Original Article

PERFORMANCE EVALUATION OF VITEK 2 AST-YS08 FOR ANTIFUNGAL SUSCEPTIBILITY TESTING OF YEAST: A CATEGORICAL AGREEMENT STUDY WITH LIOFILCHEM MIC STRIPS WITH 22 CLINICAL SAMPLES IN HOSPITAL SULTANAH AMINAH, JOHOR BHARU AND HOSPITAL PAKAR SULTANAH FATIMAH, MUAR, MALAYSIA

Kai Jie **Yow**[1]*, Yii Ling **Liow**[2], Farah **Nooraidil**[2], Dayangku Seritul Akmar **Abd Razak**[2], Nian Jye **Tey**[3], Mohan Rao **Nageswara Rao**[4]

1. Unit of Microbiology, Department of Pathology, Hospital Segamat, 85000 Segamat, Johor, Malaysia
2. Unit of Microbiology, Department of Pathology, Hospital Sultanah Aminah Johor Bahru, 80100 Johor Bahru, Johor, Malaysia
3. Unit of Microbiology, Department of Pathology, Hospital Pakar Sultanah Fatimah Muar, 84000 Muar, Johor, Malaysia

Submission Date:
15th April 2025

Acceptance Date:
19th May 2025

Publication Date:
30th May 2025

***Corresponding author:**
Kai Jie Yow
dryowkaijie@moh.gov.my
Tel: +607 9433333

DOI:
https://doi.org/10.63719/imrj.2025.11.01.008

ABSTRACT

Invasive fungal infections caused by *Candida* spp. and *Cryptococcus neoformans* present significant clinical challenges. This study evaluates the Vitek 2 AST-YS08 system against Liofilchem MIC strips to determine minimal inhibitory concentrations (MICs) of antifungal agents in two hospitals in Johor. A total of 22 clinical isolates from *Candida* spp. and *Cryptococcus neoformans* were tested. MIC values for antifungals were determined via the Vitek 2 system and Liofilchem MIC strips. Results were compared for concordance using the latest Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. The Vitek 2 system demonstrated an overall categorical agreement of 91% with MIC strips. Categorical agreements were achieved for all isolates of *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, and *Cryptococcus neoformans*. Lower concordance rates were observed for *C. tropicalis* (80%) and *C. glabrata* (75%). Two discrepancies were identified in Sample 5 (*C. glabrata*), micafungin MIC values differed between systems (0.006 µg/mL vs. 0.12 µg/mL), and in Sample 16 (*C. tropicalis*), fluconazole MIC values varied (1 µg/mL vs. 8 µg/mL). The Vitek 2 method provides quick results and matches well with manual testing. The discrepancy of the results with different methods may affect the treatment outcome for the patient. The system's automation and efficiency make it a valuable tool for high-throughput clinical microbiology laboratories. The Vitek 2 AST-YS08 system is a fast and reliable way to test the antifungal sensitivity of yeast. More studies are needed to improve its use in different medical situations.

KEYWORDS: Antifungal susceptibility testing, *Candida* spp., *Cryptococcus neoformans*, minimal inhibitory concentration (MIC), automated AS

INTRODUCTION

Invasive fungal infections, most commonly caused by *Candida* species and *Cryptococcus neoformans*, remain a significant public health challenge, especially among immunocompromised individuals (1). These infections are associated with considerable morbidity and mortality, highlighting the importance of timely and targeted antifungal therapy. While current treatment strategies for invasive Candida infections are guided by the latest IDSA (Infectious Diseases Society of America) guidelines, knowledge of local susceptibility patterns is essential. Understanding how clinical isolates respond to antifungal agents in a specific setting can help physicians select the most appropriate therapy, improving treatment outcomes and combating the emergence of antifungal resistance (2).

Traditional AFST methods, such as the broth microdilution techniques recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), are the gold standards for their accuracy. However, their complexity and extended turnaround times—sometimes requiring up to 48 hours—limit their immediate clinical applicability (3). Furthermore, in Malaysia, even tertiary hospitals often face significant financial constraints, struggling to maintain the highest standards of care due to laboratory resources and infrastructure limitations. This situation is compounded by the growing burden of infectious diseases, where rapid, reliable diagnostics are crucial but not always feasible.

