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Multicenter Evaluation of the New Vitek 2 Yeast Susceptibility Test Using New CLSI Clinical Breakpoints for Fluconazole

M. A. Pfaller,[a] D. J. Diekema,[a] G. W. Procop,[b] N. P. Wiederhold[c]

University of Iowa College of Medicine, Iowa City, Iowa, USA[a]; Cleveland Clinic Foundation, Cleveland, Ohio, USA[b]; University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA[c]

A fully automated antifungal susceptibility test system recently updated to reflect the new species-specific clinical breakpoints (CBPs) of fluconazole for *Candida* (Vitek 2 AF03 yeast susceptibility test; bioMérieux, Inc., Durham, NC) was compared in three different laboratories with the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method by testing 2 quality control strains, 10 reproducibility strains (4 *Candida* species and 6 *Cryptococcus neoformans* strains), and 746 isolates of *Candida* species (702 isolates, 13 species) and 44 isolates of *C. neoformans* against fluconazole. Excellent essential agreement (EA) (within 2 dilutions) between the reference and Vitek 2 MICs was observed for fluconazole and *Candida* species (94.0%). The EA was lower for fluconazole and *C. neoformans* at 86.4%. The mean times to a result with the Vitek 2 test were 9.1 h for *Candida* species and 12.1 h for *C. neoformans*. Categorical agreement (CA) between the two methods was assessed by using the new species-specific CBPs. For less common species without fluconazole CBPs, the epidemiological cutoff values (ECVs) were used to differentiate wild-type (WT; MIC, ≤ECV) from non-WT (MIC, >ECV) strains. The CAs between the two methods were 92.0% for *Candida* species (0.3% very major errors [VME] and 2.6% major errors [ME]) and 84.1% for *C. neoformans* (4.5% VME and 11.4% ME). The updated Vitek 2 AF03 IUO yeast susceptibility system is comparable to the CLSI BMD reference method for testing the susceptibility of clinically important yeasts to fluconazole when using the new (lower) CBPs and ECVs.

The automation of laboratory testing has clear advantages in test standardization, reproducibility and timeliness of results, and savings in labor and reagents (1, 2). Whereas total laboratory automation has been slow to arrive in clinical microbiology laboratories, automated microbial identification and antimicrobial susceptibility testing systems are widely used in microbiology laboratories (1, 2). These systems have been broadly applied in the testing of bacteria but, aside from the identification of yeasts, have not been useful for fungi (2).

Antifungal susceptibility testing of *Candida* species and *Cryptococcus neoformans* has been standardized by the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Testing (3), and until recently, the only commercially available methods for performing such testing have been manual methods such as the Sensititre Yeast One (TREK Diagnostic Systems, Cleveland, OH) and the Etest (bioMérieux, Inc., Durham, NC). The recent introduction of the Vitek 2 yeast susceptibility test (bioMérieux, Inc.) has provided a completely automated means of performing antifungal susceptibility testing of yeasts that has been shown to produce reproducible, rapid, and accurate results consistent with those obtained with the CLSI reference broth microdilution (BMD) method for amphotericin B, flucytosine, fluconazole, and voriconazole (4–9). The ease of performing antifungal susceptibility testing provided by the Vitek 2 system offers the possibility of performing this testing to laboratories that previously had not done so because of the manual nature of the available methods. Indeed, in the 2013 F1-A challenge by the College of American Pathologists (CAP) proficiency testing program, 168 laboratories out of 424 participants (39.6%) reported results obtained with the Vitek 2 yeast susceptibility test (CAP data on file).

Several studies have demonstrated excellent essential agreement (EA; within 2 dilutions) and categorical agreement (CA; susceptibility results that fall within the same interpretive category) between the Vitek 2 and CLSI BMD methods for testing fluconazole against *Candida* species and *C. neoformans* (4–9). All of these studies were performed prior to the adoption by the CLSI of new (lower), species-specific clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) of fluconazole for several species of *Candida* and *C. neoformans* (10–14). Pfaller et al. (15) reanalyzed the original data from a previous study (7) by using the new CBPs and ECVs of fluconazole and found that the CA between the Vitek 2 and CLSI BMD methods was 96.8% with no very major errors (VME) and only 1.3% major errors (ME) (15). Limitations of this reanalysis included a rather small number of isolates that were either resistant (R) or non-wild type (non-WT) to fluconazole ($n = 20$; 4.7% of the isolates tested) and the fact that the lower end of the dilution series in the Vitek 2 test stopped at 1 μg/ml (16). Subsequently, the Vitek 2 yeast susceptibility test was reconfigured to extend the fluconazole dilution range to 0.5 μg/ml and to employ new species-specific CBPs of susceptible (S) of ≤2 μg/ml, susceptible dose dependent (SDD) of 4 μg/ml, and resistant (R) of ≥8 μg/ml for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* and SDD of ≤32 μg/ml and R of ≥64 μg/ml for *C. glabrata*. The purpose of the present study was to validate the performance of the Vitek 2 AF03 IUO yeast susceptibility test with the new interpretive criteria for fluconazole against a broad range of *Candida* species and *C. neoformans* in three independent laboratories.

