AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

CrossMark click for updates

Intra- and Interlaboratory Agreement in Assessing the *In Vitro* Activity of Micafungin against Common and Rare *Candida* Species with the EUCAST, CLSI, and Etest Methods

J. Meletiadis,[a,b] E. Geertsen,[c] I. Curfs-Breuker,[c] J. F. Meis,[b,c,d] J. W. Mouton[b]

Clinical Microbiology Laboratory, Attikon University Hospital, National and Kapodistrian University of Athens, Athens, Greece[a]; Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, The Netherlands[b]; Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands[c]; Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands[d]

The emergence of resistant strains among common and rare *Candida* species necessitates continuous monitoring of the *in vitro* susceptibilities of those isolates. We therefore assessed the *in vitro* activities of micafungin against 1,099 molecularly identified isolates belonging to 5 common and 20 rare *Candida* species by the EUCAST, CLSI, and Etest methods, assessing both the intralaboratory agreement and the interlaboratory agreement for two centers. The median micafungin EUCAST MICs were as follows, from the lowest to the highest: for *Candida albicans*, 0.004 mg/liter; for *C. glabrata*, 0.016 mg/liter; for *C. tropicalis*, 0.031 mg/liter; for *C. krusei*, 0.125 mg/liter; for *C. parapsilosis*, 2 mg/liter. Among rare *Candida* species, high MICs were found for *C. guilliermondii*, *C. lipolytica*, *C. orthopsilosis*, *C. metapsilosis*, and *C. fermentati*. No resistant isolates were found by the CLSI method, whereas resistance rates of 1 to 2% were found by the EUCAST method. Overall, the EUCAST method resulted in MICs 1 to 2 dilutions higher than those found by the CLSI and Etest methods. The intra- and interlaboratory agreement between methods was >92%, except for the interlaboratory agreement between the EUCAST and CLSI methods (81%), where 17 to 31% of the differences were >2 2-fold dilutions for *C. albicans*, *C. glabrata*, *C. tropicalis*, and other rare *Candida* species and <6% for *C. parapsilosis* and *C. krusei*. For the other interlaboratory comparisons, the EUCAST method resulted in higher MICs than the Etest method for all species, but <7% of these differences were >2 2-fold dilutions. Overall, the CLSI method resulted in lower MICs than the Etest method, with 11% of all isolates demonstrating >2 2-fold-dilution differences (6 to 20% for *C. albicans*, *C. tropicalis*, and rare *Candida* species; <5% for *C. glabrata*, *C. krusei*, and *C. parapsilosis*) and smaller differences found after 24 h. Despite these differences, categorical agreement was excellent (>97%), with only 1 to 2% very major errors between the EUCAST method and the other two methods.

Micafungin is an echinocandin used for the treatment and prophylaxis of *Candida* infections in both neutropenic and nonneutropenic patients (1, 2). Although micafungin has a broad spectrum of activity against different *Candida* species, including azole-resistant isolates, the emergence of resistance in common and rare *Candida* species necessitates *in vitro* susceptibility testing of those isolates (3). Methods for the determination of MICs have recently been published by the Clinical and Laboratory Standards Institute (CLSI) (4) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (5). Both of these methods are broth microdilution methods; they differ in glucose concentration, inoculum size, the shape of microplates, and the mode of reading. In addition, the Etest is often used to determine susceptibility in routine laboratories (6).

Although all these methods are reproducible and reliable in testing the *in vitro* susceptibility of *Candida* isolates, there are few comparative studies exploring the differences between them (6, 7). Most of those studies utilized small collections of isolates for common species tested in a single lab. In addition, in light of species-specific breakpoints, correct species identification is of paramount importance both for assessing *in vitro* susceptibility and for comparing methods. Correct identification is particularly challenging for rare species, where phenotypic tests have usually failed (8). We therefore assess the *in vitro* activities of micafungin against 1,099 molecularly identified isolates belonging to 5 common and 20 rare *Candida* species with the EUCAST, CLSI, and Etest methods, measuring both intralaboratory agreement (in one lab) and interlaboratory agreement (between two labs).