Several studies have evaluated the performance of the Vitek 2 system in comparison with other antifungal susceptibility testing methods beyond Liofilchem. Posteraro et al. reported that Vitek 2 yielded results comparable to the CLSI broth microdilution method for fluconazole and voriconazole against *Candida albicans* and *Candida glabrata* (4). Peterson et al. compared Vitek 2 with Etest, Sensititre YeastOne, and both CLSI and EUCAST reference methods, observing high levels of agreement across various antifungal agents and yeast species (5). More recently, Siopi et al. investigated *Candida auris* and found that while Vitek 2 performed reliably with echinocandins, it tended to miss fluconazole resistance and overestimate amphotericin B resistance unless breakpoints were adjusted (6). These findings underscore that although Vitek 2 offers rapid and convenient testing, its results should be interpreted cautiously, particularly for less common species or when resistance is suspected.

In response to these challenges, automated systems like the Vitek 2 AST-YS08 card have emerged as a promising alternative, offering the potential for faster, more efficient testing. These systems are particularly relevant in resource-limited settings, where they can significantly improve diagnostic turnaround times, directly impacting patient outcomes. In this study, fluconazole, variconazole, micafungin and amphotericin B were tested for *Candida* spp. while amphotericin B was tested for *Cryptococcus spp*.

This study seeks to assess the performance of the Vitek 2 AST-YS08 system compared to Liofilchem MIC strips, focusing on its reliability for antifungal susceptibility testing of *Candida spp.* and *Cryptococcus neoformans*. By comparing the concordance of minimum inhibitory concentration (MIC) values between these methods, we aim to evaluate the Vitek 2 system's clinical utility and its potential to enhance diagnostic workflows in settings like Malaysia, where financial and logistical constraints often limit access to optimal care.

MATERIALS AND METHODS

Study Design

Twenty-two clinical isolates were obtained from patients admitted to Hospital Sultanah Aminah in Johor Bahru and Hospital Pakar Sultanah Fatimah in Muar. The isolates comprised *Candida albicans* (n=8), *Candida tropicalis* (n=5), *Candida glabrata* (n=4), *Candida parapsilosis* (n=2), *Candida duobushaemulonii* (n=1), *Candida guilliermondii* (n=1), and *Cryptococcus neoformans* (n=1). A quality control strain, *Candida krusei* ATCC 6258, was also included. These samples include tissue, blood, peritoneal fluid, sputum and urine, which were randomly collected between Aug 2023 and April 2024. The methods of organism identification include Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF), Vitek 2 identification and biochemical tests.

Sample Preparation

All isolates were cultivated on Columbia sheep blood agar to ensure purity and viability. Each isolate was suspended in sterile saline to attain turbidity matching a 2.0 McFarland standard, utilising the DensiChek device.

Vitek 2 AST-YS08 Method

The Vitek 2 compact 30 system was utilised in accordance with the manufacturer's instructions. The inoculum suspensions for the VITEK 2 were prepared in sterile saline at a turbidity equal to a 2.0 McFarland standard, as measured using a DensiChek instrument. The MICs were measured on AST-YS08 cards that had been inoculated with the prepared suspension and incubated for 14-27 hours. Results were interpreted using the manufacturer's breakpoints. Furthermore, a quality control strain was included to check system accuracy (*Candida Krusei* ATCC 6258).

Liofilchem MIC Strips

For comparison, minimum inhibitory concentrations (MICs) were manually assessed using Liofilchem MIC strips on Sabouraud dextrose agar (SDA). The inoculum suspensions for using Liofilchem MIC strips were prepared in sterile saline at a turbidity equal to a 0.5 McFarland standard, as measured using a DensiChek instrument before inoculation on the SDA. The MIC was established as the minimum concentration of the antifungal drug that produced an 80% decrease in growth relative to the control group, except for amphotericin B, which will be read at the point where 100% inhibition of growth intersects the strip. Results were analysed following 48 hours of incubation at 35°C. Furthermore, a quality control strain was included to check system accuracy (*Candida Krusei* ATCC 6258).

