Identification of *Candida* species isolated from clinical samples by Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis

Running Title: Speciation of *Candida* by MALDI-TOF

Kavitha Prabhu
MD Medical Microbiology, Department of Microbiology, Father Muller Medical College, Mangalore, India.
Email: kaavitaramesh@yahoo.co.in ORCID:0000-0002-7012-8101

Rashmi Theresa Dsouza
JRF/CRC. Father Muller Research Centre, Mangalore, India.
Email: rashmidsouza15@gmail.com

Santhosha Devadiga
Department of Clinical Virology, Father Muller Research Centre, Mangalore, India.
Email: santhoshbailur123@gmail.com

Beena Antony
Department of Microbiology, Father Muller Research Centre, Mangalore, India.
Email: beenafmmc@gmail.com ORCID:0009-0004-1149-1527

Meena Dias
Department of Microbiology, Father Muller Medical College, Mangalore, India.
Email: drmeenadias@gmail.com ORCID: 0000-0002-9213-8909

Corresponding author: Beena Antony
Address: Department of Microbiology and Father Muller Research Centre, Father Muller Medical College Mangalore, Karnataka, India.
Mobile number: +919448024684
Email: beenafmmc@gmail.com
E mail: kaavitaramesh@yahoo.co.in
Tel: +98917760216542

Abstract

Background: *Candida* species cause significant morbidity and mortality in vulnerable populations. Recent reports suggest a shift in the epidemiological trend to non-albicans *Candida* (NAC) infections from *C. albicans*. This study aimed to identify *Candida* species and evaluate the performance of Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) in the identification of *Candida* species.

Methods: All routine clinical samples received in the bacteriology and mycology sections were processed using the standard protocol. *Candida* species recovered from various clinical samples were identified using MALDI-TOF MS from September 2022 to November 2023, and the results were tabulated and analysed statistically.

Results: A total of 342 *Candida* species were isolated during the study period. The median age of the patients presenting with candidiasis was 46.5 years, with a male predominance. NAC species were predominant with *C. tropicalis* (30.40%), being the most common species followed by *C. parapsilosis* (14.32%). Forty strains of *C. auris* isolates were found during this 15-month study period (11.69%).

Conclusion: The role of *Candida* species, including NAC and newly emerging drug resistant *Candida* strains should be approached with caution. With its rapid and accurate results, MALDI-TOF MS can be useful compared to conventional and automated machines that are based on biochemical reactions for the speciation of *Candida* isolates.

Key words: Candidiasis, NAC, MALDI-TOF.

Introduction

Candida species are common organisms that colonize the mucous membranes of the gastrointestinal tract, genitourinary tract, and skin (1). They can cause significant morbidity and mortality in patients with breached epithelial barriers and impaired immune mechanisms (2). Genus *Candida* is a yeast, comprising more than 150 species, and among them, a few species like *C. albicans*, *C. tropicalis, C. parapsilosis,* and *C. glabrata*, are frequently pathogenic (1). Candidiasis is common in immunocompromised patients with AIDS and after prolonged antibiotic therapy, invasive surgery, indwelling intravenous catheters, prosthetic devices, hyperalimentation fluids, chemotherapy, etc. *Candida* species can cause infections of the mucosa and skin, like oral candidiasis, esophagitis, gastrointestinal candidiasis, cutaneous candidiasis, and chronic mucocutaneous candidiasis. *Candida* can cause more serious systemic infections; among them invasive bloodstream infections are frequent and the most important (1).

Many countries around the world reported a change in the epidemiology of *candida* infections, characterized by a progressive shift from a predominance of *C. albicans* to non-albicans *Candida* (NAC) species such as *C. tropicalis, C. glabrata,* and *C. krusei* (2-5). As there is variation in pathogenicity, and treatment depends on the species, species identification is essential (1). The isolation of this organism from various hospital environments and the hands of healthcare workers suggests hospital spread. Among these NAC, *C. auris* is a newly noticed, multidrug-resistant yeast leading to outbreaks in various geographical locations. Prompt and precise identification of *C. auris* is essential to enforce strict infection control practices in the hospital (6).

Laboratory diagnosis of *Candida* is not so difficult as yeast forms; pseudo-hyphae are found microscopically. They grow well on routine culture media and blood culture bottles, and do not require any special ingredients for the culture. Conventional tests like the Germ tube test, a rapid, presumptive test, and chlamydospore formation are used to differentiate between *C. albicans* and NAC species, but further species differentiation of NAC is not possible with these tests, and both false positive and false negative results may occur. Various other phenotypic tests based on biochemical reactions are available, such as the Analytical Profile Index (API), VITEK, and PHOENIX, but they require 18-24 hours for identification (1). Molecular methods based on the polymerase chain reaction and sequencing are accurate but need considerable processing time and are costly (7).

A new method, Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) , which identifies microorganisms by their protein composition can be used for the identification of the yeast within 20 minutes from the culture plate with greater accuracy (8,9). This study was conducted in a tertiary care hospital in Karnataka to identify *Candida* species isolated from clinical specimens using MALDI-TOF analysis.

