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ORIGINAL RESEARCH

Comparative Accuracy of Vitek-2 and MALDI-TOF for prevalence of various *CandidaSpecies* in Critical Care

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ABSTRACT

Introduction: Candidemia is a severe bloodstream infection caused by various *Candida* species, prevalent in adult intensive care units (ICUs) where patients are often critically ill. Rapid and accurate identification of *Candida* species is crucial for effective treatment and improved patient outcomes. This study compares the efficacy of Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and the VITEK-2 Compact system in identifying *Candida* species from blood cultures in a tertiary care setting in Uttarakhand, India.**Methods:** A prospective cross-sectional study was conducted in the Microbiology Laboratory of AIIMS Rishikesh from November 2022 to March 2024. The study enrolled ICU patients with sepsis and blood cultures positive for *Candida* species. The *Candida* isolates were identified using the VITEK-2 Compact system and MALDI-TOF MS. The results from the two methods were compared to determine concordance and discrepancies in species identification.**Results:** VITEK-2 and MALDI-TOF identified *C. tropicalis* and *C. albicans* as the most common species. VITEK-2 identified 19.4% (n=20) as *C. albicans* and 35% (n=36) as *C. tropicalis*, alongside species like *C. auris* and *C. parapsilosis*. MALDI-TOF results included 18.4% (n=19) for *C. albicans* and 34% (n=35) for *C. tropicalis*, additionally detecting species like *C. orthopsilosis* and *Pichia* species. A significant concordance rate of 98.1% (p=0.001) was found between the two methods. However, MALDI-TOF was more precise in identifying less common species and highlighted discrepancies, such as the misidentification of *C. orthopsilosis* as *C. parapsilosis* by VITEK-2.**Conclusion:** The study underscores the importance of accurate species-level identification of *Candida* in clinical settings. MALDI-TOF offers rapid and reliable identification, which is crucial for managing antifungal therapy and monitoring resistant species. While both methods demonstrated high concordance rates, the complementary use of these technologies, along with potential molecular confirmation, could enhance the precision of *Candida* species identification, aiding in better infection control and patient care.

Keywords: CANDIDA, CRITICAL CARE, MALDI-TOF,VITEK-2, WHO PRIORITY PATHOGEN

INTRODUCTION

Candidemia is a critical bloodstream infection brought on by various species of *Candida*, a type of yeast commonly found on the skin and mucous membranes. When *Candida* enters the bloodstream, it can cause systemic infections. (1)In adult intensive care units (ICUs), patients who are critically ill often face compromised immune responses due to existing health issues, recent surgeries, or immune-suppressing medications. Candidemia can originate from several sources, such as the use of central lines, postoperative complications, or prolonged use of broad-spectrum antibiotics. The significance of promptly addressing candidemia lies in its association with increased complication rates, longer hospital stays, and higher medical costs. Early detection and management are crucial in preventing the infection's progression and avoiding severe health outcomes. (2)

In ICUs, candidemia is a major health concern linked to high morbidity and mortality rates. Accurate and swift identification of *Candida* species is essential for selecting the appropriate antifungal treatment and improving patient survival. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has become a key tool for quickly identifying pathogens, including *Candida* species. (3) Retrospective studies have identified several factors that predispose ICU patients to *Candida* infections, including intravenous nutritional support, extensive antibiotic use, major

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surgeries, central venous lines, urinary catheters, mechanical ventilation, neutropenia, kidney dysfunction, corticosteroid usage, severe burns, and dialysis. (4–6)

Infections can be endogenous or exogenous and are particularly dangerous for immunosuppressed patients, with mortality rates ranging from 30% to 90% due to often delayed diagnoses. (7) Certain fungal species naturally resist specific antifungal drugs, such as *C. krusei's* resistance to fluconazole and *C. luisitaniae's* to amphotericin B, while emerging species like *C. auris* show resistance to multiple antifungal classes. The prevalence of non-albicans species causing infections varies by region and is influenced by the prescribing habits of healthcare providers. (8,9)

Technologies such as the VITEK-2 Compact system and advanced molecular detection methods offer rapid and accurate identification of *Candida* species. (9,10) MALDI-TOF MS is increasingly vital in clinical microbiology labs worldwide due to its cost-efficiency, rapid analysis, and high accuracy. It produces unique protein profiles from whole cells, facilitating early and direct identification of yeasts in positive blood cultures. (11,12)This technology significantly reduces the time needed for results, improving the diagnosis of bloodstream fungal infections and holding potential for strain categorisation and antifungal resistance assessment. This paper discusses the evolving role of MALDI-TOF MS in the daily diagnostics of fungal infections in comparison to VITEK-2 compact for rapid and accurate speciation of various candida spp in a tertiary care institute of Uttarakhand, India.