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Address correspondence to Daniel J. Diekema, daniel-diekema@uiowa.edu.

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Notably, the collection of 746 isolates in this study contained 86 isolates (82 isolates of *Candida* and 4 isolates of *C. neoformans*; 11.5% of the total) that were either R or non-WT to fluconazole according to the new interpretive criteria. The Vitek 2 results were compared to those from a frozen reference BMD panel performed according to CLSI guidelines.

MATERIALS AND METHODS

Study design. This study was designed to compare the MICs of fluconazole obtained by the Vitek 2 AF03 IUO yeast susceptibility test with the new species-specific CBPs to those obtained by the CLSI M27-A3 BMD method (3) in the three laboratories. Each laboratory tested approximately 200 clinical isolates of *Candida* species and *C. neoformans* (range, 199 to 212 isolates) by the Vitek 2 system and the CLSI frozen reference BMD panel (a total of 620 clinical isolates). In addition, a challenge set of 126 well-characterized stock isolates was tested by both methods in one of the laboratories. Intra- and interlaboratory reproducibility was determined by testing a panel of four *Candida* species isolates and six *C. neoformans* isolates in triplicate on 3 separate days in each of the participating laboratories. The MICs obtained with the Vitek 2 system after 5 to 17 h of incubation (depending on the organism growth rate) were compared to those obtained with the reference BMD read after 24 h of incubation for *Candida* species and after 72 h of incubation for *C. neoformans*.

Test organisms. The test organisms included two American Type Culture Collection (ATCC) strains that have been established as quality control (QC) strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) by the CLSI (3, 10). A challenge set of 118 isolates of *Candida* species and 8 isolates of *C. neoformans* selected to provide strains with on-scale MICs and to represent both clinically important species and resistance mechanisms (19 resistant isolates; 15.1% of the total) were tested in one of the participating laboratories. The challenge set of *Candida* isolates included 41 isolates of *C. albicans*, 8 isolates of *C. dubliniensis*, 33 isolates of *C. glabrata*, 5 isolates of *C. guilliermondii*, 5 isolates of *C. lusitaniae*, 1 isolate of *C. norvegensis*, 10 isolates of *C. parapsilosis*, 2 isolates of *C. pelliculosa*, and 13 isolates of *C. tropicalis*. An additional 584 clinical isolates of *Candida* species and 36 clinical isolates of *C. neoformans* were also tested. The clinical isolates of *Candida* species included 174 isolates of *C. albicans*, 5 isolates of *C. dubliniensis*, 148 isolates of *C. glabrata*, 3 isolates of *C. guilliermondii*, 1 isolate of *C. haemulonii*, 3 isolates of *C. kefyr*, 30 isolates of *C. krusei*, 23 isolates of *C. lusitaniae*, 99 isolates of *C. parapsilosis*, 2 isolates of *C. pelliculosa*, 94 isolates of *C. tropicalis*, and 2 isolates of *C. utilis*. These were all recent clinical isolates and were selected to represent the clinically prevalent species, including 63 (10.8%) fluconazole-resistant strains. Reproducibility within and among laboratories was assessed by using a panel of 4 *Candida* isolates (*C. guilliermondii* strain 304202, *C. krusei* strain 304204, *C. krusei* strain 304850, and *C. lusitaniae* strain 304205) and 6 *C. neoformans* isolates (strains 304213, 304214, 304215, 304216, 304217, and 304218). These isolates were selected to provide on-scale fluconazole MICs ranging from 1 to 32 μg/ml. All of the isolates were identified by standard methods used routinely in each laboratory (16). Prior to testing, each isolate was passaged at least twice on Sabouraud dextrose agar (Thermo Fisher Scientific) to ensure purity and viability.