Methods

Study setting and design

A retrospective laboratory-based study was conducted using data from the Department of Microbiology, Father Muller Medical College Hospital, Mangalore, Karnataka, India, from September 2022 to November 2023. All clinically significant Candida isolates recovered from routine clinical specimens were included.

Specimen collection and processing

Blood specimens were collected in BacT/ALERT aerobic culture bottles (bioMérieux, France) and incubated at 37°C for up to 5 days. Bottles flagged positive for yeast growth underwent Gram staining, followed by subculture onto blood agar and Sabouraud's dextrose agar (SDA) (both HiMedia Laboratories Pvt. Ltd., Mumbai, India).

All other clinical specimens-including 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)-were processed using standard microbiological protocols. These were inoculated onto appropriate media and incubated at 37°C for 24–48 hours.

Specimens submitted specifically for fungal culture were inoculated onto two slopes of SDA supplemented with gentamicin (without cycloheximide) and incubated at 25°C and 37°C for up to 4 weeks. Direct microscopy using Gram stain and/or 10% potassium hydroxide (KOH) wet mounts was performed on all specimens to detect yeast cells.

Isolation and preliminary identification

After 24–48 hours of incubation, culture plates showing smooth, cream-to-white, glabrous colonies morphologically suggestive of *Candida* were selected. Gram staining from colonies was performed to confirm yeast morphology. Presumptive isolates were then subcultured onto HiChrome™ Candida Differential Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for phenotypic color-based differentiation.

Species identification by MALDI-TOF MS

Definitive species-level identification was performed using the Bruker Microflex LT/SH MALDI-TOF MS system (Bruker Daltonics, Bremen, Germany) with MALDI Biotyper software (MBT Compass version 12.0.0.0_10833). The system identifies organisms by comparing unique ribosomal protein spectra against a reference database (10).

Sample preparation (Extended direct transfer method)

A single pure colony was smeared as a thin film onto a polished steel MALDI target plate. One microliter of 70% formic acid was overlaid and air-dried at room temperature. Subsequently, 1 µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was added and allowed to dry completely.

Data acquisition and interpretation

Spectra were acquired in automatic mode using the MALDI Biotyper software, and identification scores were interpreted according to the manufacturer's criteria: scores ≥ 2.0 indicated high-confidence species-level identification, scores between 1.7 and 1.99 denoted genus-level identification with low confidence, and scores <1.7 were considered unreliable. Candida albicans ATCC 90028 was used as the positive control, while matrix-only spots served as the negative control (11).

Statistical analysis: Categorical variables (e.g., specimen type, species distribution, and demographic data) were expressed as frequencies and percentages. Continuous variables (e.g., patient age) were reported as median with interquartile range (IQR) where applicable. Data were analyzed using descriptive statistics in Microsoft Excel.

Results

A total of 342 *Candida* isolates recovered from clinical samples were analysed during the study period from September 2022 to November 2023. The median age of infection was 46.5 years with a predominance of males. The majority of the isolates were obtained from blood (136/342, 39.76%) (Table 1). NAC species were predominant (254/342, 72.84%) compared to *C. albicans*. *C. tropicalis* (30.40%) was the most common NAC species isolated followed by *C. parapsilosis* (14.32 %). During the 15 months study period, 40 strains of *C. auris* were obtained (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)

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

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

All *Candida* species were identified by the MALDI-TOF, with a log score >1.7 for 314/342 (91.81%) isolates. The mass spectra of the most frequently isolated *Candida* species are shown below (Figure 1-4). Out of 40 isolates, 37 (92.5%) were identified as *C. auris* with a log score of >=1.7. Of the 40 cases of *C. auris*, 50% (20/40) were from the burns unit, and all 40 cases were associated with prolonged hospital stays.

Discussion

As with any infectious process, prompt and rapid identification of yeast plays a very important role in the successful management of the patient. To add to that, an increasing prevalence of NAC can complicate the selection of antifungal therapy (3-6).

The infections caused by the NAC are indistinguishable from those of *C. albicans,* but the antifungal resistance is more commonly observed in NAC (1). Our present study showed a predominance of NAC with 72.84% causing various clinical conditions of candidiasis. Similar to our study, a study by Umamaheshwari et al in Southern India, found that 73.64 % of candidiasis was due to NAC species (5). A study by Singh DP et al also indicated the emergence of non-albicans *Candida* and their predominance in various body fluids (12).

C. tropicalis (30.40%) was the most common species isolated in this study. Our results were concordant with other studies conducted by Gautam et al with an isolation rate of 26.72% and a study by Anita et al an isolation rate of 37.09% for *C. tropicalis* (13,14). While phenotypic identification based on the biochemical reactions is cheap but time-consuming and techniques based on nucleic acid detection are expensive. MALDI-TOF MS is a newly emerged technique, for the identification and speciation of yeast like *Candida* (9,11). We employed MALDI-TOF MS for the identification of *Candida* species in this study. The on-plate extraction method gives accurate identification in most cases, and it has eased the procedure. The reagents used for the MALDI-TOF MS analysis are cheap, 96 isolates can be identified by each run, so the cost of the test will be less compared to other tests based on the biochemical or nucleic acid detection methods. All suspected candida species were identified to the species level by the MALDI-TOF, with the log score >1.7 for 314/342 (91.81%) isolates which is similar to a study conducted by Periera et al (10).

