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Maldi - TOF and Vitek-2 Compact - A Revolutionary Tools For Rapid Diagnosis and Antifungal Susceptibility Tests for Clinically Isolated Candida

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ABSTRACT

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Candida species are common fungal commensals in humans but can cause a spectrum of infections collectively known as candidiasis, ranging from superficial mucocutaneous disease to life-threatening invasive infections, particularly in immunocompromised individuals. In recent years, the epidemiology of candidemia has shifted, with non-albicans *Candida* (NAC) species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* being increasingly reported. Rapid and accurate species identification is essential, and MALDI-TOF MS has emerged as a powerful tool by generating species-specific protein spectra. This cross-sectional, hospital-based study was conducted from February to May 2025. Clinical specimens including blood, urine, CSF, pus, sputum, endotracheal aspirates, and bronchoalveolar lavage were processed using Hichrome *Candida* agar, MALDI-TOF MS, and the VITEK 2 Compact system. Of 400 samples analyzed, 74 (18.5%) yielded *Candida* isolates. The highest positivity was observed in sputum (29.72%), followed by urine and ETA (20.27% each). *Candida albicans* was the predominant species (62.16%), followed by *C. tropicalis* (29.72%). Less frequent species included *C. kefyr* and *C. glabrata* (2.7% each). Amphotericin B showed high susceptibility across species, while fluconazole resistance was notable in *C. albicans* (30%). MALDI-TOF MS is crucial for timely species-level identification and supports targeted antifungal therapy, especially amid rising NAC prevalence and antifungal resistance.

Introduction

Candida species are ubiquitous fungi that can exist as commensals in the human body but have the potential to cause a range of infections, collectively termed candidiasis. These infections can vary from superficial mucocutaneous conditions to invasive systemic diseases, particularly in immunocompromised individuals [1]. The increasing prevalence of candidemia, a bloodstream infection caused by *Candida* species, is a significant

concern in healthcare settings due to its association with high morbidity and mortality rates. [1,2]

A growing concern in recent years is the shifting epidemiology of candidemia, where non-albicans *Candida* (NAC) species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* are being isolated with increasing frequency.[1] These NAC species often exhibit reduced susceptibility to standard antifungal drugs, complicating treatment protocols and highlighting

the need for accurate, species-level identification.^[2] *Candida* infections are increasing in hospital settings, especially among immunocompromised patients due to prolonged use of antibiotic, immunosuppressive therapy, disruption of skin or mucosal barriers, corticosteroids, chronic illnesses, malignancies, organ transplantation, hemodialysis, premature birth, recent surgery, trauma, and the presence of central vascular catheters.^[3]

The accurate and timely identification of *Candida* species is paramount for effective patient management. Different *Candida* species exhibit varying antifungal susceptibility profiles; thus, species-level identification informs appropriate antifungal therapy, which is crucial for improving patient outcomes and reducing mortality^[2]. Traditional identification methods, such as culture-based techniques and biochemical assays, are often time-consuming and may lack the specificity required for precise species differentiation.^[3]

To address these diagnostic challenges, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has revolutionized yeast identification. Unlike conventional culture-based or biochemical techniques, which are time-consuming and less specific, MALDI-TOF MS enables rapid and accurate species-level identification by analyzing the unique protein spectra of yeast-like pathogens.^[3] Its integration into diagnostic workflows, particularly in tertiary care centers, can significantly enhance timely antifungal therapy which is critical in managing candidemia thus reduce mortality. Vitek 2 compact gives both ID and antifungal susceptibility with MIC values which is very helpful for patient management specially in candidemia cases.

Hence this study was done for phenotypic identification of *Candida* isolates by Sabouraud Dextrose Agar (SDA) medium, Hichrome *Candida* agar medium, MALDI-TOF MS and antifungal susceptibility by Vitek 2 compact. So that knowledge of local epidemiology of *Candida* infections aids in monitoring trends, detecting outbreaks, and formulating effective infection control policies. Thus will help in treatment as non *albicans* *Candida* show varying resistance pattern.