MATERIAL AND METHODS

This prospective cross-sectional study was conducted in the Microbiology Laboratory, Department of Microbiology, AIIMS, Rishikesh (India), from November 2022 to March 2024.

STUDY PLACE: All India Institute of Medical Sciences, Rishikesh

STUDY POPULATION FROM TARGET POPULATION: Patients admitted in Intensive Care Units with sepsis whose routinely requested blood culture gave an isolation of *Candida* spp.

INCLUSION CRITERIA: All patients admitted with candidemia in intensive care units will be included whose blood sample is positive for *Candida* spp.

EXCLUSION CRITERIA

- Patients with *candida* infection with samples other than blood
- Patients who are unwilling to participate in the study.

SAMPLE SIZE: Consecutive cases meeting the inclusion and exclusion criteria.

STUDY DURATION: 18 months from the date of approval from the ethics committee

STUDY DESIGN: Hospital-based Prospective observational study

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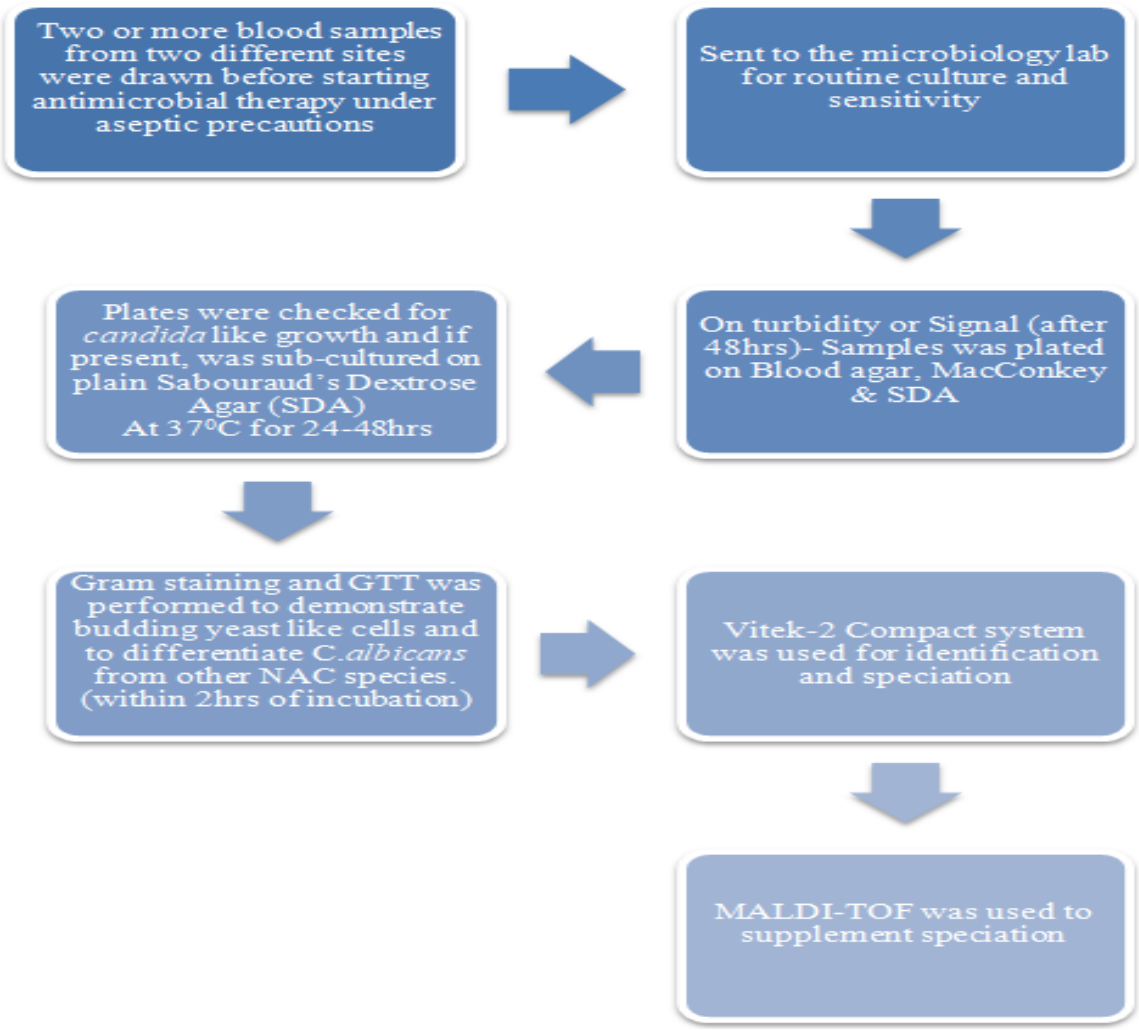
METHODOLOGY