Categorical Agreement Analysis

The MIC data derived from the Vitek 2 system were compared with those from the Liofilchem MIC strips. The categorical agreement was determined as the percentage of isolates for which both methods produced identical susceptibility classifications (sensitive, intermediate, or resistant). CLSI breakpoints were preferred over the EUCAST breakpoints in discordant cases. Statistical analysis using the two-proportion z-test and one-sample binomial test to calculate the p-value.

RESULTS

Using the Vitek 2 AST-YS08 card system, 22 clinical isolates were assessed and matched with MIC values derived from Liofilchem MIC strips. The isolates consisted of *Candida albicans* (n=8), *Candida tropicalis* (n=5), *Candida glabrata* (n=4), *Candida parapsilosis* (n=2), *Candida duobushaemulonii* (n=1), *Candida guilliermondii* (n=1), and *Cryptococcus neoformans* (n=1).

Minimal Inhibitory Concentration (MIC) Analysis

MIC values for multiple antifungal agents were determined, including fluconazole, amphotericin B, and micafungin. Results showed high concordance between the Vitek 2 AST-YS08 system and Liofilchem MIC strips, with an overall agreement rate of 91% across all tested isolates (21/23 concordant results).

Discrepant results were observed for two isolates (Table 1):

1. **Sample 5 (*Candida glabrata*)**: MIC for micafungin was 0.006 µg/mL (sensitive) using the MIC strip but 0.12 µg/mL (intermediate) with Vitek 2.
2. **Sample 16 (*Candida tropicalis*)**: MIC for fluconazole was 1 µg/mL (sensitive) with the MIC strip but 8 µg/mL (resistant) with Vitek 2.

The discrepancies may have resulted from differences in interpretation thresholds or human error in reading MIC values, particularly for Liofilchem strips.

Performance by Species

The system showed 100% concordance for *Candida albicans*, *Candida parapsilosis*, *Candida guilliermondii*, and *Cryptococcus neoformans*. However, lower concordance was observed for *Candida tropicalis* (80%) and *Candida glabrata* (75%).

Overall Performance

Overall, categorical agreement was 88.2%. The results are summarised in Table 2 and Table 3, which outline the performance evaluation of Vitek 2 AST-YS08 for Antifungal Susceptibility Testing for different species of yeast.

Table 1. Performance Evaluation of Vitek2 AST-YS08 for Antifungal Susceptibility Testing of Yeast

Sample	Organism	Antifungal Agent	MIC (Liofilchem)	MIC (Vitek 2 AST-YS08)	Breakpoints (CLSI/ EUCAST)	Interpretation	Discrepant Results
1–8	*Candida albicans*	Fluconazole	0.25–0.5 µg/mL (S)	<0.5 µg/mL (S)	≤2 (S), 4 (SDD), ≥8 (R)	✅ Concordant	-
		Voriconazole	<0.12 µg/mL (S)	<0.12 µg/mL (S)	≤1 (S), 2 (I), ≥4 (R)	✅ Concordant	-
		Amphotericin B	0.25–1 µg/mL (S)	0.5–1 µg/mL (S)	No official breakpoints, ≤1 (S)	✅ Concordant	-
9–12	*Candida glabrata*	Fluconazole	8–16 µg/mL (SDD)	-	≤2 (S), 4 (SDD), ≥8 (R)	✅ Concordant	-
		Micafungin	0.006 µg/mL (S)	0.12 µg/mL (I)	≤0.006 (S), 0.12 (I), ≥0.25 (R)	❌ Discrepant	Sample 5
13–17	*Candida tropicalis*	Fluconazole	1–2 µg/mL (S)	8 µg/mL (R)	≤2 (S), 4 (SDD), ≥8 (R)	❌ Discrepant	Sample 16
		Micafungin	0.016–0.12 µg/mL (S)	0.12 µg/mL (S)	≤0.25 (S), 0.5 (I), ≥1 (R)	✅ Concordant	-
18–19	*Candida parapsilosis*	Fluconazole	1–2 µg/mL (S)	<0.5–1 µg/mL (S)	≤2 (S), 4 (SDD), ≥8 (R)	✅ Concordant	-
20	*Candida duobushaemulonii*	Amphotericin B	>16 µg/mL (R)	8 µg/mL (R)	No official breakpoints	✅ Concordant	-
21	*Cryptococcus neoformans*	Amphotericin B	0.064 µg/mL (S)	0.5 µg/mL (S)	No official breakpoints	✅ Concordant	-
22	*Candida guilliermondii*	Micafungin	1.5 µg/mL (S)	0.5 µg/mL (S)	≤0.03 (S), >0.25 (R)	✅ Concordant	-

