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Evaluation of Two Commercial Broth Microdilution Methods Using Different Interpretive Criteria for the Detection of Molecular Mechanisms of Acquired Azole and Echinocandin Resistance in Four Common *Candida* Species

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ABSTRACT   The abilities of the new Vitek 2 AST-YS08 (YS08) and Sensititre Yeast-One (SYO) systems to detect the resistances of *Candida* isolates to azoles and echinocandins were evaluated. In total, 292 isolates, including 28 *Candida albicans* (6 Erg11 and 2 Fks mutants), 57 *Candida parapsilosis* (26 Erg11 mutants), 24 *Candida tropicalis* (10 Erg11 and 1 Fks mutants), and 183 *Candida glabrata* (39 Pdr1 and 13 Fks mutants) isolates, were tested. The categorical agreements (CAs) between the Clinical and Laboratory Standards Institute (CLSI) method and YS08 fluconazole MICs obtained using clinical breakpoints were 92.4% (*C. albicans*), 96.5% (*C. parapsilosis*), and 87.0% (*C. tropicalis*), and the CAs between the CLSI and SYO MICs were 92.3% (*C. albicans*), 77.2% (*C. parapsilosis*), 100% (*C. tropicalis*), and 98.9% (*C. glabrata*). For *C. glabrata*, the CAs with the CLSI micafungin MICs were 92.4% and 55.5% for the YS08 micafungin and caspofungin MICs, respectively; they were 100%, 95.6%, and 98.9% for the SYO micafungin, caspofungin, and anidulafungin MICs, respectively. YS08 does not provide fluconazole data for *C. glabrata*; the CA with the CLSI fluconazole MIC was 97.8% for the YS08 voriconazole MIC, using an epidemiological cutoff value (ECV) of 0.5 μg/ml. Increased CAs with the CLSI MIC were observed for the YS08 MIC using CLSI ECVs (for fluconazole and *C. tropicalis*, 100%; for micafungin and *C. glabrata*, 98.9%) and for the SYO MIC using method-specific ECVs (for fluconazole and *C. parapsilosis*, 91.2%; for caspofungin and *C. glabrata*, 98.9%). Therefore, the YS08 and SYO systems may have different abilities to detect mechanisms of azole and echinocandin resistance in four *Candida* species; the use of method-specific ECVs may improve the performance of both systems.

KEYWORDS *Candida*, azole, echinocandin, Vitek 2 AST-YS08, Sensititre YeastOne

Antifungal susceptibility testing (AFST) against *Candida* is useful for optimizing the treatment of *Candida* bloodstream infections (BSIs) (1, 2). In the clinical microbiology laboratory, two commercial broth microdilution (BMD)-based AFSTs, the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and the Sensititre YeastOne (SYO) system (Thermo Scientific, Cleveland, OH, USA), are widely used, particularly for *Candida* BSI isolates (3–6). The Vitek 2 system is a fully automated susceptibility testing system used for both bacteria and yeasts in many laboratories and has the advantages of rapidity, reduced costs, and ease of use (5–7). A new Vitek 2 AST-YS08 yeast susceptibility system (referred to as YS08) was recently updated to reflect the revised species-specific clinical

breakpoints (CBPs) defined by the Clinical and Laboratory Standards Institute (CLSI) for common *Candida* species, but YS08 does not provide fluconazole MIC data for any of the *Candida glabrata* isolates (7, 8). On the other hand, SYO, the colorimetric broth microdilution method, has good concordance with the CLSI reference method for *Candida* susceptibility testing (4, 9) but requires manual setup and visual reading of the results (10, 11). To date, only one study has evaluated the new YS08 using revised CLSI CBPs (8). In this study, the essential agreement (EA) and categorical agreement (CA) between YS08 and SYO were suboptimal using 68 clinical *Candida* isolates skewed for resistant isolates. They suggest that validation data with resistant isolates need to be obtained (8).

Candida develops resistance to echinocandins through point mutations in the *FKS1* (all *Candida* species) or *FKS2* (*C. glabrata* only) gene, which are linked to resistance to all three echinocandins, including micafungin, caspofungin, and anidulafungin (12, 13). Several studies have evaluated the performance of echinocandin susceptibility testing by using a panel of *Candida* isolates with and without known *FKS* mutation-related echinocandin resistance mechanisms (14, 15), but there are limited data regarding the evaluation of AFSTs for the detection of azole-resistant *Candida* isolates using molecularly defined isolates (16). Multiple mechanisms of azole resistance, such as *ERG11* mutations and overexpression of efflux pumps, have been reported for *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* (17–19); however, the overwhelming singular mechanism of resistance identified in clinical isolates of *C. glabrata* is mutation of the transcription factor *PDR1*, which leads to increased expression of multidrug transporters that act as efflux pumps (20, 21).

The evaluation of AFST methods for *Candida* species is difficult, partly because the results can be influenced by the composition of resistant *Candida* isolates within a collection. When evaluation studies are performed using an abundance of antifungal-susceptible isolates in the collection, very major errors (VMEs) and substantial major discrepancies are uncommon, while errors are more common when studies are performed using an abundance of resistant isolates (22, 23). In addition, CA between two systems may vary depending on the interpretive criteria of the MICs used. Although most commercial AFST systems use CLSI CBPs for categorical interpretation of MICs (24, 25; bioMérieux), method-specific epidemiological cutoff values (ECVs) have recently been proposed as the interpretive criteria for SYO or Etest (26, 27). Given that the performance of AFST relies on categorical interpretive criteria of the MIC results, we performed a comparative evaluation of two commercial AFSTs (YS08 and SYO) and the reference CLSI BMD method for the detection of azole- and echinocandin-resistant isolates of four common *Candida* species, applying different MIC interpretive criteria, including CLSI CBPs, CLSI ECVs, or the method-specific ECVs of SYO (SYO ECVs). We tested a panel of well-characterized *Candida* strains from a South Korean collection composed of 97 azole- or echinocandin-resistant isolates with molecularly defined resistance mechanisms that reflect the antifungal resistance mechanisms most commonly identified in isolates of four common *Candida* species from South Korean hospitals (19, 28); we also tested 195 control isolates without defined resistance mechanisms. Furthermore, we evaluated the ability of voriconazole MIC determination using YS08 to correctly detect fluconazole-resistant (FR) *C. glabrata* isolates.

