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Multicenter Comparison of the Etest and EUCAST Methods for Antifungal Susceptibility Testing of *Candida* Isolates to Micafungin

M.-E. Bougnoux,[a] E. Dannaoui,[b] I. Accoceberry,[c] A. Angoulvant,[d] E. Bailly,[e] F. Botterel,[f] S. Chevrier,[g] T. Chouaki,[h] M. Cornet,[i] F. Dalle,[j] A. Datry,[k] A. Dupuis,[a] A. Fekkar,[k,l] J. P. Gangneux,[g] J. Guitard,[l,m] C. Hennequin,[l,m] Y. Le Govic,[n] P. Le Pape,[o] D. Maubon,[i] S. Ranque,[p] M. Sautour,[k] B. Sendid,[q] J. Chandenier[e,r]

Université Paris-Descartes, Faculté de Médecine, and Unité de Parasitologie-Mycologie, Service de Microbiologie, Hôpital Necker Enfants Malades, Assistance Publique—Hôpitaux de Paris, Paris, France[a]; Université Paris-Descartes, Faculté de Médecine, and Unité de Parasitologie-Mycologie, Service de Microbiologie, Hôpital Européen Georges Pompidou, Assistance Publique—Hôpitaux de Paris, Paris, France[b]; Laboratoire de Parasitologie-Mycologie, Centre hospitalier Universitaire de Bordeaux, Bordeaux, France[c]; Assistance Publique—Hôpitaux de Paris, Hôpital de Bicêtre, Laboratoire de Parasitologie-Mycologie, and Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, CNRS UMR 8621, Génétique Quantitative et Evolution, Le Moulon, France[d]; Service de Parasitologie-Mycologie-Médecine tropicale Centre Hospitalier Régional Universitaire, Tours, France[e]; Hôpital Henri Mondor, DHU VIC, APHP, and EA 7380, DYNAMYC, Université Paris-Est Créteil, Créteil, France[f]; Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire, and Université Rennes 1 Inserm U1085—IRSET, Rennes, France[g]; Centre Hospitalo-Universitaire, Amiens, France[h]; Laboratoire de Parasitologie-Mycologie, Centre Hospitalo-Universitaire Grenoble, and Université Grenoble Alpes, Grenoble, France[i]; Laboratoire de Parasitologie Mycologie, Centre Hospitalo-Universitaire, and Université Bourgogne—Franche Comté, UMR PAM, Dijon, France[j]; AP-HP, Groupe Hospitalier La Pitié-Salpêtrière, Service de Parasitologie Mycologie, Paris, France[k]; Sorbonne Universités, UPMC Univ Paris 06, INSERM U1135, CNRS ERL 8255, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Paris, France[l]; AP-HP, Hôpital St Antoine, Service de Parasitologie-Mycologie, Paris, France[m]; Laboratoire de Parasitologie-Mycologie, Institut de Biologie en Santé-PBH, Centre Hospitalier Universitaire, Angers, France[n]; Laboratoire de Parasitologie-Mycologie, Institut de Biologie, Centre Hospitalier Universitaire de Nantes, Nantes, France[o]; Parasitologie & Mycologie, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, and IP-TPT UMR MD3, Aix-Marseille Université, Marseille, France[p]; Centre Hospitalier Universitaire de Lille, Université de Lille, Inserm U995-Team 2, and Inflammation Research International Center (LIRIC), Lille, France[q]; Université François Rabelais, INSERM U1100, Centre d'Étude des Pathologies Respiratoires (CEPR), Tours, France[r]

***In vitro* susceptibility of 933 *Candida* isolates, from 16 French hospitals, to micafungin was determined using the Etest in each center. All isolates were then sent to a single center for determination of 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.**

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 in 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 those published by 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 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.

(This study was presented in part at the 25th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Copenhagen, Denmark, 25 to 28 April 2015.)

Sixteen centers (6 in the Paris area and 10 elsewhere 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 (*Candida 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 sent to 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 strains (14). For comparison purposes, Etest MICs were increased to the

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Address correspondence to E. Dannaoui, eric.dannaoui@egp.aphp.fr.

TABLE 1 Distribution of micafungin MICs for different *Candida* species determined by the EUCAST broth microdilution method

Species (no. of isolates)	No. of isolates with an MIC (μg/ml) of:									% R/non-WT[a]
	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[b]
C. krusei (127)	3	1	59	56	8					0
C. lusitaniae (23)		5	16	2						ND
Other *Candida* spp. ($n = 32$)[c]	11	6	3	1	1	5	5			ND
All isolates ($n = 933$)	412	137	138	73	14	19	86	52	2	

[a] Resistance (R) and non-wild-type susceptibility (WT) were defined based on EUCAST clinical breakpoints or ECOFFs when clinical breakpoints were not available.
[b] ND, not determined.
[c] Other *Candida* spp. included *C. guilliermondii* (9 isolates), *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), and *C. nivariensis* (1).