Material and Methods

This was a cross-sectional hospital-based study done for a period of 4 Months i.e. February 2025 to May 2025 in

department of microbiology at tertiary Care hospital. All clinical samples like blood, urine, CSF, Pus, Sputum, Endotracheal aspirate, BAL (Brochoalveolar lavage) and other sterile body fluids were received and processed conventionally as per standard laboratory protocol^[13]. All yeast isolates were subcultured on Sabouraud Dextrose Agar (SDA) & HiChrome-Agar. Gram stain and Germ tube test were done from culture growth. All yeast isolates were further identified and confirmed by MALDI-TOF MS method and Vitek 2 compact. Antifungal susceptibility were done for only 20 *Candida* isolates (10 blood, 9 Pus, 1 CSF) by Vitek 2 compact by using YST08 cards. MIC values were given for six antifungal drugs i.e.: Amphotericin B, Fluconazole, Voriconazole, Caspofungin, Micafungin, and Flucytosine.

Results and Discussion

Total of 400 samples were collected and processed for culture during study time period. Out of 400 sample, 74(18.50%) samples were positive for *Candida* growth. Out of 74 positive isolates, 41(55.41%) were male and 33(44.59%) were female, 43(58.11%) were urban and 31(41.89%) were from rural areas. 21 (23.38) were from OPD and 53 (71.62%) were from IPD. This is showing high rate of *Candida* infection among hospitalized patients

Among all *Candida* isolates maximum positivity was seen in the age group ≤ 10 years (33.78%) followed by >60 years (27.02) & 41-50 years age group (5.40%). (as shown in Graph 1)

Table 1 presents sample-wise distribution of *Candida* Species. Maximum cases were seen in sputum (29.72%) followed by urine (20.27%), ETA (20.27%) & blood (14.86%).

Table no.2 provides a detailed breakdown of *Candida* species distribution across various clinical sample types, identified using MALDI-TOF, Vitek 2 compact and Hichrome *Candida* Agar. The identification results were concordant by all three methods. *Candida albicans* was the predominant species across most sample types, representing 81.81% (18 isolates) in sputum, 60% (9 isolates) in ETA, and 100% (1 isolate) in CSF. It also formed a significant proportion in blood (54.54%, 6 isolates) and pus (50%, 5 isolates). *Candida tropicalis* was the second most common species, showing notable presence in sputum (18.18%, 4 isolates), urine (33.33%,

5 isolates), blood (36.36%, 4 isolates), ETA (33.33%, 5 isolates), and pus (40%, 4 isolates). Other *Candida* species were detected in smaller numbers. *Candida kefyr* was found in urine (1 isolate, 6.66%) and ETA (1 isolate, 6.66%). *Candida glabrata* was identified in urine (2 isolates, 13.33%). *Candida parapsilosis* was present in pus (1 isolate, 10%), and *Candida krusei* was detected in blood (1 isolate, 9.09%). Overall, sputum samples yielded the highest number of isolates (22, 29.72%), followed by urine (15, 20.27%) and ETA (15, 20.27%). Blood samples accounted for 11 isolates (14.86%), while pus samples contributed 10 isolates (13.52%). A single isolate was found in CSF (1.36%).

This table 3 presents the susceptibility (S) and resistance (R) patterns of 20 *Candida* species to different antifungal agents. Amphotericin B generally shows high susceptibility across *Candida* species, with 90% of *C. albicans* and 100% of *C. tropicalis* and *C. parapsilosis* isolates being susceptible. However, 100% of the single *C. krusei* isolate was resistant to Amphotericin B.