RESULTS

Fluconazole testing for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. When 42 FR isolates (6 FR *C. albicans* isolates harboring the K143R mutation in Erg11p, 26 *C. parapsilosis* isolates harboring the Y132F mutation in Erg11p, and 10 FR *C. tropicalis* isolates with *ERG11* overexpression or the Y132F mutation in Erg11p) and 64 fluconazole-susceptible (FS) isolates (20 *C. albicans*, 31 *C. parapsilosis*, and 13 *C. tropicalis* isolates without defined resistance mechanisms) were tested, the EAs between the CLSI M27 method and YS08 with fluconazole were 96.2%, 96.5%, and 100% for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, respectively; the EAs between CLSI M27 and SYO were 92.3%, 100%, and 95.7%, respectively (Table 1). When the categorical results

TABLE 1 CAs between fluconazole MICs obtained by the CLSI BMD method and those obtained by each of the two commercial BMD assays for 197 molecularly defined isolates of four common *Candida* species according to different MIC interpretive criteria[a]

Species (no. of isolates tested)	Antifungal agent	Test method	Interpretive criterion[b]	% of isolates with the following categorical MIC interpretation[c]:			CA (%)	No. (%) of isolates with discrepancies[d]			EA (%)[e]
				R or non-WT	SDD	S or WT		VME	ME	Minor	
C. albicans (26)	Fluconazole	CLSI M27	CLSI CBP (8)	23.1	0.0	76.9					
		YS08	CLSI CBP (8)	15.4	3.8	80.8	92.4	1 (3.8)	0 (0.0)	1 (3.8)	96.2
		YS08	CLSI ECV (1)	42.3		57.7	73.1	1 (3.8)	6 (23.1)	0 (0.0)	
		SYO	CLSI CBP (8)	30.8	0.0	69.2	92.3	0 (0.0)	2 (7.7)	0 (0.0)	92.3
		SYO	SYO ECV (2)	34.6		65.4	88.5	0 (0.0)	3 (11.5)	0 (0.0)	
C. parapsilosis (57)	Fluconazole	CLSI M27	CLSI CBP (8)	45.6	0.0	54.4					
		YS08	CLSI CBP (8)	42.1	3.5	54.4	96.5	0 (0.0)	0 (0.0)	2 (3.5)	96.5
		YS08	CLSI ECV (2)	49.1		50.9	96.5	0 (0.0)	2 (3.5)	0 (0.0)	
		SYO	CLSI CBP (8)	24.6	15.8	59.6	77.2	4 (7.0)	0 (0.0)	9 (15.8)	100.0
		SYO	SYO ECV (4)	40.4		59.6	91.2	4 (7.0)	1 (1.8)	0 (0.0)	
C. tropicalis (23)	Fluconazole	CLSI M27	CLSI CBP (8)	43.5	0.0	56.5					
		YS08	CLSI CBP (8)	30.5	13.0	56.5	87.0	0 (0.0)	0 (0.0)	3 (13.0)	100.0
		YS08	CLSI ECV (2)	43.5		56.5	100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (8)	43.5	0.0	56.5	100.0	0 (0.0)	0 (0.0)	0 (0.0)	95.7
		SYO	SYO ECV (8)	43.5		56.5	100.0	0 (0.0)	0 (0.0)	0 (0.0)	
C. glabrata (91)	Fluconazole	CLSI M27	CLSI CBP (64)	42.9	57.1						
		SYO	CLSI CBP (64)	44.0	56.0		98.9	0 (0.0)	0 (0.0)	1 (1.1)	95.6
		SYO	SYO ECV (128)	40.7		59.3	97.8	2 (2.2)	0 (0.0)	0 (0.0)	
	Voriconazole	YS08	P-CLSI ECV (1)	42.9		57.1	97.8	1 (1.1)	1 (1.1)	0 (0.0)	NA
		YS08	CLSI ECV (0.5)	58.2		41.8	82.4	1 (1.1)	15 (16.5)	0 (0.0)	

[a]CLSI, Clinical and Laboratory Standards Institute; CLSI M27, CLSI M27-ED4 broth microdilution method (BMD); YS08, Vitek 2 AST-YS08 yeast susceptibility system; SYO, Sensititre YeastOne system; CLSI CBP, clinical breakpoint provided by CLSI; ECV, epidemiological cutoff value; SYO ECV, method-specific ECV of SYO; P-CLSI ECV, previous CLSI epidemiological cutoff value; R, resistant; non-WT, non-wild type; SDD, susceptible, dose dependent; S, susceptible; WT, wild type; CA, categorical agreement; VME, very major error; ME, major error; minor, minor error; EA, essential agreement; NA, not available.

[b]The values in parentheses denote interpretive fluconazole MIC criteria (in micrograms per milliliter) from the CLSI CBPs (MICs greater than or equal to the MIC for resistance), CLSI ECVs (MICs greater than or equal to the non-WT MIC), or SYO ECVs (MICs greater than or equal to the non-WT MIC) against each *Candida* species (27, 30, 34). The results for voriconazole obtained with YS08 were alternatively evaluated for *C. glabrata* isolates because YS08 does not provide fluconazole results for *C. glabrata*. Although a CLSI clinical breakpoint is not yet proposed for voriconazole and *C. glabrata*, both the previous CLSI ECV (0.5 μg/ml) and the revised CLSI ECV (0.25 μg/ml) were used (34, 45).

