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UNVEILING PROFILE OF CANDIDEMIA IN A TERTIARY CARE HOSPITAL IN BIHAR AND COMPARING FLUCONAZOLE AND AMPHOTERICIN B SUSCEPTIBILITY TESTING METHODS IN *CANDIDA SPECIES* BY BROTH MICRODILUTION VS VITEK2 SYSTEM

Clinical Microbiology

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ABSTRACT

Background: Candidemia is a major healthcare concern, particularly in immunocompromised patients, with an increasing prevalence of non-albicans *Candida species* demonstrating antifungal resistance . Rapid and accurate identification of *Candida species* and antifungal susceptibility testing (AFST) are critical for appropriate management[1-6]. This study aims to characterize the species distribution of *Candida* isolates in a tertiary care hospital in Bihar and compare the essential and categorical agreement of Fluconazole and Amphotericin B susceptibility testing using the broth microdilution method versus the automated VITEK 2 system [7-9]. **Methods:** A total of 126 *Candida* isolates from bloodstream infections were identified using both conventional methods (Gram staining, germ tube test, CHROMagar, Dalmau plate using cornmeal agar, and carbohydrate assimilation and fermentation profiles) and the automated VITEK 2 system (bioMérieux, version 9.02) [1,2]. AFST for Fluconazole and Amphotericin B was performed using the Clinical and Laboratory Standards Institute (CLSI) M27/A3 broth microdilution method and the VITEK 2 system (bioMérieux, version 9.02) [8]. Essential and categorical agreement between the two methods was assessed [10]. **Results:** Among the isolates, 11.9% were *Candida albicans*, while 88.1% were *non-albicans Candida*, with *Candida parapsilosis* and *Candida tropicalis* being the most prevalent. The VITEK 2 system demonstrated high reliability, with 100% categorical agreement for Fluconazole and 95.4% for Amphotericin B compared to broth microdilution. Essential agreement was 98.4% for Fluconazole and 60.3% for Amphotericin B. The VITEK 2 system significantly reduced the time required for *Candida* speciation and susceptibility testing, enabling timely clinical decision-making. **Conclusion:** *Candida* speciation and antifungal susceptibility testing are crucial for guiding appropriate therapy and preventing resistance [7]. Non-albicans *Candida* species are emerging as significant pathogens, necessitating accurate identification and susceptibility testing [4-6]. The VITEK 2 system enhances *Candida* identification and antifungal susceptibility determination, offering a rapid and reliable alternative to conventional methods [8-10]. With high agreement rates for Fluconazole and Amphotericin B, it serves as an efficient tool for clinical decision-making. However, further research is needed to refine susceptibility testing methods and ensure optimal antifungal therapy.

KEYWORDS

Candidemia, *Candida* speciation , Antifungal susceptibility testing (AFST), Fluconazole, Amphotericin B, Broth microdilution, VITEK2 system.

INTRODUCTION:

Candida species are ubiquitous fungi that commonly inhabit various mucosal surfaces and can occasionally cause opportunistic infections, especially in immunocompromised individuals. *Candida* encompasses a diverse group of yeast-like fungi, with over 20 species known to cause human infections. Among these, *Candida albicans* is the most prevalent, but non-albicans species such as *Candida glabrata*, *Candida tropicalis*, and *Candida auris* are increasingly encountered, exhibiting varying degrees of antifungal resistance [1,2]. The rise of antifungal resistance among *Candida* strains poses a significant challenge to clinical management, necessitating accurate speciation and susceptibility testing to guide appropriate treatment strategies [3-6].