Fluconazole demonstrates variable susceptibility. While *C. tropicalis* (87.50%) and *C. parapsilosis* (100%) show high susceptibility, *C. albicans* has a 30% resistance rate. The *C. krusei* isolate was resistant to Fluconazole. Voriconazole, Caspofungin, and Miconazole exhibit similar patterns. They show high susceptibility for *C. tropicalis* and *C. parapsilosis* (100% for both), but a notable resistance in *C. albicans* (60% for all three agents). The single *C. krusei* isolate was susceptible to Voriconazole and Caspofungin, but resistant to Miconazole. Flucytosine shows the lowest susceptibility among the tested antifungals for *C. albicans*, with 70% of isolates being resistant. Conversely, *C. tropicalis* and *C. parapsilosis* are 100% susceptible. The *C. krusei* isolate was resistant to Flucytosine. This data highlights significant differences in antifungal susceptibility among *Candida* species, emphasizing the importance of species identification and susceptibility testing for effective treatment.

The present cross-sectional study aimed to investigate the phenotypic identification and species distribution of *Candida* isolates using both conventional methods, MALDI-TOF MS and Vitek 2 compact. This analysis sheds light on the evolving epidemiology of candidiasis, particularly in Western Rajasthan. Out of 400 clinical samples, 74 cases (18.5%) tested positive for *Candida* infections (Table 1). However, other studies like Makled

et al.,^[8] found positivity rate of 15.5% and Nandini D *et al.*,^[17] recorded lower prevalence rate of 6.5%. The variation in the finding could be influenced by factors such as local healthcare practices, environmental conditions, and population vulnerability.

A significant majority (71.62%) of *Candida*-positive cases were detected in hospitalized (IPD) patients, affirming findings by Rajni E *et al.*,^[2] and Tajane SB *et al.*,^[4], who documented elevated candidiasis prevalence among ICU and hospitalized populations due to immunosuppressive therapies, invasive procedures, and prolonged hospital stays. Such patients are often subjected to multiple risk factors, including prior antibiotic use, steroid therapy, and the presence of indwelling medical devices, which facilitate *Candida* colonization and infection.

A bimodal age distribution was noted, with the highest incidence in the pediatric age group (<10 years, 33.78%) and older adults (>60 years, 27.02%). While candidiasis is typically more common in the elderly due to immunocompromise status and chronic illness, the spike in pediatric cases may reflect heightened diagnostic scrutiny or underlying pediatric conditions. Similar age-related vulnerability was noted in studies by Rajni E^[2], and M A *et al.*,^[5] who reported increased incidence among children.^[5]

In this study Sputum samples yielded the highest positivity rate (29.72%, 22 Isolates), followed by urine and ETA (Endotracheal Aspirate) samples, both contributing 20.27% (15 isolates each). Blood samples accounted for 14.86% (11 isolates), while pus samples comprised 13.51% (10 isolates). Notably, only one *Candida* isolate (1.35%) was detected from CSF.

This distribution concordant with findings from other study Malani AN *et al.*,^[21] in tertiary care settings where respiratory tract samples (sputum, ETA) and urinary samples are frequently implicated in candidiasis, often due to colonization in critically ill or catheterized patients. The presence of *Candida* in blood and CSF, though less frequent in our study, signifies invasive candidiasis, which is associated with high morbidity and mortality^[2,16,].

In our study identified *Candida albicans* as the most prevalent species (62.16%, 46 isolates) on CHROME agar.

Table.1 Sample-wise Distribution of Candida Species

Sample type	Total	Positive	%
Sputum	173	22	29.72
Urine	97	15	20.27
Blood	40	11	14.86
ETA	43	15	20.27
Pus	37	10	13.51
CSF	10	1	1.35
Total	400	74	100

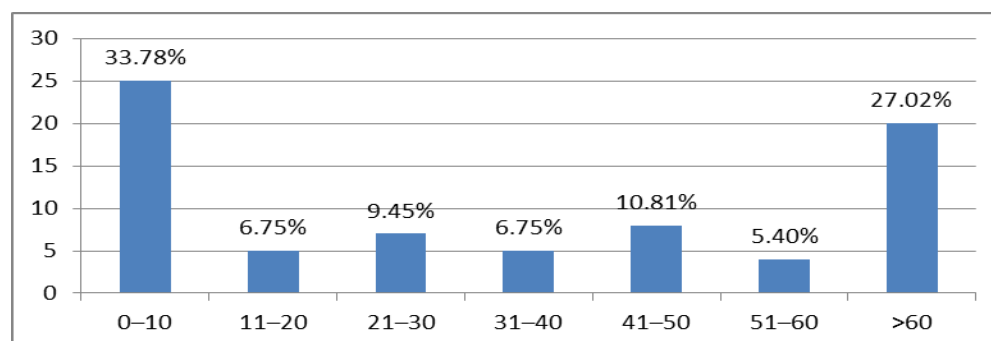
Table.2 Species wise distribution of Candida isolates.

