



## Comparison of Vitek 2C antifungal susceptibility testing with broth microdilution testing for *Candida* species

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### Abstract

**Background:** The reference method for antifungal susceptibility testing is broth microdilution according to Clinical and Laboratory Standard Institute (CLSI) guidelines. However, the fully automated system, Vitek 2C system may reduce the workload and observer bias associated with manual broth microdilution. This study aimed to compare the results of YS08 card with the results of the broth microdilution (BMD) method.

**Methods:** A total of 50 clinical *Candida* isolates were included in the study. The susceptibility testing was done by Vitek 2C using the YS08 card. Broth microdilution was done according to CLSI guidelines M27M44S-Ed3.

**Results:** For *C. albicans*, the categorical agreement was 85.8%, 71.5%, 85.8%, and 100% for fluconazole, voriconazole, caspofungin, and micafungin, respectively. The minor errors (MiE) of 14.2% for fluconazole and caspofungin, 28.5% for voriconazole, were detected in *C. albicans*. In *C. glabrata*, the categorical agreement (CA) was 100% for micafungin, voriconazole, but 63.7% for caspofungin. An MiE of 36.3% was detected for caspofungin. *C. parapsilosis* showed a 100% CA for fluconazole, caspofungin, and micafungin, and 85.8% for voriconazole. There were 14.2% of MiEs for voriconazole. In *C. tropicalis*, 100% CA was observed for fluconazole, micafungin, and caspofungin, and 88.9% for voriconazole. Moreover, 11.1% (1/9) of MiEs was observed for voriconazole. In *C. auris*, there was a 100% CA for caspofungin and micafungin, 77.8% for fluconazole, and 66.7% for amphotericin B. There was a major error of 22.2% for fluconazole and 33.3% for amphotericin B.

**Conclusion:** The majority of Vitek 2C showed comparable results with the broth microdilution (BMD) method. Only minor errors (MiEs) were observed in the tested *Candida* species.

### Introduction

*Candida* infections are one of the most common causes of invasive fungal infections in hospitals, leading to high morbidity and mortality, particularly in patients in intensive care units (1,2). In recent years, azole-resistant non-*Candida albicans* and multidrug-resistant *Candida auris* have emerged as important pathogens, resulting in treatment failures (2,3). Hence antifungal susceptibility testing (AFST) is important to monitor the development of resistance in these species. We need a faster and more reliable method for AFST. According to the Clinical and Laboratory Standard Institute (CLSI) guidelines, the reference method for AFST is Broth microdilution (BMD). However, the fully automated system, Vitek 2C system may reduce the workload and observer bias associated with manual BMD. It has also been updated for AFST of *Candida* species and interpreted as per the latest CLSI guidelines. The aim of this study was to compare the antifungal susceptibility results of *Candida* spp. with Vitek 2 AST-YS08 card and standard BMD method.

### Methods

A total of 50 isolates of *Candida* spp. from blood cultures were included in the study from January 2022 to December 2022. Identification was done by the Vitek 2C system using the YST card (bioMérieux, Marcy l'Etoile, France). The antifungal susceptibility testing was done both by Vitek 2C using the YS08 (bioMérieux, Marcy l'Etoile, France) card, and BMD was done according to the CLSI guidelines M27M44S-Ed3 (4). The CLSI quality control isolate *Candida parapsilosis* ATCC 22019 was used as a control for each run.

The following drugs were tested, and the range of the concentration was 0.125-64 µg/L for fluconazole, and 0.03-16 µg/L for voriconazole, caspofungin, micafungin, and amphotericin B. The antifungal powders were procured from Sigma-Aldrich, United States. The test was done in duplicate. Discrepant results were repeated by both Vitek 2C and BMD. The BMD plates were incubated at 35 °C for 24 hrs. The results were interpreted as per CLSI guidelines M27M44S-Ed3 (5). Vitek 2C does not display MIC for *C. auris*. The results of MIC were extrapolated in Vitek 2C by choosing any non-*Candida albicans*. The breakpoints recommended by the CDC were used for *C. auris* (6).

Discrepancies among MIC endpoints of more than two dilutions (Two wells) were used to calculate the essential agreement (EA) between the MICs determined with the Vitek 2 system and by the reference BMD (2). Categorical agreement (CA) was defined as the percentage of isolates classified in the same category by the reference procedures and the test method (7). Very major errors (VME) were defined when the isolate was considered resistant by the reference

procedure but susceptible by the VITEK 2 system. Major errors (ME) occurred when the isolate was susceptible by the reference method but resistant by the VITEK 2 system, and minor error (MiE) were identified when the results by one of the methods included susceptible or resistant and susceptible-dose dependent/intermediate by the other method (8).