MATERIALS AND METHODS

Isolates. A total of 1,099 *Candida* isolates were collected from 871 patients with no prior echinocandin exposure who entered into clinical trials of invasive and esophageal candidiasis from 2002 to 2004. The isolates were all identified using molecular techniques, including amplified fragment length polymorphism (AFLP) and internal transcribed spacer (ITS) analysis, as described elsewhere (9). They included 584 *Candida albicans*, 180 *C. tropicalis*, 122 *C. parapsilosis*, 86 *C. glabrata*, and 30 *C. krusei* isolates, as well as 97 isolates belonging to other *Candida* species (20 *C. guilliermondii*, 15 *C. orthopsilosis*, 11 *C. lusitaniae*, 8 *C. dubliniensis*, 7 *C. rugosa*, 7 *C. kefyr*, 6 *C. pelliculosa*, 5 *C. fabianii*, 3 *C. lipolytica*, 3 *C. metapsilosis*, 2 *C. utilis*, 2 *C. fermentati*, 2 *C. intermedia*, 1 *C. inconspicua*, 1 *C. pararugosa*, 1 *C. famata*, 1 *C. palmioleophila*, 1 *C. xestobii*, and 1 *C. viswanathii* isolate).

Received 13 May 2016 Returned for modification 29 June 2016
Accepted 29 July 2016

Accepted manuscript posted online 1 August 2016

Citation Meletiadis J, Geertsen E, Curfs-Breuker I, Meis JF, Mouton JW. 2016. Intra- and interlaboratory agreement in assessing the *in vitro* activity of micafungin against common and rare *Candida* species with the EUCAST, CLSI, and Etest methods. Antimicrob Agents Chemother 60:6173–6178. doi:10.1128/AAC.01027-16.

Address correspondence to J. Meletiadis, jmeletiadis@med.uoa.gr.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

The quality control (QC) strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used in each experiment.

Susceptibility testing. *In vitro* testing of susceptibility to the antifungal micafungin was performed according to the EUCAST method (EDef 7.2) and the CLSI M27-A3 method (4, 10). The Etest (bioMérieux, Solna, Sweden) was performed according to the manufacturer's instructions and was read after 24 h and 48 h.

Analysis. Micafungin MICs were determined by each method in two different laboratories for all 1,099 isolates. The intralaboratory agreement among the three methods was calculated for a selection of 200 isolates (50 *C. albicans*, 40 *C. parapsilosis*, 40 *C. glabrata*, 40 *C. tropicalis*, and 30 *C. krusei* isolates), which were tested in parallel by each method. The median MIC and the range of MICs were calculated for each species separately and for all isolates together in both the intralaboratory and the interlaboratory comparison. The percentages of susceptible (S), intermediate (I), and resistant (R) isolates were calculated for each method and for the five common species *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei* using the EUCAST and CLSI breakpoints. For *C. tropicalis* and *C. krusei*, for which no EUCAST breakpoints have been determined, the epidemiological cutoff values of 0.064 and 0.25 mg/liter, respectively, were used. For the Etest, given the lack of method-specific breakpoints, the susceptibility categories were determined using either CLSI or EUCAST breakpoints solely for comparison, and the essential agreement rates between the Etest and each of the two reference methods were also included in the analysis.

To compare the methods, MIC values for each strain were transformed by taking the $\log_2$. For a proper comparison, Etest values were rounded to the next highest value in a 2-fold dilution range. The differences were assessed statistically with a paired *t* test adjusted for multiple comparisons using Bonferroni's adjustment. The *t* test assessed whether the differences observed between MICs were systematic or were due to random error. In order to assess the microbiological significance of these differences, the percentage of isolates with >2 2-fold differences was calculated. Finally, in order to assess the potential clinical significance of these differences, the percentages of minor, major, and very major errors were calculated as the percentage of isolates classified either S or R with one method and I with the reference comparator method (or vice versa), R with one method and S with the reference comparator method, and S with one method and R with the reference comparator method, respectively. The reference method compared to the Etest method was either the EUCAST or the CLSI method; the reference method compared to the EUCAST method was the CLSI method. For the Etest method, analysis was performed for 24-h and 48-h results.