Species	<i>Candida albicans</i>		<i>Candida tropicalis</i>		<i>Candida kefir</i>		<i>Candida glabrata</i>		<i>Candida parapsilosis</i>		<i>Candida krusei</i>		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Sputum	18	81.81%	4	18.18%	0	0%	0	0%	0	0%	0	0%	22	29.72%
Urine	7	46.66%	5	33.33%	1	6.66%	2	13.33%	0	0%	0	0%	15	20.27%
Blood	6	54.54%	4	36.36%	0	0%	0	0%	0	0%	1	9.09%	11	14.86%
ETA	9	60%	5	33.33%	1	6.66%	0	0%	0	0%	0	0%	15	20.27%
Pus	5	50%	4	40%	0	0%	0	0%	1	10%	0	0%	10	13.52%
CSF	1	100%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1.36%
Total	46	62.16%	22	29.72%	2	2.70%	2	2.70%	1	1.35%	1	1.35%	74	100%

Table.3 Antifungal susceptibility pattern of *Candida albicans* and non-*albicans* *Candida*

Antifungal agents	<i>C. albicans</i> (n=10)		<i>C. tropicalis</i> (n=8)		<i>C. parasilopsis</i> (n=1)		<i>C. krusei</i> (n=1)	
	S	R	S	R	S	R	S	R
Amphotericin B	9 (90%)	1 (10%)	8 (100%)	0 (0%)	1(100%)	0 (0%)	0 (0%)	1 (100%)
Fluconazole	7 (70%)	3 (30%)	7 (87.50%)	1 (12.50%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Voriconazole	4 (40%)	6 (60%)	8 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Caspofungin	4 (40%)	6 (60%)	8 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Micafungin	4 (40%)	6 (60%)	8 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Flucytosine	3 (30%)	7 (70%)	8 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)

Graph.1 Age wise distribution of Candida isolates



This finding is consistent with global epidemiology, where *C. albicans* traditionally remains the most common *Candida* species causing human infections.^[9,10,11] *Candida tropicalis* was the second most common species (29.72%, 22 isolates), showed increasing prevalence of non-*albicans Candida* (NAC) species, including *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*, has been a growing concern in recent years, as they often exhibit reduced susceptibility to commonly used antifungals^[1,5,15]. The detection of *C. kefyr* (2.70%), *C. glabrata* (2.70%), *C. parapsilosis* (1.35%), and *C. krusei* (1.35%) in our study, although in smaller numbers, underscores the importance of accurately identifying these species for guiding appropriate antifungal therapy.^[5,6]

MALDI-TOF is a rapid, accurate, and cost-effective method for identifying microorganisms, significantly improving the turnaround time for pathogen identification compared to traditional methods.^[1,3] Our MALDI-TOF results largely corroborated the phenotypic identifications from Candichrome agar, while providing a more precise breakdown of species distribution across different sample types.

Consistent with the overall prevalence, *Candida albicans* was the dominant species in sputum (81.81%) and ETA (60%) samples, reinforcing its role as a significant colonizer and pathogen in the respiratory tract [9]. Its 100% presence in the single CSF isolate is clinically relevant, indicating its potential to cause severe central nervous system infections. In urine samples, *C. albicans* was still the most common (46.66%), but *C. tropicalis* (33.33%) and *C. glabrata* (13.33%) also contributed significantly, reflecting the varied etiology of candiduria^[22].

