ORIGINAL ARTICLE MYCOLOGY

# Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections

**A. K. Ghosh, S. Paul, P. Sood, S. M. Rudramurthy, A. Rajbanshi, T. J. Jillwin and A. Chakrabarti**
*Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India*

## Abstract

Few studies have systematically standardised and evaluated matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of yeasts from bloodstream infections. This is rapidly becoming pertinent for early identification of yeasts and appropriate antifungal therapy. We used 354 yeast strains identified by polymerase chain reaction (PCR) sequencing for standardisation and 367 blind clinical strains for validation of our MALDI-TOF MS protocols. We also evaluated different sample preparation methods and found the on-plate formic acid extraction method as most cost- and time-efficient. The MALDI-TOF assay correctly identified 98.9% of PCR-sequenced yeasts. Novel main spectrum projections (MSP) were developed for *Candida auris*, *C. viswanathii* and *Kodamaea ohmeri,* which were missing from the Bruker MALDI-TOF MS database. Spectral cut-offs computed by receiver operating characteristics (ROC) analysis showed 99.4% to 100% accuracy at a log score of $\geq$1.70 for *C. tropicalis, C. parapsilosis, C. pelliculosa, C. orthopsilosis, C. albicans, C. rugosa, C. guilliermondii, C. lipolytica, C. metapsilosis, C. nivariensis*. The differences in the species-specific scores of our standardisation and blind validation strains were not statistically significant, implying the optimal performance of our test protocol. The MSPs of the three new species also were validated. We conclude that MALDI-TOF MS is a rapid, accurate and reliable tool for identification of bloodstream yeasts. With proper standardisation, validation and regular database expansion, its efficiency can be further enhanced.


**Keywords:** Bloodstream infection, *Candida*, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, rapid diagnosis, yeast
**Original Submission:** 15 August 2014; **Revised Submission:** 7 November 2014; **Accepted:** 11 November 2014
Editor: E. Roilides
**Article published online:** 20 November 2014

**Corresponding author:** Arunaloke Chakrabarti, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh - 160012, India
**E-mail:** arunaloke@hotmail.com

## Introduction

*Candida* species are among the most common fungi causing nosocomial bloodstream infections and are associated with high mortality [1,2]. *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata* are the predominant candidemia agents, but other less common *Candida* spp. and yeasts also are emerging as significant pathogens [3]. Aside from emergence of new pathogenic species, increasing antifungal resistance, especially to azoles, is a growing concern [4]. Bloodstream fungal infections exert a substantial toll in terms of prolonged hospital stay, health care costs, morbidity and mortality [5]. Rapid species identification can facilitate early institution of suitable antifungal therapy and expedite recovery in high-risk patients [6]. This is even more pertinent for emerging rare yeasts resistant to multiple antifungal agents [7].

Conventional phenotyping, although useful for routine identification of common *Candida* species, is time consuming and inept at identifying rare yeasts. In contrast, identification by



http://dx.doi.org/10.1016/j.cmi.2014.11.009

molecular techniques such as rDNA sequencing offers a rapid and reliable alternative [8]. However, despite its advantages, it entails a steep learning curve, need for expertise, higher costs and relatively longer run time than other faster techniques. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is rapidly becoming popular for routine identification of clinical pathogens [9]. MALDI-TOF MS circumvents many challenges posed by other diagnostics and is being adapted for identification of fungi [10,11]. Standardisation and validation of any novel technique is essential before adopting it for routine high-throughput diagnostic use. We undertook this study to standardise and validate MALDI-TOF MS for routine identification of bloodstream yeasts, especially *Candida* species.

## Materials and methods

### Yeast isolates and reference strains

*C. albicans* (ATCC-90028) and *C. tropicalis* (ATCC-6258) reference strains were used for the initial assessment of different MALDI-TOF MS extraction protocols and as internal controls for subsequent runs. Two sets of yeast isolates were used in this study. A total of 354 strains (standardisation set) retrieved from the National Culture Collection of Pathogenic Fungi (www.nccpf.com), established at our department, were used for optimisation, standardisation and performance assessment of our MALDI-TOF MS protocols. These strains were isolated from intensive care unit–acquired candidemia patients admitted at 27 centres across India. Each strain was tested for purity, identified by conventional biotyping [12] and confirmed by sequencing of the ITS2 and large subunit 28S ribosomal RNA gene's D1/D2 regions [13]. A second set of 367 strains (validation set) isolated from nosocomial candidemia patients admitted to our institution from October 2013 to March 2014 were included to validate our established MALDI-TOF MS protocols. These isolates were directly subjected to MALDI-TOF MS analysis without prior confirmation by polymerase chain reaction (PCR) sequencing. In all, a total of 723 *Candida* strains were used in this study.