Antifungal susceptibility testing (AFST) is crucial for guiding the selection of appropriate therapy in the management of *Candida* infections, particularly in the face of increasing antifungal resistance [7-9]. Two commonly used methods for determining susceptibility to antifungal agents, like Fluconazole and Amphotericin B, are broth microdilution and the VITEK system [10,11]. The gold standard method for AFST is broth microdilution, which determines the minimum inhibitory concentration (MIC) of antifungal drugs against *Candida* isolates [12]. MIC values help clinicians interpret the susceptibility of *Candida* strains to various antifungal agents, informing therapeutic decisions and minimizing the risk of treatment failure or resistance development [13]. The VITEK system is an automated platform commonly used for microbial identification and susceptibility testing [14-16]. It employs a colorimetric method based on the growth of microorganisms in predefined wells containing various concentrations of antimicrobial agents [17]. The system measures the optical density of the wells to determine the MIC, providing results in a shorter time frame compared to broth microdilution. However, there have been concerns regarding the accuracy and reproducibility of VITEK results, particularly for certain antimicrobial agents and microorganisms [18].

Understanding the agreement between these methods is vital for accurate clinical decision-making. Categorical agreement refers to the degree of agreement between two testing methods regarding the interpretation of susceptibility categories (e.g., susceptible, intermediate, or resistant) [19]. High categorical agreement indicates consistent categorization of isolates as susceptible, intermediate, or resistant by both methods. Studies evaluating the categorical agreement between broth microdilution and the VITEK system for Fluconazole and Amphotericin B have shown variable results, with some demonstrating good agreement and others reporting discrepancies, particularly for specific *Candida species* [8,9]. Categorical agreement assesses the degree of concordance between the MIC results obtained by two testing methods. High essential agreement indicates consistent MIC values between methods, allowing for reliable interpretation of susceptibility. While both broth microdilution and the VITEK system are capable of determining MIC values, differences in testing conditions and interpretive criteria may result in discrepancies in categorical agreement, especially for borderline MIC values [10].

In this article, we delve into the intricacies of *Candida* speciation and antifungal susceptibility testing, shedding light on their importance in clinical practice and to explore the essential and categorical agreement of Fluconazole and Amphotericin B susceptibility testing in *Candida*

species when comparing broth microdilution and the VITEK system.

MATERIALS AND METHODS:

Yeast Identification by Manual Methods - A total of 126 clinical isolates of *Candida* species from blood samples were initially identified using conventional methods, including Gram staining, germ tube test, colony morphology on Sabouraud Dextrose Agar (SDA), chromogenic differentiation on CHROMagar, micromorphology by Dalmau plate culture on cornmeal agar, and carbohydrate assimilation and fermentation profiling [1,2].

Yeast identification by VITEK 2 system (bioMérieux, version 9.02) - All the 126 clinical isolates of *Candida sp.* were identified parallelly along with manual method by automated VITEK 2 system (bioMérieux) using yeast ID card (VITEK 2 YST) [15-17].

Antifungal Susceptibility Testing of Yeasts by VITEK 2 system (bioMérieux, version 9.02) - Antifungal susceptibility testing for all 126 clinical isolates of *Candida sp.* were done by automated VITEK 2 system (bioMérieux, version 9.02) using yeast AST card (VITEK 2 AST-YS08) [8-10].

Antifungal Susceptibility Testing of Yeasts by Broth Microdilution - Broth microdilution was performed for Amphotericin B and Fluconazole following M27/A3 protocol of CLSI. All antifungal susceptibility tests were validated using ATCC control strains (*C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) [12]. In instances where discrepancies in minimum inhibitory concentrations (MICs) were noted between the two methods, AFST was repeated independently.

RESULTS:

A. *CANDIDA* SPECIATION AND DISTRIBUTION IN CANDIDEMIA PATIENTS

In this study of 126 blood isolates of *Candida sp.* 15 (11.90%) were *Candida albicans* and 111 (88.10%) Non-albicans *Candida* as identified by automated VITEK 2 system (bioMérieux). The identification results obtained from the VITEK 2 YST card were validated against conventional gold standard methods, including germ tube testing, chromogenic agar (CHROMagar Candida) for colony morphology, cornmeal agar for micromorphological features, and carbohydrate assimilation and fermentation profiles using standard sugar panels.