### Results

The isolates of *Candida* included seven *Candida albicans*, nine *Candida tropicalis*, fourteen *Candida parapsilosis*, eleven *Candida glabrata*, and nine *Candida auris*. For *C. albicans*, the categorical agreement was 85.8%, 71.5%, 85.8%, and 100% for fluconazole, voriconazole, caspofungin, and micafungin, respectively. The MiE of 14.2% was detected for fluconazole and caspofungin, as well as 28.5% for voriconazole, in *C. albicans*. In *C. glabrata*, the CA was 100% for micafungin and voriconazole, while 63.7% for caspofungin. An MiE of 36.3% for caspofungin were detected. *C. parapsilosis* showed 100% CA for fluconazole, caspofungin, micafungin and 85.8% for voriconazole. There were 14.2% of MiEs for voriconazole. In *C. tropicalis*, a 100% CA was observed for fluconazole, micafungin, and caspofungin, and 88.9% for voriconazole. Also, an MiE of 11.1% (1/9) was observed for voriconazole. In *C. auris*, there was a 100% CA observed for caspofungin and micafungin, 77.8% for fluconazole, and 66.7% for amphotericin B. There was an ME of 22.2% for fluconazole and 33.3% for amphotericin B.

The susceptibility pattern of the *Candida* spp. by BMD and Vitek 2C is shown in Table 1. The agreement between the results of Vitek 2C antifungal susceptibility testing with broth microdilution was high for all tested *Candida* spp. (Table 2).

### Discussion

Antifungal susceptibility testing will help clinicians start appropriate treatment for invasive *Candidal* infections. The standard method for AFST is BMD, which is time-consuming and difficult to perform (9). The Vitek 2 YS08 card has been updated for AFST according to the CLSI guidelines. It is easy to perform and the time consumed is shorter compared to BMD (1). In the present study, AFST was done for 50 isolates of *Candida* spp. against fluconazole, voriconazole, caspofungin, and micafungin. Many studies have compared Vitek 2 results with CLSI BMD at 24 hrs and 48 hrs of incubation (9,10,11). In the present study, the BMD plates were read at 24hrs as per CLSI guidelines (6). In the present study, the EA for fluconazole, caspofungin and micafungin was 100% across all tested *Candida* spp., while it was lower for voriconazole in *C. albicans* and *C.*

parapsilosis. The comparison of the EA, CA, and various errors from different studies is shown in Table 3.

There was an MiE of 14.2% in case of *C. albicans* and ME of 22.2% in case of *C. auris* for fluconazole. Vitek 2 does not show the MIC for fluconazole in *C. glabrata* as the modified fluconazole formulation has not been validated for the species. Hence, this drug was not compared in the present study for *C. glabrata* (1). In the present study, we reported MiE for voriconazole for the *Candida* spp. Other studies have reported both minor and major errors for voriconazole (Table 3). For voriconazole, there are no breakpoints for *C. glabrata* in CLSI (5) and for *C. auris* in CDC (4). In the case of *C. glabrata*, there is insufficient data to correlate between in vitro susceptibility testing and clinical outcomes for voriconazole (5).

Minor errors (MiEs) were observed with caspofungin in *C. albicans* and *C. glabrata* in the present study. In other studies, there were both minor and major errors for caspofungin (Table 3). In the case of *C. glabrata*, micafungin results are more reliable in YS08 than caspofungin as there is a high chance of false resistance (12). Eight isolates of *C. glabrata* were resistant and one isolate had intermediate susceptibility to caspofungin in our study. According to the CLSI guidelines, caspofungin resistance should be confirmed with additional testing

such as micafungin/anidulafungin and DNA sequence analysis of FKS genes (5). In the current study, no minor or major errors were observed with micafungin for other *Candida* spp., while there was an ME with micafungin in a study from Korea (1). The breakpoints for *C. parapsilosis* are applicable only in regions with a low prevalence of cryptic species (5).

In the case of *C. auris*, the EA was low for amphotericin B and there was an ME of 33.3%. Hence, automated systems should be used with caution and results should be confirmed with BMD while doing AFST for *C. auris* (13). There are only two studies that assessed the clinical performance of YS08 cards compared with BMD using clinical isolates from the world (1,14,15). Two studies had compared YS08 with sensitizer (12,16). To the best of our knowledge, this is probably the second study from India.

### Conclusion

The majority of Vitek 2C antifungal susceptibility testing results were consistent with those obtained using BMD. There were MiE in the tested *Candida* spp. except for *C. auris*, which showed ME for fluconazole and amphotericin B. Thus, the Vitek 2 system provides reliable results in a shorter time, aiding clinicians in initiating timely and appropriate antifungal therapy.

