

Multicenter comparison of the etest and EUCAST methods for antifungal susceptibility testing of *Candida* isolates to micafungin

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1 **Multicenter comparison of the Etest® and EUCAST for antifungal**

2 **susceptibility testing of *Candida* isolates to micafungin**

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63

64 **Abstract**

65 *In vitro* susceptibility of 933 *Candida* isolates, from 16 French hospitals, to micafungin was
66 determined using the Etest® in each center. All isolates were centralized for determination of
67 minimum inhibitory concentrations (MICs) by the EUCAST reference method. Overall
68 essential agreement between the two tests was 98.5% at $\pm 2 \log_2$ dilutions and 90.2% at ± 1
69 \log_2 dilutions. Categorical agreement was 98.2%. The Etest® is a valuable alternative to
70 EUCAST for the routine determination of micafungin MICs in medical mycology laboratories.
71

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

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

85 With this aim, a prospective, multicenter French study was performed to compare the
86 EUCAST and Etest® methods for micafungin susceptibility testing of a large panel of clinical
87 isolates of different *Candida* species. Sixteen centers (six in Paris area and 10 across France)
88 participated in the study. Over a 2-month period, each center was asked to test 64 *Candida*
89 isolates, from any clinical sample, of the following species: 10 isolates of each of the six most
90 common pathogenic species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr*
91 and *C. krusei*) and four isolates belonging to other *Candida* species. Species identification
92 was performed in each center according to the currently recommended phenotypic methods
93 (12). Micafungin susceptibility testing was performed using the Etest® (Biomérieux, Marcy
94 l'Etoile, France), according to the manufacturer's instructions. *Candida* isolates were then
95 centralized in a single center for MIC determination by the EUCAST reference method (13).
96 *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 $\mu\text{g/ml}$,
104 ≤ 0.03 / >0.03 $\mu\text{g/ml}$, and ≤ 0.002 / >2 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$ and ≤ 0.06 / >0.06 $\mu\text{g/ml}$, respectively. MIC results obtained by the two methods were
107 considered to be in essential agreement when they were within $\pm 2 \log_2$ dilutions. Agreement
108 at $\pm 1 \log_2$ 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 $\mu\text{g/ml}$) were several dilutions higher than for the other common species (modal MIC of 0.015
117 $\mu\text{g/ml}$ for *C. albicans*, *C. tropicalis*, and *C. glabrata* and 0.03 and 0.06 $\mu\text{g/ml}$ 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

122 0.7% for *C. tropicalis* and 0% for *C. krusei*. The overall essential agreement between
123 EUCAST and Etest® results was high (98.5% at $\pm 2 \log_2$ dilutions and 90.2% at $\pm 1 \log_2$
124 dilution) (Figure 1) with minor differences between species (Table 2). The lowest essential
125 agreement (96.7% at $\pm 2 \log_2$ dilutions) was observed for *C. parapsilosis*. An overall
126 categorical agreement of 98.2% was observed for the 742 isolates belonging to the five
127 species for which clinical breakpoints or ECOFFs are available (Table 3). The highest (100%)
128 and lowest (96.7%) categorical agreements were found for *C. krusei* and *C. glabrata*,
129 respectively. Major errors were observed in six cases (three *C. albicans*, two *C. tropicalis*, and
130 one *C. glabrata*) and very major errors in six cases (two *C. albicans* and four *C. glabrata*).
131 These 12 discrepancies were observed for strains isolated and tested in eight different centers.
132 The Etest® has been used in several studies for micafungin susceptibility testing of *Candida*
133 spp. (17-22), but only a few comparative studies with a reference method have been
134 performed (17, 20-22). In one of these previous studies, Marcos-Zambrano *et al.* (21) tested
135 160 yeast isolates with both the Etest® and EUCAST methods and reported an essential
136 agreement of 90.3% at $\pm 2 \log_2$ dilutions (85.8% at $\pm 1 \log_2$ dilution) and a categorical
137 agreement of >90%. Similarly, in another study, a comparison between Etest® and CLSI
138 methods showed an overall essential agreement of 94.7% and a categorical agreement of
139 97.2% (20). The ability of the Etest® to detect micafungin resistance, for most of the species,
140 has also been demonstrated previously by testing *FKS* mutant isolates (17, 21, 22). We
141 enrolled 16 centers and demonstrated that under real-life conditions the Etest® gave very
142 similar micafungin susceptibility results to the EUCAST reference method.
143 Altogether, our results show that the Etest® is a valuable and reliable method to routinely test
144 the *in vitro* susceptibility of clinical *Candida* isolates to micafungin. *In vitro* micafungin
145 resistance among the main *Candida* species isolated from clinical samples remains
146 uncommon in France.