27 (21.43%) *Candida parapsilosis*, 27 (21.43%) *Candida tropicalis*, 21 (16.67%) *Candida pelliculosa*, 15 (11.90%) *Candida guilliermondii*, 10 (7.94%) *Candida auris*, 7 (5.56%) *Candida glabrata*, 2 (1.59%) *Candida rugosa*, 1 (0.79%) *Candida krusei*, and 1 (0.79%) *Candida lusitaniae* were among the 111 Non-albicans *Candida*. Both *Candida parapsilosis* and *Candida tropicalis* made up the majority of the isolates.

The VITEK 2 YST identification card reduced time-to-identification to 18 hours from 48 to 72 hours required by the conventional tests. Furthermore, amongst the clinical isolates identification card correctly identified 100% of the clinical isolates. As per VITEK 2 System Identification levels, of the 126 total isolates, 70 isolates had - excellent confidence levels (96-99% probability), 34 isolates had - very good confidence level (93-95% probability), 9 isolates had - good confidence level (89-92% probability), and 13 isolates had - acceptable confidence level (85-88% probability).

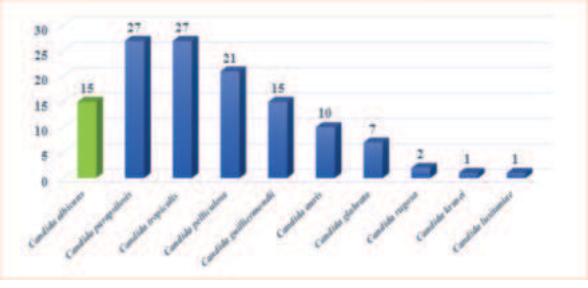

Figure 1: Species wise distribution of *Candida* isolates

Figure 1 shows proportion of different species of *Candida* which were isolated. A total of 126 *Candida* isolates were obtained of which 15 (11.90%) were *Candida albicans* and 111 (88.10%) Non-albicans *Candida*. The Non-albicans *Candida* again comprises of 27 (21.43%) *Candida parapsilosis*, 27 (21.43%) *Candida tropicalis*, 21 (16.67%) *Candida pelliculosa*, 15 (11.90%) *Candida guilliermondii*, 10 (7.94%) *Candida auris*, 7 (5.56%) *Candida glabrata*, 2 (1.59%) *Candida rugosa*, 1 (0.79%) *Candida krusei*, and 1 (0.79%) *Candida lusitaniae.*

B. ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA SP.* BY VITEK 2 TO COMMONLY USED ANTIFUNGAL AGENTS

Out of 15 *Candida albicans* isolates, all were susceptible to fluconazole, voriconazole, caspofungin, micafungin, and flucytosine, while 14 were susceptible and 1 was resistant to amphotericin B. Among the 27 *Candida parapsilosis* isolates, 17 were susceptible to fluconazole, 26 to voriconazole, 24 to amphotericin B, and all were susceptible to caspofungin, micafungin, and flucytosine; fluconazole resistance was observed in 10 isolates (37.04%), one isolate showed susceptible dose-dependent response to voriconazole, and three were resistant to amphotericin B. Of the 27 *Candida tropicalis* isolates, 25 were susceptible to fluconazole and all were susceptible to the remaining five antifungal agents tested. Among the 21 *Candida pelliculosa* isolates, 18 were susceptible to fluconazole and 20 to flucytosine, while 3 isolates (14.3%) were resistant to fluconazole and 1 showed an intermediate response to flucytosine; susceptibility data for voriconazole, amphotericin B, caspofungin, and micafungin were not reported due to the absence of standardized CLSI or EUCAST interpretive criteria. In 15 *Candida guilliermondii* isolates, 12 were susceptible to fluconazole, 14 to caspofungin and flucytosine, and all to micafungin; one isolate each showed intermediate response to caspofungin and flucytosine, and three (20%) were resistant to fluconazole. Among the 7 *Candida glabrata* isolates, all were susceptible to amphotericin B, micafungin, and flucytosine, while five showed intermediate and two showed resistant responses to caspofungin; fluconazole susceptibility was dose-dependent in four isolates, and three (42.86%) were resistant. Voriconazole susceptibility was not reported for *C. glabrata* due to lack of interpretive standards. Both *Candida rugosa* isolates were susceptible to fluconazole and showed intermediate susceptibility to flucytosine; data for voriconazole, amphotericin B, caspofungin, and micafungin were not included due to the absence of standardized breakpoints. The single *Candida krusei* isolate was susceptible to amphotericin B and micafungin but resistant to the remaining four antifungals tested; as expected, it exhibited intrinsic resistance to fluconazole. One isolate of *Candida lusitaniae* was found to be susceptible to both fluconazole and flucytosine, while data for the remaining antifungals were not reported due to non-availability of standard breakpoints. For *Candida auris*, species-specific breakpoints are not defined by CLSI or EUCAST; therefore, CDC-recommended tentative breakpoints were used. Of the 10 *C. auris* isolates, 9 were susceptible to caspofungin and micafungin and 1 to amphotericin B, while all 10 (100%) were resistant to fluconazole, 9 to amphotericin B, and 1 each to caspofungin and micafungin as per CDC guidance.

Table 1: Antifungal Susceptibility Profile (In Percentage) And Breakpoints For *Candida Species*

SPECIES	Antifungal	Susceptibility Pattern	Breakpoints (µg/ml)	Reference
C. albicans (n=15)	Fluconazole	100% S	S ≤ 2, SDD = 4, R ≥ 8	CLSI M60
	Voriconazole	100% S	S ≤ 0.12, I = 0.25–0.5, R ≥ 1	CLSI M60
	Amphotericin B	93.3% S, 6.7% R	S ≤ 1, R > 1	EUCAST v10
	Caspofungin	100% S	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Micafungin	100% S	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Flucytosine	100% S	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
C. parapsilosis (n=27)	Fluconazole	62.96% S, 37.04% R	S ≤ 2, SDD = 4, R ≥ 8	CLSI M60
	Voriconazole	96.3% S, 3.7% SDD	S ≤ 0.12, I = 0.25–0.5, R ≥ 1	CLSI M60
	Amphotericin B	88.9% S, 11.1% R	S ≤ 1, R > 1	EUCAST v10
	Caspofungin	100% S	S ≤ 2, I = 4, R ≥ 8	CLSI M60
	Micafungin	100% S	S ≤ 2, I = 4, R ≥ 8	CLSI M60

