fact that 100% of the intermediate isolates grew on the plates, this procedure cannot be recommended for *C. parapsilosis*, *C. albicans*, or *C. tropicalis*.

Our main limitation is the low number of *C. albicans* and *C. tropicalis FKS* mutant isolates. However, the acquisition of echinocandin resistance involves *C. glabrata* to a greater extent than other *Candida* spp. (11, 12), and the number of *C. glabrata* mutant isolates tested was moderately high. Furthermore, the positive results reported here reinforce our previous observation of basidiomycete yeast being correctly classified as caspofungin resistant (2).

We conclude that the ET_{DIR} for micafungin and anidulafungin is a reliable and fast procedure when screening for the presence of echinocandin resistance in *Candida* species causing candidemia and can be easily implemented in the routine of the microbiology laboratory.

MATERIALS AND METHODS

Samples. We prospectively collected 80 positive blood cultures (Bactec-FX system, Becton-Dickinson, Cockeysville, MD, USA) to screen for echinocandin-susceptible *Candida* spp. (*C. albicans*, $n = 20$; *C. tropicalis*, $n = 20$; *C. glabrata*, $n = 20$) and echinocandin-intermediate *C. parapsilosis* ($n = 20$) from patients with candidemia admitted to Gregorio Marañón Hospital (Madrid, Spain) between 2010 and 2013. A total of 1 to 2 ml of broth from each bottle was stored at −70°C. Additionally, 0.5-ml (0.5 McFarland) suspensions of 35 echinocandin-resistant *Candida* species isolates obtained in previous studies (2, 27, 30–32) were artificially spiked in nonfungemic/bacteremic Bactec bottles until they were flagged as positive (Table 3). All 115 isolates were identified by amplification and sequencing of the ITS1-5.8S-ITS2 regions (33).

EUCAST antifungal susceptibility testing and ET_{SD}. All isolates were tested for susceptibility to micafungin (Astellas Pharma, Inc., Tokyo, Japan) and anidulafungin (Pfizer Pharmaceutical Group, New York, NY, USA) according to the EUCAST E.DEF 7.3.1 microdilution procedure (34–36). The echinocandin concentrations ranged from 0.015 to 8 mg/liter. Inoculated plates were incubated for 24 h at 35°C.

ET_{SD} of micafungin and anidulafungin was performed on the isolates according to the manufacturer's instructions. Briefly, the suspensions were prepared, adjusted to 0.5 McFarland, and stroked on RPMI 1640 agar plates supplemented with 2% glucose (bioMérieux, Marcy-l'Etoile, France). The strips were placed on the agar surfaces of the plates, which were then incubated at 35°C for 24 h. The MIC was set when the fungal elliptic growth intersected the plastic strip.

Antifungal susceptibility testing performed directly on blood samples using ET_{DIR} and anidulafungin-containing agar plates. A total of 2 to 4 drops of the broth medium (stored broth from the 80 isolates preincubated overnight at 37°C and positive flagged bottles of the 35 echinocandin-resistant isolates) were stroked on RPMI 1640 agar plates on which Etest strips of micafungin and anidulafungin had been placed; 2 to 4 drops of the broth medium were also stroked on Sabouraud agar plates containing 2 mg/liter of anidulafungin. The plates were incubated at 35°C for 24 h.

Data analysis. The geometric mean (GM) MICs of micafungin and anidulafungin against the isolates obtained by the three methods were calculated and compared using the *t* test, with a *P* value of <0.05 considered statistically significant. MICs obtained using EUCAST 7.3.1 were considered the gold standard and were compared with those obtained by ET_{DIR} and ET_{SD} to calculate the essential agreement between the methods (percentage of isolates in which MIC differed by $\pm$2-log dilutions over the reference method). All isolates were classified as resistant or susceptible according to the clinical breakpoints proposed by EUCAST for any of the three methods (37). Given the lack of clinical breakpoints for micafungin against *C. tropicalis*, we tentatively considered isolates showing an MIC above the ECOFF ($>$0.06 mg/liter) to be resistant in order to avoid the term "non-wild-type," which is used exclusively for *FKS* mutants (34). The procedures were in categorical agreement when the results were in the same susceptibility category (2) based on two gold standards: the EUCAST method and *FKS* sequence (regardless of the MIC). The anidulafungin-containing plate-screening procedure was in categorical agreement with the EUCAST method when resistant isolates or *FKS* mutants were able to grow on the plates and susceptible isolates or *FKS* wild types were unable to grow visibly on the plates. Errors were categorized as very major (agar diffusion methods indicated susceptibility and the EUCAST method/*FKS* sequences indicated resistance or mutations) or major (agar diffusion methods indicated resistance and the EUCAST method/*FKS* sequence indicated susceptibility or wild type).

Ethical considerations. This study was approved by the ethics committee of Hospital Gregorio Marañón (CEIC-A1, study no. 208/16).

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