[c]Each of the categorical results were determined to indicate resistant, susceptible, dose dependent, and susceptible using speciesspecific CLSI CBPs of the CLSI M60, 1st edition, method (30) and as non-WT and WT using CLSI ECVs of the CLSI M59, 2nd edition, method (34) or the SYO ECVs (27).

[d]Details regarding isolates with categorical errors obtained using CLSI CBPs were as follows. For *C. albicans*, YS08 misclassified two fluconazole-resistant (FR) isolates harboring a K143R mutation in Erg11 as fluconazole susceptible, dose dependent (F-SDD), and fluconazole susceptible (FS; MIC, ≤0.5 μg/ml), respectively. SYO misclassified two FS isolates (MICs, 1 and 2 μg/ml) as FR (MIC, >256 μg/ml). For *C. parapsilosis*, YS08 misclassified two FR isolates harboring Y132F in Erg11 as F-SDD, and SYO misclassified four and eight FR isolates as FS (MIC, 2 μg/ml) and F-SDD, respectively, and one FS isolate (MIC, 2 μg/ml) as FR. For *C. tropicalis*, YS08 misclassified 3 FR isolates as F-SDD. For *C. glabrata*, SYO misclassified one F-SDD isolate (MIC, 8 μg/ml) as FR (MIC, 64 μg/ml).

[e]Essential agreement (± 2 $\log_2$ dilutions) between the MICs obtained by the CLSI M27 method and each of the Vitek 2 YS08 and YeastOne MICs.

for fluconazole with YS08 determined using two different interpretive criteria (CLSI CBPs or ECVs) were compared to those produced by the CLSI method, the agreements of YS08 using CLSI CBPs for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were 92.4%, 96.5%, and 87.0%, respectively; agreements determined using CLSI ECVs were 73.1%, 96.5%, and 100%, respectively. When the results for fluconazole produced by SYO were compared to those produced by the CLSI method, the CAs of the fluconazole MICs produced by SYO using CLSI CBPs for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were 92.3%, 77.2%, and 100%, respectively; the CAs obtained using SYO ECVs were 88.5%, 91.2%, and 100%, respectively.

Azole testing for *C. glabrata*. When the fluconazole MIC results for 91 isolates of *C. glabrata* (39 FR isolates harboring a Pdr1 mutation and 52 fluconazole-susceptible, dose dependent [F-SDD], isolates without Pdr1 mutations) obtained with SYO were compared to those produced by the CLSI method, the CAs for fluconazole produced by SYO using the CLSI CBPs and the SYO ECVs were 98.9% and 97.8%, respectively (Table 1). The EA for fluconazole between CLSI M27 and SYO was 95.6%. Because YS08 does not provide fluconazole data for *C. glabrata*, the results for voriconazole obtained with YS08 were used to predict the fluconazole susceptibility category for *C. glabrata*.
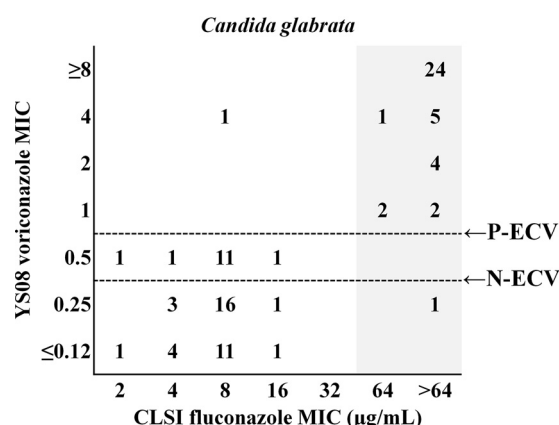
FIG 1 A scattergram comparing the fluconazole MICs obtained by the Clinical and Laboratory Standards Institute (CLSI) method and voriconazole MICs obtained with the Vitek 2 AST-YS08 (YS08) system. Numbers represent the number of isolates of *Candida glabrata* at each MIC pair. The gray zone indicates fluconazole-resistant Pdr1 mutants of *C. glabrata*. The voriconazole MIC results obtained with YS08 with the previous CLSI ECV (P-ECV; 0.5 μg/ml) showed a clearer separation between Pdr1 mutants and non-Pdr1 mutants than those obtained with the new CLSI ECV (N-ECV; 0.25 μg/ml).

Although a species-specific CLSI CBP has not yet been proposed for voriconazole and *C. glabrata*, categorical results for the YS08 voriconazole MICs based on the previous CLSI ECV of 0.5 μg/ml showed 97.8% agreement with the results of the CLSI method for fluconazole, whereas the categorical result of YS08 for voriconazole based on the new CLSI ECV (0.25 μg/ml) showed 82.4% agreement with the results of the CLSI method for fluconazole. Figure 1 shows that the voriconazole results obtained with YS08 with the previous CLSI ECV (P-ECV; 0.5 μg/ml) demonstrated a clearer separation between Pdr1 mutants and non-Pdr1 mutants than the results obtained with the new CLSI ECV (N-ECV; 0.25 μg/ml).