VITEK-2 COMPACT IDENTIFICATION PROCEDURE:(9)

1. The VITEK®-2 Compact System (bioMérieux, France) employs the ID-YST card for the swift identification of significant Candida species within 15 hours. This calorimetric card has 64 wells with 47 fluorescent biochemical assays, and the system's database includes 51 yeast taxa.
2. In the procedure, the inoculum is standardised to a 0.5 McFarland density. The ID-YST card is then filled, sealed, incubated at 35°C for 24 hours, and read automatically every 15 minutes.
3. The analysis involves fluorescence measurement with results interpreted via an algorithm and compared to the system's software (database v.3.01) for final *Candida* identification.

MALDI TOF - MS (MATRIX-ASSISTED LASER DESORPTION IONISATION TIME OF FLIGHT - MASS SPECTROMETRY IDENTIFICATION PROCEDURE(13)

1. Add one pure colony from a freshly sub-cultured plate of SDA onto a MALDI plate in duplicate and let dry.
2. Add 0.5 µL of 70% formic acid to each spot and dry againapply 0.8 µL of the matrix solution. α-cyano-4-hydroxycinnamic acid (CHCA)
3. Identify the spots using MALDI-TOF MS, acquire spectra with FLEX software, andcompare them to the MALDI BioTyper database.
4. Interpret scoresas per Bruker guidelines:

- **<1.7 is unreliable**
- **>1.7 to 1.99 is the genus level**
- **>2.0 is species level**.

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STATISTICAL METHODS

1. All the data was entered in an Excel spreadsheet and was analysed using SPSS version 26.0 for Windows OS.
2. The difference in the distribution of categorical variables between 2 groups was tested for statistical significance using the Fisher exact test / Chi-square test as applicable.
3. P-value < 0.05 was considered statistically significant.

RESULTS

Automated identification of various *candida spp*. (Quality control for identification of yeast: ATCC *C.albicans* 14053)

The advent of automated and molecular methods has revolutionised the identification of *Candida* and other clinically relevant yeasts.The performance of two such systems, VITEK 2 and MALDI-TOF,for identifying the *Candida* isolates in this study. Both systems identified *C. tropicalis*, *C. albicans*, and *C. parapsilosis* as the most common species, consistent with the phenotypic findings from culture morphology and biochemical tests. However, there were some notable discrepancies between the two methods.

When isolates were tested using VITEK-2 Compact using YST cards (n=103), the most common species was identified as *C. tropicalis* (n=36), followed by *C. albicans* (n=20) and *C. parapsilosis* (n=17). Alarming, this automated system identified *C.auris* in about 10.7% of isolates(n=11), which is a Multi-drug Resistant species of *candida* typicallyseen in immunocompromised and critical patients.

Other less commonly isolated species include *C.glabrata*(n=6),*C.pelliculosa*(n=5), *C. guilliermondii*(n=4),*C.krusei*(n=2) and*C.rugosa* (n=1) as seen in **Figure 1**.

Simultaneously, these yeast isolates were also tested by MALDI-TOF MS system for speciation of various candida spp, with a turnaround time of less than an hour from processing to results. The most commonly isolated species by this automated system were similar to the VITEK-2 system *C. tropicalis* (n=35) followed by *C. albicans* (n=19). Notably, *C.orthopsilosis* was seen in about 9.7% (n=10) isolates, which were misidentified as *C. parapsilosis* by the VITEK-2 compact system. *C.auris* was identified in about 10.7% (n=11) of isolates along with *C.pelliculosa*(n=5), *C. guilliermondii*(n=4), *C.krusei*(n=2) and *C.rugosa* (n=1) identical to the VITEK-2 compact system. Other commonly isolated species were *C.glabrata*(n=7) and *C. parapsilosis* (n=7).