RESULTS

Tables 1 and 2 show the micafungin MIC data for *Candida* species for each method and the intra- and interlaboratory agreement, respectively. The median micafungin MICs were ranked from low to high as follows: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*. This order was more pronounced with EUCAST (0.004, 0.016, 0.031, 0.125, and 2 mg/liter, respectively) than with the CLSI and Etest methods. The median MICs for the other *Candida* species were close to the *C. krusei* MICs. Table 3 shows the MICs of rare *Candida* species, with the highest MICs (EUCAST MICs, >0.25 mg/liter) observed for *C. guilliermondii*, *C. lipolytica*, *C. orthopsilosis*, *C. metapsilosis*, *C. fermentati*, and *C. xestobii*, followed by *C. lusitaniae*, *C. rugosa*, and *C. pararugosa* (EUCAST MICs, 0.125 to 0.25 mg/liter), whereas the other *Candida* species had lower MICs (EUCAST MICs, <0.125 mg/liter). Similar differences were found with the CLSI and Etest methods.

Intralaboratory agreement. In the intralaboratory comparison, there were statistically significant differences between the EUCAST and CLSI methods, which were most pronounced for *C. albicans* and *C. parapsilosis*, for which average EUCAST MICs

TABLE 1 Intralaboratory agreement between test methods[a]

Species (no. of isolates)	Median (range) MIC (mg/liter) by:				Mean (range) difference (% of isolates with differences of >2 2-fold dilutions)				
	EUCAST	CLSI	Etest (24 h)	Etest (48 h)	EU vs CL	EU vs Et24	EU vs Et48	CL vs Et24	CL vs Et48
C. albicans (50)	0.016 (0.004–0.016)	0.004 (≤0.002–0.016)	0.008 (0.004–0.016)	0.012 (0.008–0.016)	1.1 (−1 to 3)* (4)	0.3 (−1 to 2) (0)	0 (−2 to 1) (0)	0.8 (−1 to 3)* (6)	−1.1 (−3 to 1)* (8)
C. glabrata (40)	0.016 (0.008–0.016)	0.016 (0.016–0.031)	0.008 (0.004–0.008)	0.008 (0.004–0.016)	−0.3 (−1 to 0)* (0)	0.9 (0 to 2)* (0)	0.8 (0 to 2)* (0)	−0.8 (−2 to 6)* (5)	1 (0 to 2)* (0)
C. tropicalis (40)	0.063 (0.016–0.125)	0.031 (0.008–0.031)	0.016 (0.008–0.032)	0.016 (0.016–0.032)	0.5 (−1 to 2) (0)	1.3 (0 to 3)* (3)	0.9 (0 to 2)* (0)	−0.8 (−2 to 1)* (0)	0.4 (−1 to 1)* (0)
C. parapsilosis (40)	1 (1–4)	1 (0.25–1)	0.5 (0.25–1)	1 (0.5–2)	0.9 (0 to 2)* (0)	1.3 (0 to 3)* (3)	0.7 (0 to 2)* (0)	−0.4 (−2 to 2) (0)	−0.2 (−2 to 1) (0)
C. krusei (30)	0.125 (0.063–0.25)	0.125 (0.031–0.25)	0.063 (0.032–0.125)	0.063 (0.032–0.125)	0.2 (−1 to 2) (0)	1.2 (0 to 2)* (0)	1 (0 to 2)* (0)	−0.9 (−3 to 1)* (3)	0.8 (−1 to 2)* (0)
All (200)	0.016 (0.004–4)	0.016 (0.002–1)	0.016 (0.004–1)	0.016 (0.004–2)	0.5 (−1 to 3)* (1)	0.9 (−1 to 3)* (1)	0.6 (−2 to 2)* (0)	0.1 (−3 to 2) (2)	−0.3 (−3 to 6)* (3)

[a] Comparison of results obtained by the EUCAST method (EU), the CLSI method (CL), and the Etest read after 24 h (Et24) or 48 h (Et48) in one laboratory. Median (range) MICs are presented for each method, whereas for the comparison, the average (range) 2-fold-dilution difference is shown (together with the percentage of isolates with differences of ≥2 2-fold dilutions). Asterisks indicate a *P* value of <0.001 by a paired *t* test.

