Identification of *Candida* species isolated from clinical samples by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis

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Abstract

Background: *Candida* species are responsible for substantial morbidity and mortality among susceptible populations. Contemporary epidemiological data indicate a noticeable shift in infection trends away from *Candida albicans* (*C. albicans*) toward non-albicans *Candida* (NAC) species. The primary objectives of this research were therefore to identify the prevalent *Candida* species and to assess the efficacy of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of these species.

Methods: The standard protocol was employed for processing all routine clinical samples submitted to the bacteriology and mycology departments. Identification of *Candida* species isolated from diverse clinical specimens was performed using MALDI-TOF MS between September 2022 and November 2023. Subsequently, these findings were recorded in tables and subjected to statistical analysis.

Results: During the study period, a total of 342 *Candida* species were isolated. Patients diagnosed with candidiasis had a median age of 46.5 years and exhibited a male predominance. NAC species were the most frequently isolated group, with *Candida tropicalis* (*C. tropicalis*) (30.40%) as the most common species, followed by *Candida parapsilosis* (*C. parapsilosis*) (14.32%). Furthermore, 40 strains of *Candida auris* (*C. auris*) were identified over the 15-month duration of the study (11.69%).

Conclusion: The significance of *Candida* species, encompassing both NAC and novel, emerging drug-resistant strains, necessitates a cautious approach. Compared to conventional and automated systems that rely on biochemical reactions for the speciation of *Candida* isolates, MALDI-TOF MS offers a potentially advantageous alternative due to its rapid and accurate results.

Introduction

Candida species are prevalent organisms commonly found colonizing the mucous membranes of the gastrointestinal tract, genitourinary tract, and skin (1). These fungi are capable of causing substantial morbidity and mortality, particularly in patients who have compromised epithelial barriers and underlying impairments in their immune defense mechanisms (2). The genus *Candida* is a type of yeast that encompasses over 150 different species. Among these, a limited number are frequently recognized as pathogenic, including *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida parapsilosis* (*C. parapsilosis*), and *Candida glabrata* (*C. glabrata*) (1). Candidiasis frequently occurs in individuals who are immunocompromised, such as patients with acquired immunodeficiency syndrome (AIDS), and following extensive medical interventions, including prolonged antibiotic therapy, invasive surgery, use of indwelling intravenous catheters or prosthetic devices, administration of hyperalimentation fluids, or chemotherapy. *Candida* species are responsible for a range of mucosal and cutaneous infections, which include oral candidiasis, esophagitis, gastrointestinal candidiasis, cutaneous candidiasis, and chronic mucocutaneous candidiasis. Furthermore, *Candida* can lead to more critical systemic infections, with invasive bloodstream infections being the most prevalent and clinically significant among them (1).

Reports from numerous countries worldwide indicate an alteration in the epidemiology of *Candida* infections, marked by a gradual transition from the dominant species, *C. albicans*, to a prevalence of non-albicans *Candida* (NAC) species, including *C. tropicalis*, *C. glabrata*, and *Candida krusei* (*C. krusei*) (2-5). Given that both pathogenicity and appropriate therapeutic approaches vary by species,

accurate species identification is crucial (1). The recovery of these NAC organisms from diverse hospital settings and on the hands of healthcare personnel suggests nosocomial transmission. Noteworthy among these NAC species is *Candida auris* (*C. auris*), a recently recognized, multidrug-resistant yeast that has been responsible for outbreaks across several geographic regions. To implement stringent infection control measures within a hospital setting, rapid and accurate identification of *C. auris* is crucial (6).

The laboratory diagnosis of *Candida* is generally straightforward due to the microscopic presence of yeast forms and pseudo-hyphae. These organisms exhibit robust growth on standard culture media and in blood culture bottles, eliminating the need for specialized culturing ingredients. Conventional phenotypic assays, such as the rapid, presumptive germ tube test and chlamydospore formation, are employed to distinguish *C. albicans* from NAC species. However, these methods are incapable of providing further species-level differentiation among NAC isolates and are subject to inherent limitations of false positive and false negative results. Other phenotypic identification methods, which rely on biochemical reactions, include systems like the Analytical Profile Index (API), VITEK, and PHOENIX. However, these methods typically require 18-24 hours to yield identification results (1). Conversely, molecular approaches such as those based on polymerase chain reaction (PCR) and sequencing offer high accuracy but are often associated with significant processing time and considerable expense (7).

A novel approach employing matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a rapid and highly accurate method for identifying yeasts directly from culture plates based on their unique protein profile, typically within 20

minutes (8,9). This technique was utilized in the current study, which was executed at a tertiary care hospital in Karnataka, with the objective of identifying *Candida* species that were isolated from various clinical specimens through MALDI-TOF MS analysis.