### Standardisation of sample extraction protocol

We initially evaluated three different MALDI-TOF MS sample preparation protocols including the direct on-plate, on-plate formic acid and off-plate extraction methods. For this standardisation we used the *C. albicans* (ATCC-90028) and *C. tropicalis* (ATCC-6258) reference strains. The strains were subcultured on Sabouraud dextrose agar (SDA) at 37°C for 16 to 20 hours and fresh growth was used for further extraction.

The direct on-plate method was carried out as described by the manufacturer. One to two isolated colonies of *Candida* were spotted and air dried on a 96-target, polished steel MALDI plate (Bruker Daltonik GmbH, Germany). The on-plate formic acid extraction was carried out with some modifications [11]. A loop-full of fresh *Candida* culture was emulsified in 1-mL sterile double-distilled water (DDW) and vortexed in 1.5-mL microcentrifuge tubes. The suspension was centrifuged at 13 000 rpm for 2 minutes, and the supernatant was discarded. This was repeated twice. The pellet was re-suspended in 50 µL sterile DDW. One microlitre of this suspension was spotted and air dried in duplicate on the MALDI plate. The spots were overlaid with 0.5 µL of 98% formic acid and again air dried. The off-plate method also was carried out as previously described [11]. A loop-full of fresh *Candida* culture was mixed in 300 µL sterile DDW and briefly vortexed; then 900 µL absolute ethanol was mixed into this suspension and spun at 13 000 rpm for 2 minutes. The supernatant was discarded, and the pellet was air dried. It was resuspended in 50 µL of 70% formic acid and 50 µL acetonitrile. The suspension was recentrifuged at 13 000 rpm for 2 minutes. One microlitre of suspension was placed on the MALDI plate and air dried. Finally, for every extraction method, 0.8 µL of matrix solution (100 µL of matrix solution containing: 50 µL acetonitrile, 2.5 µL trifluoroacetic acid, 1 mg α-cyano-4-hydroxycinnamic acid and 47.4 µL sterile DDW) was added on each spot and allowed to air dry prior to MALDI-TOF MS analysis.

### Mass spectrum acquisition and analysis

All MALDI-TOF MS assays were carried out on a MALDI Microflex LT mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Ionized spectra from each spot were acquired in a linear positive mode at a laser frequency of 20 Hz within a mass range from 2 to 20 kDa. Instrument calibration was performed using the Bruker Bacterial Test Standard *Escherichia coli* (#255343). MALDI BioTyper v1.1 was used for automated smoothing, normalisation, baseline subtraction and peak selection of different spectra. Significant peaks were selected to create sample profiles that were compared with the MALDI BioTyper database using pattern-matching algorithms. Results were expressed as log scores ranging from a value of 0 to 3. The manufacturer recommends $\geq$2 log score for species identification; 1.7 to 1.99 for genus identification; and <1.7 as unreliable. Any species misidentified or unavailable in the manufacturer's database was resolved by PCR sequencing [14].

### Development of new mass spectrum profiles

*Candida* species unavailable in the MALDI Bio Typer database were further analysed for constructing their mass spectrum profiles. The new main spectrum projections (MSP) were developed after identifying these strains by PCR sequencing. In brief, the samples were extracted by on-plate formic acid methods in triplicate. Eight technical replicates of these

 

triplicates, i.e. a total of 24 spots, were used for any given strain. Accordingly 24 spectra were acquired for every strain and subjected to flex analysis, smoothening and baseline subtraction. After analysis, the best 20 spectra were saved to the MALDI Bio Typer database as the MSP of the new species.

### Statistical analysis

Analysis and presentation of results were carried out as per the Standards for Reporting of Diagnostic Accuracy guidelines. PCR sequencing was used as the reference standard for all comparisons. Diagnostic test accuracy and reproducibility of MALDI-TOF MS was assessed by computing species-specific sensitivity, specificity, area under the curve and test efficiency (kappa). To assess protocol performance, species-specific receiver operating characteristics (ROC) cut-offs were computed. Poststandardisation performance was assessed by Student *t* test of species-specific MALDI-TOF log scores between standardisation and validation strains.