147

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169

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270
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272 **Table 1: Distribution of micafungin MICs ($\mu\text{g/ml}$) for different *Candida* species (n=933)**
 273 **determined by the EUCAST broth microdilution method**

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

274

275 ND: not determined.

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

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

281 **Table 2** *In vitro* susceptibilities of the 933 *Candida* isolates to micafungin as determined by the Etest® method and EUCAST broth
 282 microdilution method
 283

Species (number of isolates)	Etest® MIC (µg/ml)				EUCAST MIC (µg/ml)				Essential agreement [#]
	Range	MIC ₅₀	MIC ₉₀	GM	Range	MIC ₅₀	MIC ₉₀	GM	
<i>C. albicans</i> (159)	≤0.015 - 0.06	0.015	0.015	0.016	≤0.015 - 0.06	0.015	0.015	0.016	100
<i>C. glabrata</i> (152)	≤0.015 - 0.125	0.015	0.015	0.016	≤0.015 - 1	0.015	0.015	0.018	98.7
<i>C. parapsilosis</i> (152)	0.06 - 4	0.5	2	0.63	≤0.125 - 4	1	2	1.15	96.7
<i>C. tropicalis</i> (152)	≤0.015 - 0.5	0.015	0.03	0.019	≤0.015 - 1	0.015	0.03	0.021	99.3
<i>C. kefyr</i> (136)	≤0.015 - 0.25	0.03	0.125	0.036	≤0.015 - 0.125	0.03	0.06	0.044	97.8
<i>C. krusei</i> (127)	≤0.015 - 0.25	0.125	0.125	0.084	≤0.015 - 0.25	0.125	0.125	0.089	98.4
Other <i>Candida</i> 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

284 **C. lusitanae* (23), *C. guilliermondii* (9), *C. norvegensis* (5), *C. inconspicua* (5), *C. famata* (3), *C. pelliculosa* (2), *C. lambica* (2), *C. sphaerica*
 285 (1), *C. cijferrii* (1), *C. catenulata* (1), *C. utilis* (1), *C. colliculosa* (1), *C. nivariensis* (1).

286 [#] ± 2 log₂ 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	%
<i>C. albicans</i> (159)	154	96.9	-	-	3	1.9	2	1.2
<i>C. glabrata</i> (152)	147	96.7	-	-	1	0.7	4	2.6
<i>C. parapsilosis</i> (152)	151	99.3	1	0.7	0	0	0	0
<i>C. tropicalis</i> (152)	150	98.7	-	-	2	1.3	0	0
<i>C. krusei</i> (127)	127	100	-	-	0	0	0	0
All isolates (742)	729	98.2	1	0.1	6	0.8	6	0.8

290

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

294

Etest MIC (µg/ml)	EUCAST MIC (µg/ml)									Total
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	
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

295

*: number of isolates with more than 2 Log₂ dilution differences between Etest® and EUCAST

Etest MIC ($\mu\text{g/ml}$)	EUCAST MIC ($\mu\text{g/ml}$)									Total
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0.015	381	63	18	3*		1*				466
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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 Log₂ dilution differences between Etest® and EUCAST