Methods

Study setting and design

This research, structured as a retrospective, laboratory-based study, utilized data procured from the Department of Microbiology at Father Muller Medical College Hospital in Mangalore, Karnataka, India, from September 2022 to November 2023. The study's cohort comprised all clinically significant *Candida* isolates that were successfully recovered from routine clinical specimens during the specified timeframe.

Specimen collection and processing

Blood samples were obtained and inoculated into BacT/ALERT aerobic culture bottles (bioMérieux, France). These bottles were subsequently incubated at a temperature of 37°C for a maximum duration of 5 days. Any bottles indicating a positive signal for yeast proliferation were subjected to Gram staining, followed by subculturing onto both blood agar and Sabouraud's dextrose agar (SDA) media (Both supplied by HiMedia Laboratories Pvt. Ltd., Mumbai, India).

All remaining clinical samples, encompassing high vaginal swabs, urine, pus, central venous catheter (CVC) tips, bronchoalveolar lavage (BAL) fluid, nail clippings, ascitic fluid, wound swabs, tissue, bile, and cerebrospinal fluid (CSF), underwent processing in accordance with standard microbiological protocols. These specimens were inoculated onto suitable culture media and subsequently incubated at 37°C for 24–48 hours.

Specimens specifically requested for fungal culture were inoculated onto two separate slopes of SDA supplemented with gentamicin (Omitting cycloheximide). These cultures were then incubated at 25°C and 37°C for a maximum duration of four weeks. Direct examination of all specimens was conducted utilizing Gram stain and/or 10% potassium hydroxide (KOH) wet mounts for the primary purpose of detecting yeast cells.

Isolation and preliminary identification

Following an incubation period of 24–48 hours, culture plates exhibiting smooth, cream-to-white, and glabrous colonies-morphologically indicative of *Candida* species-were chosen for further analysis. A Gram stain was subsequently performed on these colonies to confirm the characteristic yeast morphology. These presumptive isolates were then subcultured onto HiChrome™ Candida Differential Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for a reliable, phenotypic differentiation based on colony color.

Species identification by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

Species-level identification was definitively achieved utilizing the Bruker Microflex LT/SH MALDI-TOF MS system (Bruker Daltonics, Bremen, Germany) in conjunction with MALDI Biotyper software (MBT Compass version 12.0.0.0_10833). This system operates by comparing the distinctive ribosomal protein spectra of the isolates against a specialized reference database for organism identification (10).

Sample preparation (Extended direct transfer method)

A pure colony was thinly smeared onto a polished steel MALDI target plate. Subsequently, 1 μL of a 70% formic acid solution was applied to the sample and permitted to air-dry at room temperature. Following this step, 1 μL of the α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was added and allowed to completely dry.

Data acquisition and interpretation

The MALDI Biotyper software was utilized in automatic mode to acquire the spectra. Interpretation of the resulting identification scores adhered to the manufacturer's established criteria: A score of ≥ 2.0 signified a high-confidence identification at the species level; scores ranging from 1.7 to 1.99 indicated genus-level identification with lower confidence; and scores of < 1.7 were classified as unreliable. The *C. albicans* ATCC 90028 functioned as the positive control, while spots containing matrix only were employed as the negative control (11).

Statistical analysis

Categorical data (Such as specimen type, species distribution, and demographic information) were presented using frequencies and percentages. Continuous variables (e.g., patient age) were summarized by the median accompanied by the interquartile range (IQR), as appropriate. Descriptive statistics in Microsoft Excel were employed for data analysis.

Results

A comprehensive analysis was conducted on 342 *Candida* isolates derived from clinical specimens over a 15-month period, spanning from September 2022 to November 2023. The median age of patients at the time of infection was 46.5 years, with a notable male predominance. The primary source of the isolates was blood (136/342, 39.76%) (Table 1). NAC species constituted the majority (254/342, 72.84%), outnumbering *C. albicans*. Among the NAC group, *C. tropicalis* was the most prevalent isolate (30.40%), followed by *C. parapsilosis* (14.32%). Furthermore, 40 strains of *C. auris* were identified during the study (11.69%) (Table 2).