Echinocandin testing with YS08 and SYO. Table 2 summarizes the agreements between the micafungin susceptibility results obtained by the CLSI method and those obtained by each of the echinocandin tests with the YS08 or SYO method for 95 isolates of three common *Candida* species. Two micafungin-resistant (MR) *C. albicans* isolates and one MR *C. tropicalis* isolate harboring an Fks1 mutation were correctly classified as resistant or non-wild type (non-WT) to both micafungin and caspofungin with YS08 or SYO, irrespective of the interpretive criteria. Notably, one *C. albicans* isolate harboring S645P in Fks1 showed an anidulafungin MIC value of 0.25 μg/ml, which was misclassified as susceptible in the anidulafungin test of SYO using the CLSI CBP; however, it was correctly classified as non-WT to anidulafungin using the SYO ECVs. For 92 isolates of *C. glabrata* (13 MR isolates harboring Fks mutations and 79 micafungin-susceptible [MS] isolates without Fks mutations), the EA for micafungin between the CLSI M27 method and YS08 was 92.4%, while the EA between CLSI M27 and SYO was 97.8%. Figure 2 depicts two scattergrams comparing the micafungin MICs for *C. glabrata* isolates obtained by the CLSI method with the micafungin or caspofungin MICs obtained with YS08, using the CLSI CBPs or ECVs. Because YS08 does not show micafungin MIC results of ≤0.03 μg/ml, a modified ECV of 0.06 μg/ml instead of the CLSI ECV (0.03 μg/ml) was used for the micafungin and *C. glabrata* combination (Fig. 2A). For assessment of the caspofungin MICs obtained with YS08, only the CLSI CBP was used because of the lack of a CLSI ECV for caspofungin (Fig. 2B). The categorical results for the YS08 micafungin MIC obtained using the CLSI CBP showed that 7 out of 13 MR *C. glabrata* isolates with Fks mutation were misclassified as micafungin intermediate (six isolates) or MS (one isolate), whereas all 79 MS isolates were correctly classified as susceptible, resulting in a CA of 92.4% with the CLSI micafungin MIC. The categorical results for the YS08 micafungin MIC obtained using a modified ECV of 0.06 μg/ml showed that 12 of 13 MR isolates were correctly classified as non-WT, resulting in a CA of 98.9% with the CLSI micafungin MIC results. The categorical results for the YS08

TABLE 2 CAs of micafungin results obtained by the reference CLSI BMD method and echinocandin (micafungin, caspofungin, or anidulafungin) results obtained by two commercial BMD assays for 95 isolates of three common *Candida* species according to different MIC interpretive criteria[a]

Species (no. of isolates tested)	Antifungal agent	Test method	Interpretive criterion[b]	% of isolates with the following categorical MIC interpretation[c]:			CA (%)	No. (%) of isolates with discrepancies			EA (%)[e]
				R or non-WT	I	S or WT		VME	ME	Minor	
C. albicans (2)	Micafungin	CLSI M27	CLSI CBP (1)	100.0							
		YS08	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	100.0
		YS08	M-CLSI ECV (0.12)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	100.0
		SYO	SYO ECV (0.12)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
	Caspofungin	YS08	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	SYO ECV (0.5)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
	Anidulafungin	SYO	CLSI CBP (1)	50.0		50.0	50.0	1 (50.0)[d]	0 (0.0)	0 (0.0)	
		SYO	SYO ECV (0.25)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
C. tropicalis (1)	Micafungin	CLSI M27	CLSI CBP (1)	100.0							
		YS08	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	100.0
		YS08	CLSI ECV (0.12)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	100.0
		SYO	SYO ECV (0.12)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
	Caspofungin	YS08	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	SYO ECV (0.5)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
	Anidulafungin	SYO	CLSI CBP (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
		SYO	SYO ECV (1)	100.0			100.0	0 (0.0)	0 (0.0)	0 (0.0)	
C. glabrata (92)	Micafungin	CLSI M27	CLSI CBP (0.25)	14.1		85.9					
		YS08	CLSI CBP (0.25)	6.5	6.5	87.0	92.4	1 (1.1)	0 (0.0)	6 (6.5)	92.4
		YS08	M-CLSI ECV (0.12)	13.0		87.0	98.9	1 (1.1)	0 (0.0)	0 (0.0)	
		SYO	CLSI CBP (0.25)	14.1		85.9	100.0	0 (0.0)	0 (0.0)	0 (0.0)	97.8
		SYO	SYO ECV (0.06)	14.1		85.9	100.0	0 (0.0)	0 (0.0)	0 (0.0)	
	Caspofungin	YS08	CLSI CBP (0.5)	13.0	40.2	46.8	55.5	0 (0.0)	4 (4.3)	37 (40.2)	
		SYO	CLSI CBP (0.5)	15.2	3.3	81.5	95.6	0 (0.0)	1 (1.1)	3 (3.3)	
		SYO	SYO ECV (0.5)	15.2		84.8	98.9	0 (0.0)	1 (1.1)	0 (0.0)	
	Anidulafungin	SYO	CLSI CBP (0.5)	13.0		87.0	98.9	1 (1.1)	0 (0.0)	0 (0.0)	
		SYO	SYO ECV (0.25)	13.0		87.0	98.9	1 (1.1)	0 (0.0)	0 (0.0)	

[a]CLSI, Clinical and Laboratory Standards Institute; CLSI M27, CLSI M27-ED4 broth microdilution method; YS08, Vitek 2 AST-YS08 yeast susceptibility system; SYO, Sensititre YeastOne system; CLSI CBP, clinical breakpoint provided by the CLSI; ECV, epidemiological cutoff value; M-CLSI ECV, modified CLSI ECV; SYO ECV, SYO-specific ECV; R, resistant; non-WT, non-wild type; I, intermediate; S, susceptible; WT, wild type; CA, categorical agreement; VME, very major error; ME, major error; minor, minor error; EA, essential agreement.

[b]The values in parentheses denote interpretive echinocandin MIC criteria (in micrograms per milliliter) from the CLSI CBPs (MICs greater than or equal to the MIC for resistance), CLSI ECVs (MICs greater than or equal to the non-WT MIC), or SYO ECVs (MICs greater than or equal to the non-WT MIC) against each *Candida* species (26, 30, 34). Because YS08 does not give the micafungin MIC results of ≥ 0.03 μg/ml, a modified ECV of 0.06 μg/ml instead of the CLSI ECV (0.03 μg/ml) was used for micafungin for YS08.

