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Original Research Article

Comparison of MALDI-TOF MS and VITEK 2 for Identification of *Candida* Species in Clinical Isolates

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Abstract

Background: Fungal infections are an increasing cause of morbidity and mortality, particularly in immunocompromised patients. *Candida* spp. are among the most frequently isolated fungal pathogens. Therefore, early, and accurate species-level identification is essential for a timely and effective treatment.

Objective: To compare the performance of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (VITEK MS, bioMérieux, France) and VITEK 2 (bioMérieux, France) systems for identification of *Candida* species isolated from clinical samples in a tertiary care hospital.

Materials and Methods: A cross-sectional descriptive study was conducted at the Department of Microbiology, LHMC, from September 2022 to February 2024. In total, 210 clinical isolates of *Candida* spp. were analyzed. Identification was performed using both MALDI-TOF MS and VITEK 2, and the results were compared using categorical agreement (CA), Cohen's kappa coefficient, and p-values. Isolates with discrepant results were further evaluated using conventional phenotypic methods.

Results: *Candida albicans* (30%) and *Candida tropicalis* (30%) were the most frequently isolated species. The overall categorical agreement between VITEK 2 and MALDI-TOF MS was 86.6%. For all identified species, kappa values exceeded 0.8, and p-values were <0.01, indicating strong agreement and statistical significance.

Conclusion: Both MALDI-TOF MS and VITEK 2 are reliable methods for yeast identification. However, MALDI-TOF MS is faster, more cost-effective, and more accurate. Incorporating MALDI-TOF MS into routine fungal diagnostics can significantly reduce turnaround time, hospital stay duration, and healthcare costs.

Keywords: MALDI-TOF MS, VITEK 2, *Candida*, species Identification, Antifungal Stewardship, Diagnostic Accuracy.

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Introduction

Fungal infections have become an increasingly significant cause of morbidity and mortality, particularly in immunocompromised patients. *Candida* species are among the most frequently isolated fungal pathogens and are a major cause of hospital-acquired infections [1]. According to the Centers for Disease Control and Prevention (CDC) and the National Healthcare Safety Network (NHSN), *Candida* ranks fifth among hospital-acquired pathogens and fourth among causative agents of bloodstream infections (BSIs) [2].

Although *Candida albicans* remains the predominant species causing infections, recent decades have witnessed a notable epidemiological shift toward non-albicans *Candida* (NAC) species [3]. The prevalence of invasive infections caused by NAC has increased from 10–40% before the 1990s to 35–65% in recent years [4].

Early and accurate identification of the infecting *Candida* species is crucial because antifungal susceptibility varies substantially among species, with some demonstrating intrinsic resistance to specific antifungal agents. Therefore, accurate species-level diagnosis informs targeted antifungal therapy and supports effective antifungal stewardship. Reliance solely on conventional phenotypic methods for *Candida* identification is often challenging and time-consuming [5]. To address these limitations, advanced diagnostic techniques, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the VITEK 2 system, have been developed for rapid and reliable species identification. The VITEK 2 system offers an additional advantage of antifungal susceptibility testing. In this study, we compared the performance of MALDI-TOF MS (VITEK MS, bioMérieux, France) and VITEK 2 (bioMérieux, France) for the

identification of *Candida* species isolated from various clinical samples in a tertiary care hospital.

Materials and Methods

This descriptive cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology, Lady Hardinge Medical College (LHMC), and associated hospitals in New Delhi, India, from September 2022 to February 2024. Ethical approval was obtained from the Institutional Ethics Committee (LHMC/IEC/2022/PG Thesis/72, 09/27/2022).

Study Location: Mycology Laboratory, Department of Microbiology, LHMC, and associated hospitals in New Delhi, India.

Sample Size: 210 *Candida* isolates obtained from 210 patients.

Study Population: All clinical samples received for fungal culture at the Mycology Laboratory during the study period.

Inclusion Criteria: Pure cultures of *Candida* species determined to be clinically relevant based on patient clinical history, sample type, site of infection, and associated risk factors.

Exclusion Criteria

1. Samples showing polymicrobial growth.
2. Multiple isolates from the same infection site in the same patient.

Sample Processing: A total of 210 clinical samples yielding pure cultures of *Candida* spp. that met the inclusion criteria were analyzed.