*MIC= Minimum Inhibitory Concentration, S= Susceptible, I- Intermediate, R= Resistant, SDD= Susceptible Dose-dependent

Table 2. Categorical agreement (CA) of *in vitro* susceptibilities between Vitek 2 AST-YS08 yeast susceptibility system MICs and Etest MICs using CLSI M27M44S & EUCAST for 22 *Candida spp*. Isolates

Species (No. of isolates)	Antifungal agent	Test method	MIC, µg/ml		MICs by category (n)			CA (%)	Errors (%)		
			Reference Breakpoints	Range	S	I / SDD	R		Very Major Error	Major Error	Minor Error
Candida albicans **(n=8)**	Amphotericin B	Vitek 2 AST-YS08	S ≤1; R >1	0.5- 1	8	0	0	100	0	0	0
		E Test		0.064 - 0.5	8	0	0				
	Fluconazole	Vitek 2 AST-YS08	S ≤2; SDD 4; R >8	<0.5	8	0	0	100	0	0	0
		E Test		0.094 - 0.5	8	0	0				
	Voriconazole	Vitek 2 AST-YS08	S ≤0.12; I = 0.25-0.5; R ≥1	< 0.12	8	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Caspofungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	< 0.12 - 0.25	8	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Micafungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	<0.06 - 0.12	8	0	0	100	0	0	0
		E Test		0.006 - 0.012	8	0	0				
	Anidulafungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		0.012 - 0.032	8	0	0				

***Candida glabrata* (n=4)**	Amphotericin B	Vitek 2 AST-YS08	S ≤1; R >1	1	4	0	0	100	0	0	0
		E Test		0.5	4	0	0				
	Fluconazole	Vitek 2 AST-YS08	SDD ≤32; R ≥64	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		16	4	4	0				
	Voriconazole	Vitek 2 AST-YS08	Not Determined	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Caspofungin	Vitek 2 AST-YS08	S ≤0.12; I = 0.25; R ≥0.5	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Micafungin	Vitek 2 AST-YS08	S ≤0.06; I = 0.12; R ≥0.25	<0.06 - 0.12	1	3	0	75	0	0	25
		E Test		<0.06 - 0.12	2	2	0				
	Anidulafungin	Vitek 2 AST-YS08	S ≤0.12; I = 0.25; R ≥0.5	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		0.064	4	0	0				
Candida tropicalis (n=5)	Amphotericin B	Vitek 2 AST-YS08	S ≤1; R >1	<0.25 - 0.5	5	0	0	100	0	0	0
		E Test		0.5 - 1	5	0	0				
	Fluconazole	Vitek 2 AST-YS08	S ≤2; SDD 4; R ≥8	<0.5 - 16	4	0	1	80	20	0	0
		E Test		1 - 1.5	5	0	0				
	Voriconazole	Vitek 2 AST-YS08	S ≤0.12; I = 0.25-0.5; R ≥1	<0.12 - 1	4	0	1	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				

	Caspofungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	<0.12	5	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Micafungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	<0.06 - 0.12	5	0	0	100	0	0	0
		E Test		0.016 - 0.023	5	0	0				
	Anidulafungin	Vitek 2 AST-YS08	S ≤0.25; I = 0.5; R ≥1	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		0.016 - 0.047	5	0	0				
Candida parapsilosis (n=2)	Amphotericin B	Vitek 2 AST-YS08	S ≤1; R >1	0.5	2	0	0	100	0	0	0
		E Test		0.5	2	0	0				
	Fluconazole	Vitek 2 AST-YS08	S ≤2; SDD 4; R ≥8	<0.5 - 2	2	0	0	100	0	0	0
		E Test		1	2	0	0				
	Voriconazole	Vitek 2 AST-YS08	S ≤0.12; I = 0.25-0.5; R ≥1	<0.12	2	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Caspofungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	0.25 - 0.05	2	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Micafungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	0.5	2	0	0	100	0	0	0
		E Test		0.05	2	0	0				
	Anidulafungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		4	0	2	0				