[c]Each of the categorical results were determined to indicate resistant, intermediate, and susceptible using the species-specific CLSI CBPs of the CLSI M60, 1st edition, method (30) and as non-WT and WT using the CLSI ECVs of the CLSI M59, 2nd edition, method (34) or SYO ECVs (26).

[d]One *C. albicans* harboring Fks1 S645P was misclassified as susceptible in the SYO anidulafungin test using the CLSI CBP.

[e]Essential agreement (± 2 $\log_2$ dilutions) between the MICs obtained by the CLSI M27 method and each of the Vitek 2 YS08 and YeastOne MICs.

caspofungin MIC obtained using the CLSI CBP did not differentiate between MR and MS isolates of *C. glabrata*, resulting in a CA of 55.5% with the CLSI micafungin MIC results. Figure 3 demonstrates that all three echinocandin (micafungin, caspofungin, and anidulafungin) tests with SYO showed excellent performance (>95%) to differentiate between MR Fks mutants of *C. glabrata* and MS non-Fks mutants, irrespective of the MIC interpretive criteria. The CA of SYO for micafungin was 100% using both the CLSI CBP and SYO ECV, while the CA of SYO for caspofungin was 95.6% using the CLSI CBP and 98.9% using the SYO ECV. In addition, the CAs of anidulafungin for *C. glabrata* obtained with SYO using the CLSI CBP and the SYO ECV were both 98.9%.

DISCUSSION

By testing 292 isolates of four common *Candida* species with or without molecular mechanisms of acquired azole and echinocandin resistance, the present study revealed
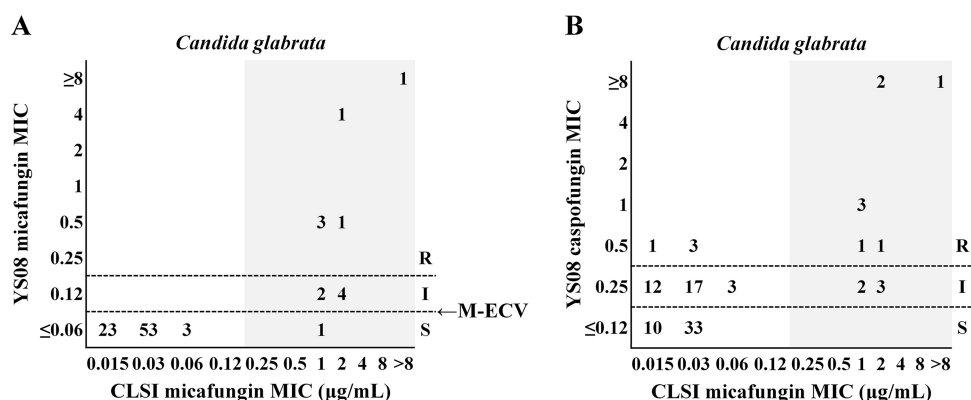
FIG 2 Two scattergrams comparing the micafungin MICs obtained by the CLSI method and the micafungin (A) or caspofungin (B) MICs obtained with the Vitek 2 AST-YS08 (YS08) system. Numbers represent the number of isolates of *Candida glabrata* at each MIC pair. The gray zone indicates micafungin-resistant Fks mutants of *C. glabrata*. The resistant (R), intermediate (I), and susceptible (S) designations represent categorical MIC interpretations determined by the use of CLSI clinical breakpoints (CLSI CBPs). The micafungin MIC results obtained with YS08 with the modified CLSI ECV (M-ECV; 0.06 μg/ml) showed a clearer separation between Fks mutants and nonmutants than those obtained with YS08 with the CLSI CBP (A), whereas the caspofungin MIC results obtained with YS08 did not discriminate between Fks mutants and nonmutants (B).

the different abilities of the YS08 and SYO systems to detect various mechanisms of azole and echinocandin resistance. YS08 showed performance characteristics superior to those of SYO for the detection of FR *C. parapsilosis* isolates harboring the Y132F mutation in Erg11p, but caspofungin testing with YS08 seemed to be unreliable for *C. glabrata*. SYO showed excellent performance for all three echinocandins, irrespective of the interpretive criteria used, CLSI CBPs or SYO ECVs. This is the first study to show that YS08 voriconazole testing may be used as a surrogate marker for fluconazole testing of *C. glabrata* and that the use of method-specific ECVs may improve the performance of commercial AFST systems.

We found excellent (>90%) EAs and CAs for the fluconazole MICs between the CLSI method and the YS08 or SYO method for three species, including *C. albicans*, *C. parapsilosis*, and *C. tropicalis*; however, the CA of YS08 was 87.0% for *C. tropicalis* and the CA of SYO was 77.2% for *C. parapsilosis*. The only minor errors (13%) of YS08 with fluconazole were observed with *C. tropicalis* due to the misclassification of 3 of 10 FR isolates as F-SDD, suggesting that the F-SDD results from the fluconazole test of YS08 should be interpreted cautiously for *C. tropicalis*. A Y132F amino acid substitution in Erg11p is reportedly the most frequent fluconazole resistance mechanism in clinical
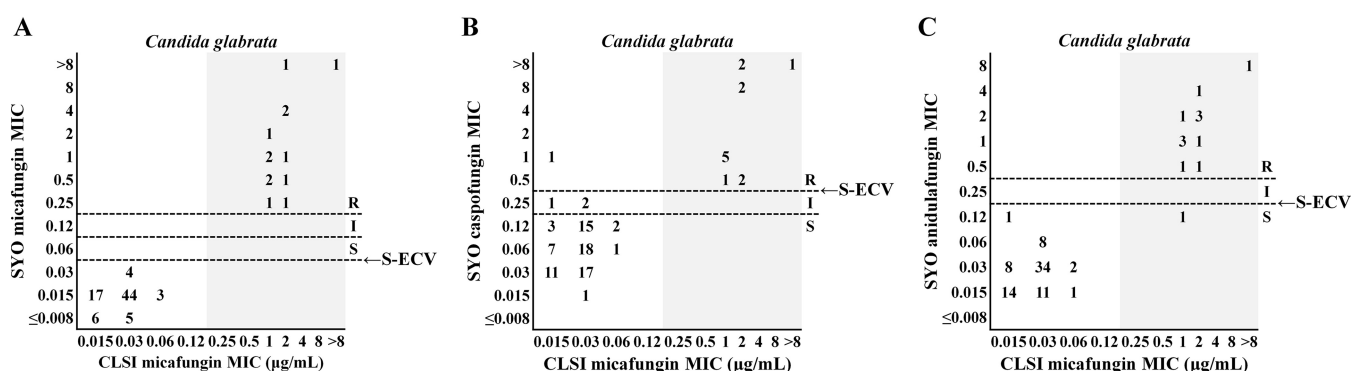