- All samples, except nail specimens, were inoculated onto two tubes of Sabouraud's Dextrose Agar (SDA; HiMedia, India) supplemented with gentamicin (HiMedia, India) and chloramphenicol (HiMedia, India), and an additional tube of Brain Heart Infusion (BHI; HiMedia, India) medium.
- The nail samples were inoculated into two SDA tubes containing gentamicin, chloramphenicol, and cycloheximide.
- One SDA and BHI tube were incubated at 25 °C, and the second SDA tube was incubated at 37 °C for 6 weeks before being reported as negative.

Identification Methods: Species identification was performed using MALDI-TOF MS (VITEK MS, bioMérieux, France) and VITEK 2 (bioMérieux, France). For comparison, the MALDI-TOF MS results were used as reference standards.

Resolution of Discrepancies: Isolates with discrepant identifications between the two systems were further analyzed using conventional methods, including growth on *Candida* CHROMagar (HiMedia, India), morphological assessment on Cornmeal Agar (HiMedia, India), and the germ tube test.

MALDI-TOF MS: Pure cultures of the test isolates incubated for 24–48 h were used for analysis. The sample preparation and analysis were performed according to the method described in [6]. A small portion of a single, well-isolated colony was smeared onto a designated spot on the target plate and overlaid with 0.5 µL of formic acid (bioMérieux, France). The preparation was air-dried at room temperature, followed by the addition of 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA, bioMérieux, France) matrix solution.

After drying at room temperature, the target plate was loaded into a VITEK MS system (bioMérieux, France). Escherichia coli ATCC 8739 (bioMérieux, France) was applied to the central calibration spot as a control strain. When the identification results were unreliable the species was considered 'unidentified'.

VITEK 2: The samples were analyzed following the procedure described in [7]. Pure cultures of the test yeasts, incubated for 24–48 h, were prepared as suspensions in 3 mL of 0.45–0.5% NaCl solution (pH 4.5–7.0) using disposable plastic tubes supplied by the manufacturer. One to two colonies were emulsified into the suspension with a sterile inoculation loop, and turbidity was adjusted to 1.8–2.2 McFarland units using a densitometer (DensiCheckT; bioMérieux, France). The prepared tubes were placed into the VITEK 2 cassette along with yeast identification and antifungal susceptibility testing (AST) cards for each isolate.

The cassette was inserted into the loading chamber of the VITEK 2 instrument, and the "Start Fill" function was initiated. After the system beeped, indicating that the suspension had been drawn into the cards by the chamber vacuum, the cassette was transferred to the incubation chamber. The tubes were discarded, and after the incubation for approximately 15 h at 35^0C to complete the analysis, the data were obtained using the VITEK 2 software interface upon.

Conventional Methods

A. *Candida* CHROMagar (Figure 1): Identification of *Candida* strains on CHROMagar [8, 9] was performed as follows. Colonies obtained from Sabouraud Dextrose Agar (SDA) were subcultured onto *Candida* CHROMagar plates (HiMedia, India). Plates were incubated aerobically at 37 °C for 36–48 h to allow optimal color development. Colony colors were recorded and compared with the manufacturer's reference chart for species identification.