Candida guilliermondii (n=1)	Amphotericin B	Vitek 2 AST-YS08	Not Determined	0.5	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		0.0094	N/A	N/A	N/A				
	Fluconazole	Vitek 2 AST-YS08	Not Determined	2	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		24	N/A	N/A	N/A				
	Voriconazole	Vitek 2 AST-YS08	Not Determined	<0.12	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Caspofungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	0.5	1	0	0	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Micafungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	0.5	1	0	0	100	0	0	0
		E Test		1.5	1	0	0				
	Anidulafungin	Vitek 2 AST-YS08	S ≤2; I = 4; R ≥8	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		>32	0	0	1				
Cryptococcus neoformans (n=1)	Amphotericin B	Vitek 2 AST-YS08	S ≤1; R >1	0.5	1	0	0	100	0	0	0
		E Test		0.064 - 0.5	1	0	0				
	Fluconazole	Vitek 2 AST-YS08	Not Determined	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Voriconazole	Vitek 2 AST-YS08	Not Determined	0.12	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Caspofungin	Vitek 2 AST-YS08	Not Determined	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				

	Micafungin	Vitek 2 AST-YS08	Not Determined	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Anidulafungin	Vitek 2 AST-YS08	Not Determined	N/A	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				
	Flucytosine	Vitek 2 AST-YS08	Not Determined	2	N/A	N/A	N/A	ND	ND	ND	ND
		E Test		N/A	N/A	N/A	N/A				

*MIC= Minimum Inhibitory Concentration, N/A= Not Available, ND= Not Determined, S= Susceptible, I- Intermediate, R= Resistant

Table 3. Categorical agreement (CA) of in vitro susceptibility between Vitek 2 AST-YS08 yeast susceptibility system MICs and Etest MICs using CLSI M59 Epidemiological Cutoff Values (ECV) for *Candida duobushaemulonii* with no clinical breakpoint

Species (no. of isolates)	Antifungal agent	Test method	MIC, µg/ml		WT	NWT	CA (%)	Error (%)		
			ECV	MIC VALUE				Very Major Error	Major Error	Minor Error
C. duobushaemulonii **(n=1)**	Amphotericin B	Vitek 2 AST-YS08	ND	8	0	0	N/A	0	0	0
		E Test		>16	0	0				
	Fluconazole	Vitek 2 AST-YS08	32	8	1	0	100	0	0	0
		E Test		8	1	0				
	Voriconazole	Vitek 2 AST-YS08	0.5	1	0	1	100	0	0	0
		E Test		>8	0	1				
	Caspofungin	Vitek 2 AST-YS08	0.25	0.25	1	0	100	0	0	0
		E Test		0.25	1	0				
	Micafungin	Vitek 2 AST-YS08	0.5	<0.06	1	0	100	0	0	0
		E Test		<0.06	1	0				
	Anidulafungin	Vitek 2 AST-YS08	1	N/A	0	0	N/A	ND	ND	ND
		E Test		N/A	0	0				

*MIC= Minimum Inhibitory Concentration, N/A= Not Available, ND= Not Determined

DISCUSSION

The Vitek 2 AST-YS08 system demonstrated excellent overall agreement with the MIC strip method, particularly for common yeast species such as *Candida albicans* (7). Its rapid processing time (14–27 hours compared to the 48 hours typically required for CLSI or EUCAST methods) makes it a clinically beneficial alternative for antifungal susceptibility testing. This rapid turnaround is especially crucial in settings where timely diagnosis and treatment can significantly impact patient outcomes, such as in Malaysia's tertiary hospitals, where financial and resource limitations often hinder the ability to provide the highest standard of care. The Vitek 2 system offers an efficient solution for overburdened laboratories, where antifungal susceptibility testing is essential to managing invasive fungal infections (8).