TABLE 2 Interlaboratory agreement between test methods[a]

Species (no. of isolates)	Median (range) MIC (mg/liter) by:				Mean (range) difference (% of isolates with differences of >2 2-fold dilutions)				
	EUCAST	CLSI	Etest (24 h)	Etest (48 h)	EU vs CL	EU vs Et24	EU vs Et48	CL vs Et24	CL vs Et48
C. albicans (584)	0.004 (≤0.002–0.016)	0.002 (≤0.002–0.016)	0.008 (0.004–0.016)	0.008 (0.008–0.016)	1.1 (−2 to 4)* (17)	0.1 (−2 to 2) (0)	−0.1 (−2 to 2)* (0)	−1 (−9 to 1)* (6)	−1.2 (−4 to 1)* (11)
C. glabrata (86)	0.016 (0.004–0.031)	0.008 (≤0.002–0.016)	0.008 (0.004–0.008)	0.008 (0.004–0.016)	1.2 (−2 to 4)* (19)	1 (−1 to 2)* (0)	0.9 (−1 to 2)* (0)	−0.5 (−9 to 2) (5)	−0.2 (−2 to 2) (0)
C. tropicalis (180)	0.031 (0.031–0.031)	0.008 (≤0.002–0.031)	0.016 (0.008–0.016)	0.016 (0.008–0.032)	1.7 (−5 to 6)* (31)	1.1 (0 to 3)* (2)	0.7 (0 to 3)* (1)	−0.6 (−3 to 6)* (17)	−0.9 (−4 to 5)* (20)
C. parapsilosis (122)	2 (1–2)	0.5 (0.25–2)	0.5 (0.25–1)	1 (0.5–4)	1.1 (0 to 8)* (6)	1.4 (0 to 3)* (4)	0.8 (−1 to 2)* (0)	0.3 (−7 to 2) (1)	−0.3 (−8 to 2) (2)
C. krusei (30)	0.125 (0.063–0.25)	0.094 (0.031–0.125)	0.064 (0.032–0.125)	0.064 (0.032–0.125)	0.8 (0 to 2)* (0)	1.2 (0 to 2)* (0)	1 (0 to 2)* (0)	0.4 (−1 to 2) (0)	0.2 (−1 to 2) (0)
Other *Candida* spp. (97)	0.1875 (0.016–1)	0.094 (≤0.002–4)	0.032 (0.008–0.5)	0.063 (0.008–1)	1.4 (−5 to 9)* (28)	1.5 (−1 to 4)* (7)	0.9 (−1 to 3)* (4)	0 (−7 to 7) (15)	−0.5 (−8 to 6) (20)
All strains (1,099)	0.016 (0.004–2)	0.008 (≤0.002–2)	0.012 (0.008–1)	0.016 (0.008–2)	1.2 (−5 to 9)* (19)	0.6 (−2 to 4)* (1)	0.3 (−2 to 3)* (1)	−0.6 (−9 to 7)* (8)	−0.9 (−8 to 6)* (11)

[a] Comparison of results by the EUCAST method (EU), the CLSI method (CL), and the Etest read after 24 h (Et24) and 48 h (Et48), determined in different laboratories. Median (range) MICs are presented for each method, whereas for the comparison, the average (range) 2-fold-dilution difference is shown (together with the percentage of isolates with differences of >2 2-fold dilutions). Asterisks indicate a *P* value of <0.001 by a paired *t* test.

were 1 2-fold dilution higher than average CLSI MICs, whereas for the other *Candida* species, the average differences were <0.5 2-fold dilution (Table 1). However, <4% of those differences were >2 2-fold dilutions. EUCAST MICs were ca. 1 dilution higher than Etest method MICs for the non-*C. albicans* species. In contrast, MICs for non-*C. albicans* species by the CLSI method were lower than those obtained by the Etest method after 24 h, whereas the opposite was observed for *C. albicans*. However, <6% of those differences were >2 2-fold dilutions.

Interlaboratory agreement. The differences found in the intralaboratory comparison were consistent and enlarged in the interlaboratory comparison (Table 2). Overall, the average MICs were 1 to 2 2-fold dilutions higher for the EUCAST method than for the CLSI method, with 19% of the isolates demonstrating differences of >2 2-fold dilutions. This percentage ranged from 0% for *C. krusei* to 31% for *C. tropicalis*. With the exception of C. *albicans*, where similar MICs were found, the average EUCAST MICs were 0.9 to 1.7 dilutions higher than the Etest MICs, particularly after 24 h. However, <7% of those differences were >2 2-fold dilutions. Compared to the CLSI method, the Etest yielded higher MICs for *C. albicans* and *C. tropicalis*, with 8% and 11% of the isolates showing differences of >2 2-fold dilutions after 24 h and 48 h, respectively. Of note are the differences for *C. tropicalis* and rare *Candida* spp., where 15 to 20% of the differences were >2 2-fold dilutions.