Antifungal agents and microdilution panels. The Vitek 2 AF03 IUO cards containing serial 2-fold dilutions of fluconazole (range, 0.5 to 64 μg/ml) and the frozen BMD panels containing serial 2-fold dilutions of fluconazole (range, 0.25 to 128 μg/ml) were provided by the manufacturer. The Vitek 2 cards were shipped in sealed packages and stored at 2 to 8°C until testing was performed. The BMD panels were shipped frozen in sealed packages and stored at −70°C until the day of the test.

Inoculum preparation. Stock inoculum suspensions of the yeast isolates were obtained from 24-h cultures (48 to 72 h for *C. neoformans*) on Sabouraud dextrose agar at 35°C. The inoculum suspensions for the Vitek 2 test were prepared in sterile saline to a turbidity equal to a 2.0 McFarland standard with the bioMérieux DensiCHEK Plus instrument. The inoculum suspensions for the reference BMD were prepared by diluting a portion of the 2.0 McFarland suspension prepared for the Vitek 2 test to match the turbidity of a 0.5 McFarland standard.

CLSI BMD method. Reference BMD testing was performed exactly as outlined in CLSI document M27-A3 (3), with a final inoculum concentration of $1.5 \times 10^3 \pm 1.0 \times 10^3$ cells/ml and RPMI 1640 medium with 0.2% glucose buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer. The panels were incubated in air at 35°C and observed for the presence or absence of growth at 24 h (*Candida*) and 72 h (*C. neoformans*). The fluconazole MIC was defined as the lowest concentration that produced a prominent decrease in turbidity (ca. 50% growth reduction) relative to that of the drug-free control (3).

Vitek 2 AF03 IUO yeast susceptibility test. The standardized 2.0 McFarland inoculum suspension was placed into a Vitek 2 cassette along with a sterile polystyrene test tube and a yeast susceptibility test card for each organism. The loaded cassettes were then placed into the Vitek 2 instrument, and the respective yeast suspensions were diluted appropriately, after which the cards were filled, incubated, and read automatically. The time of incubation varied from 5 to 17 h, depending on the rate of growth in the drug-free control well, and the results were expressed as MICs in micrograms per milliliter.

QC. QC was ensured by testing the CLSI-recommended QC strains *C. parapsilosis* ATCC 2209 and *C. krusei* ATCC 6258 (3, 10). These isolates were tested between 32 and 64 times in each of the three laboratories (total number of results = 260), and 259 (99.6%) MICs were in the respective reference ranges.

Analysis of results. The MICs obtained with the Vitek 2 AF03 IUO yeast susceptibility test were compared with those obtained with the reference BMD panels read at 24 h (*Candida* species) and 72 h (*C. neoformans*). High off-scale MICs were converted to the next highest concentration, and low off-scale results were left unchanged. Discrepancies among MIC endpoints of more than two dilutions (two wells) were used to calculate the EA. Interlaboratory and intralaboratory agreement, assessed with the 10-isolate reproducibility panel, was defined as MICs that were within a 3-dilution range. The new species-specific CBPs for *Candida* species were used to obtain percentages of CA between the MICs determined with the Vitek 2 system and the 24-h CLSI BMD method. The fluconazole susceptibility categories for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were as follows: ≤2 μg/ml, S; 4 μg/ml, SDD; ≥8 μg/ml, R. Those for *C. glabrata* were as follows: ≤32 μg/ml, SDD; ≥64 μg/ml, R (10, 13, 14). All isolates of *C. krusei* are considered to be R to fluconazole, irrespective of the MIC. Because of the lack of CBPs for the less common species of *Candida* and *C. neoformans* (14), the ECVs of fluconazole were used for *C. lusitaniae* (1 μg/ml), *C. dubliniensis* (0.5 μg/ml), *C. guilliermondii* (8 μg/ml), *C. pelliculosa* (4 μg/ml), *C. kefyr* (1 μg/ml), and *C. neoformans* (16 μg/ml) to categorize isolates of these species as wild type (WT; MIC, ≤ECV) or non-WT (MIC, >ECV) (11, 12, 14). VME were identified when the reference CLSI BMD MIC indicated an R or non-WT result and the Vitek 2 MIC was S or WT. ME were identified when the isolate was classified as R or non-WT by the Vitek 2 system and S or WT by the CLSI BMD method. Minor errors were identified when the isolate was classified as SDD by one system and S or R by the other system.