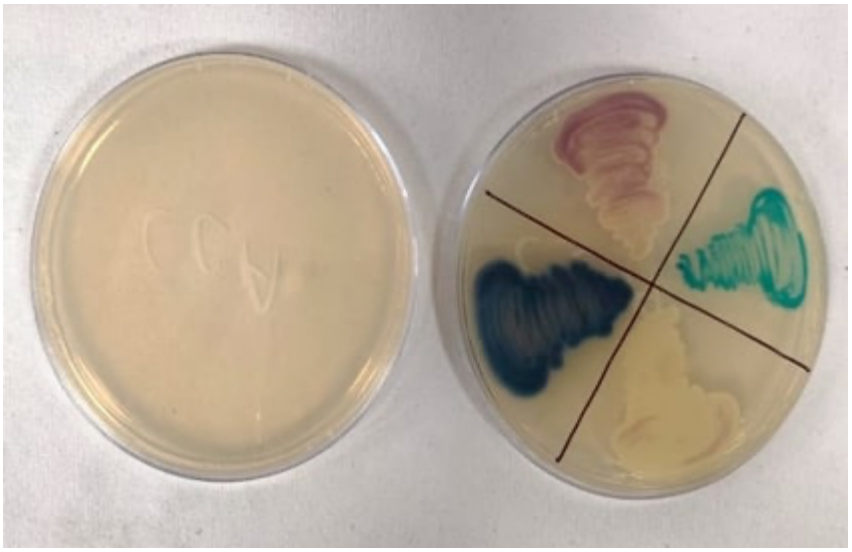


Figure 1. *Candida* CHROMagar showing colonies of various species with distinct pigmentation.

B. Germ Tube Test: Also known as the Reynolds–Braude phenomenon, the germ tube test [8] was performed for the preliminary identification of *Candida albicans*.

Procedure:

1. Fresh growth medium was obtained from a pure isolate.
2. A light suspension was prepared using sterile human serum (pooled serum, 0.5 mL).
3. The suspension was then incubated at 37 °C for 2 h.
4. A drop of the incubated suspension was placed on a clean glass slide, covered with a coverslip, and examined under a light microscope at ×40 magnification.
5. The presence of germ tubes - tube-like projections without constriction at the junction with the yeast cells - was noted.
6. Positive (*C. albicans*) and negative (*C. tropicalis*) controls were included for each test.

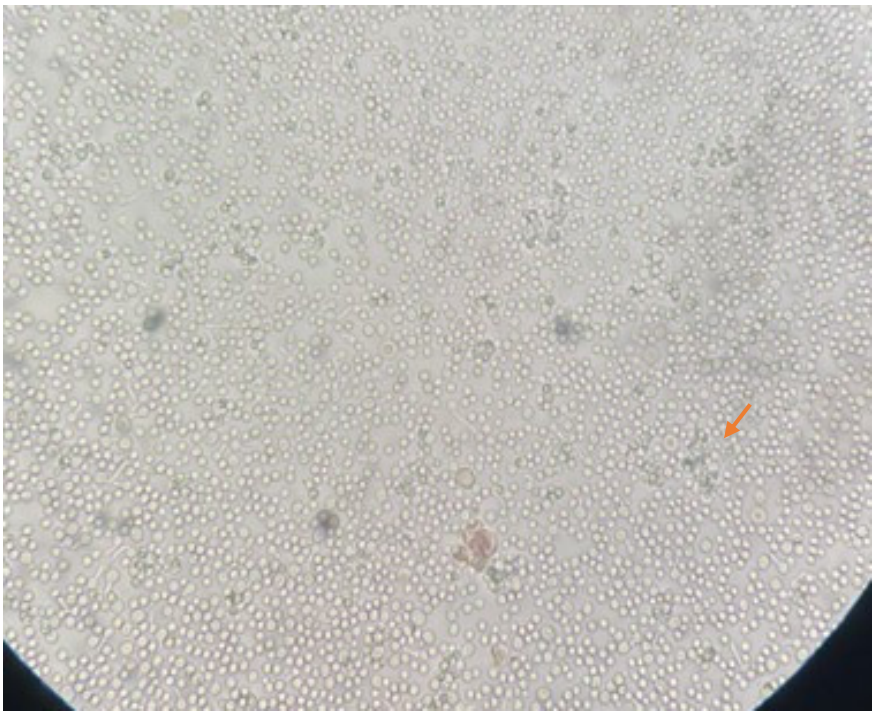


Figure 2: Positive germ tube formation was observed at ×40 magnification.

C. Morphology on Corn Meal Agar (CMA): The Dalmau plate method [8] was used for microscopic morphological assessments.

Procedure:

1. The CMA plate was divided into four quadrants and labelled.
2. Using a sterile straight wire, yeast colonies were lightly touched and streaked (2–3 streaks of approximately 4 cm length) in each quadrant.
3. A flame-sterilized cooled coverslip was placed over the inoculated streaks.
4. The plates were incubated at 25 °C for 3–5 d.
5. After incubation, coverslip edges were examined microscopically at ×10 and ×40 magnification to identify morphological features, such as chlamydospores, pseudohyphae, and blastoconidia.