Categorical agreement. Overall, most isolates were susceptible (>98%) with each method and breakpoint, with the exception of *C. parapsilosis* and EUCAST, where most isolates were intermediate because of the wide range of the intermediate susceptibility breakpoint (0.004 to 2 mg/liter). More resistant isolates were detected with EUCAST breakpoints (1 to 2%) than with CLSI breakpoints (0%). The resistant isolates belonged to *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. Table 4 shows the percentages of categorical agreement between the methods. Overall, the percentage of agreement between methods was >97%, with 1 to 2% very major errors between the EUCAST and CLSI methods. The same error rates were found with Etest when EUCAST breakpoints were applied. Between the CLSI and Etest methods, 1 to 2% minor errors and no very major errors were found.

DISCUSSION

Micafungin demonstrated potent *in vitro* activity against most *Candida* species except *C. parapsilosis*, followed by *C. krusei*, among the common *Candida* species and *C. guilliermondii*, *C. lipolytica*, *C. orthopsilosis*, *C. metapsilosis*, *C. fermentati*, and *C. xestobii*, followed by *C. lusitaniae*, *C. rugosa*, and *C. pararugosa*, among the rare *Candida* species. Overall, the EUCAST method resulted in MICs 1 to 2 dilutions higher than the CLSI and Etest MICs. These differences were more pronounced when tests were performed in different labs, with 19% of all isolates having MIC differences of >2 2-fold dilutions. The smallest differences were found for *C. krusei* and the highest with *C. tropicalis*. Although for *C. albicans*, micafungin MICs were similar between the EUCAST and the Etest methods, for most *Candida* species, the MICs with the EUCAST method were around 1 dilution higher than that with the Etest method. Overall, the CLSI method resulted in lower MICs than the Etest method, with 11% of all isolates demonstrating differences of >2 2-fold dilutions. These differences were pronounced after 48 h. Despite these differences, categorical agreement was excellent (>97%), with only 1 to 2% very major errors

TABLE 3 *In vitro* susceptibilities of rare *Candida* species to micafungin

Species	No. of isolates	MIC (mg/liter)[a] by:			
		EUCAST	CLSI	Etest (24 h)	Etest (48 h)
C. guilliermondii	20	1 (0.25–8)	0.375 (0.002–0.5)	0.25 (0.125–1)	0.5 (0.25–2)
C. orthopsilosis	15	0.5 (0.25–1)	0.25 (0.031–1)	0.25 (0.125–0.5)	0.25 (0.125–1)
C. lusitaniae	11	0.125 (0.125–0.25)	0.063 (0.031–0.063)	0.032 (0.016–0.063)	0.063 (0.031–0.063)
C. dubliniensis	8	0.031 (0.016–0.063)	0.004 (0.002–0.008)	0.012 (0.008–0.016)	0.016 (0.008–0.031)
C. kefyr	7	0.063 (0.016–0.125)	0.031 (0.002–0.063)	0.031 (0.016–0.031)	0.063 (0.032–0.063)
C. rugosa	7	0.25 (0.031–0.25)	0.5 (0.5–8)	0.031 (0.016–0.064)	0.032 (0.031–0.125)
C. pelliculosa	6	0.031 (0.016–0.063)	0.005 (0.002–0.031)	0.012 (0.008–0.031)	0.012 (0.008–0.031)
C. fabianii	5	0.031 (0.031–0.125)	0.031 (0.004–0.063)	0.008 (0.008–0.031)	0.016 (0.008–0.032)
C. lipolytica	3	1 (0.5–1)	0.5 (0.25–0.5)	0.25 (0.25–0.5)	0.5 (0.25–0.5)
C. metapsilosis	3	0.5 (0.25–1)	0.25 (0.063–0.5)	0.25 (0.125–0.5)	0.25 (0.125–0.5)
C. fermentati	2	0.5, 0.5	0.1875 (0.125–0.25)	0.1875 (0.125–0.25)	0.25 (0.25–0.25)
C. intermedia	2	0.016, 0.031	0.008, 0.008	0.008, 0.032	0.016, 0.063
C. utilis	2	0.016, 0.016	0.002, 0.002	0.008, 0.008	0.008, 0.016
C. famata	1	0.063	0.002	0.031	0.031
C. inconspicua	1	0.063	0.002	0.016	0.016
C. palmioleophila	1	0.063	0.002	0.031	0.031
C. pararugosa	1	0.125	0.031	0.032	0.125
C. viswanathii	1	0.016	0.008	0.016	0.016
C. xestobii	1	0.5	0.125	0.25	0.25