The distribution in blood samples is particularly important given the severity of candidemia. Here, *C. albicans* (54.54%) and *C. tropicalis* (36.36%) were the primary isolates. While *C. albicans* is traditionally the leading cause of candidemia, the high proportion of *C. tropicalis* aligns with global trends showing an increasing incidence of *C. tropicalis* candidemia, which is often associated with higher mortality rates in some settings^[1,2,5]. The detection of *Candida krusei* (9.09%) in blood, though only one isolate, is notable because this species is intrinsically resistant to fluconazole, a commonly used antifungal^[13,15]. Similarly, *C. parapsilosis* in pus samples highlights its potential role in wound infections and device-related infections^[3,10].

This study underscores the critical need for species-level identification in managing candidiasis, especially given the varying antifungal susceptibility profiles of Non *albicans Candida* (NAC) species. Timely identification supports appropriate antifungal therapy and resistance prevention. These observations are in line with recommendations by Tajane SB *et al.*,^[4] and Tiwari & Verma,^[6] who advocate for integrated diagnostics combining phenotypic and molecular tools for effective fungal management.

The present study findings on the antifungal susceptibility of *Candida* species are crucial for guiding effective treatment strategies, especially given the global rise in candidemia and the emergence of drug-resistant strains (Shetty *et al.*,^[1] Rajni *et al.*,^[2]). The observed variations in susceptibility across different *Candida* species and antifungal agents underscore the importance of species-level identification and antifungal susceptibility testing (AFST) in clinical settings (Mathur *et al.*,^[16] Nandini *et al.*,^[17]). Though in our study only 20 candidaisolates antifungal susceptibility were done due to limited availability of YST08 card. Our data for *C. albicans* (n=10) indicates high susceptibility to Amphotericin B (90%), consistent with its established efficacy as a broad-spectrum antifungal (Chander *et al.*,^[13]). However, the observed resistance rates to Fluconazole (30%), Voriconazole (60%), Caspofungin (60%), Micafungin (60%), and Flucytosine (70%) are concerning. This trend aligns with other studies from India and globally, reporting increasing resistance to azoles and echinocandins in *C. albicans* (Tiwari & Verma *et al.*,^[6] Gonçalves *et al.*,^[15] Makled *et al.*,^[8]). The relatively high resistance to Flucytosine could be attributed to its rapid development of resistance when used as monotherapy, often necessitating its use in combination with other antifungals (Chander *et al.*,^[13]).

Non-*albicans Candida* (NAC) species are increasingly recognized as significant pathogens in candidemia and other invasive fungal infections, often exhibiting intrinsic or acquired resistance to common antifungals (Shetty *et al.*,^[1] Rajni *et al.*,^[2]). Our results show excellent susceptibility of *C. tropicalis* (n=8) to Amphotericin B, Voriconazole, Caspofungin, Micafungin, and Flucytosine (all 100% susceptible). While Fluconazole susceptibility was also high at 87.50%, a small percentage (12.50%) showed resistance. This generally high susceptibility profile for *C. tropicalis* to most tested antifungals is a positive finding, although regional variations in susceptibility patterns

exist and warrant continuous surveillance (Thean Yen Tan *et al.*,^[23]).

The single *C. parapsilosis* isolate in our study demonstrated 100% susceptibility to all tested antifungal agents. While this is a promising observation, it's important to note that *C. parapsilosis* complex species are known for their varying susceptibility patterns, and often require molecular methods for accurate identification within the complex (Samantaray & Singh *et al.*,^[3]). Further studies with a larger number of *C. parapsilosis* isolates would be beneficial to confirm this trend. The *C. krusei* isolate (N=1) in our study showed intrinsic resistance to Amphotericin B, Fluconazole, and Micafungin (100% resistance for each). This is a well-documented characteristic of *C. krusei*, which is inherently resistant to Fluconazole and often exhibits reduced susceptibility to other azoles and certain echinocandins (Chander *et al.*,^[13], Gonçalves *et al.*,^[15]). Notably, our *C. krusei* isolate was susceptible to Voriconazole and Caspofungin, which aligns with their established activity against this species. The management of *C. krusei* infections often necessitates the use of echinocandins or newer generation azoles like Voriconazole (Hoenigl *et al.*,^[14]).