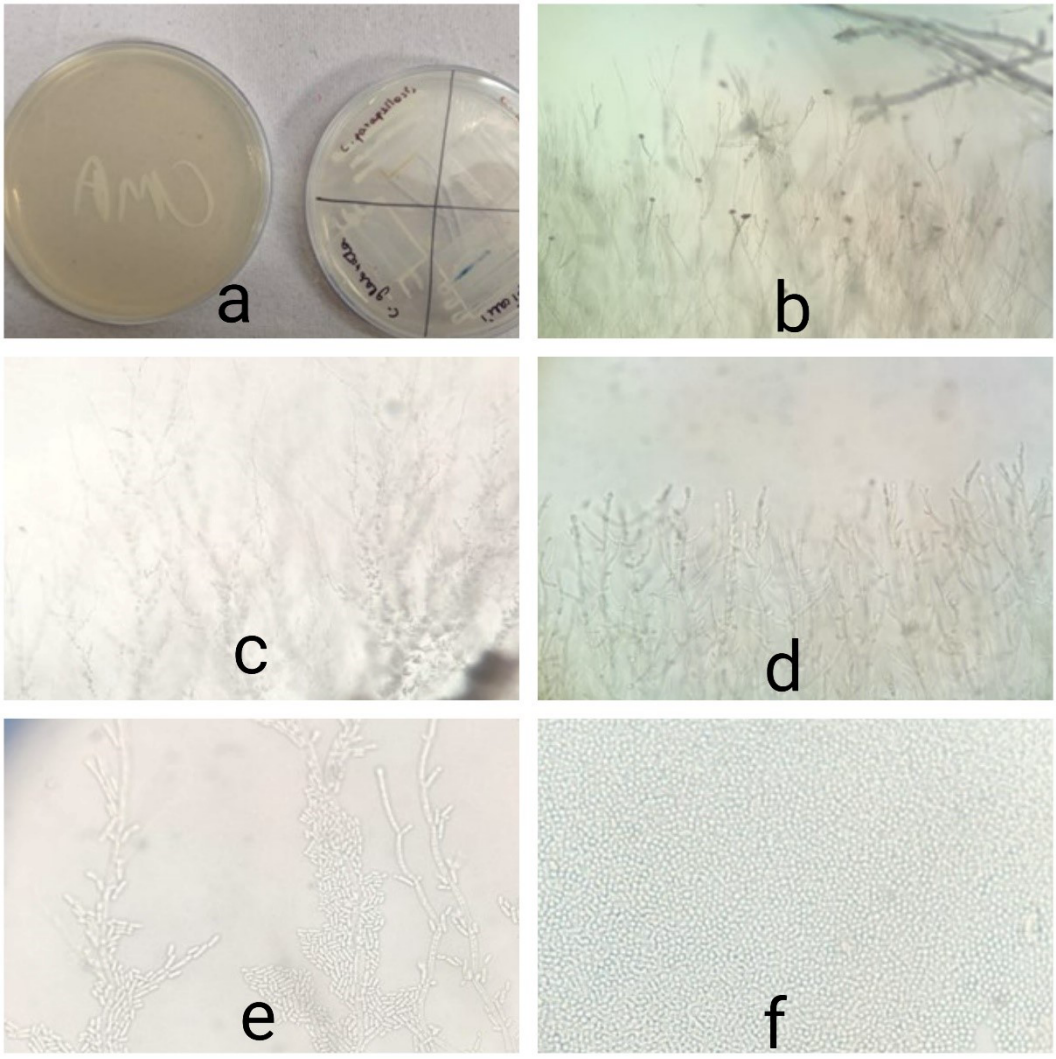


Figure 3: a) Corn Meal Agar: Dalmau Plate Method; (b)–(f) Morphological features of *Candida* species on CMA at ×40 magnification. b) *C. albicans*: pseudohyphae with clusters of round blastoconidia at septa and single, terminal chlamydospores; c) *C. tropicalis*: long, slender pseudohyphae with blastoconidia singly or in small groups; occasional teardrop chlamydospores; d) *C. parapsilosis*: short, curved pseudohyphae with single or clustered blastoconidia; e) *C. krusei*: pseudohyphae with elongated blastoconidia, producing a "crossed matchstick" or "tree-like" appearance; f) *C. glabrata*: no pseudohyphae; only small, oval yeast cells with single terminal budding.