[a] Where two isolates were tested, MICs are separated by a comma; where more than two isolates were tested, the median (range) MIC is given.

between EUCAST and the other two methods; EUCAST detected more resistant isolates.

To our knowledge, this is the largest comparative study of these three methods assessing both intra- and interlaboratory agreement. In a comparative study of the three methods for 133 *Candida* isolates, the essential agreement (differences within 2 2-fold dilutions) was >90%, with the EUCAST method identifying more resistant strains (6). This is in agreement with the present study, where the EUCAST method detected more resistant strains, and most (>92%) differences in the intralaboratory comparison were within 2 2-fold dilutions for the five common species. However, in the interlaboratory comparison, using a larger collection of isolates belonging to common and rare *Candida* species, the overall essential agreement dropped to 81% between the EUCAST and CLSI methods. Noticeable were the differences for *C. tropicalis* and rare *Candida* species, where 31% and 28% of the differences were >2 2-fold dilutions. The corresponding rates were 17% for *C. albicans* and 19% for *C. glabrata*, whereas for *C. parapsilosis* and *C. krusei*, these differences were <6%. In another comparative study with 357 *Candida* isolates, the modal MICs of the EUCAST method were 1 to 2 dilutions lower than the CLSI modal MICs for all five *Candida* common species, with >94% essential agreement and >91% categorical agreement (7). Unfortunately, a paired comparison was not made to assess whether the EUCAST method consistently resulted in lower micafungin MICs than the CLSI method (6). Finally, in a larger study with 584 *Candida* isolates belonging to common species, the essential/categorical agreement between the EUCAST and CLSI methods was 93%/>90% for micafungin, with the EUCAST method resulting in modal MICs for *C. albicans* ($n = 251$) and *C. parapsilosis* ($n = 224$)—but not *C. tropicalis* ($n = 46$), *C. glabrata* ($n = 37$), and *C. krusei* ($n = 11$)—that were 1 2-fold-dilution higher and detecting more resistant isolates than the CLSI method (2.7% versus 1.5%, respectively), in agreement with our study (11).

When the Etest method was compared with the EUCAST method for micafungin against 160 *Candida* isolates, the MIC_{90} with the EUCAST method was 2 dilutions higher than the MIC_{90} with the Etest method after 24 h, whereas the opposite was observed with the Etest after 48 h, in agreement with the present findings (12). However, most (>93%) differences between the EUCAST and Etest methods were within 2 2-fold dilutions in the present study. In another intralaboratory study, the Etest method gave MICs similar to those with the EUCAST and CLSI methods; the only remarkable difference was the 1-to-2 2-fold dilution-lower modal MICs for *C. parapsilosis* and *C. guilliermondii* with the Etest method than with the other methods (6). In the present

TABLE 4 Categorical agreement between the different methods

Species (no. of isolates)	% of agreement/minor errors/major errors/very major errors for comparison of the following methods:				
	EUCAST vs:			CLSI vs:	
	CLSI	Etest (24 h)	Etest (48 h)	Etest (24 h)	Etest (48 h)
C. albicans (584)	99/0/0/1	100/0/0/0	100/0/0/0	100/0/0/0	100/0/0/0
C. glabrata (86)	100/0/0/0	100/0/0/0	100/0/0/0	100/0/0/0	100/0/0/0
C. tropicalis (180)	97/1/0/2	98/0/0/2	98/0/0/2	99/1/0/0	99/1/0/0
C. parapsilosis (122)	99/0/0/1	99/0/0/1	99/0/0/1	100/0/0/0	98/2/0/0
C. krusei (30)	100/0/0/0	100/0/0/0	100/0/0/0	100/0/0/0	100/0/0/0