Table 1. Distribution of Candida species by specimen

Serial No.	Specimen	Number (%)
1	Blood	136 (39.76)
2	High vaginal swab	60 (17.52)
3	Urine	54 (15.78)
4	Pus	41 (11.98)
5	CVC tip	12 (3.5)
6	BAL	11 (3.21)
7	Nail clipping	9 (2.63)
8	Ascitic fluid	7 (2.04)
9	Wound swab	5 (1.46)
10	Tissue	3 (0.8)
11	Bile	3 (0.8)
12	CSF	1 (0.29)
	Total	342 (100)

CVC: Central Venous Catheter; BAL: Bronchoalveolar Lavage; CSF: Cerebrospinal Fluid

Table 2. Distribution of Candida species isolated from different clinical samples

Serial No.	*Candida* Species	Number (%)
1	*Candida albicans*	88 (27.16)
2	*Candida tropicalis*	104 (30.40)
3	*Candida parapsilosis*	49 (14.32)
4	*Candida auris*	40 (11.69)
5	*Candida glabrata*	29 (8.47)
6	*Candida orthopsilosis*	18 (5.26)
7	*Candida metapsilosis*	4 (1.16)
8	*Candida krusei*	3 (0.87)
9	*Candida haemolunii*	2 (0.58)
10	*Candida dubliensis*	2 (0.58)
11	*Candida nivariensis*	2 (0.58)
12	*Candida utilis*	1 (0.29)
	Total	342 (100)

All *Candida* species were identified using MALDI-TOF MS, achieving a log score of > 1.7 for 314/342 (91.81%) isolates. The mass spectra of the most prevalent *Candida* species isolated are presented subsequently (Figure 1-4). Specifically, among the 40 isolates, 37 (92.5%) were identified as *C. auris* with a log score of >= 1.7. Regarding the 40 cases of *C. auris*, 50% (20/40) originated from the burn's unit, and all 40 cases were linked to extended hospitalizations.
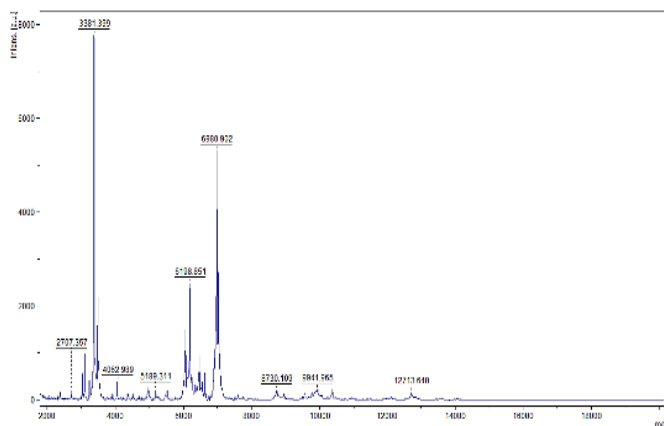
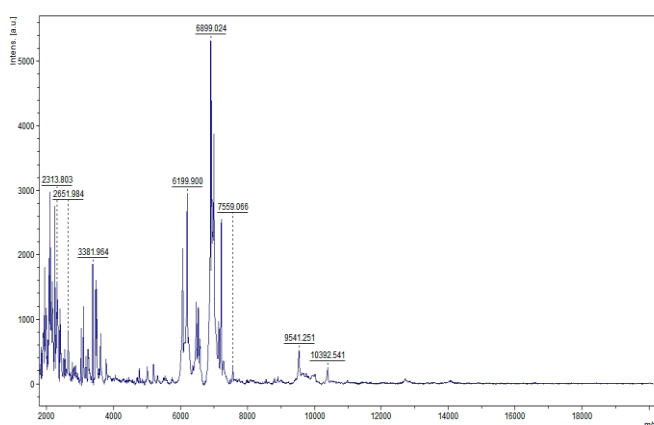**Figure 1.** Mass spectra graph of *Candida albicans*
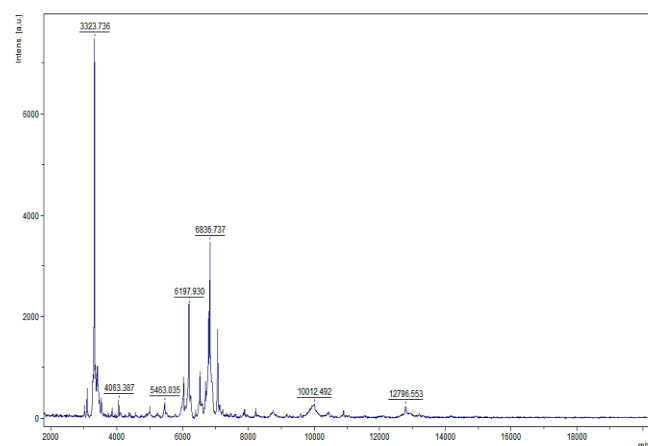