D. Sugar Assimilation Test: The sugar assimilation test [8] was performed using the disc impregnation–pour plate auxanographic method described by Wickerham et al. [10].

Preparation of the yeast nitrogen base (YNB)

- YNB (HiMedia, India): 6.7 g dissolved in 100 mL distilled water, sterilized by filtration, and stored at 4 °C.
- Agar (20 g) was dissolved in 980 mL distilled water, dispensed into 18 × 150 mm screw-capped tubes, autoclaved at 121 °C, and stored at 4 °C.

- Commercial carbohydrate discs were obtained from HiMedia, India.

Procedure:

1. A heavy inoculum from a 24–48 h culture was suspended in 2 mL of YNB.
2. This suspension was added to 18 mL of molten agar (cooled to 45 °C) and mixed thoroughly.
3. The mixture was poured into 90 mm Petri dishes and allowed to solidify at room temperature.
4. Carbohydrate-impregnated discs were placed on the agar surface.
5. The plates were incubated aerobically at 37 °C for 3–4 days and examined for growth around the discs.
6. Growth around the glucose disc was recorded first and used as a control to confirm yeast viability.

Statistical Analysis: The obtained data were coded, entered into Microsoft Excel, and analyzed using SPSS software (version 25.0). Normality of distribution was assessed using the Shapiro–Wilk test. Quantitative variables are expressed as mean ± standard deviation, while qualitative variables are presented as frequencies. Categorical agreement and the percentage of discrepancies were calculated. Cohen's kappa coefficient and p-values were determined to assess statistical agreement, with significance set at $p < 0.05$.

Results

A total of 210 clinically relevant samples from 210 patients at the Lady Hardinge Medical College associated hospitals were included in the study. *Candida* species were identified using two methods - MALDI-TOF MS and VITEK 2 - and the results were compared using categorical agreement (CA). Based on MALDI-TOF MS identification, *Candida albicans* (30.0%) and *Candida tropicalis* (30.0%) were the most frequently isolated species, followed by *Candida parapsilosis* (12.4%), *Candida pelliculosa* (6.7%), and *Candida glabrata* (5.2%). Candida auris was isolated from eight samples (3.8%). Less frequently detected species included *C. duobushaemulonii*, *C. guilliermondii*, *C. haemulonii*, *C. kefyr*, *C. krusei*, *C. lusitaniae, C. orthopsilosis, C. rugosa, and C. utilis* (Table 1, Figure 4).

A comparison of species identification by MALDI-TOF MS and VITEK 2 showed an overall CA of 86.6% (Table 2). Cohen's kappa coefficients for all identified species were greater than 0.8, indicating very good agreement between the two methods. The calculated p-values were <0.01 for all species, confirming that the observed agreement was statistically significant.

Conventional identification methods were employed to resolve the discrepancies between the MALDI-TOF MS and VITEK 2 results (Table 3).

Table 1: Distribution of *Candida* species identified using MALDI-TOF in the clinical samples (n=210)

Species identified by MALDI	No.	%
Candida albicans	63	30.0
Candida auris	8	3.8
Candida duobushaemulonii	1	0.5
Candida glabrata	11	5.2
Candida guilliermondii	2	1.0
Candida haemulonii	1	0.5
Candida kefyr	1	0.5
Candida krusei	9	4.3
Candida lusitaniae	1	0.5
Candida orthopsilosis	2	1.0
Candida parapsilosis	26	12.4
Candida pelliculosa	14	6.7
Candida rugosa	1	0.5
Candida tropicalis	63	30.0
Candida utilis	7	3.3

