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Abstract

In vitro susceptibility of 933 *Candida* isolates, from 16 French hospitals, to micafungin was determined using the Etest® in each center. All isolates were centralized for determination of minimum inhibitory concentrations (MICs) by the EUCAST reference method. Overall essential agreement between the two tests was 98.5% at ± 2 $\log_2$ dilutions and 90.2% at ± 1 $\log_2$ dilutions. Categorical agreement was 98.2%. The Etest® is a valuable alternative to EUCAST for the routine determination of micafungin MICs in medical mycology laboratories.

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The echinocandin antifungal drug micafungin is highly effective *in vitro* against most *Candida* species (1-3). Micafungin is now widely used for prophylaxis and treatment of invasive candidiasis (IC) (4, 5). During the last decade, acquired resistance of various *Candida* species to echinocandins has emerged worldwide, including France, and may become an important issue in the therapeutic management of IC (6-10).

In vitro antifungal susceptibility testing is currently recommended to detect resistance in *Candida* species and to guide antifungal treatment (6, 11). Microdilution broth methods such as EUCAST and CLSI are the reference methods for antifungal susceptibility testing. Nevertheless, because these reference methods are labor intensive and time-consuming, most clinical microbiology laboratories use commercial methods, such as the Etest®, for routine determination of minimum inhibitory concentrations (MICs). It is therefore essential to evaluate these commercial tests and to determine their ability to give MIC values that agree with those from the reference methods.

With this aim, a prospective, multicenter French study was performed to compare the EUCAST and Etest® methods for micafungin susceptibility testing of a large panel of clinical isolates of different *Candida* species. Sixteen centers (six in Paris area and 10 across France) participated in the study. Over a 2-month period, each center was asked to test 64 *Candida* isolates, from any clinical sample, of the following species: 10 isolates of each of the six most common pathogenic species (*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. kefyr* and *C. krusei)* and four isolates belonging to other *Candida* species. Species identification was performed in each center according to the currently recommended phenotypic methods (12). Micafungin susceptibility testing was performed using the Etest® (Biomérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. *Candida* isolates were then centralized in a single center for MIC determination by the EUCAST reference method (13). *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were included as quality control

97 strains (14). For comparison purposes, Etest® MICs were increased to the next higher
98 corresponding EUCAST concentration (15). Resistance was based on EUCAST clinical
99 breakpoints. When clinical breakpoints were not available (*i.e.* for *C. krusei* and *C.*
100 *tropicalis*), ECOFFs were used to categorize isolates as non-wild-type (16). The same
101 ECOFFs (defined by EUCAST) were used for analyzing results of Etest® as specific ECOFFs
102 have not been determined yet. *C.albicans*, *C. glabrata*, and *C. parapsilosis* isolates were
103 considered susceptible / resistant to micafungin when MICs were ≤0.016 / >0.016 µg/ml,
104 ≤0.03 / >0.03 µg/ml, and ≤0.002 / >2 µg/ml, respectively. *C. krusei* and *C. tropicalis* isolates
105 were considered wild-type / non-wild-type to micafungin when MICs were ≤0.25 / >0.25
106 µg/ml and ≤0.06 / >0.06 µg/ml, respectively. MIC results obtained by the two methods were
107 considered to be in essential agreement when they were within ± 2 $\log_2$ dilutions. Agreement
108 at ± 1 $\log_2$ dilution was also calculated. Categorical agreement was defined as the percentage
109 of isolates classified in the same category (i.e. susceptible, intermediate, and resistant or wild-
110 type and non-wild-type) by both techniques (15). Discrepancies (very major, major, and
111 minor errors) were defined as described previously (15).

112 Results from antifungal susceptibility testing were available for 933 *Candida* isolates,
113 including 878 isolates of the six most medically important *Candida* species and 55 other
114 *Candida* species. Table 1 shows the micafungin MICs for the 933 isolates determined by the
115 EUCAST reference method. Micafungin MICs for *C. parapsilosis* isolates (modal MIC of 1
116 µg/ml) were several dilutions higher than for the other common species (modal MIC of 0.015
117 µg/ml for *C. albicans, C. tropicalis*, and *C. glabrata* and 0.03 and 0.06 µg/ml for *C. kefyr* and
118 *C. krusei*, respectively). MICs for rare species were similar than those of the common species
119 except for *C. colliculosa* and some isolates of *C. guilliermondii* and *C. famata*. According to
120 the current clinical breakpoints (16), the micafungin resistance rate was <2% for *C. albicans*
121 and *C. parapsilosis*, and 3.9% for *C. glabrata*. Based on ECOFFs, the non-wild-type rate was