A multidrug-resistant yeast, *C. auris* is often misidentified as *C. haemulonii, C. famata, C. sake, C. lusitanie*, etc. by common phenotypic automated systems. Definitive confirmation of the *C. auris* can be done using either MALDI-TOF or DNA sequencing (15). We found a high number of *C. auris.* (11.69%), especially from burn patients, which is a great concern. Burn patients with impaired immune defence and large wounds are at high risk of acquiring *C. auris* infection from the hospital environment (16). The dominance of *C. auris was* also seen in a study conducted by Prayag PS from western India (6).

The rapid and accurate identification of NAC will help choose empirical antifungal therapy. Strict infection prevention and control measures should be instituted for the patients infected or colonized with *C. auris* for the containment of multidrug-resistant yeast and the prevention of any outbreak.

Conclusion

The role of *Candida* species, including NAC and newly emerging drug-resistant *Candida* strains should be approached with caution. MALDI-TOF MS can replace conventional and automated machines which are based on biochemical reactions for the speciation of *Candida* isolates. Although the initial cost is high, the cheaper reagents and easy protocols make it a cost-effective test that can be used to identify yeast isolates from routine clinical specimens.

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Ethical statement

Ref. No. FMIEC/CCM/550/2023 (Protocol No-512/2023)

Conflicts of interest

There are no conflicts of interest to declare by any of the authors.

Author contribution

KP contributed to the study concept, study design, data analysis, and manuscript writing and review. RTP contributed to data collection and manuscript writing. SD and BA contributed to data analysis and manuscript review. MD reviewed the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

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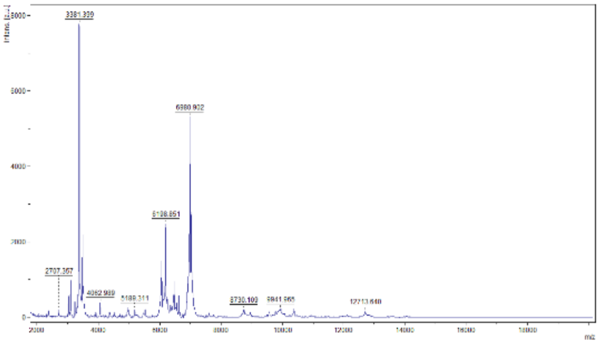

Figure 1: Mass spectra graph of *C.albicans*

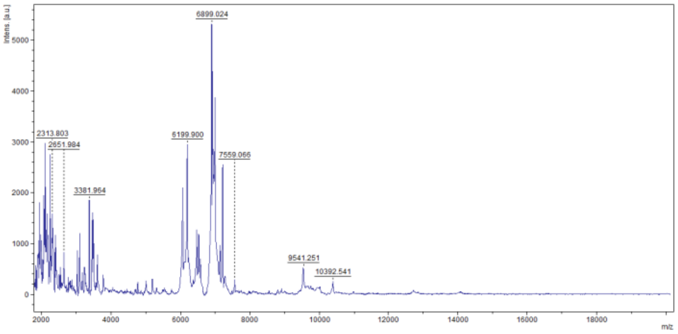

Figure 2: Mass spectra graph of *C.tropicalis*

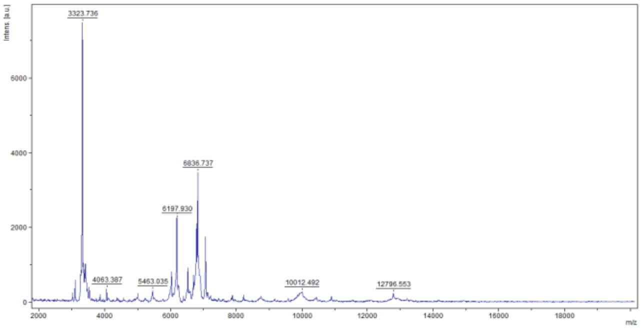

Figure 3: Mass spectra graph of *C. parapsilosis*

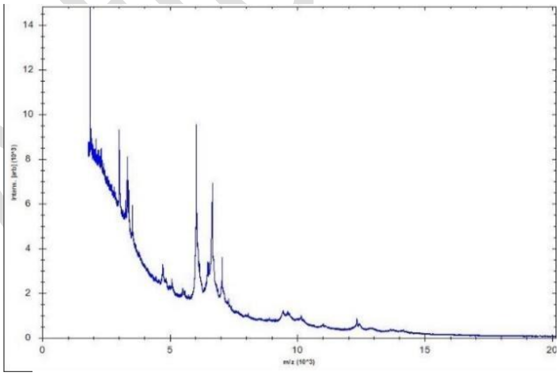

Figure 4: Mass spectra graph of *C. auris*