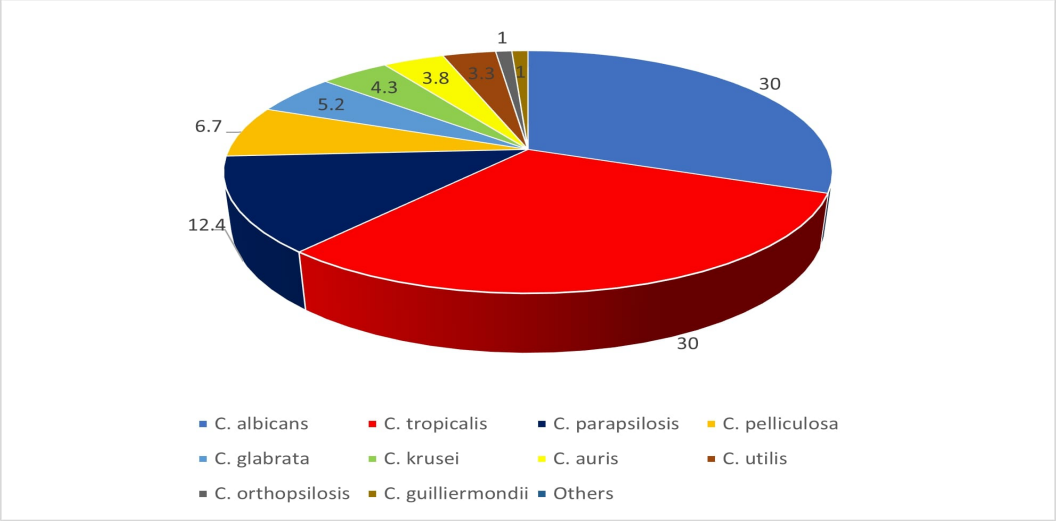


Figure 4: Percentage of different *Candida* species isolated in the present study

Table 2: Concordance in *Candida* species identification using different techniques (n=210)

Species identified by MALDI-TOF MS	Number of isolates identified by MALDI-TOF MS (Total)	Concordant results by VITEK	CA%	Kappa value	P value
Candida albicans	63	61	96.82	**0.97**	**<0.001**
Candida auris	8	6	75.00	**0.85**	**<0.001**
Candida duobushaemulonii	1	0	0	-	
Candida glabrata	11	8	72.72	**0.83**	**<0.001**
Candida guilliermondii	2	2	100.00	**1.00**	**<0.001**
Candida haemulonii	1	1	100.00	**1.00**	**<0.001**
Candida kefyr	1	1	100.00	**1.00**	**<0.001**
Candida krusei	9	9	100.00	**1.00**	**<0.001**
Candida lusitaniae	1	1	100.00	**1.00**	**<0.001**
Candida orthopsilosis	2	0	0	-	
Candida parapsilosis	26	22	84.61	**0.95**	**<0.001**
Candida pelliculosa	14	13	92.86	**0.96**	**<0.001**
Candida rugosa	1	1	100.00	**1.00**	**<0.001**
Candida tropicalis	63	52	82.54	**0.86**	**<0.001**
Candida utilis	7	5	71.43	**0.90**	**<0.001**
Total	210	182	86.60		

Table 3: Identification of *Candida* species by conventional methods in isolates that showed different results when analyzed by MALDI-TOF MS and VITEK 2

Species identified by MALDI-TOF MS	Species identified by VITEK 2 for the same isolate	Species identified by Conventional Methods for the same isolate
C. albicans	*C. ciferrii*	*C. albicans*
C. albicans	*C. parapsilosis*	*C. albicans*
C. tropicalis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. tropicalis*
C. tropicalis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. tropicalis*
C. tropicalis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. tropicalis*
C. tropicalis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. tropicalis*
C. tropicalis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. tropicalis*
C. tropicalis	*Low discrimination 50% C. famata, 50% C. sake*	*C. tropicalis*
C. tropicalis	*C. parapsilosis*	*C. tropicalis*

C. tropicalis	*C. parapsilosis*	*C. tropicalis*
C. tropicalis	*C. parapsilosis*	*C. tropicalis*
C. tropicalis	*C. albicans*	*C. tropicalis*
C. tropicalis	*C. krusei*	*C. tropicalis*
C. parapsilosis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. parapsilosis*
C. parapsilosis	*Low discrimination 50% C. tropicalis, 50% C. parapsilosis*	*C. parapsilosis*
C. parapsilosis	*C. tropicalis*	*C. parapsilosis*
C. parapsilosis	*C. tropicalis*	*C. parapsilosis*
C. pelliculosa	*C. tropicalis*	*C. pelliculosa*
C. glabrata	*C. albicans*	*C. glabrata*
C. glabrata	*C. albicans*	*C. glabrata*
C. glabrata	*C. tropicalis*	*C. glabrata*
C. auris	*C. tropicalis*	*C. auris*
C. auris	*Millerozyma farinosa*	*C. auris*
C. utilis	*C. pelliculosa*	*Candida non albicans*
C. utilis	*Candida magnoliae*	*Candida non albicans*