	Flucytosine	100% S	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
C. tropicalis (n=27)	Fluconazole	92.6% S, 7.4% R	S ≤ 2, SDD = 4, R ≥ 8	CLSI M60
	Voriconazole	100% S	S ≤ 0.12, I = 0.25–0.5, R ≥ 1	CLSI M60
	Amphotericin B	100% S	S ≤ 1, R > 1	EUCAST v10
	Caspofungin	100% S	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Micafungin	100% S	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Flucytosine	100% S	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
C. pelliculosa (n=21)	Fluconazole	85.7% S, 14.3% R	S ≤ 2, R > 4	EUCAST v10
	Flucytosine	95.2% S, 1 I	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
	Others	Not reported	No standardized breakpoints	–
C. guilliermondii (n=15)	Fluconazole	80% S, 20% R	S ≤ 2, R > 4	EUCAST v10
	Caspofungin	93.3% S, 1 I	S ≤ 2, I = 4, R ≥ 8	CLSI M60
	Micafungin	100% S	S ≤ 2, I = 4, R ≥ 8	CLSI M60
	Flucytosine	93.3% S, 1 I	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
	Others	Not reported	No standardized breakpoints	–
C. glabrata (n=7)	Fluconazole	57.1% SDD, 42.9% R	SDD ≤ 32, R ≥ 64	CLSI M60
	Voriconazole	Not reported	No standardized breakpoints	–
	Amphotericin B	100% S	S ≤ 1, R > 1	EUCAST v10
	Caspofungin	71.4% S, 28.6% R/I	S ≤ 0.12, I = 0.25, R ≥ 0.5	CLSI M60
	Micafungin	100% S	S ≤ 0.12, I = 0.25, R ≥ 0.5	CLSI M60
	Flucytosine	100% S	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
C. rugosa (n=2)	Fluconazole	100% S	S ≤ 2, R > 4	EUCAST v10
	Flucytosine	100% I	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
	Other drugs	Not reported	NA	–
C. krusei (n=1)	Fluconazole	Intrinsically R	-	CLSI M60
	Voriconazole	100% R	S <= 0.5, I = 1, R >= 2	CLSI M60
	Amphotericin B	100% S	S ≤ 1, R > 1	EUCAST v10
	Caspofungin	100% R	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Micafungin	100% S	S ≤ 0.25, I = 0.5, R ≥ 1	CLSI M60
	Flucytosine	100% R	S <=4, I : 8-16, R >= 32	CLSI M27-A3
C. lusitaniae (n=1)	Fluconazole	100% S	S ≤ 2, R > 4	EUCAST v10
	Flucytosine	100% S	S ≤ 4, I = 8–16, R ≥ 32	CLSI M27-A3
	Others	Not reported	NA	–
C. auris (n=10)	Fluconazole	100% R	R ≥ 32	CDC
	Amphotericin B	10% S, 90% R	R ≥ 2	CDC
	Caspofungin	90% S, 10% R	R ≥ 2	CDC
	Micafungin	90% S, 10% R	R ≥ 4	CDC

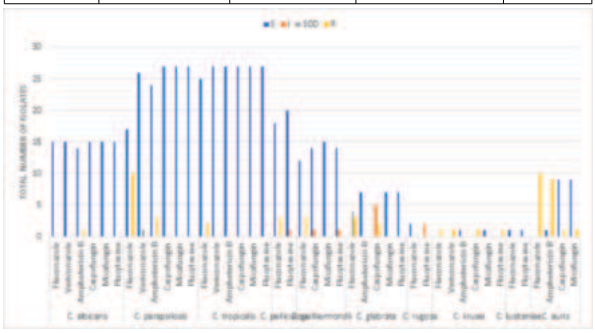

Figure 2: Antifungal susceptibility pattern of *Candida* isolates

C. FLUCONAZOLE AND AMPHOTERICIN B VITEK 2 AST VS BROTH MICRODILUTION

Fluconazole Susceptibility of *Candida species* by VITEK 2 and Broth Microdilution -

The number of isolates susceptible by both methods were found to be 90, susceptible dose dependent were 4 and resistant were 32. No minor, major or very major error were observed.

Table 2: Fluconazole Susceptibility Of *Candida species* By VITEK 2 And Broth Microdilution

Fluconazole Susceptibility by VITEK 2		Fluconazole Susceptibility by Broth Microdilution		
		S	SDD	R
S	Observed	90	0	0
	% of total	71.4 %	0.0 %	0.0 %
SDD	Observed	0	4	0
	% of total	0.0 %	3.2 %	0.0 %
R	Observed	0	0	32
	% of total	0.0 %	0.0 %	25.4 %

Overall essential agreement between the VITEK 2 AST MIC and the Broth Microdilution MIC with the 24 hour broth microdilution result was found to be 98.4 % for Fluconazole.