0.7% for *C. tropicalis* and 0% for *C. krusei*. The overall essential agreement between EUCAST and Etest® results was high (98.5% at ± 2 $\log_2$ dilutions and 90.2% at ± 1 $\log_2$ dilution) (Figure 1) with minor differences between species (Table 2). The lowest essential agreement (96.7% at ± 2 $\log_2$ dilutions) was observed for *C. parapsilosis*. An overall categorical agreement of 98.2% was observed for the 742 isolates belonging to the five species for which clinical breakpoints or ECOFFs are available (Table 3). The highest (100%) and lowest (96.7%) categorical agreements were found for *C. krusei* and *C. glabrata*, respectively. Major errors were observed in six cases (three *C. albicans*, two *C. tropicalis*, and one *C. glabrata*) and very major errors in six cases (two *C. albicans* and four *C. glabrata*). These 12 discrepancies were observed for strains isolated and tested in eight different centers.

The Etest® has been used in several studies for micafungin susceptibility testing of *Candida* spp. (17-22), but only a few comparative studies with a reference method have been performed (17, 20-22). In one of these previous studies, Marcos-Zambrano *et al*. (21) tested 160 yeast isolates with both the Etest® and EUCAST methods and reported an essential agreement of 90.3% at ± 2 $\log_2$ dilutions (85.8% at ± 1 $\log_2$ dilution) and a categorical agreement of >90%. Similarly, in another study, a comparison between Etest® and CLSI methods showed an overall essential agreement of 94.7% and a categorical agreement of 97.2% (20). The ability of the Etest® to detect micafungin resistance, for most of the species, has also been demonstrated previously by testing *FKS* mutant isolates (17, 21, 22). We enrolled 16 centers and demonstrated that under real-life conditions the Etest® gave very similar micafungin susceptibility results to the EUCAST reference method.

Altogether, our results show that the Etest® is a valuable and reliable method to routinely test the i*n vitro* susceptibility of clinical *Candida* isolates to micafungin. *In vitro* micafungin resistance among the main *Candida* species isolated from clinical samples remains uncommon in France.

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Conflicts of interest: MEB received grants from Astellas, Gilead and Merck and speaker's fees from Astellas and Merck. ED has received grants from Gilead, Ferrer, and Biorad, payment for lectures from Gilead, MSD, and Schering, and has been consultant for Astellas and Innothera. IA received speaker's fees from Merck. AA received funds for speaking from Merck and for travel from Astellas, Merck and Pfizer. EB received grants from Merck. FB received grants from Astellas and speaker's fees from Merck. TC received speaker's fees from Merck and Gilead. MC received travel grants from Gilead, Pfizer and Merck and received remuneration for talks on behalf of Pfizer. FD received grants from Astellas, Pfizer and Merck. AF received funds for speaking from Merck; for consultancy from Pfizer; and for travel from Astellas, Gilead, Merck and Pfizer. JPG received speaker's fees from Astellas, MSD, Gilead and Pfizer. CH received travel grants from MSD, Astellas, Pfizer and Gilead and fees for oral speaker by MSD and Astellas. YLG received grant support from Merck. PLP is a consultant to Basilea and received grants from Astellas and Pfizer and speaker's fees from Merck and Gilead. DM received travel grants from Pfizer. BS received grant support from Astellas, Merck, and bio-Mérieux. JC received grants from Astellas, Pfizer and Merck and speaker's fees from Astellas and Pfizer. SC, TC, ADa, ADu, JG, SR, and MS declare no conflict of interest.

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Table 1: Distribution of micafungin MICs (µg/ml) for different *Candida* species (n=933) determined by the EUCAST broth microdilution method

Species (number of isolates)	Number of isolates with an MIC (µg/ml) of									% R/non WT*
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	
C. albicans (159)	157	1	1							1.3
C. glabrata (152)	137	9	4			1	1			3.9
C. parapsilosis (152)				1	5	13	79	52	2	1.3
C. tropicalis (152)	97	48	6				1			0.7
C. kefyr (136)	7	67	49	13						ND
C. krusei (127)	3	1	59	56	8					0
C. lusitaniae (23)		5	16	2						ND
Other *Candida* spp.# (32)	11	6	3	1	1	5	5			ND
All isolates (933)	412	137	138	73	14	19	86	52	2	

ND: not determined.

* Resistance (R) or non-wild-type (WT) was defined based on EUCAST clinical breakpoints or ECOFFs when clinical breakpoints were not available.