Using the VITEK 2 method, one *Candida albicans* isolate was misidentified as *Candida ciferrii* and another as *Candida parapsilosis*. Both strains were correctly identified as *C. albicans* using conventional methods.

For *Candida tropicalis*, five isolates were reported by VITEK 2 as "low discrimination" between 50% *C. tropicalis* and 50% *C. parapsilosis*. Additionally, three isolates were misidentified as *C. parapsilosis*, and one each as *C. albicans*, *C. krusei*, and as "low discrimination" between 50% *C. famata* and 50% *C. sake*. All 11 isolates were confirmed to be *C. tropicalis* by conventional methods.

In the case of C. parapsilosis, two isolates were reported as "low discrimination" between 50% *C. tropicalis* and 50% *C. parapsilosis*, and two were misidentified as *C. tropicalis* by the VITEK 2. All four strains were correctly identified as *C. parapsilosis* using conventional methods.

One *C. pelliculosa* isolate was misidentified as C. tropicalis by VITEK 2 but was correctly identified as *C. pelliculosa* by conventional methods. Two *C. glabrata* isolates were incorrectly identified as *C. albicans* and one as *C. tropicalis* by VITEK 2; all were confirmed as *C. glabrata* by conventional methods.

Among the eight *C. auris* isolates identified by MALDI-TOF MS, one was misidentified by VITEK 2 as *C. tropicalis* and the other as Millerozyma farinosa. Using conventional methods, both were confirmed to be *C. auris*.

One isolate identified as *C. utilis* by MALDI-TOF MS was reported as *C. magnoliae* by VITEK 2, and another as *C. pelliculosa*. Neither could be identified at the species level using conventional methods.

Discussion and Conclusion

In the present study, 63 isolates were identified as *Candida albicans* and the remaining 147 isolates were identified as non-albicans *Candida* (NAC) species. Similar to our findings, a marked shift towards increased isolation of NAC species from infected patients has been reported in recent publications. More et al., Ahmed et al., Chakraborty et al., Chaudhary et al., Rajgopal S.K. et al., and Jaya et al. all observed a higher proportion of NAC isolates compared to *C. albicans* in their studies [1, 11, 12, 13, 14, 15].

While studies by Jaggi et al., Mokaddas et al., and Gupta et al. reported *C. albicans* as the most frequently isolated species [16, 17], other investigations by Ahmed et al., Chaudhary et al., Jaya et al., and Rajgopal S.K. identified *C. tropicalis* as the predominant species [11, 13, 14, 15]. In the present study, *C. albicans* and *C. tropicalis* were isolated in equal numbers. Within the NAC group, *C. tropicalis* (30%) was the most prevalent, followed by *C. parapsilosis* (12.4%), *C. pelliculosa* (6.7%), *C. glabrata* (5.2%), *C. krusei* (4.3%), *C. auris* (3.8%), *C. utilis* (3.3%), *C. orthopsilosis* (1%), and *C. guilliermondii* (1%). Less frequently isolated species included *C. haemulonii*, *C. kefyr*, *C. lusitaniae*, and *C. rugosa* (each 0.5%).

In our study, the overall agreement between VITEK 2 and MALDI-TOF MS for Candida species identification was 86.6%. Low categorical agreement (<90%) was observed for *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida auris*, and for less frequently isolated species, such as *Candida duobushaemulonii*, *Candida orthopsilosis*, and *Candida utilis*.

Isolates with discordant results were reidentified using conventional methods. All results obtained using conventional methods were concordant with MALDI-TOF MS, except for two *C. utilis* isolates, which VITEK 2 had identified as *Candida magnoliae* and *Candida pelliculosa*; these could not be identified to the species level using conventional methods.