study, the average Etest MIC for *C. parapsilosis* was lower than the EUCAST MICs after both 24 and 48 h, whereas compared to the CLSI MICs, lower Etest MICs were found after 24 h. No significant differences was found in the classification of isolates between the Etest and the two reference methods. Because of the lack of specific breakpoints for the Etest and the use of EUCAST and CLSI breakpoints, Etest classification may not reflect the accurate susceptibility for all isolates. The essential (within 2 dilutions) intra- and interlaboratory agreement between the Etest and the two reference methods was >92% and >89%, respectively, with most differences observed between the Etest read at 48 h and the CLSI method for *C. tropicalis* and other *Candida* species. A recently published multicenter study showed >90% essential and categorical agreement between Etest and EUCAST testing of the susceptibilities of 933 *Candida* isolates to micafungin (13).

Few studies have included such a large collection of isolates, including rare *Candida* species for which *in vitro* susceptibility data are limited. The higher MICs (EUCAST MICs, >0.25 mg/liter) for the *C. parapsilosis* complex and *C. guilliermondii* have been described previously (6, 11, 14). The present study shows also that *C. lipolytica*, *C. fermentati*, and *C. xestobii* also had similarly high MICs (15). Reduced susceptibility to micafungin was found for *C. krusei*, *C. lusitaniae*, *C. rugosa*, and *C. pararugosa* (EUCAST MICs, 0.125 to 0.25 mg/liter), whereas for all the other *Candida* species, lower MICs were found.

A limitation of the present study is the fact that most of the isolates were susceptible, since the collection of isolates came from the first clinical trials with micafungin, where the prevalence of echinocandin resistance was low. Therefore, no information is available for the performance of each test at detecting resistant mutants.

Thus, although the three methods tend to give similar results for common *Candida* species in intralaboratory comparisons, testing larger collections of isolates of common and rare species and in different centers may result in differences of >2 2-fold dilutions, as we found in the present study. *In vitro* conditions and interlaboratory experimental variation are well known to have a major impact on antifungal susceptibility testing (16). The error rate for the classification of isolates was very low, <2%, using either the EUCAST or the CLSI breakpoints. Low error rates were previously found between the three methods for common *Candida* species, except for *C. glabrata*, where 6 to 9% very major errors were found using the epidemiological cutoff values (6). Overall, the EUCAST method consistently gave MICs 1 to 2 2-fold dilutions higher, whereas the CLSI resulted in lower MICs, with the Etest MICs lying between the EUCAST and CLSI MICs. These differences had only a minor impact on the final classification of the isolates.

FUNDING INFORMATION

Part of this study was sponsored by an unrestricted grant from Astellas Pharma Europe, Inc.