Table 3: *Fluconazole MIC Essential Aggrement Between VITEK 2 And Broth Microdiution*

	FLUCONAZOLE VITEK 2 MIC WITHIN TWO-FOLD DILUTION OF BROTH MICRODILUTION	FLUCONAZOLE VITEK 2 MIC BEYOND TWO-FOLD DILUTION OF BROTH MICRODILUTION
Total number	124	2
Percentage	98.4 %	1.6 %

All 126 isolates were falling under same susceptibility category with 90 isolates susceptible, 4 susceptible dose dependent, and 32 resistant by both methods. Hence, overall categorical agreement between the VITEK 2 AST system and the Broth Microdilution with the 24 hour broth microdilution result was found to be 100 % for Fluconazole.

Amphotericin B Susceptibility of *Candida species* by VITEK 2 and Broth Microdilution–

The number of isolates susceptible by both methods were found to be 70, and resistant by both methods were 13. However, 4 isolates were found susceptible by VITEK 2 and resistant by broth microdilution thereby showing very major error. In instances where discrepancies in minimum inhibitory concentrations (MICs) were noted between the two methods, AFST was repeated independently. The repeated results were found to be consistent with the initial findings, supporting the reliability of the observed susceptibility patterns.

Table 4: *Amphotericin B Susceptibility Of Candida Species By VITEK 2 and Broth Microdilution*

Amphotericin B Susceptibility by VITEK 2		Amphotericin B Susceptibility by Broth Microdilution	
		S	R
S	Observed	70	4
	% of total	55.6 %	3.2 %
R	Observed	0	13
	% of total	0.0 %	10.3 %

Overall essential agreement between the VITEK 2 AST MIC and the Broth Microdilution MIC with the 24 hour broth microdilution result was found to be 60.3 % for Amphotericin B.

Table 5: ***Amphotericin B MIC Essential Aggrement Between VITEK 2 and Broth Microdiution***

	AMPHOTERICIN B VITEK 2 MIC WITHIN TWO-FOLD DILUTION OF BROTH MICRODILUTION	AMPHOTERICIN B VITEK 2 MIC BEYOND TWO-FOLD DILUTION OF BROTH MICRODILUTION
Total number	76	11
Percentage	60.3%	8.7%

For Amphotericin B of the 87 isolates, 83 isolates belonged to same susceptibility category with 70 susceptible and 13 resistant by both methods. Hence, overall categorical agreement between the VITEK 2 AST system and the Broth Microdilution with the 24 hour broth microdilution result was found to be 95.4 % for Amphotericin B.

To assess the diagnostic performance of the VITEK 2 system in comparison to the reference broth microdilution (BMD) method, statistical parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's kappa coefficient were calculated for both Fluconazole and Amphotericin B.

For Fluconazole, complete agreement was observed between the two methods for all 126 isolates. Specifically, 90 isolates were categorized as susceptible, 4 as susceptible dose dependent, and 32 as resistant by both methods. There were no discrepancies in susceptibility interpretation. Therefore, the sensitivity, specificity, PPV, and NPV of the VITEK 2 system for Fluconazole were all 100%. Additionally, the Cohen's kappa coefficient was 1.00, indicating perfect agreement between the two methods.

For Amphotericin B, susceptibility results were available for 87 isolates. Among these, 70 isolates were categorized as susceptible and 13 as resistant by both VITEK 2 and broth microdilution methods. However, 4 isolates were reported as susceptible by VITEK 2 but resistant by broth microdilution, representing very major errors. Based on this data, the sensitivity of the VITEK 2 system for Amphotericin B was calculated to be 100%, while the specificity was 76.47%. The PPV was 94.59%, and the NPV was 100%, indicating high reliability in detecting true resistant and susceptible isolates, respectively. The Cohen's kappa coefficient was 0.89, denoting almost perfect agreement between the two methods.

These findings support the validity of the VITEK 2 system as a rapid and reliable method for antifungal susceptibility testing of Candida species, particularly for Fluconazole. However, the observed discrepancies with Amphotericin B highlight the need for cautious interpretation in certain clinical contexts.