Figure 2. Mass spectra graph of *Candida tropicalis*

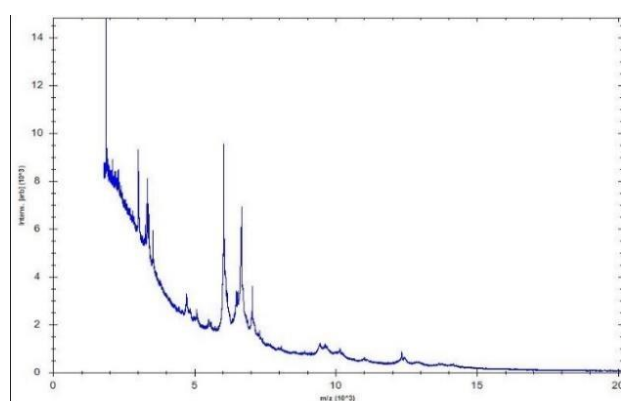**Figure 3.** Mass spectra graph of *Candida parapsilosis*

Figure 4. Mass spectra graph of *Candida auris*

Discussion

Prompt and accurate identification of yeast is critical for effective patient management in any infectious process. Furthermore, the rising prevalence of NAC species can complicate the selection of appropriate antifungal therapy (3-6).

Infections caused by NAC species are clinically indistinguishable from those caused by *C. albicans*; however, antifungal resistance is more frequently associated with NAC species (1). Our current research demonstrated a predominance of NAC, accounting for 72.84% of the isolates responsible for various clinical candidiasis conditions. This finding aligns with a study conducted by Umamaheshwari et al. in Southern India, which reported that 73.64% of candidiasis cases were attributable to NAC species (5). Furthermore, the emergence and predominance of NAC in various body fluids have also been highlighted by Singh DP et al. (12).

In the current study, *C. tropicalis* (30.40%) was determined to be the most frequently isolated species. The finding of our study aligned with the results reported by Gautam et al. (26.72%) and Anita et al. (37.09%) for the isolation frequency of *C. tropicalis* (13,14). While traditional phenotypic identification methods, which rely on biochemical reactions, are cost-effective, they are inherently time-consuming. Conversely, techniques involving nucleic acid detection, although highly specific, are often expensive. Consequently, MALDI-TOF MS has emerged as a novel and efficient method for the identification and speciation of yeasts, such as *Candida* (9,11). MALDI-TOF MS was utilized for accurate species-level identification of *Candida* isolates. The on-plate extraction method simplified the procedure, and the technique demonstrated high throughput and cost-effectiveness. Specifically, its ability to analyze 96 isolates per run using inexpensive reagents makes it significantly more economical than traditional biochemical or nucleic acid-based methods. MALDI-TOF MS successfully identified all suspected *Candida* species. A high degree of confidence was achieved for 91.81% (314/342) of the isolates, with log scores > 1.7, which is consistent with findings reported by Periera et al. (10).

The multidrug-resistant yeast *C. auris* is frequently misidentified by conventional phenotypic automated systems as other *Candida* species, including *Candida haemulonii* (*C. haemulonii*), *Candida famata* (*C. famata*), *Candida sake* (*C. sake*), and *Candida lusitanie* (*C. lusitaniae*). Definitive confirmation of *C. auris* requires advanced methods, MALDI-TOF MS or DNA sequencing (15). In this study, a concerningly high prevalence of *C. auris* (11.69%) was observed, particularly among burn patients. These patients are highly susceptible to acquiring *C. auris* infections from the hospital environment due to their impaired immune defense and extensive wounds (16). This observed dominance of *C. auris* is consistent with findings from a previous study conducted in western India by Prayag PS (6).

Accurate and timely diagnosis of NAC is crucial for the selection of appropriate empirical antifungal treatment. Furthermore, the implementation of stringent infection prevention and control (IPC) protocols is essential for patients colonized or infected with *C. auris* to effectively contain this multidrug-resistant yeast and mitigate the risk of institutional outbreaks.

Conclusion

The role of *Candida* species, encompassing NAC and novel drug-resistant strains, necessitates a cautious approach in clinical and laboratory settings. MALDI-TOF MS offers a viable alternative to conventional and automated identification systems that rely on biochemical reactions for *Candida* speciation. Despite the significant initial capital investment, the use of less expensive reagents and the implementation of simplified protocols establish MALDI-TOF MS as a cost-effective method for the routine identification of yeast isolates from clinical specimens.

Ethical statement

Reference No. FMIEC/CCM/550/2023 (Protocol No. 512/2023)

Conflicts of interest

No conflict of interest.

Author contributions

K.P.: Study concept, Study design, Data analysis, and Manuscript writing and Review; R.T.P.: Data collection and Manuscript writing; S.D. and B.A.: Data analysis and Manuscript review; M.D.: Manuscript review.

Data availability statement

The data supporting the findings of this study will be made available by the corresponding author to any interested party upon presentation of a reasonable request.

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