next higher corresponding EUCAST concentration (15). Resistance was based on EUCAST clinical breakpoints. When clinical breakpoints were not available (i.e., for *C. krusei* and *C. tropicalis*), epidemiological cutoff values (ECOFFs) were used to categorize isolates as non-wild-type isolates (16). The same ECOFFs (defined by EUCAST) were used for analyzing Etest results, as Etest-specific ECOFFs have not yet been determined. *C. albicans*, *C. glabrata*, and *C. parapsilosis* isolates were considered susceptible or resistant to micafungin when MICs were ≤0.016 or >0.016 μg/ml, ≤0.03 or >0.03 μg/ml, and ≤0.002 or >2 μg/ml, respectively. *C. krusei* and *C. tropicalis* isolates were considered wild-type isolates or non-wild-type isolates with respect to micafungin susceptibility when MICs were ≤0.25 or >0.25 μg/ml and ≤0.06 or >0.06 μg/ml, respectively. MIC results obtained by the two methods were considered to be in essential agreement when they were within ±2 $\log_2$ dilutions. Agreement at ±1 $\log_2$ dilutions was also calculated. Categorical agreement was defined as the percentage of isolates classified in the same category (i.e., as susceptible, intermediate, or resistant isolates and wild-type or non-wild-type isolates) by both techniques (15). Discrepancies (very major, major, and minor errors) were defined as described previously (15).

Results from antifungal susceptibility testing were available for 933 *Candida* isolates, including 878 isolates of the six most medically important *Candida* species and 55 other *Candida* species. Table 1 shows the micafungin MICs for the 933 isolates determined by the EUCAST reference method. Micafungin MICs for *C. parapsilosis* isolates (modal MIC of 1 μg/ml) were several dilutions higher than for the other common species (modal MIC of 0.015 μg/ml for *C. albicans*, *C. tropicalis*, and *C. glabrata* and 0.03 and 0.06 μg/ml for *C. kefyr* and *C. krusei*, respectively). MICs for rare species were similar to those of the common species except for *C. colliculosa* and some isolates of *C. guilliermondii* and *C. famata*. According to the current clinical breakpoints (16), the micafungin resistance rates were <2% for *C. albicans* and *C. parapsilosis* and 3.9% for *C. glabrata*. Based on ECOFFs, the rates for the non-wild-type isolates were 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 $\log_2$ dilutions) (Fig. 1), with minor differences between species (Table 2). The lowest essential agreement (96.7% at ±2 log 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 were 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* isolates, two *C.*

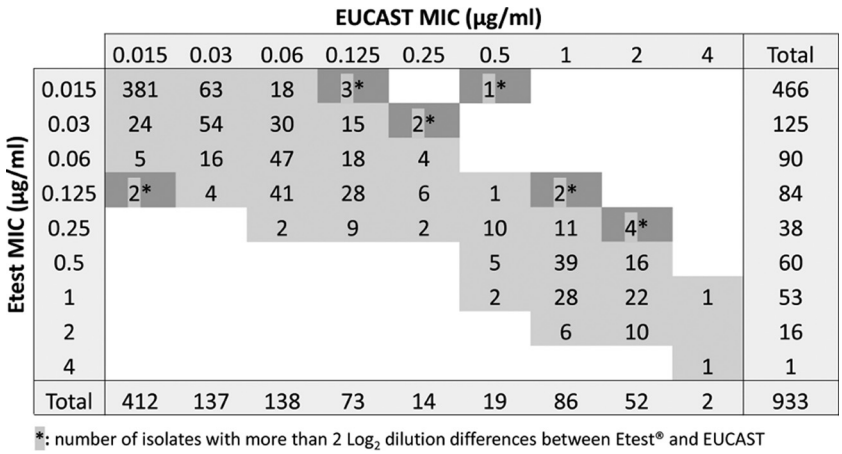

FIG 1 Correlation between the EUCAST and Etest methods for *in vitro* testing of the susceptibility of 933 *Candida* isolates to micafungin.

TABLE 2 *In vitro* susceptibilities of the 933 *Candida* isolates to micafungin as determined by the Etest method and EUCAST broth microdilution method[a]

Species (no. of isolates)	Etest MIC (μg/ml)				EUCAST MIC (μg/ml)				% essential agreement
	Range	MIC_{50}	MIC_{90}	GM	Range	MIC_{50}	MIC_{90}	GM	
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.[b] (55)	≤0.015–1	0.03	0.25	0.057	≤0.015–1	0.06	0.5	0.068	98.2
Total (933)	≤0.015–4	0.03	0.5	0.046	≤0.015–4	0.03	1	0.054	98.5

[a] GM, geometric mean. Percent essential agreement data represent ±2 $\log_2$ dilutions.
[b] Other *Candida* spp. included *C. lusitaniae* (23 isolates), *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 3 Categorical agreement between the EUCAST and Etest methods for *in vitro* testing of susceptibility of the major pathogenic *Candida* species to micafungin[a]

Species (total no. of isolates)	Categorical agreement		Minor error		Major error		Very major error	
	No. of isolates	% of isolates	No. of isolates	% of isolates	No. of isolates	% of isolates	No. of isolates	% of isolates
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

[a] For both techniques, categorization of isolates as resistant or non-wild-type isolates was performed based on EUCAST endpoints (clinical breakpoints or ECOFFs when clinical breakpoints were not available).

tropicalis isolates, and one *C. glabrata* isolate) and very major errors in six cases (two *C. albicans* isolates and four *C. glabrata* isolates). 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 those 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 $\log_2$ dilutions) and 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 the Etest gave micafungin susceptibility results that were very similar to those given by the EUCAST reference method under real-life conditions.

Taken together, our results show that the Etest is a valuable and reliable method for routine testing of the *in 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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