RESULTS AND DISCUSSION

Table 1 summarizes the *in vitro* fluconazole susceptibilities of 702 isolates of *Candida* species as determined by the Vitek 2 AF03 IUO system and by the reference BMD read at 24 h. Because of the similarity of the results obtained with the Vitek 2 test and the 24-h BMDs for both the challenge isolates (118 isolates, 97.5% EA) and the clinical isolates (584 isolates, 93.3% EA), the results for the two organism sets are combined in Table 1. In general, the MICs of fluconazole were typical for each *Candida* species (17), with the lowest MICs obtained with both the Vitek 2 and BMD methods for *C. albicans* and *C. dubliniensis* and the highest MICs obtained

TABLE 1 Fluconazole susceptibilities of 702 isolates of *Candida* species as determined by the Vitek 2 AF03 IUO yeast susceptibility test and the CLSI BMD method[a]

Species (no. of isolates tested) and test method	MIC (μg/ml) Range	50% of strains	90% of strains	EA (%)
C. albicans (215)				
Vitek 2	≤0.5–≥64	≤0.5	1	95.8
BMD	≤0.25–≥256	≤0.25	1	
C. glabrata (181)				
Vitek 2	1–≥64	8	≥64	88.4
BMD	0.5–≥256	4	64	
C. parapsilosis (109)				
Vitek 2	0.5–≥64	≤0.5	8	99.1
BMD	≤0.25–64	0.5	8	
C. tropicalis (107)				
Vitek 2	≤0.5–≥64	1	8	94.4
BMD	≤0.25–≥256	≤0.25	4	
C. krusei (30)				
Vitek 2	4–≥64	16	32	93.3
BMD	2–≥256	16	32	
C. lusitaniae (28)				
Vitek 2	≤0.5–≥64	≤0.5	2	92.9
BMD	≤0.25–128	≤0.25	1	
C. dubliniensis (13)				
Vitek 2	≤0.5–1	≤0.5	1	100
BMD	≤0.25	≤0.25	≤0.25	
C. guilliermondii (8)				
Vitek 2	≤0.5–2	2		100
BMD	≤0.25–4	2		
C. pelliculosa (4)				
Vitek 2	2	2		100
BMD	1–2	2		
C. kefyr (3)				
Vitek 2	1–2	1		66.7
BMD	≤0.25–0.5	≤0.25		
C. utilis (2)				
Vitek 2	2–8	2		100
BMD	2–4	2		
C. haemulonii (1)				
Vitek 2	≤0.5			100
BMD	≤0.25			
C. norvegensis (1)				
Vitek 2	8			100
BMD	8			
All *Candida* species (702)				
Vitek 2	≤0.5–≥64	1	32	94.0
BMD	≤0.25–≥256	0.5	16	

[a] Isolates included both clinical (n = 584) and challenge (n = 118) sets. BMD results were read at 24 h of incubation. Percent, EA (±2 $\log_2$ dilutions) between Vitek 2 and CLSI BMD MICs is shown.

TABLE 2 Fluconazole susceptibilities of 44 isolates of *C. neoformans* as determined by the Vitek 2 AF03 IUO yeast susceptibility test and the CLSI BMD method[a]

Test method	MIC (μg/ml) Range	50% of strains	90% of strains	EA (%)
Vitek 2	1–32	2	32	
BMD	0.5–128	4	16	86.4

[a] Isolates include both clinical (n = 36) and challenge (n = 8) sets. BMD results were read at 72 h of incubation. Percent EA (±2 $\log_2$ dilutions) between Vitek 2 and CLSI BMD MICs is shown.

for *C. glabrata* and *C. krusei*. In general, the MICs generated by the Vitek 2 system were slightly higher than those generated by BMD.

The overall EA between the Vitek 2 AF03 IUO and 24-h BMD MICs was 94.0% (92.4% for on-scale results). Of the discrepancies noted between the Vitek 2 test and the BMD, the MICs generated by the Vitek 2 test were higher than those obtained by BMD in 40 (95.2%) of 42 instances. The EA was greater than 90% for all of the species, with the exception of *C. glabrata* (EA = 88.4%) and *C. kefyr* (EA = 66.7%).