Some of the rare species of *candida* were also identified by the MALDI-TOF MS database as *Pichia/ wickerhamomycesanomalus*(n=5), *Pichia occidentalis* (n=2), *C. dubliniensis* (n=1) and *C.doubushaemulonii*(n=1) as depicted in **Figure 2**

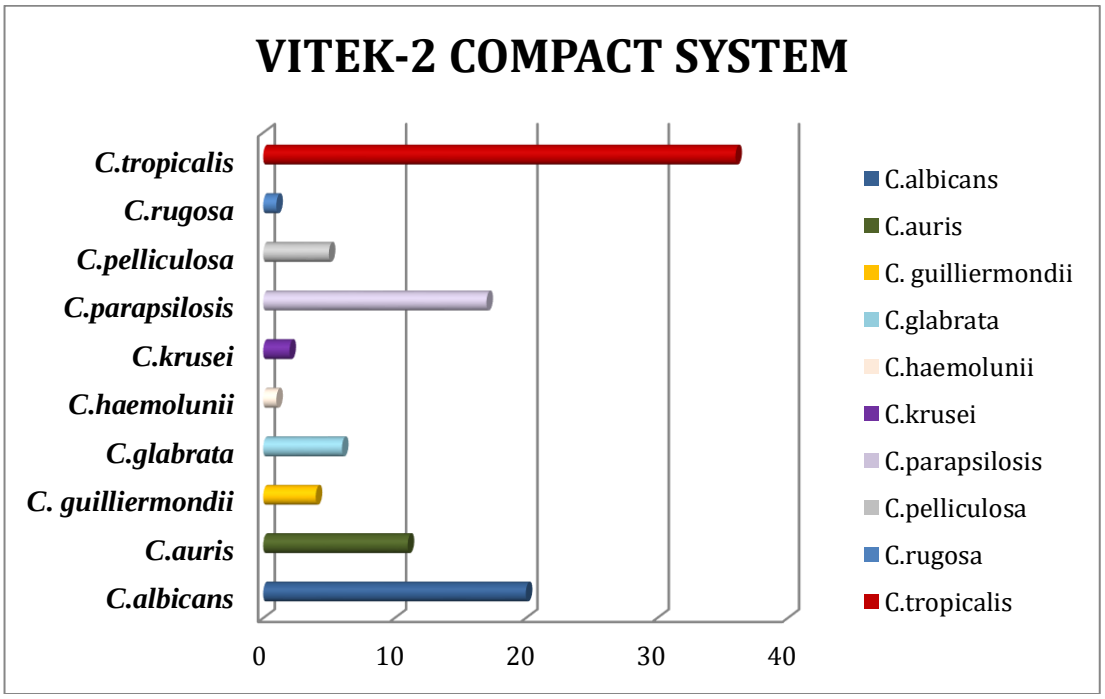

Figure 1: Identification of Candida species by automated VITEK system (n=103)