Table 1. Susceptibility pattern of *Candida* spp. by BMD and Vitek 2C

Organism	Antifungal drugs	Methods	No. of isolates			
			Susceptible	Susceptible dose-dependent	Intermediate	Resistant
<i>C. Albicans</i> , n=7	Fluconazole	BMD	6	1	-	-
		Vitek 2C	6	-	-	1
	Voriconazole	BMD	5	-	2	-
		Vitek 2C	6	-	-	1
	Caspofungin	BMD	7	-	-	-
		Vitek 2C	6	-	1	-
Micafungin	BMD	7	-	-	-	
	Vitek 2C	7	-	-	-	
<i>C. Tropicalis</i> , n=9	Fluconazole	BMD	8	-	-	1
		Vitek 2C	8	-	-	1
	Voriconazole	BMD	8	-	1	-
		Vitek 2C	7	-	2	-
	Caspofungin	BMD	9	-	-	-
		Vitek 2C	9	-	-	-
Micafungin	BMD	9	-	-	-	
	Vitek 2C	9	-	-	-	
<i>C. Parapsilosis</i> , n=14	Fluconazole	BMD	13	-	-	1
		Vitek 2C	13	-	-	1
	Voriconazole	BMD	12	-	2	-
		Vitek 2C	11	-	3	-
	Caspofungin	BMD	14	-	-	-
		Vitek 2C	14	-	-	-
Micafungin	BMD	14	-	-	-	
	Vitek 2C	14	-	-	-	
<i>C. Glabrata</i> , n=11	Caspofungin	BMD	2	-	1	8
		Vitek 2C	1	-	3	7
	Micafungin	BMD	11	-	-	-
		Vitek 2C	11	-	-	-
<i>C. Auris</i> , n=9	Fluconazole	BMD	4	-	-	5
		Vitek 2C	2	-	-	7
	Caspofungin	BMD	9	-	-	-
		Vitek 2C	9	-	-	-
	Micafungin	BMD	9	-	-	-
		Vitek 2C	9	-	-	-
Amphotericin B	BMD	4	-	-	5	
	Vitek 2C	1	-	-	8	

Table 2. Comparison of results of Vitek 2C antifungal susceptibility testing with BMD

Organism	Antifungal drugs	No. of isolates (%)					
		EA*	CA†	VME‡	ME§	MiE	
<i>C. Albicans</i> , n=7	Fluconazole	7 (100)	6 (85.8)	0	0	1 (14.2)	
	Voriconazole	6 (85.7)	5 (71.5)	0	0	2 (28.5)	
	Caspofungin	7 (100)	6 (85.8)	0	0	1 (14.2)	
	Micafungin	7 (100)	7 (100)	0	0	0	
<i>C. Tropicalis</i> , n=9	Fluconazole	9 (100)	9 (100)	0	0	0	
	Voriconazole	9 (100)	8 (88.9)	0	0	1 (11.1)	
	Caspofungin	9 (100)	9 (100)	0	0	0	
	Micafungin	9 (100)	9 (100)	0	0	0	
<i>C. Parapsilosis</i> , n=14	Fluconazole	14 (100)	9 (100)	0	0	0	
	Voriconazole	13 (92.8)	12 (85.8)	0	0	2 (14.2)	
	Caspofungin	14 (100)	14 (100)	0	0	0	
	Micafungin	14 (100)	14 (100)	0	0	0	
<i>C. Glabrata</i> , n=11	Caspofungin	11 (100)	7 (63.7)	0	0	4 (36.3)	
	Micafungin	11 (100)	11 (100)	0	0	0	
<i>C. Auris</i> , n=9	Fluconazole	9 (100)	7 (77.8)	0	2 (22.2)	0	
	Caspofungin	9 (100)	9 (100)	0	0	0	
	Micafungin	9 (100)	9 (100)	0	0	0	
	Amphotericin B	BMD	6 (66.7)	6 (66.7)	0	3 (33.3)	0
		Vitek 2C	6 (66.7)	6 (66.7)	0	3 (33.3)	0

\*EA: Essential Agreement, †CA: Categorical Agreement, ‡VME: Very Major Error, §ME: Major Error, ||MiE: Minor Error