Puig et al. [18] evaluated the VITEK MS system (based on MALDI-TOF MS) for the identification of *Candida* isolates from bloodstream infections. Using specific PCR and ITS sequencing as reference methods, they found that VITEK MS correctly identified 279 of 298 isolates at the species level. Misidentifications included five *C. albicans*, six *C. parapsilosis*, four *C. tropicalis*, one *C. glabrata*, and two *C. lusitaniae* isolates. The system correctly identified all isolates of *C. krusei*, *C. guilliermondii*, *C. lipolytica*, and *C. haemulonii*.

Kaur et al. [19], using molecular methods as the gold standard for *Candida* species identification, reported that VITEK 2 correctly identified 155 of 172 isolates, compared to 171 of 172 by conventional methods. VITEK 2 misidentified eleven *C. albicans* isolates as *C. famata* (n=6), *C. tropicalis* (n=1), C. glabrata (n=1), *C. krusei* (n=1), *G. capitatum* (n=1), and *K. ohmeri* (n=1). Additionally, one *C. tropicalis* isolate was misidentified as *K. ohmeri* and one *C. parapsilosis* as *C. famata*. Four isolates were reported as "low discrimination." In contrast, the conventional methods misidentified only one *C. albicans* isolate as *C. tropicalis*. Meena et al. [20], using sequencing as the reference method, compared conventional methods with MALDI-TOF MS and found substantial agreement ($\kappa = 0.763$). Three discrepant isolates underwent ITS region sequencing, which revealed that two *C. krusei* isolates were misidentified as Wickerhamomyces anomalus and one as *T. ovoides* by MALDI-TOF MS; all were correctly identified by conventional methods. These misidentifications were attributed to the use of a simple extraction method rather than a more complex, multipart protocol [21]. Yaman et al. [22] compared *Candida* species identification by MALDI-TOF MS and VITEK 2, finding that both methods achieved 94% accuracy. However, there were 16 discordant results in the data obtained using the two systems. Sequencing of these isolates confirmed MALDI-TOF MS identification.

Garza-González et al. [23] compared yeast identification by MALDI-TOF MS and VITEK 2, reporting 100% agreement for *C. glabrata*, C. krusei, and Cryptococcus neoformans, and >90% agreement for *C. albicans*, *C. tropicalis*, and *C. orthopsilosis*.

Dhiman et al. [24] found that MALDI-TOF MS accurately identified 96.3% of common and 84.5% of archived yeast strains to the species level, and was capable of differentiating *C. albicans from* C. dubliniensis. Graf et al. [25] analyzed 241 *Candida* isolates, of which VITEK 2 unequivocally identified 222 (92.1%) to the species level, including 11 (4.6%) isolates initially showing low discrimination, which were resolved by additional tests. They reported complete accuracy for *C. tropicalis*, *C. albicans*, and *C. glabrata* using VITEK 2—findings that contrast with our results, where 2 *C. albicans*, 3 *C. glabrata*, and 11 *C. tropicalis* isolates were misidentified.

In our study, MALDI-TOF MS provided *Candida* species identification within approximately 15 min, which was significantly faster than VITEK 2, which required approximately 15 h. Conventional methods took 24–48 h for identification and up to 72 h for germ tube-negative *Candida* species. Similar observations were reported by Yaman et al. [22], Meena et al. [20], and other researchers.

Although conventional methods have long been regarded as reliable for yeast identification, they are labor-intensive, time-consuming, and less reliable for identifying uncommon species. Molecular methods are rapid and highly accurate but remain costly and technically demanding.

Based on our findings and the published literature, both MALDI-TOF MS and VITEK 2 are effective options for *Candida* identification, with MALDI-TOF MS offering greater speed, cost efficiency, and accuracy. The adoption of MALDI-TOF MS holds strong promise for precise species-level identification, significantly reducing turnaround time, shortening hospital stay, lowering patient care costs, and supporting more effective antifungal stewardship programs. This study has several limitations. The number of isolates for certain species was small, and molecular methods, such as sequencing, were not performed to confirm species identity in cases where MALDI-TOF MS and VITEK 2 results were discordant.

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