## Results

All three extraction methods correctly identified the *C. albicans* (ATCC-90028) and *C. tropicalis* (ATCC-6258) reference strains. However, statistical comparison of log scores revealed significant differences in direct on-plate extraction from the on-plate formic acid extraction (*C. albicans*: 1.65 vs. 2.02, p 0.002; *C. tropicalis*: 1.55 vs. 2.16, p 0.003) and the off-plate extraction (*C. albicans*: 1.65 vs. 2.10, p 0.002; *C. tropicalis*: 1.55 vs. 2.07, p 0.002). There were no significant log score differences between the latter two methods. Given its ease and efficiency, we adopted on-plate formic acid extraction for the remaining study.

Standardisation of MALDI-TOF MS using the 354 strains yielded a 98.9% concordance rate, with misidentification of four (1.1%) strains (Table 1, Fig. 1). We updated MALDI Bio Typer database for missing species including *C. auris*, *C. viswanathii and Kodamaea ohmeri*. MALDI-TOF correctly identified all of these strains in the standardisation set using the new MSP profiles incorporated into our instrument with log score >2 (Table 1). Distribution of species-specific log scores (Table 1) showed that the majority of strains (236 of 350; 67.5%) could be successfully identified between scores of 1.70 and 1.99. The four strains that were missed by MALDI-TOF included *C. rugosa* (2), *C. tropicalis* (1) and *Pichia jadinii* (1).

In comparison to PCR sequencing, MALDI-TOF MS identified yeasts with 100% accuracy with ROC cut off log score >2.60 for *C. auris, C. catenulate, C. glabrata, C. krusei, C. viswanathii, K. ohmeri* and *Trichosporon asahii.* Spectral cut-off computed analysis showed 99.4% to 100% accuracy with ROC cut-off log score of $\geq$1.70 for *C. albicans, C. guilliermondii,* C. *lipolytica, C. metapsilosis, C. nivariensis, C. orthopsilosis, C. parapsilosis, C. pelliculosa, C. rugosa* and *C. tropicalis* (Table 2). The overall predictive power, accuracy and efficiency of MALDI-TOF MS were uniformly high and comparable to sequencing, barring slight underperformance for *C. tropicalis*, *C. rugosa* and *C. parapsilosis*.

MALDI-TOF analysis of the 367 validation set clinical isolates successfully identified 359 (97.8%) isolates. A comparison of species-specific log scores between the validation and standardisation sets showed no statistical difference, indicating

**TABLE 1. Species-wise distribution of isolates across various MALDI Bruker scores for the standardisation set**

| Species | Total number of isolates | Missed by MALDI-TOF | Number of isolates with different Bruker score | | | | |
|---|---|---|---|---|---|---|---|
| | | | <1.7 | 1.7–1.799 | 1.8–1.899 | 1.9–1.999 | ≥2.0 |
| *C. tropicalis* | 165 | 1 | 0 | 40 | 30 | 56 | 38 |
| *C. parapsilosis* | 49 | 0 | 0 | 7 | 6 | 19 | 17 |
| *C. pelliculosa* | 23 | 0 | 0 | 6 | 6 | 6 | 5 |
| *C. krusei* | 21 | 0 | 0 | 0 | 2 | 4 | 15 |
| *C. glabrata* | 20 | 0 | 0 | 2 | 0 | 3 | 15 |
| *C. orthopsilosis* | 14 | 0 | 0 | 7 | 4 | 2 | 1 |
| *C. albicans* | 12 | 0 | 0 | 2 | 3 | 4 | 3 |
| *C. rugosa* | 11 | 2 | 0 | 5 | 2 | 2 | 0 |
| *C. auris* | 6 | 0 | 0 | 0 | 0 | 0 | 6 |
| *C. guilliermondii* | 6 | 0 | 0 | 3 | 1 | 2 | 0 |
| *Kodamaea ohmeri* | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| *Trichosporon asahii* | 5 | 0 | 0 | 1 | 0 | 1 | 3 |
| *C. catenulata* | 4 | 0 | 0 | 0 | 2 | 0 | 2 |
| *C. lipolytica* | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| *C. metapsilosis* | 2 | 0 | 0 | 1 | 1 | 0 | 0 |
| *C. nivariensis* | 2 | 0 | 0 | 2 | 0 | 0 | 0 |
| *C. viswanathii* | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| *C. intermedia* | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *C. neoformans* | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| *C. pararugosa* | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| *C. utilis* | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Pichia jadinii* | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Total | 354 | 04 | 0 | 77 | 59 | 100 | 114 |

MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

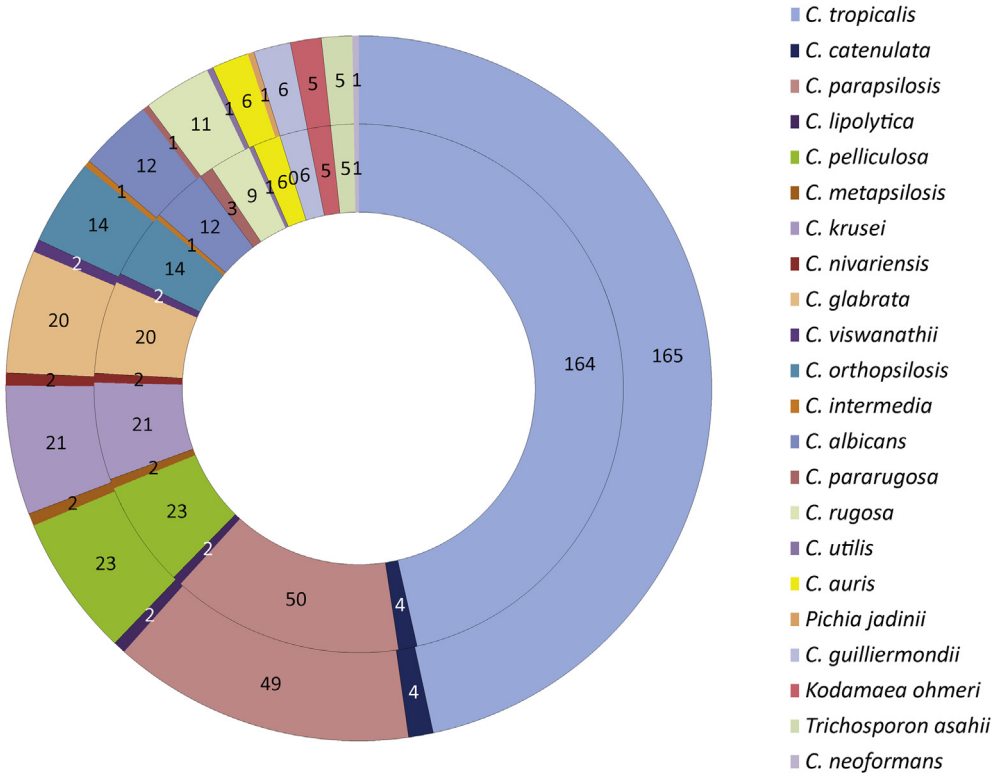


**FIG. 1.** A doughnut chart depicting the species-specific distribution and performance of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (inner ring) in comparison to polymerase chain reaction sequencing (outer ring) for the standardisation set of yeast strains. Numbers indicate the number of strains identified by respective techniques.

optimum performance of our standardised protocols (Table 3). The MALDI-TOF MS assay accurately identified not only common yeasts but also most of the uncommon ones, including *C. robusta*, *C. auris*, *C. utilis*, *C. lipolytica*, *C. kefyr*, *C. haemulonii*, *C. fabianii*, *Magnusiomyces capitatus*, *Trichosporon asahii*, *Pseudozyma aphidis*, *Rhodotorula mucilaginosa* and *K. ohmeri*. The eight isolates that could not be reliably identified by MALDI-TOF MS had log scores varying from 1.03 to 1.66. These isolates were identified by sequencing ITS2 and D1/D2 region and included four isolates of *Wickerhamomyces anomalus*, and two each of *Meyerozyma guilliermondii*, and *Cyberlindnera fabianii*. None of these species have their MSP in the Bruker BioTyper database.

**TABLE 2. Efficacy of MALDI-TOF identification in comparison to ITS2 and D1/D2 sequence identification of select *Candida* species**