Table 3. Comparison with other similar studies

Study	<i>C. albicans</i>				<i>C. tropicalis</i>				<i>C. parapsilosis</i>				<i>C. glabrata</i>				<i>C. auris</i>				
	flu*	vor†	cas‡	mic§	flu*	vor†	cas‡	mic§	flu*	vor†	cas‡	mic§	flu*	vor†	cas‡	mic§	flu*	cas‡	mic§		
Pfaller MA, USA, 2007 (10)	n-198				n-238				n-43				n-83				-				
	EA	97	NT <sup>  </sup>	NT <sup>  </sup>	100	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	93.3	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	98.8	NT <sup>  </sup>							
	CA	99	NT <sup>  </sup>	NT <sup>  </sup>	100	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	97	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	89.2	NT <sup>  </sup>							
	VME	0	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>							
	ME	0	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>							
	MiE	1	NT <sup>  </sup>	NT <sup>  </sup>	0	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	2.3	NT <sup>  </sup>	NT <sup>  </sup>	NT <sup>  </sup>	10.8	NT <sup>  </sup>							
Bourgeois N, France, 2009 (11)	n-84				n-17				n-22				n-56				-				
	EA	98.8	100	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	95.4	100	NT <sup>  </sup>	NT <sup>  </sup>	94.6	89.3	NT <sup>  </sup>						
	CA	100	98.8	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	78.6	87.5	NT <sup>  </sup>						
	VME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>						
	ME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	1	0	NT <sup>  </sup>						
	MiE	0	1	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	11	5	NT <sup>  </sup>						
Cejudo MA, Spain, 2010 (8)	n-97				n-21				n-8				n-29				-				
	EA	92.8	95.8	NT <sup>  </sup>	85.7	95.2	NT <sup>  </sup>	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	72.4	89.6	NT <sup>  </sup>						
	CA	85.6	97	NT <sup>  </sup>	80.9	95.2	NT <sup>  </sup>	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	69	96.5	NT <sup>  </sup>						
	VME	2	1	NT <sup>  </sup>	0	4.8	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	3.5	0	NT <sup>  </sup>						
	ME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>						
	MiE	12.4	2	NT <sup>  </sup>	19.1	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	27.5	3.5	NT <sup>  </sup>						
Borghini E, Italy, 2010 (9)	n-324				n-37				n-142				n-74				-				
	EA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CA	100	100	NT <sup>  </sup>	94.6	100	NT <sup>  </sup>	NT <sup>  </sup>	97.2	98.8	NT <sup>  </sup>	NT <sup>  </sup>	51.4	90.5	NT <sup>  </sup>						
	VME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	12.2	0	NT <sup>  </sup>						
	ME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	4	NT <sup>  </sup>						
	MiE	0	0	NT <sup>  </sup>	2.8	0	NT <sup>  </sup>	NT <sup>  </sup>	5.4	1.4	NT <sup>  </sup>	NT <sup>  </sup>	36.6	0	NT <sup>  </sup>						
Rajmane SV, India, 2018 (14)	n-12				n-10				n-2				n-2				-				
	EA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CA	100	100	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	100	100	NT <sup>  </sup>	NT <sup>  </sup>	100	100	NT <sup>  </sup>						
	VME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>						
	ME	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>						
	EA	0	0	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	NT <sup>  </sup>						
Lee H, Korea, 2022 (1)	n-24				n-21				n-19				n-20				n-45				
	EA	83.3	91.7	95.8	76.3	100	100	100	89.5	100	100	100	NT <sup>  </sup>	NT <sup>  </sup>	45	100	42	44	44		
	CA	79.2	91.7	95.8	66.7	100	100	100	84.2	100	94.7	100	NT <sup>  </sup>	NT <sup>  </sup>	15	100	-	-	-		
	VME	12.5	4.2	4.2	0	0	0	0	5.3	0	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	-	-	-		
	ME	0	4.2	0	0	0	0	0	0	0	0	0	NT <sup>  </sup>	NT <sup>  </sup>	10	0	-	-	-		
	MiE	0	0	0	33.3	0	0	0	10.5	0	10.5	0	NT <sup>  </sup>	NT <sup>  </sup>	75	0	-	-	-		
The present study	n-7				n-9				n-14				n-11				n-9				
	EA	100	85.7	100	100	100	100	100	100	92.8	100	100	NT <sup>  </sup>	NT <sup>  </sup>	100	100	100	100	100		
	CA	85.8	71.5	85.8	100	100	88.9	100	100	85.8	100	100	NT <sup>  </sup>	NT <sup>  </sup>	63.7	100	77.8	-	-		
	VME	0	0	0	0	0	0	0	0	0	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	-	-	-		
	ME	0	0	0	0	0	0	0	0	0	0	0	NT <sup>  </sup>	NT <sup>  </sup>	0	0	22.2	-	-		
	MiE	14.2	28.5	14.2	0	0	11.1	0	0	14.2	0	0	NT <sup>  </sup>	NT <sup>  </sup>	36.3	0	-	-	-		

flu\*-fluconazole, vor†-voriconazole, cas‡-casopfungin, mic§-micafungin, NT<sup>||</sup> -Not tested  
 ■ Shaded areas indicate errors in the studies

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**Ethical statement**

Since this study included isolates from samples routinely sent to the Department of Microbiology and did not involve any patient details or clinical trial, ethical approval was not applicable.

**Conflicts of interest**

The authors declare no conflict of interest.

**Author contributions**

SS: designed the concept, conducted the literature review, wrote the manuscript, analyzed the results, and edited the manuscript. NARS: handled the technical aspects and reviewed the manuscript. UP: contributed to the concept design and reviewed the manuscript.

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