REFERENCES

1. **Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ.** 2012. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect **18:**19–37. http://dx.doi.org/10.1111/1469-0691.12039.
2. **Ullmann AJ, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli S, Bassetti M, Bille J, Calandra T, Castagnola E, Cornely OA, Donnelly JP, Garbino J, Groll AH, Hope WW, Jensen HE, Kullberg BJ, Lass-Flörl C, Lortholary O, Meersseman W, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Cuenca-Estrella M.** 2012. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect **18:**53–67. http://dx.doi.org/10.1111/1469-0691.12041.
3. **Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M.** 2013. Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic. J Clin Microbiol **51:**2571–2581. http://dx.doi.org/10.1128/JCM.00308-13.
4. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 3nd ed. CLSI document M27-A3. CLSI, Wayne, PA.
5. **EUCAST.** 2008. EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. Clin Microbiol Infect **14:**398–405. http://dx.doi.org/10.1111/j.1469-0691.2007.01935.x.
6. **Pfaller MA, Castanheira M, Diekema DJ, Messer SA, Moet GJ, Jones RN.** 2010. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Etest methods with the CLSI broth microdilution method for echinocandin susceptibility testing of *Candida* species. J Clin Microbiol **48:**1592–1599. http://dx.doi.org/10.1128/JCM.02445-09.
7. **Pfaller MA, Castanheira M, Messer SA, Rhomberg PR, Jones RN.** 2014. Comparison of EUCAST and CLSI broth microdilution methods for the susceptibility testing of 10 systemically active antifungal agents when tested against *Candida* spp. Diagn Microbiol Infect Dis **79:**198–204. http://dx.doi.org/10.1016/j.diagmicrobio.2014.03.004.
8. **Meletiadis J, Arabatzis M, Bompola M, Tsiveriotis K, Hini S, Petinaki E, Velegraki A, Zerva L.** 2011. Comparative evaluation of three commercial identification systems using common and rare bloodstream yeast isolates. J Clin Microbiol **49:**2722–2727. http://dx.doi.org/10.1128/JCM.01253-10.
9. **Prakash A, Sharma C, Singh A, Kumar Singh P, Kumar A, Hagen F, Govender NP, Colombo AL, Meis JF, Chowdhary A.** 2016. Evidence of genotypic diversity among *Candida auris* isolates by multilocus sequence typing, matrix-assisted laser desorption ionization time-of-flight mass spectrometry and amplified fragment length polymorphism. Clin Microbiol Infect **22:**277.e1–277.e9. http://dx.doi.org/10.1016/j.cmi.2015.10.022.
10. **Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope W; EUCAST-AFST.** 2012. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clin Microbiol Infect **18:**E246–E247. http://dx.doi.org/10.1111/j.1469-0691.2012.03880.x.
11. **Montagna MT, Lovero G, Coretti C, Martinelli D, De Giglio O, Iatta R, Balbino S, Rosato A, Caggiano G.** 2015. Susceptibility to echinocandins of *Candida* spp. strains isolated in Italy assessed by European Committee for Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute broth microdilution methods. BMC Microbiol **15:**106. http://dx.doi.org/10.1186/s12866-015-0442-4.
12. **Marcos-Zambrano LJ, Escribano P, Rueda C, Zaragoza O, Bouza E, Guinea J.** 2013. Comparison between the EUCAST procedure and the Etest for determination of the susceptibility of *Candida* species isolates to micafungin. Antimicrob Agents Chemother **57:**5767–5770. http://dx.doi.org/10.1128/AAC.01032-13.
13. **Bougnoux ME, Dannaoui E, Accoceberry I, Angoulvant A, Bailly E, Botterel F, Chevrier S, Chouaki T, Cornet M, Dalle F, Datry A, Dupuis A, Fekkar A, Gangneux JP, Guitard J, Hennequin C, LeGovic Y, Le Pape P, Maubon D, Ranque S, Sautour M, Sendid B, Chandenier J.** 2016. Multicenter comparison of the Etest and EUCAST for antifungal susceptibility testing of *Candida* isolates to micafungin. Antimicrob Agents Chemother **60:**5088–5091. http://dx.doi.org/10.1128/AAC.00630-16.
14. **Espinel-Ingroff A, Canton E, Pelaez T, Peman J.** 2011. Comparison of micafungin MICs as determined by the Clinical and Laboratory Standards Institute broth microdilution method (M27-A3 document) and Etest for

Candida spp. isolates. Diagn Microbiol Infect Dis **70**:54–59. http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.010.

15. **Dannaoui E, Lortholary O, Raoux D, Bougnoux ME, Galeazzi G, Lawrence C, Moissenet D, Poilane I, Hoinard D, Dromer F.** 2008. Comparative in vitro activities of caspofungin and micafungin, determined using the method of the European Committee on Antimicrobial Susceptibility Testing, against yeast isolates obtained in France in 2005-2006. Antimicrob Agents Chemother **52**:778–781. http://dx.doi.org/10.1128/AAC.01140-07.
16. **Odds FC, Motyl M, Andrade R, Bille J, Cantón E, Cuenca-Estrella M, Davidson A, Durussel C, Ellis D, Foraker E, Fothergill AW, Ghannoum MA, Giacobbe RA, Gobernado M, Handke R, Laverdière M, Lee-Yang W, Merz WG, Ostrosky-Zeichner L, Pemán J, Perea S, Perfect JR, Pfaller MA, Proia L, Rex JH, Rinaldi MG, Rodriguez-Tudela J-L, Schell WA, Shields C, Sutton DA, Verweij PE, Warnock DW.** 2004. Interlaboratory comparison of results of susceptibility testing with caspofungin against *Candida* and *Aspergillus* species. J Clin Microbiol **42**:3475–3482. http://dx.doi.org/10.1128/JCM.42.8.3475-3482.2004.