The fluconazole susceptibilities of 44 isolates of *C. neoformans* determined by the Vitek 2 AF03 IUO system are compared with those obtained with the reference BMD read at 72 h in Table 2. The EA between the Vitek 2 test and BMD of fluconazole for *C. neoformans* was 86.4%. Again, the MICs generated by the Vitek 2 system were generally 1 dilution higher than those obtained with BMD.

The mean time to a result with the Vitek 2 system was 9.1 h for *Candida* species (range, 7.5 h [*C. glabrata*] to 11.2 h [*C. parapsilosis*]) and 12.1 h for *C. neoformans* (data not shown). All of the isolates grew sufficiently well in both the Vitek 2 and BMD systems. Similar results were obtained at all three study sites.

The Vitek 2 fluconazole MICs were highly reproducible, as determined by replicate testing of a panel of four *Candida* species isolates and six *C. neoformans* isolates in the three laboratories (Table 3). Both intra- and interlaboratory reproducibility was 100% for all 10 organisms. This is in agreement with previous evaluations of the Vitek 2 system (6, 7, 9) and underscores the high level of test standardization achieved with this automated microbiology system.

The CA between the results obtained with the Vitek 2 AF03 IUO system and those obtained by 24-h CLSI BMD (72 h for *C. neoformans*) was assessed by combining the data obtained with the clinical and challenge organism collections in all three laboratories (Table 4). The MICs for a total of 698 isolates of *Candida* (2 isolates of *C. utilis* and 1 each of *C. haemulonii* and *C. norvegensis* were omitted because of a lack of CBPs and ECVs) and 44 isolates of *C. neoformans* were used to determine the CA.

The CA was 92.0% for *Candida* species (0.3% VME, 2.6% ME, and 5.1% minor errors) and 84.1% for *C. neoformans* (4.5% VME and 11.4% ME). The CA was greater than 90% for all of the species except *C. glabrata* (87.8%), *C. lusitaniae* (85.7%), *C. dubliniensis* (84.6%), *C. kefyr* (66.7%), and *C. neoformans* (84.1%). The only VME in the entire study were seen with *C. parapsilosis* (2 isolates) and *C. neoformans* (2 isolates). The remainder of the discrepancies between the two systems were either ME or minor errors reflecting the tendency of the Vitek 2 results to be slightly higher than the BMD results. The ME seen with *C. lusitaniae*, *C. dubliniensis*, *C. kefyr*, and *C. neoformans* may be due in part to the use of ECVs to

TABLE 3 Vitek 2 fluconazole MIC reproducibility within and among three different laboratories

Species, strain, and study site(s)	No. of results at MIC (μg/ml) of:					
	1	2	4	8	16	32
C. guilliermondii 304202						
1		9				
2		9				
3		9				
All		27				
C. krusei 304204						
1					9	
2					9	
3					9	
All					27	
C. krusei 304850						
1					1	8
2						9
3						9
All					1	26
C. lusitaniae 304205						
1		9				
2		9				
3		9				
All		27				
C. neoformans 304213						
1		8	1			
2		9				
3		9				
All		26	1			
C. neoformans 304214						
1		9				
2		7	2			
3		9				
All		25	2			
C. neoformans 304215						
1		9				
2	3	6				
3	1	8				
All	4	23				
C. neoformans 304216						
1		9				
2		9				
3	1	8				
All	1	26				
C. neoformans 304217						
1		9				
2		9				
3		9				
All		27				
C. neoformans 304218						
1		8	1			
2		9				
3		9				
All		26	1			

TABLE 4 CA between Vitek 2 AF03 IUO yeast susceptibility test and CLSI BMD MICs of fluconazole for 698 *Candida* and 44 *C. neoformans* isolates when using new CBPs and ECVs