The increasing prevalence of NAC species and their diverse susceptibility patterns highlight the urgent need for robust diagnostic capabilities that can rapidly identify *Candida* species and perform AFST (Mathur *et al.*,^[16], Shetty *et al.*,^[1]). Techniques like MALDI-TOF MS have proven invaluable for rapid and accurate species identification, aiding in timely appropriate antifungal therapy (Shetty *et al.*,^[1]; Samantaray & Singh *et al.*,^[3]).

The observed resistance, particularly in *C. albicans* to multiple agents and the intrinsic resistance of *C. krusei*, underscores the challenge of antifungal resistance. This phenomenon is influenced by various factors, including widespread antifungal use, inappropriate dosing, and patient-specific factors such as immunosuppression and prolonged hospitalization (Gonçalves *et al.*,^[15]; Thomas-Rüddel *et al.*,^[19]).

The findings from our study are crucial for developing effective antifungal stewardship programs and improving patient outcomes in our tertiary care hospital. The prevalence of *C. albicans* reaffirms the need for continued surveillance and appropriate treatment protocols for this species. However, the significant

presence of NAC species, particularly *C. tropicalis*, necessitates vigilant identification and antifungal susceptibility testing to guide targeted therapy, especially considering their varying susceptibility profiles.^[6,13]

The application of advanced diagnostic techniques like MALDI-TOF MS in routine laboratory practice provides rapid and accurate identification, facilitating timely intervention and reducing the reliance on empirical antifungal therapy^[1,3]. This is particularly important in managing critically ill patients with underlying risk factors such as diabetes mellitus, immunosuppression (e.g., HIV, organ transplantation), and malignancy, who are highly susceptible to invasive candidiasis^[18,19,20].

Limitations and Future Directions

Our study was done for short period of time (4 months) and is limited by the small sample size. Therefore, these findings should be interpreted with caution, and larger cohort studies are needed to confirm the observed susceptibility patterns. While the VITEK 2 system is a commonly used method for AFST, its accuracy for certain *Candida* species and antifungal combinations has been debated, with some studies suggesting potential overestimation or underestimation of resistance (Lee *et al.*, 2022; Siopi *et al.*, 2024; Kaur *et al.*, 2016)^[24,25,26]. Future research should consider employing broth microdilution as the gold standard method for AFST to validate these findings and explore the molecular mechanisms underpinning the observed resistance patterns (Lee *et al.*, 2022; Siopi *et al.*, 2024)^[24,25]. Also we have done AFST for only 20 candida isolates due to limited AFST vitek card due to this limited sensitive and resistance profile was generated.

In conclusion, this study provides valuable insights into the epidemiological profile of *Candida* infections and understanding of antifungal susceptibility patterns in a tertiary care setting. The data underscores the continued dominance of *C. albicans* while highlighting the significant contribution of Non Albicans *Candida* species, particularly *C. tropicalis*, across various clinical samples. The use of advanced identification methods like MALDI-TOF is indispensable for accurate and timely diagnosis, which is paramount for effective antifungal management and combating the evolving challenge of candidiasis in healthcare. The findings highlight the critical need for species-specific identification and AFST to guide appropriate antifungal

therapy and combat the growing challenge of antifungal resistance. There is need of continued surveillance and research to monitor changes in *Candida* epidemiology and antifungal resistance patterns to inform clinical guidelines and improve patient care.

Author Contributions

Richa Agrawal: Investigation, formal analysis, writing—original draft. Sanju Pannu: Validation, methodology, writing—reviewing. Ravindra Singh Rathore:—Formal analysis, writing—review and editing. Swati Duggal: Investigation, writing—reviewing. Praveen Rathore: Resources, investigation writing—reviewing. Prabhu Prakash: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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