DISCUSSION:

A. *CANDIDA* SPECIATION AND DISTRIBUTION IN CANDIDEMIA PATIENTS

In present study Non-albicans *Candida* was the primary cause of candidemia in majority of patients 111 of 126 (88.10%), indicating an increase in non-albicans candidemia [3-5]. *Candida parapsilosis* and *Candida tropicalis*, both constituting 21.43% of total *Candida* isolates were the most common isolates. Numerous factors, including severe immunosuppression or illness, premature birth, the use of broad-spectrum antibiotics, the indiscriminate use of antimycotic medications, the use of catheters in hospitalised patients, cancer, age, and geographic location, are linked to the rising frequency of isolation of non-albicans *Candida sp.* [4-6]. It was also suggested that a warmer environment may be linked to *Candida tropicalis* pathogenicity. This may explain why it was found to be the most common *Candida sp.* in our study. In Asia and North America, the prevalence of *Candida parapsilosis* is also region dependent [23]. For instance, it is the second most commonly identified species in the hospital environment in Japan and China, being responsible for 20 to 23% of all *Candida* bloodstream infections, while it is the third most frequently identified *Candida sp.* in India and other tropical regions of Asia [21-23].

Conventional identifying techniques are time consuming [1]. Commercially available biochemical and molecular techniques that enable identification in a matter of hours have been developed and assessed [2,24]. In this study, automated VITEK 2 ID system identified all 126 *Candida* isolates correctly thereby reducing time for *Candida* speciation [15-17].

B. ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA SP.* BY VITEK 2 TO COMMONLY USED ANTIFUNGAL AGENTS

With the increased incidence of candidemia and the growing number of antifungal agents, laboratory plays a crucial role for accurate selection of antifungal therapy [19,20]. The standardised broth microdilution method is expensive, laborious, and difficult to implement in clinical microbiology facilities [11,12]. In this study, we used the automated VITEK 2 system to determine antifungal susceptibility pattern. The VITEK 2 system is a reliable method for determining the antifungal susceptibility of yeast species, and it can be used clinically to determine the susceptibility of *Candida sp.* and other yeast species [8,9,19]. Additionally, it is a reliable technique for determining Azole and Amphotericin B resistance in vitro. It has the advantage of being easier and more rapid than the substitute methods developed by either the CLSI or the EUCAST and the disk diffusion method [12,13].

In present study organisms were found to be susceptible most commonly to Flucytosine (88.10%). Although Azoles, Amphotericin B, and Flucytosine continue to exhibit good efficacy, the decline in the sensitivity of *Candida* isolates to Fluconazole is a cause for worry [20,21]. Despite all *Candida* isolates having strong Amphotericin B susceptibility, Indian studies have found very high Fluconazole resistance, which is consistent with our findings [3,20,21]. Caspofungin has a 100% sensitivity pattern to few NAC species in this study, which may help medical professionals manage the *Candida* infection brought on by NAC. The primary reason NAC dominates *Candida albicans* is the widespread usage of Fluconazole in numerous clinical conditions [6,7]. The changing epidemiology of candidemia stresses how important it is to closely monitor the distribution and susceptibility of *Candida sp.* in order to improve therapy and outcomes [5-7]. In order to begin the appropriate antifungal as an empirical treatment, it is crucial to identify isolates down to the species level because the drug-resistant non-albicans *Candida* is developing as an opportunistic pathogen [22]. Conventional identification and antifungal susceptibility testing methods take a long period, approximately 24 to 48 hours, which may delay the start of treatment [1,11]. Guidelines for empirical therapy should therefore be developed based on the epidemiology of the condition. In present study, organisms were found to be susceptible most commonly to Flucytosine making it an option for empirical therapy in our institute.