Species (no. of isolates tested) and test method	% of MICs by category[a]			CA (%)	% VME	% ME	% Minor errors
	S	SDD	R				
C. albicans (215)							
Vitek 2	93.0	0.5	6.5				
BMD[b]	94.4	3.3	2.3	94.9	0.0	2.3	2.8
C. glabrata (181)							
Vitek 2		71.8	28.2				
BMD		85.6	14.4	87.8	0.0	0.0	12.2
C. parapsilosis (109)							
Vitek 2	85.3	0.9	13.8				
BMD	83.5	3.7	12.8	94.5	1.8	0.9	2.8
C. tropicalis (107)							
Vitek 2	86.0	1.9	12.1				
BMD	87.9	6.5	5.6	90.6	0.0	4.7	4.7
C. krusei (30)							
Vitek 2			100				
BMD			100	100	0.0	0.0	0.0
C. lusitaniae (28)							
Vitek 2	82.1		17.9				
BMD	96.4		3.6	85.7	0.0	14.3	
C. dubliniensis (13)							
Vitek 2	84.6		15.4				
BMD	100		0.0	84.6	0.0	15.4	
C. guilliermondii (8)							
Vitek 2	100		0.0				
BMD	100		0.0	100	0.0	0.0	
C. pelliculosa (4)							
Vitek 2	100		0.0				
BMD	100		0.0	100	0.0	0.0	
C. kefyr (3)							
Vitek 2	66.7		33.3				
BMD	100		0.0	66.7	0.0	33.3	
C. neoformans (44)							
Vitek 2	84.1		15.9				
BMD	90.9		9.1	84.1	4.5	11.4	

[a] The CBPs for S, SDD, and R, respectively, were those of the CLSI of fluconazole for *C. albicans*, *C. tropicalis*, and *C. parapsilosis* (S, ≤2 μg/ml; SDD, 4 μg/ml; R, ≥8 μg/ml) and *C. glabrata* (SDD, ≤32 μg/ml; R, ≥64 μg/ml). There are no CBPs of fluconazole for *C. krusei*; all isolates are categorized as R irrespective of the MIC. Because of the lack of CBPs, the ECV was used to determine fluconazole S (WT) and R (non-WT) of *C. lusitaniae* (≤1 and ≥2 μg/ml), *C. dubliniensis* (≤0.5 and ≥1 μg/ml), *C. guilliermondii* (≤8 and ≥16 μg/ml), *C. pelliculosa* (≤4 and ≥8 μg/ml), *C. kefyr* (≤1 and ≥2 μg/ml), and *C. neoformans* (≤16 and ≥32 μg/ml).

[b] The CLSI BMD test result was read at either 24 h (*Candida* species) or 72 h (*C. neoformans*) of incubation.

assess the CA where only two categories, WT and non-WT, were employed, as opposed to the use of CBPs with other comparisons where the S and R categories were buffered by the SDD category. This provides the opportunity for minor errors rather than ME or VME for some comparisons. If the previous CBPs of ≤8 μg/ml

(S), 16 to 32 μg/ml (SDD), and ≥64 μg/ml (R) were applied to these species of *Candida*, the CAs improved to 92.9% for *C. lusitaniae* and 100.0% for *C. dubliniensis* and *C. kefyr* (data not shown).

These results provide further support for our reanalysis of data from a previous multicenter study (15) indicating that the new (lower) CBPs and ECVs do not adversely affect the performance of the Vitek 2 system for testing fluconazole against *Candida*. We demonstrate that the excellent EA and reproducibility found in the earlier study (7) can also be achieved with the new Vitek AF03 IUO card. In our previous evaluation of the Vitek 2 test using the new CBPs and ECVs, we found an overall CA of 96.8% with 0.0% VME and 1.3% ME for nine different species of *Candida*, compared with a CA of 92.0%, 0.3% VME, and 2.6% ME in the present study with the Vitek AF03 IUO card. The previous study was limited by the lack of fluconazole-resistant isolates, whereas the present study included 82 fluconazole-resistant *Candida* isolates (11.7% of the total) and four non-WT strains of *C. neoformans* (9.1% of the total). Although the present study is still limited by the fact that none of the isolates were characterized with respect to azole resistance mechanisms, the inclusion of a larger number of isolates for which the fluconazole BMD MICs were elevated provides additional support to the findings of others that the Vitek 2 system is able to accurately detect fluconazole-resistant yeasts (9) and that the new AF03 IUO card and updated software correctly categorize these resistant strains according to the new CBPs. Notably, the Vitek 2 system was not as reliable in differentiating WT from non-WT *C. neoformans* isolates, with the tendency toward higher MICs leading to a high rate of ME.

The Vitek 2 AF03 IUO yeast susceptibility test using the new CBPs reliably identifies fluconazole resistance among *Candida* species isolates and demonstrates excellent quantitative and qualitative agreement with the reference BMD method when testing fluconazole in three independent laboratories. The use of the Vitek 2 system provides a highly automated, rapid, and standardized means of performing antifungal susceptibility testing in the clinical microbiology laboratory.

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