The two observed discrepancies highlight potential challenges when transitioning between automated systems and manual MIC interpretation. We repeated the test for both isolates with both methods. In Sample 5 (*Candida glabrata*), the difference in micafungin MIC values (0.006 µg/mL vs. 0.12 µg/mL) may reflect variations in the endpoint definition for 80% growth inhibition. In Sample 16 (*Candida tropicalis*), the eightfold difference in fluconazole MIC values raises concerns about possible human error in interpreting the MIC strip result. Both samples with discrepancy results were isolated from the blood culture. However, the MIC from the E-test may be more sensitive than the MIC from Vitek-2 AST, which is similar to the findings in a study done in Kuwait. The author also suggests that it may be due to the limited spectrum of dilutions employed in E-test strips compared to Vitek cards (9). These cases underscore the need for standardisation and training in interpreting MIC values to reduce variability. This issue is especially relevant in resource-constrained environments such as Malaysia, where dependence on manual interpretation due to budgetary limitations may result in outcome variations.

Furthermore, discrepancies in MIC results where one method indicates susceptibility and another suggests intermediate resistance can have important implications for patient treatment decisions, potentially affecting the choice and effectiveness of antifungal therapy.

Strengths and Limitations

The primary strength of the Vitek 2 system lies in its automation and ease of use, allowing laboratories to process large sample volumes efficiently (8). Additionally, its integration with clinical workflows supports rapid decision-making in antifungal therapy, which is vital in environments where diagnostic resources are stretched. However, its reliance on manufacturer-defined breakpoints and occasional discrepancies with manual methods may sometimes limit its utility. Furthermore, manual, labour-intensive methods like MIC testing can still be prone to errors, such as when the lawn is too thick or too thin, leading to misinterpretation of results (10). These challenges can significantly affect test accuracy and treatment decisions in Malaysia, where laboratory technicians may handle high volumes of samples under pressure. Due to financial constraints, we did not proceed with some of the antifungal (e.g. Voriconazole, Caspofungin) for *Candida spp*. with Liofilchem MIC strips. We do Anidulafungin for *Candida spp*., however, Vitek 2 AST-YS08 did not include this antifungal in the antifungal panel. Another limitation of this study was the small sample size, mainly because some fungal species like *Cryptococcus neoformans*, *Candida guilliermondii*, and *Candida duobushaemulonii* are rarely encountered in clinical settings. Since this is an exploratory study, the focus was more on evaluating how well the testing method performs across different types of fungi rather than running detailed statistical comparisons for each species. Even with just a few samples, including these uncommon organisms, it helps provide valuable early insights and adds to the limited research on antifungal testing for rare fungal infections. Besides that, one of the most significant *Candida spp*. (*Candida krusei*) was not included in this evaluation study because it was not isolated during the study period.

Clinical Implications

The Vitek 2 AST-YS08 system reliably determines antifungal susceptibility for most yeast species, including emerging pathogens such as *Candida duobushaemulonii* (11). Providing MIC values more quickly than traditional methods enhances the ability to manage invasive fungal infections, often requiring prompt and accurate treatment. In resource-constrained settings like India, where the demand for rapid diagnostics is growing, but financial and infrastructure limitations remain, this system could play a pivotal role in improving patient outcomes by facilitating quicker, more reliable antifungal susceptibility testing (12), which is similar to the situation in Malaysia.

Future Directions

Further studies should include a larger sample size and incorporate isolates with known resistance mechanisms to better evaluate the system's performance. Additionally, external validation of manufacturer-defined MIC breakpoints against CLSI and EUCAST standards may improve confidence in its use for clinical decision-making.

CONCLUSION

The findings of this study reinforce the value of the Vitek 2 AST-YS08 system as a reliable and efficient tool for antifungal susceptibility testing in clinical practice. Its high categorical agreement with Liofilchem MIC strips, especially across a wide range of antifungal agents, supports its use in guiding timely treatment decisions for invasive yeast infections. However, the few observed discrepancies, particularly near clinical breakpoints, highlight the need for cautious interpretation and possible confirmatory testing in critical cases. These limitations underscore the importance of continued evaluation, especially in diverse clinical settings and with emerging or less common fungal species. Future studies with larger sample sizes and broader species representation are essential to further validate the system's accuracy and refine breakpoints for improved diagnostic consistency.

Author Contributions: All authors contributed equally to the study's conception, design, data collection, analysis, and interpretation. The manuscript was collaboratively drafted and reviewed, with each author approving the final version for submission.

.**Funding:** The authors entirely funded this research. No external financial support was provided, ensuring that the study was conducted independently and without any influence from third-party funding sources.