C. guilliermondii (9), *C. norvegensis* (5), *C. inconspicua* (5), *C. famata* (3), *C. pelliculosa* (2), *C. lambica* (2), *C. sphaerica* (1), *C. ciferrii* (1), *C. catenulata* (1), *C. utilis* (1), *C. colliculosa* (1), *C. nivariensis* (1).

Table 2 *In vitro* susceptibilities of the 933 *Candida* isolates to micafungin as determined by the Etest® method and EUCAST broth microdilution method

	Etest® MIC (µg/ml)				EUCAST MIC (µg/ml)				
Species (number of isolates)	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	**Essential agreement[#]**
C. albicans (159)	≤0.015 - 0.06	0.015	0.015	0.016	≤0.015 - 0.06	0.015	0.015	0.016	100
C. glabrata (152)	≤0.015 - 0.125	0.015	0.015	0.016	≤0.015 - 1	0.015	0.015	0.018	98.7
C. parapsilosis (152)	0.06 - 4	0.5	2	0.63	≤0.125 - 4	1	2	1.15	96.7
C. tropicalis (152)	≤0.015 - 0.5	0.015	0.03	0.019	≤0.015 - 1	0.015	0.03	0.021	99.3
C. kefyr (136)	≤0.015 - 0.25	0.03	0.125	0.036	≤ 0.015 - 0.125	0.03	0.06	0.044	97.8
C. krusei (127)	≤0.015 - 0.25	0.125	0.125	0.084	≤0.015 - 0.25	0.125	0.125	0.089	98.4
Other *Candida* spp.* (55)	≤0.015 - 1	0.03	0.25	0.057	≤0.015 - 1	0.06	0.5	0.068	98.2
Total number (933)	≤0.015 - 4	0.03	0.5	0.046	≤0.015 - 4	0.03	1	0.054	98.5

**C. lusitaniae* (23), *C. guilliermondii* (9), *C. norvegensis* (5), *C. inconspicua* (5), *C. famata* (3), *C. pelliculosa* (2), *C. lambica* (2), *C. sphaerica* (1), *C. ciferrii* (1), *C. catenulata* (1), *C. utilis* (1), *C. colliculosa* (1), *C. nivariensis* (1).

[#]± 2 $\log_2$ dilutions.

287 **Table 3 Categorical agreement between the EUCAST and Etest® methods for *in vitro* susceptibility testing of the major pathogenic**
288 ***Candida* species to micafungin***
289

Species (number of isolates)	Categorical agreement		Minor error		Major error		Very major error	
	n	%	n	%	n	%	n	%
C. albicans (159)	154	96.9	-	-	3	1.9	2	1.2
C. glabrata (152)	147	96.7	-	-	1	0.7	4	2.6
C. parapsilosis (152)	151	99.3	1	0.7	0	0	0	0
C. tropicalis (152)	150	98.7	-	-	2	1.3	0	0
C. krusei (127)	127	100	-	-	0	0	0	0
All isolates (742)	729	98.2	1	0.1	6	0.8	6	0.8

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291 * For both techniques, categorization of isolates as resistant or non-wild-type was defined based on EUCAST endpoints (clinical breakpoints or
292 ECOFFs when clinical breakpoints were not available)

293 **Figure 1 Correlation between EUCAST and Etest® methods for *in vitro* susceptibility testing of 933 *Candida* isolates to micafungin**

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Etest MIC (µg/ml) \ EUCAST MIC (µg/ml)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	Total
0.015	381	63	18	3*		1*				466
0.03	24	54	30	15	2*					125
0.06	5	16	47	18	4					90
0.125	2*	4	41	28	6	1	2*			84
0.25			2	9	2	10	11	4*		38
0.5						5	39	16		60
1						2	28	22	1	53
2							6	10		16
4									1	1
Total	412	137	138	73	14	19	86	52	2	933

*: number of isolates with more than 2 Log_2 dilution differences between Etest® and EUCAST

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Etest MIC (µg/ml) \ EUCAST MIC (µg/ml)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	Total
0.015	381	63	18	3*		1*				466
0.03	24	54	30	15	2*					125
0.06	5	16	47	18	4					90
0.125	2*	4	41	28	6	1	2*			84
0.25			2	9	2	10	11	4*		38
0.5						5	39	16		60
1						2	28	22	1	53
2							6	10		16
4									1	1
Total	412	137	138	73	14	19	86	52	2	933

*: number of isolates with more than 2 Log_2 dilution differences between Etest® and EUCAST