| Species | Number of isolates | Correctly identified *n* (%) | Misidentified *n* (%) | Area under the curve (95% CI) | Receiver-operator characteristic cut-off log score | Kappa (95% CI) |
|---|---|---|---|---|---|---|
| *C. tropicalis* | 165 | 164 (99.3%) | 1 (0.6%) | 0.99 (0.98–1.00) | ≥1.70 | 0.99 (0.98–1.00) |
| *C. parapsilosis* | 49 | 49 (100%) | 0 | 0.99 (0.98–0.99) | >2.60 | 0.99 (0.97–1.00) |
| *C. pelliculosa* | 23 | 23 (100%) | 0 | 1.00 (0.99–100) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. krusei* | 21 | 21 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |
| *C. glabrata* | 20 | 20 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |
| *C. orthopsilosis* | 14 | 14 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. albicans* | 12 | 12 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. rugosa* | 11 | 9 (81.8%) | 2 (18.2%) | 0.91 (0.87–0.94) | ≥1.70 | 0.90 (0.76–1.00) |
| *C. guilliermondii* | 06 | 06 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. auris* | 06 | 06 (100%) | 0 | 1.00 (0.99 -1.00) | >2.60 | 1.00 (1.00–1.00) |
| *Trichosporon asahii* | 05 | 05 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |
| *Kodamaea ohmeri* | 05 | 05 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |
| *C. catenulata* | 04 | 04 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |
| *C. lipolytica* | 02 | 02 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. metapsilosis* | 02 | 02 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. nivariensis* | 02 | 02 (100%) | 0 | 1.00 (0.99–1.00) | ≥1.70 | 1.00 (1.00–1.00) |
| *C. viswanathii* | 02 | 02 (100%) | 0 | 1.00 (0.99–1.00) | >2.60 | 1.00 (1.00–1.00) |

MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; CI, confidence interval.

**TABLE 3. Comparison of MALDI Bruker log scores for different species across the standardisation and validation sets**

| | Standardisation set | | Validation set | | |
|---|---|---|---|---|---|
| **Species** | **Number of isolates** | **Mean log score ± SD (range)** | **Number of isolates** | **Mean log score ± SD (range)** | **p** |
| *C. tropicalis* | 165 | 1.90 ± 0.11 (1.70–2.32) | 103 | 1.92 ± 0.13 (1.66–2.30) | 0.34 |
| *C. parapsilosis* | 49 | 1.96 ± 0.11 (1.72–2.11) | 24 | 1.95 ± 0.10 (1.75–2.12) | 0.94 |
| *C. pelliculosa* | 23 | 1.89 ± 0.13 (1.70–2.20) | 57 | 1.94 ± 0.13 (1.73–2.23) | 0.16 |
| *C. krusei* | 21 | 2.09 ± 0.14 (1.88–2.32) | 76 | 2.04 ± 0.15 (1.76–2.41) | 0.16 |
| *C. glabrata* | 20 | 2.08 ± 0.15 (1.75–2.30) | 20 | 2.06 ± 0.11 (1.84–2.21) | 0.65 |
| *C. albicans* | 12 | 1.91 ± 0.10 (1.77–2.06) | 39 | 1.87 ± 0.09 (1.71–2.12) | 0.13 |
| *C. orthopsilosis* | 14 | 1.84 ± 0.12 (1.74–2.00) | 6 | 1.81 ± 0.07 (1.73–1.91) | 0.48 |
| *C. auris* | 6 | 2.44 ± 0.11 (2.30–2.55) | 3 | 2.40 ± 0.19 (2.18–2.52) | 0.73 |
| *C. guilliermondii* | 6 | 1.83 ± 0.08 (1.76–1.95) | 11 | 1.84 ± 0.07 (1.75–1.97) | 0.88 |
| *Kodamaea ohmeri* | 5 | 2.45 ± 0.09 (2.30–2.50) | 3 | 2.37 ± 0.12 (2.30–2.50) | 0.28 |
| *C. viswanathii* | 2 | 2.55 ± 0.07 (2.50–2.60) | 6 | 2.44 ± 0.08 (2.30–2.50) | 0.14 |

MALDI, matrix-assisted laser desorption ionization.

## Discussion

MALDI-TOF MS is a robust tool for high-volume laboratory services. MALDI-TOF MS can accurately identify fastidious microorganisms in <3 hours, as compared to 2 to 7 days by other conventional and molecular techniques. MALDI-TOF MS is also being utilised for outbreak investigation [15,16]. Unlike bacteria, few studies have evaluated the performance accuracy of MALDI-TOF MS for high-throughput clinical identification of fungi. This has led to substantial variations in protocols for sample preparation, MS analysis and development of MSP databases [15]. Most studies use empiric extraction methods. Standardised protocols that can be universally applied to fungi are needed for good reproducibility [17].