Acknowledgements: We thank the Microbiology Unit, Department of Pathology, Hospital Sultanah Aminah Johor Bahru, and the Bacteriology Unit, Institute for Medical Research, for their support and facilities. We would also like to thank the Hospital Sultanah Aminah Johor Bahru director and the Director General of Health Malaysia for allowing us to publish our findings.

Conflicts of Interest: Declare any potential conflicts of interest related to the research or publication. If there are none then state “Authors have no conflict of interest to declare”

CITATION

Yow KJ, Liow YL, Nooraidil F, Abd Razak DSA, Tey NJ, Nageswara Rao MR. Performance evaluation of Vitek 2 AST-YS08 for antifungal susceptibility testing of yeast: a categorical agreement study with Liofilchem mic strips with 22 clinical samples in Hospital Sultanah Aminah, Johor Bharu and Hospital Pakar Sultanah Fatimah, Muar. International Medical Research Journal. 2025 May 1;11(1):84–97. https://doi.org/10.63719/imrj.2025.11.01.008

REFERENCES

1. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4(165):165rv13.

2. Sasoni N, Caracciolo B, Cabeza MS, Gamarra S, Carnovale S, Garcia-Effron G. Antifungal susceptibility testing following the CLSI M27 document, along with the measurement of MFC/MIC ratio, could be the optimal approach to detect amphotericin B resistance in *Clavispora (Candida) lusitaniae*. Susceptibility patterns of contemporary isolates of this species. Antimicrob Agents Chemother. 2024;68(1):e00968-23.

3. Salam MA, Al-Amin MY, Pawar JS, Akhter N, Lucy IB. Conventional methods and future trends in antimicrobial susceptibility testing. Saudi J Biol Sci. 2023;30(3):103582.

4. Posteraro B, Martucci R, La Sorda M, Fiori B, Sanglard D, De Carolis E, et al. Reliability of the Vitek 2 yeast susceptibility test for detection of in vitro resistance to fluconazole and voriconazole in clinical isolates of *Candida albicans* and *Candida glabrata*. J Clin Microbiol. 2009;47(6):1927–30.

5. Peterson JF, Pfaller MA, Diekema DJ, Rinaldi MG, Riebe KM, Ledeboer NA. Multicenter comparison of the Vitek 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing caspofungin, micafungin, and posaconazole against *Candida* spp. J Clin Microbiol. 2011;49(5):1765–71.

6. Siopi M, Pachoulis I, Leventaki S, Spruijtenburg B, Meis JF, Pournaras S, et al. Evaluation of the Vitek 2 system for antifungal susceptibility testing of *Candida auris* using a representative international panel of clinical isolates: overestimation of amphotericin B resistance and underestimation of fluconazole resistance. J Clin Microbiol. 2024;62(4):e01528-23.

7. Melhem MS, Bertoletti A, Lucca HR, Silva RB, Meneghin FA, Szeszs MW. Use of the Vitek 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Braz J Microbiol. 2013;44(4):1257–66.

8. Pages Monteiro L, Allmen N, Friesen I, Huth K, Zambardi G. Performance of the VITEK 2 Advanced Expert System for the validation of antimicrobial susceptibility testing results. Eur J Clin Microbiol Infect Dis. 2021;40:1–3.

9. Alfouzan W, Al-Enezi T, AlRoomi E, Sandhya V, Chandy R, Khan ZU. Comparison of the Vitek 2 antifungal susceptibility system with Etest using clinical isolates of *Candida* species. Rev Iberoam Micol. 2017;34(3):171–4.

10. Gajic I, Kabic J, Kekic D, Jovićević M, Milenkovic M, Mitić-Ćulafić D, et al. Antimicrobial susceptibility testing: a comprehensive review of currently used methods. Antibiotics. 2022;11(3):427.

11. Ruiz de Alegría-Puig C, Rodríguez-Lozano J, Agüero-Balbin J. Identification and antifungal susceptibility testing of *Candida haemulonii* complex isolated from clinical samples. Folia Microbiol (Praha). 2024;69(1):165–71.

12. Verma S, Nataraj G, Wanjare S, Mehta P. Utility of VITEK 2 in rapid speciation and antifungal susceptibility testing of yeasts in a resource-constrained setting. Indian J Med Microbiol. 2021;39(2):213–6.