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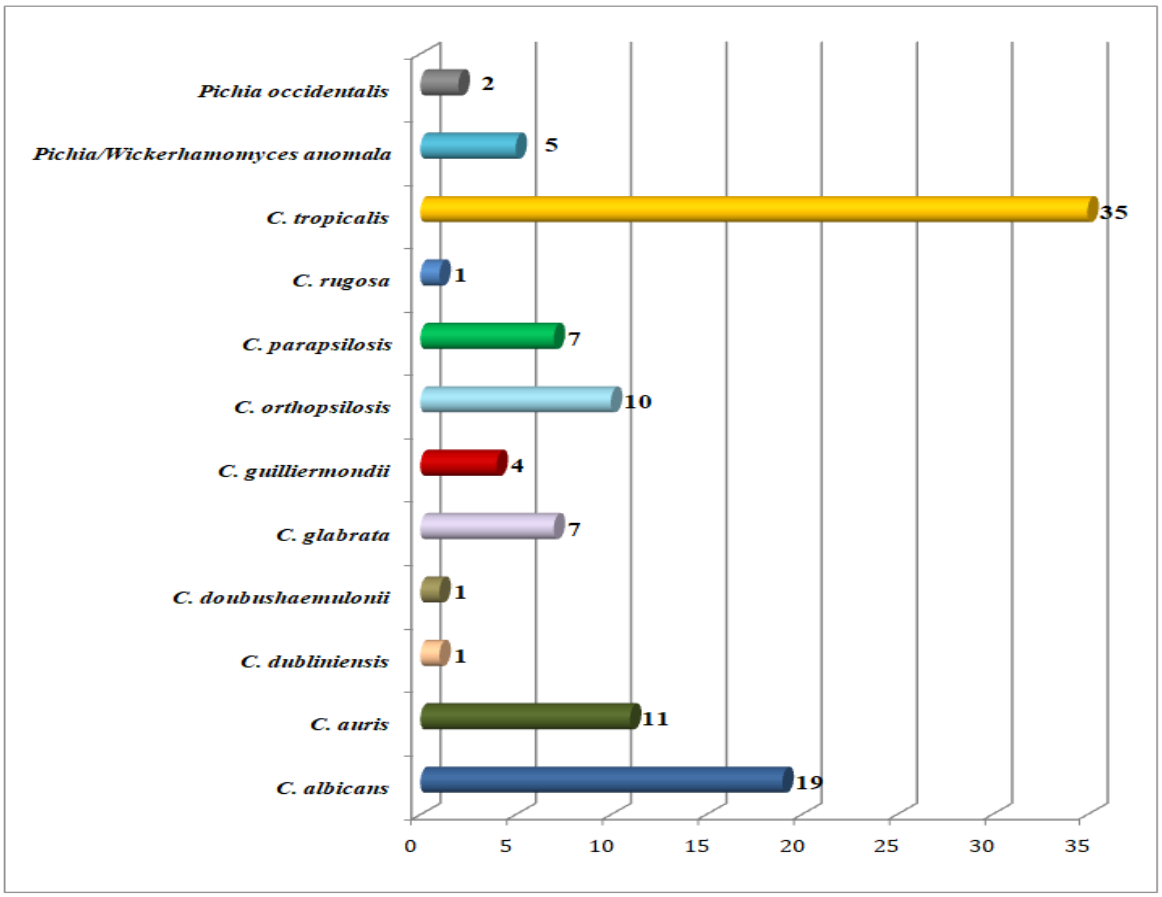

Figure 2: Identification of Candida species by MALDI TOF-MS (n=103)

Table 1: Comparison of Candida species identification by MALDI-TOF vs VITEK (n=103)

MALDI -TOF	Identification through VITEK											Total	p-value
	C. albicans	*c. auris*	*c. dubliniensis*	*c. glabrata*	*c. guilliermondii*	*c. krusei*	*c. orthopsilosis*	*c. parapsilosis*	*c. pelliculosa*	*c. rugosa*	*c. tropicalis*	*c. doubushaemulonii*	TOTAL	
C. albicans	19 (95.0%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	20 (19.40%)	0.001
C. auris	0 (0.0%)	11 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (10.70%)	
C. dubliniensis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

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C.glabrata	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (5.80%)	
C. guilliermondii	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (4.90%)	
C.krusei	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.90%)	
C. orthopsilosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (9.70%)	
C. parapsilosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (6.80%)	
C. pelliculosa	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (4.90%)	
C. rugosa	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (1.00%)	
C. tropicalis	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	35 (97.20%)	0 (0.0%)	36 (35.00%)	
C. doubushaemulonii	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1 (1.00%)	

Table 1 presents a comparison of the results for *Candida* species identification using two methods: VITEK 2 and MALDI-TOF mass spectrometry. The study demonstrates a significant level of concordance between the two methods, with an overall agreement rate of 98.1% (p=0.001). Specifically, both VITEK 2 and MALDI-TOF accurately and consistently identified *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. auris, C. guilliermondii, C. rugosa, and C. krusei*. However, there were a few discrepancies between the two methods. For instance, MALDI-TOF identified one isolate as *C. dubliniensis* and VITEK 2 was identified as *C. albicans.* Notably, MALDI-TOF identified multiple isolates of *C. orthopsilosis* that VITEK 2 misidentified as *C. parapsilosis.* Furthermore, MALDI-TOF detected certain Pichia species and *C. doubushaemulonii*, which VITEK 2 either failed to detect or misidentified. Conversely, VITEK 2 identified *C. pelliculosa* (4.9%) and *C. haemulonii* (1%), which were not detected by MALDI-TOF. These discrepancies can be attributed to differences in the detection systems, underlying principles, and databases used by each method.