FIG 3 Three scattergrams of the micafungin MICs obtained by the CLSI method versus the micafungin (A), caspofungin (B), and anidulafungin (C) MICs obtained by the Sensititre YeastOne (SYO) system. Numbers represent the number of isolates of *Candida glabrata* at each MIC pair. The gray zone indicates micafungin-resistant Fks mutant isolates of *C. glabrata*. The resistant (R), intermediate (I), and susceptible (S) designations represent the categorical MIC interpretations determined by the use of CLSI clinical breakpoints (CLSI CBPs). The SYO results obtained for all three echinocandins, including micafungin, caspofungin, and anidulafungin, correctly discriminated Fks mutants and nonmutants of *C. glabrata* (>95%), irrespective of the interpretive criteria used, i.e., CLSI CBPs or SYO-specific ECVs (S-ECV).

isolates of *C. parapsilosis* in South Korea; this substitution has also been reported in Brazil, the United States, and Kuwait (28). In a recent Italian study, SYO was able to correctly detect FR *C. parapsilosis* isolates harboring the Y132F *ERG11* gene (Y132F isolates), in which the fluconazole MICs for most (44 of 49 isolates) Y132F isolates were ≥64 μg/ml using the SYO method; all but 1 of the isolates were also resistant to voriconazole using the CLSI method (29). Notably, the fluconazole MICs of most Y132F isolates in the present study were 8 to 16 μg/ml using the CLSI (24 of 26 isolates) or YS08 (24 of 26 isolates) method, and only 7.7% of them were resistant to voriconazole and 80.8% were susceptible, dose dependent (SDD), to voriconazole by the CLSI method (data not shown); therefore, SYO does not efficiently detect Y132F isolates with lower fluconazole MICs. These results indicate that YS08 appears to be a superior method for the detection of FR *C. parapsilosis* isolates harboring a Y132F mutation in Erg11p from the South Korean collection.

According to the revised CLSI CBP, *C. glabrata* isolates are no longer considered susceptible to fluconazole but are classified only as either F-SDD or FR (30). In the present study, the fluconazole test with SYO was able to differentiate between 39 FR isolates and 52 F-SDD isolates of *C. glabrata*, resulting in a CA of 98.9% with the CLSI method. However, the new YS08 does not provide fluconazole results for *C. glabrata*. A previous study showed that the CA for fluconazole between the Vitek 2 AF03 yeast susceptibility test and the CLSI BMD method was greater than 90% for all *Candida* species except *C. glabrata* (87.8%) (23). A recent study also showed that a modified YS08 to assess the adequacy of fluconazole MICs for *C. glabrata* by setting the tested isolates to *C. albicans* within the Vitek 2 software produced VMEs (3 of 26 isolates) compared to the results obtained with the previous version of the Vitek 2 AST-YS07 card (7). This suggests the need for an alternative approach to detect FR *C. glabrata* isolates with YS08.

Acquired resistance to both fluconazole and voriconazole in *C. glabrata* is mostly governed by a single mechanism through a gain-of-function mutation in the transcription factor for Pdr1p, regulating the drug-efflux transporter genes (20). Accordingly, cross-resistance between fluconazole and voriconazole in *C. glabrata* has already been reported (31, 32); in one study, 98.8% of FR isolates were not susceptible to voriconazole (MIC > 0.5 μg/ml) (32). Given that the Infectious Diseases Society of America guidelines for the management of candidiasis recommend routinely performing AFST for determination of *C. glabrata* susceptibility to azoles and echinocandins (1), we alternatively investigated the voriconazole MIC determination of YS08 to correctly discriminate FR *C. glabrata* isolates harboring a Pdr1 mutation from F-SDD isolates without Pdr1 mutations. Because a CLSI CBP for the voriconazole-*C. glabrata* combination has not been proposed, we used both the previous and the new CLSI ECVs (0.5 and 0.25 μg/ml, respectively) to differentiate wild-type (WT) from non-WT strains of *C. glabrata*. The voriconazole result obtained with YS08 with the previous CLSI ECV (0.5 μg/ml) showed a clearer separation between Pdr1 mutants and non-Pdr1 mutants, resulting in a CA of 97.8% with the results for fluconazole by the CLSI method, while the CA of the YS08 voriconazole test using the new ECV (0.25 μg/ml) was only 82.4%. These findings indicate that the use of the YS08 voriconazole test with an ECV of 0.5 μg/ml could provide a reliable discrimination between Pdr1 mutants and non-Pdr1 mutants of *C. glabrata* for clinical laboratories.