We evaluated different extraction protocols and found on-plate formic acid extraction reliable and efficient. Fungal cells are larger and tougher than bacteria, needing stronger reagents for MALDI-TOF MS ionization [15]. The off-plate method achieves this effectively, but the extra steps render it costlier and time consuming. The direct on-plate method, on the other hand, is faster and consumes fewer resources, but inadequate fungal proteins extraction yields lower log scores. The on-plate formic acid extraction achieves a good balance between these two techniques. Whereas acetonitrile enhances protein solubility, 98% formic acid hastens extraction (3 to 5 minutes) and improves log scores, as seen in our study. Similar efficacy of this method has been noted in bacterial studies [18,19]. We found SDA an acceptable medium for MALDI-TOF MS. Thorough washing of cells prevented interference from culture medium proteins, yielding reproducible spectral scores.

Our study has several strengths, which set a precedent for MALDI-TOF MS identification of clinical yeasts. Most studies examine only reference strains without validating their protocol on blind clinical strains. We demonstrate that validation verifies standardisation and helps identify and develop MSPs missing in the MALDI Bio Typer database. Further, we have compared manufacturer's log scores against statistically generated ROC cut-offs. This clearly depicts how the identification efficiency of our assay is varying with respect to log scores, and which species need greater representation in the MSP database to further enhance performance.

Our assay missed 4 of 354 strains from the standardisation set, which included two strains of *C. rugosa*, one *C. tropicalis* and one *Pichia jadinii*. *Pichia jadinii* is poorly represented in the Bruker database, as true for other uncommon yeasts [20]. The misidentification of *C. rugosa* and *C. tropicalis* indicate subtle but significant variations in our regional strains, which are probably underrepresented in the Bruker database. Poor identification and log scores can have several causes. We maintained rigorous quality control to circumvent causes such as strain impurity, older (>48 hours) yeast colonies, culture condition differences, inefficient protein extraction and contamination by culture medium proteins [21]. Aside from technical factors, qualitative and quantitative variations in intraspecies protein expression and underrepresentation of such strain variations in MALDI Bio Typer database are significant causes of such misidentifications/nonidentifications. Such variations have been reported earlier from different regions [21]. Further, our assay failed to identify 8 of 367 isolates from the validation set. These comprised *Cyberlindnera fabianii, Meyerozyma guilliermondii* and *Wickerhamomyces anomalus*, none of which are present in the Bruker database.

Although the manufacturer recommends a log score $\geq$2.0 for species identification, ROC analysis of our data revealed that a log score cut-off $\geq$1.7 can unambiguously identify a majority of the species. However a few species, including *C. auris*, *C. catenulate, C. glabrata, C. krusei, C. viswanathii*, *Kodamaea ohmeri* and *Trichosporon asahii*, showed higher log score cut-offs ($\geq$2.60). This difference indicates that these species do not have sufficient entries in the Bruker database to capture all variations among our isolates. MSP for *C. auris, C. visvanathii* and

*Kodamaea ohmeri* were not available in the Bruker database and were constructed in this study. Furthermore, log score cut-offs for new species tend to be higher if a small number of isolates (two each in our case) are used to create the MSP. In addition, because all of these strains were from a single centre, the spectral variation expected is minimal and this may have contributed to the high score. These findings highlight two issues. First, although the manufacturer recommends a stringent score of $\geq$2.0 for reliable species identification, this does not invalidate correct identification at scores <2.0. Previous studies have shown that supplementing the Bruker database with in-house spectra enhances the specificity of identification even at lower log scores [20,22]. Increasing species-specific entries better captures proteomic variations and helps the pattern-matching algorithm to learn and become more proficient [23]. Secondly, regular, systematic expansion and calibration of the MALDI-TOF database with carefully identified strains of different species is important. This enhances the spectral profile of underrepresented species, adds completely missing uncommon species, accounts for geographical genotypic and phenotypic variations, captures emerging species and monitors time trends within species [15,24,25]. Such supplementary libraries can either be queried separately or merged with existing libraries [20].

Despite its several advantages, MALDI-TOF MS requires substantial initial set-up cost, needs molecular diagnostics for identifying missing species and is yet to develop a global database of all common and uncommon pathogens [20]. Our study also was limited by the low number of strains for uncommon yeasts. These species are being regularly monitored, and as their numbers grow their MSP profiles will be upgraded for better MALDI-TOF MS performance.

In conclusion, our study demonstrates the utility of MALDI-TOF MS for rapid and accurate identification of bloodstream yeasts. Proper standardisation, validation and statistical interpretation of MALDI-TOF MS results are essential for identifying assay deficiencies and enhancing performance. MALDI-TOF MS promises to be a useful tool for routine patient care, rapid diagnostics and outbreak investigation.

## Transparency declaration

The authors declare that they have no conflicts of interest.

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