DISCUSSION

The research aimed to compare the species-level identification of *Candida* isolates using automated systems such as VITEK 2 and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). VITEK identified 19.4% (n=20) as *C. albicans* and 35% (n=36) as *C. tropicalis*, alongside identifying *C. auris* (10.7%, n=11), *C. parapsilosis* (16.5%, n=17), and *C. glabrata* (5.8%, n=6). Similarly, MALDI-TOF results included 18.4% (n=19) for *C. albicans* and 34% (n=35) for *C. tropicalis*, additionally detecting species like *C. orthopsilosis* (9.7%, n=10) and *Pichia* species.

Comparative analysis with existing studies shows that Barantsevich N. et al. reported a 20% identification rate for *C. albicans* using VITEK, which is closely aligned with our finding of 19.4%. (14)Yadav A. et al. found *C. tropicalis* as the most prevalent species at 33%, consistent with our 35% finding. (15) The greater sensitivity of MALDI-TOF is highlighted

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through its identification of additional species like *C. orthopsilosis* (9.7%), corroborating results from Kailasa SK. et al., who emphasised its accuracy over traditional biochemical methods for less common pathogens. (16)This is further supported by Kalaivani S. et al., who noted that MALDI-TOF and VITEK effectively complement each other in identifying *Candida* species. (17)

The study established a significant difference in species identification accuracy between the two methods (P = 0.001), underlining the critical need for

Precise species identification in healthcare. Accurate species identification is essential for effectiveness.

Infection control, epidemiological monitoring, and appropriate antifungal therapy. The emergence of multidrug-resistant organisms like *Candida auris* underscores the necessity of reliable identification techniques. (18,19) Previous studies, such as those by Teke L et al. and Rudolph et al., affirm that MALDI-TOF is more precise than VITEK for identifying less common *Candida* species. (20,21)

CONCLUSION

In conclusion, this research sheds light on the speciation of candidemia from critical care units of a tertiary care institute in Uttarakhand, India. The striking predominance of non-albicans *Candida* species underlines the significance of species-level identification of clinical isolates.

The use of MALDI-TOF for *Candida* identification is a strength of this study, as this method provides rapid and accurate species-level identification, which is essential for guiding antifungal therapy and tracking the emergence of resistant species. However, it is essential to recognise the limitations of each method and consider complementary approaches when necessary, particularly in the context of rare or emerging species. Species identification differences between VITEK-2 and MALDI-TOF MS likely reflect the distinct methodologies and reference databases used by each system. MALDI-TOF relies on the unique protein spectral signatures of each species, while VITEK 2 uses a combination of biochemical reactions and substrates. The inclusion of rare and emerging species like *C. auris* in the MALDI-TOF database may explain its superior performance for these isolates. However, the lack of definitive molecular identification for all isolates limits the conclusions that can be drawn about the true accuracy of each method. The comparative data presented here highlight the strengths and weaknesses of VITEK 2 and MALDI-TOF for *Candida* identification and underscore the importance of using multiple methods for definitive species assignment.

These diverse outcomes underscored the necessity of developing more effective countermeasures to challenge candidemia's tenacious grip over time and curb its continuing impact on public health.

LIMITATIONS AND FUTURE SCOPE

- Several investigations have shown that MALDI-TOF and VITEK may misidentify or miss rare or cryptic *Candida* species, emphasising the need for molecular confirmation like gene sequencing.
- The focus of additional research on DNA barcoding techniques for the identification of fungal species has been on a variety of molecular targets, such as ITS sections or protein-coding genes. These targets have the potential to offer alternative or confirming techniques.

ETHICAL CONSIDERATIONS

- The study was conducted after approval from the Institutional Ethics Committee**(Clearance Code: No.357/IEC/PGM/2022)**. Patients were enrolled in the study only after she/he gave informed consent to participate in the study.
- This study benefited the patient in more accurate diagnosis of the causative organism, objective assessment of severity and response evaluation to pharmacotherapy, and improved the outcome of patients. The prospective patient population will benefit from the outcome of this study in the future. This study also serves the purpose of reducing the burden of antifungal resistance and helps the physicians involved in prescribing appropriate treatment.

CONFIDENTIALITY OF DATA: Confidentiality of the information obtained from the patient was maintained, and the identity of the patient was not revealed.

CONFLICT OF INTERESTS: The study had no involvement with any organisation or entity with any financial interests.

FUNDING: None

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