Because the significant interlaboratory variability of the *in vitro* caspofungin test by the reference CLSI BMD method contributes to false resistance, the echinocandin results obtainded with the two systems were compared to the micafungin results obtained using CLSI methods (30, 33). When the micafungin and caspofungin MIC results obtained with YS08 were compared to the micafungin MIC results for *C. glabrata* produced by the CLSI method, the CA of YS08 for micafungin was 92.4% using the CLSI CBP, whereas it was 55.5% for the YS08 caspofungin test. Our findings are consistent with the results of a recent study in which the YS08 caspofungin test for *C. glabrata* had low CAs (5/15 isolates) with the SYO caspofungin test, although the results for the isolates that they evaluated were not molecularly confirmed (8). In the YS08 instruc-

tions, the Vitek 2 system also recommends the use of an alternative method before reporting YS08 caspofungin test results for *C. glabrata* (bioMérieux). All of these findings confirm that YS08 micafungin test results are more reliable than YS08 caspofungin test results.

The CBPs classify isolates as susceptible or resistant, while the ECVs are useful for distinguishing between WT isolates without resistance mechanisms and non-WT isolates harboring resistance mechanisms (34). Both CBPs and ECVs can be used to determine the CA between any two methods. When the micafungin, caspofungin, and anidulafungin MIC results for all 95 isolates (16 Fks mutants and 79 nonmutants) of three *Candida* species produced by SYO were compared to the micafungin MIC results produced by the CLSI method, excellent CAs (>95%) were found for all three echinocandins, irrespective of the interpretive criteria used, i.e., CBPs or SYO ECVs. Notably, the CA of the SYO caspofungin test results for *C. glabrata* were 95.6% using the CLSI CBP and 98.9% using the SYO ECV. In addition, the CAs of the SYO fluconazole test results for *C. glabrata* were 77.2% using the CLSI CBP and 91.2% using the SYO ECV, suggesting that the use of method-specific ECVs may improve the performance of SYO.

YS08 uses CLSI CBPs for interpreting the results for azoles or echinocandins. Using CLSI CBPs, the fluconazole test for *C. tropicalis* with YS08 showed a 13.0% minor error rate without VMEs or major errors (ME); the micafungin test for *C. glabrata* with YS08 showed a 6.5% minor error rate with 1.1% VMEs. The reasons for the high rates of minor errors for YS08 are not fully clear, but the discrepancies may reflect the tendency of the YS08 MIC results to be slightly lower than the CLSI or SYO MIC results. We found that adjusting the fluconazole CLSI ECV of 1 μg/ml for *C. tropicalis* improved the performance of YS08, yielding a CA of 100% and reducing the minor error rate from 13.0% to 0%. In addition, we applied a modified ECV of 0.06 μg/ml to the YS08 micafungin test for *C. glabrata*, which reduced the minor error rate from 6.5% to 0%, while it increased the CA from 92.4% to 98.9%. Therefore, we emphasize that erroneous results may be observed if the results obtained by one method are interpreted by use of the ECVs/CBPs determined for another method. Considering that echinocandin resistance is more frequently found among *C. glabrata* isolates worldwide (35) than among other *Candida* species, we suggest that broadening the micafungin MIC detection range to 0.03 μg/ml and applying the CLSI ECV (0.03 μg/ml) for micafungin and *C. glabrata* might further improve the performance of YS08 for the detection of echinocandin resistance. In addition, YS08 will be most useful if either method- or species-dependent ECVs are available for the *Candida* isolate and antifungal agent evaluated, such as SYO (26, 27).

MATERIALS AND METHODS

Fungal isolates. A total of 292 clinical isolates of four common *Candida* species from a South Korean collection were tested. Table 3 described the 292 isolates evaluated in this study. The South Korean collection of *Candida* isolates included 284 BSI isolates and 8 isolates from other clinical specimens (4 from urine, 1 from ascitic fluid, 1 from sputum, 1 from skin, and 1 from ear pus) submitted to Chonnam National University Hospital, Gwangju, South Korea, from several South Korean hospitals during a nationwide surveillance conducted in South Korea (2003 to 2018) (19, 28, 36, 37). *Candida* isolates were identified to the species level using biochemical tests, matrix-assisted laser desorption ionization–time of flight mass spectrometry, and/or molecular methods (37, 38). For all *Candida* isolates, antifungal MICs were determined using the CLSI M27, 4th edition (M27-ED4), broth microdilution method (39). Sequence analyses of *ERG11* and three transcription factor genes (*TAC1*, *MRR1*, and *UPC2*) were performed for all 26 *C. albicans* and 57 *C. parapsilosis* isolates, irrespective of their fluconazole susceptibility (28, 40); the expression levels of *CDR1*, *MDR1*, and *ERG11* were quantified, and the *ERG11* and *UPC2* genes were sequenced for all 23 isolates of *C. tropicalis* (19). The *PDR1* genes were sequenced for all 39 FR isolates and 52 F-SDD isolates of *C. glabrata* (41). The *FKS1* (for *Candida* species) and *FKS2* (for *C. glabrata*) genes were sequenced for both the 16 MR isolates of three *Candida* species and the 79 MS *C. glabrata* isolates (42, 43). All isolates were stored at −70°C in Trypticase soy broth supplemented with 15% glycerol until they were tested. These isolates were passaged twice on potato dextrose agar at 35°C prior to testing.