C. FLUCONAZOLE AND AMPHOTERICIN B VITEK 2 AST VS BROTH MICRODILUTION

In our analysis, however, the essential agreement for Fluconazole and Amphotericin B were 98.4% and 91.3%. Overall categorical agreement between the VITEK 2 AST system and the Broth Microdilution MIC was found to be 100 % for Fluconazole and 97.6 % for Amphotericin B with the 24 h broth microdilution result [8-10]. Understanding the agreement between these methods is vital for accurate clinical decision-making.

CHALLENGES AND CONSIDERATIONS:

Despite advances in *Candida* speciation and AFST, several challenges persist in clinical practice. Interpretation of AFST results can be complex due to variations in testing methodologies, breakpoints, and clinical breakpoints [11-13]. Moreover, emerging multidrug-resistant *Candida species* such as *Candida auris* and *Candida krusei* represent a serious threat in clinical settings. *C. auris* is known for its high resistance to azoles, amphotericin B, and sometimes echinocandins, along with its ability to cause nosocomial outbreaks and persist in the hospital environment. *C. krusei*, inherently resistant to fluconazole, limits treatment choices from the outset. Additionally, increasing reports of azole and echinocandin resistance among other non-*albicans* species like *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* reflect a troubling trend. The omission of such species-specific resistance patterns may lead to inadequate empirical therapy. Therefore, ongoing surveillance, timely species identification, and antifungal susceptibility testing are essential not only for optimal patient management but also for containing the spread of these resistant pathogens. [5-7].

CONCLUSION:

Candida speciation and antifungal susceptibility testing are

indispensable tools in the management of *Candida* infections, guiding clinicians in the selection of appropriate antifungal therapy [1-3]. Accurate identification of *Candida species* and determination of their susceptibility profiles are essential for optimizing patient outcomes and minimizing the emergence of antifungal resistance [4-6]. Continued research and collaboration are essential to address challenges in *Candida* speciation and AFST, ensuring effective management of *Candida* infections in clinical practice. The main pathogens linked to different clinical forms of candidiasis in our study were Non-albicans *Candida sp.*. Its isolation from clinical samples cannot be disregarded as a non-pathogenic isolate or disregarded as a contaminant [4-6]. Additionally, with the development of drug-resistant non-albicans *Candida* as an opportunistic pathogen, it is essential to identify isolates down to the species level in order to start the proper antifungal as an empirical treatment [22].

Accurate susceptibility testing is essential for guiding antifungal therapy in the management of *Candida* infections [11]. The results of the present study showed that the VITEK 2 system enhances the rate of identification of *Candida sp.* and determining their susceptibility pattern [15-17]. However, conventional identification methods are still considered reference standard methods, despite time consuming along with ambiguities results [1,2]. Thus, the VITEK 2 system is an alternate way for identifying and prescribing the right antifungal medications for *Candida sp.* in immunosuppressed individuals [19].

While both broth microdilution and the VITEK system are widely used methods for determining susceptibility to Fluconazole and Amphotericin B, understanding the essential and categorical agreement between these methods is critical for clinical decision-making [8-10]. However further research is needed to elucidate the factors influencing agreement between testing methods and to optimize their performance in accurately predicting antifungal susceptibility in *Candida species*.

LIST OF ABBREVIATIONS:

AFST – Antifungal Susceptibility Testing**; MIC** – Minimum Inhibitory Concentration**; CLSI** – Clinical and Laboratory Standard Institute**; EUCAST** – European Committee on Antimicrobial Susceptibility Testing**; SDA** – Sabouraud Dextrose Agar**; AST** – Antifungal Susceptibility Testing (used in VITEK 2 system)**; S** – Susceptible**; R** – Resistant**; I** – Intermediate**; SDD** – Susceptible Dose Dependent**; CDC** – Centers for Disease Control and Prevention

DECLARATIONS:

Ethics approval and consent to participate – The study was approved by The Institutional Ethics Committee, All India Institute of Medical Sciences Patna, Reference number AIIMS/Pat/IEC/PGTh/Jan21/29.

Consent For Publication – Not applicable

Availability Of Data And Materials - The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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