Antifungal susceptibility testing. The antifungal MICs for each isolate were determined by three methods: by the CLSI M27-ED4 BMD method and with the Vitek 2 and Sensititre systems. For all *Candida* isolates, the MICs of fluconazole and micafungin were redetermined by the CLSI M27-ED4 broth microdilution method after 24 h of incubation; they were defined as the lowest concentrations that produced an approximately 50% growth reduction compared to the growth of a drug-free control (39). The Vitek 2 system AST-YS08 and the Sensititre YeastOne YO10 system were used following the manufacturers' instructions (25; bioMérieux). The ranges of antifungal drug concentrations used to

TABLE 3 Resistance mechanisms and categorical results for fluconazole and micafungin by the CLSI M27 method for all 292 isolates of four common *Candida* species evaluated in this study[a]

Candida species	Result by CLSI M27[b]			No. of isolates		
	Drug	Category	Defined mechanism(s) of resistance	Blood	Other	Total
C. albicans	Fluconazole	R	Erg11, K143R mutation	5	1	6
		S	No mutations (Erg11, Tac1, Mrr1, and Upc2) defined	16	4	20
	Micafungin	R	Fks1, S645P mutation	2		2
C. parapsilosis	Fluconazole	R	Erg11, Y132F mutation	26		26
		S	No mutations (Erg11, Tac1, Mrr1, and Upc2) defined	31		31
C. tropicalis	Fluconazole	R	*ERG11* overexpression	6	3	9
		R	Erg11, Y132F mutation	1		1
		S	No mutations (Erg11 and Upc2) defined	13		13
	Micafungin	R	Fks1, S654S/P	1		1
C. glabrata	Fluconazole	R	Pdr1 mutations N132S, G1099S (1),[c] S236N (1), P258L (1), P258S (2), V260A (1), L280S (1), S316I (1), P327L (1), S337F (1), E340G, D919Y (1), E340K (1), G346S (2), L347F (2), L366P, E555D (1), A731I (1), R376Q (1), S391L (1), Y556C (1), H576Y (2), Y584D (1), E714D (1), T752I (1), N768D (2), R772K (1), K776E (1), G788W (1), L825P (1), F832L, L833V (1), Y932C (1), G943S (1), S947L (1), D954E (1), T1080N (1), and G1088E (1)	39		39
		SDD	No Pdr1 mutation	52		52
	Micafungin	R	Fks mutations Fks1, S629P (1),[c] Fks1, P633T and Fks2, D666N (1), Fks2, S663P (8), Fks2, R665G (1), Fks2, I661_S662insFL (1), Fks2, and P667T (1)	13		13
		S	No Fks1 or Fks2 mutations	79		79
Total	Fluconazole	R/SDD	Presence of defined azole resistance mechanisms	77	4	81
		SDD/S	No azole mutation defined	112	4	116
	Micafungin	R	Fks1 or Fks2 mutations	16		16
		S	No Fks1 or Fks2 mutations	79		79

[a]CLSI, Clinical and Laboratory Standards Institute; R, resistant; S, susceptible; SDD, susceptible, dose dependent.
[b]Antifungal MICs were obtained by the CLSI M27-ED4 broth microdilution method (39) and categorized using the CLSI M60, 1st edition, method (30).
[c]Numbers in parentheses indicate the number of strains with that resistance mechanism. The new Pdr1 sequences of FR *C. glabrata* isolates have been deposited in GenBank with accession numbers MT077187, MT077189, MT077190, MT077193, MT077196, MT077197, MT077200, MT077202, MT077205 to MT077207, MT090722, MT090725, MT090726, MT090728 to MT090735, MT090737, and MT090738 (24 isolates); the Fks sequence has been given GenBank accession number MT090721 (1 isolate).

determine the MICs for fluconazole, voriconazole, micafungin, and caspofungin were 0.5 to 64 μg/ml, 0.12 to 8 μg/ml, 0.06 to 8 μg/ml, and 0.12 to 8 μg/ml, respectively, for YS08, and those for fluconazole, micafungin, caspofungin, and anidulafungin were 0.12 to 256 μg/ml, 0.008 to 8 μg/ml, 0.008 to 8 μg/ml, and 0.015 to 8 μg/ml, respectively, for SYO. Antifungal MICs were automatically determined with the Vitek 2 system, while the MIC endpoints in the SYO were visually determined after 24 h of incubation as changes in color (purple or blue) from that for the drug-free control growth (red). Two reference strains (*C. parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were tested in each antifungal susceptibility test as quality control isolates. The reproducibility of YS08 was determined by testing five isolates (*C. albicans* ATCC 64550, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750 and ATCC 201380, and *C. glabrata* ATCC 90030) in triplicate on three different days (44); the results showed 100% reproducibility for all azole and echinocandin agents.

Data analyses. EA was determined with respect to the MIC results of YS08 and SYO, with EA being a result within 2 dilutions (two wells) of the result obtained by CLSI BMD. The MIC results obtained by YS08 were interpreted according to two criteria, including CLSI CBPs and CLSI ECVs (Tables 1 and 2) (30, 34, 45). The MIC results obtained with SYO were interpreted according to two criteria, including CLSI CBPs and SYO ECVs (26, 27, 30). CA was defined as the percentage of isolates classified by the two commercial tests into the same susceptible (or WT) or resistant (or non-WT) category into which they were classified by the CLSI reference method. Errors were considered VMEs when an isolate classified as "resistant" by the CLSI method using the CLSI CBP was categorized as "susceptible" or "WT" by a test, MEs when an isolate classified as "susceptible" by the CLSI method using the CLSI CBP was classified as "resistant" or "non-WT" by a test, and minor errors for other discordances.

Ethics statement. This study was approved by the Institutional Review Board of Chonnam National University Hospital (approval number CNUH-2018-120).

Data availability. The new Pdr1 sequences of FR *C. glabrata* isolates have been deposited in GenBank with accession numbers MT077187, MT077189, MT077190, MT077193, MT077196, MT077197, MT077200, MT077202, MT077205 to MT077207, MT090722, MT090725, MT090726, MT090728 to MT090735, MT090737, and MT090738 (24 isolates), while the Fks sequence has been given GenBank accession number MT090721 (1 isolate) (Table 3).

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