International Journal of Research in Medical Sciences
Fonseca M et al. Int J Res Med Sci. 2020 Jul;8(7):2575-2582
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20202898

Original Research Article

MALDI-TOF MS: the proteomic approach, the future of fungal identification in India

Mallika Fonseca[1]*, Sarala Menon[1], Praveen Rahi[2], Prachi Patekar[1], Abhay S. Chowdhary[1]

[1]Department of Microbiology, Grant Government Medical College and Sir J. J. group of Hospitals, Mumbai, Maharashtra, India
[2]Department of Microbiology, National Centre for Microbial Resource, National Centre for Cell Science, Pune, Maharashtra, India

Received: 27 April 2020
Accepted: 28 May 2020

***Correspondence:**
Dr. Mallika Fonseca,
E-mail: mallikafonseca@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: India, being a country where fungal infections are rampant, is urgently in need of effective tools for early and accurate diagnosis of fungal infections. Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) is a recent method which has shown potential in identifying clinically important bacterial pathogens as well as clinically important fungi. The main objective of this study was to compare the utility of MALDI-TOF MS for the identification of fungi against that of conventional methods.
Methods: The project was carried out in a tertiary care government hospital in India. Fifty clinical isolates comprising mainly various yeast species were subjected to conventional identification (Phenotypic) as well as MALDI-TOF-MS. Their results were further compared.
Results: MALDI-TOF MS showed a high concordance with conventional methods while identifying species like C. albicans, C. tropicalis, C. parapsilosis and C. neoformans, although the concordance for species such as Rhodotorula and Trichosporon could only be matched up to genus level.
Conclusions: MALDI-TOF MS-based identification is both a rapid and a viable tool for identification of clinically relevant yeast species with good correlation to conventional methods and a quick turnaround time.

Keywords: Mycoses diagnosis, Mycological typing techniques, Molecular diagnostic techniques, Mass spectrometry, Matrix assisted laser desorption-ionization

INTRODUCTION

India, with a climate that is widely tropical and sub-tropical, is largely suited for the growth and colonization of myriad fungal species, many of which pose a health hazard to the Indian population. The Indian health system has an extremely large burden of superficial fungal infections, caused by dermatophytes and non-dermatophytic species like Candida.[1] Encountered commonly in immune-compromised patients, especially those with HIV/AIDS, diabetes, haematological malignancies and in transplant recipients, these fungal infections not only have a high mortality rate of 35-75% but also cause several underlying surgical and medical morbidities, and these patients are frequently exposed to high-risk medications and higher antibiotics.[2-4]

Currently, fungal identification relies on the conventional macroscopic and microscopic observation of colonies and is complicated because of inadequate knowledge of the whole fungal phylogeny connected with population biology, evolution and ecology.[5] Identification of fungal

pathogens using conventional phenotypic methods requires skilled mycologists, is often subjective, takes a considerable amount of time and may, sometimes, still not be conclusive for rare species of yeasts, dermatophytes and moulds.[2]

Commercial methods for rapid identification are now available which are based on the biochemical characteristics of the isolates. These include biochemical and enzymatic card panels (API ID 20C, API ID 32C), chromogenic agar media (CHROM Agar), and automated systems for molecular identification (Vitek ID YST systems).[2] However, although the identification procedures involving molecular tools take less time than a culture, it still is expensive and labour intensive. Because of the time involved in the identification of fungal species, clinicians often use empirical therapy based only on clinical observation, which at times leads to unnecessary use of antifungal agents causing toxicity and resistance in patients. We thus require a rapid and simple technique for the identification of fungal species, to optimize appropriate antifungal treatment for the specific infection.

In recent times, the proteomic approach for identification of microbial species is becoming popular as an alternative to molecular methods. One such method is Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI TOF MS). MALDI-TOF MS is an emerging tool for high-output and rapid microbial identification which has revolutionized routine identification of microorganisms. The principle behind microbial identification using MALDI-TOF MS is based on the fact that every microorganism has a specific protein composition, which gives them a characteristic and unique mass spectrum.

Accurate profiling of short proteins and whole cell peptides of microorganisms using MALDI-TOF MS approach gives a fair idea about their speciation and taxonomic affiliations, which assists in their identification. An unknown microorganism is identified by comparing its spectrum with the spectra in the reference library. MALDI-TOF MS-based identification is simple, fast, low-cost and accurate and has a high output for most bacteria.[6]

A few preliminary studies aimed to identify fungi have been carried out and were reviewed as part of this study, but as yet, a standardized protocol is not available for all fungal species. These preliminary studies carried out using MALDI-TOF MS for fungal identification had promising results, and thus, this study was devised out to identify whether this approach of identification could be used as a generalized, reliable, time-saving tool for routine identification of fungal species.

The objective of this study was to compare Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry to conventional identification for fungal isolates, by first identifying fungi (mainly yeast) from different clinical specimens using conventional method and then subjecting the same fungal isolates to Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. This study also aimed to analyse the utility of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry for the identification of fungi.

METHODS

This was an experimental Laboratory Investigation conducted in the Department of Microbiology, Tertiary care Public Hospital, Mumbai for the duration of 6 months.

Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry will be carried out at Microbial Culture Collection (MCC), Pune, Maharashtra, a national facility funded by the Department of Biotechnology (DBT), Government of India;

Necessary ethical approvals, permissions were obtained from Institutional Ethical Committee of our tertiary care public institute in Mumbai.

In this study, 50 yeast isolates from different clinical specimens were collected and processed using conventional identification techniques- both mycological and biochemical. They were further processed for MALD-TOF MS analysis and then identified using the Bruker Daltonik GmbH MALDI-TOF MS instrument. A comparison was then made between the two methods.

Table 1: The characteristic Dalmau morphology in different *Candida spp*.

Candida albicans	**Pseudo hyphae with blastoconidia formed at clustered intervals along with hyphae, Terminal chlamydoconidium**
Candida tropicalis	Multibranched pseudo hyphae with blastoconidia formed singly
Candida parapsilosis	Extensive multibranched pseudo hyphae, blastotconidaia may be formed in clusters, curved pseudo hyphae
Non-albicans candida	Germ tube negative and no characteristic structures seen on Dalmau

Conventional identification of fungi

Routine microscopic identification was carried out via direct microscopic identification using KOH mount (hair, nail, skin, tissue), India ink (CSF) and Gram staining (Pus, Sputum, CSF). All the clinical specimens were directly inoculated on Sabouraud's dextrose agar and Sabouraud’s dextrose agar with chloramphenicol and cycloheximide in duplicate.

All the culture media were incubated at two different temperatures (37 °C and 30°C). Culture readings for Yeasts were taken at 24, 48, 72 hours, 5th day and 7th day. Genus and species level of identification of the yeast colonies obtained was done by Germ tube test, testing for growth at higher temperature and by Dalmau colony morphology/assimilation and fermentation wherever required (Table 1).[7-10]

Growth at higher temperature, growth on cycloheximide containing agar, urease activity, assimilation and fermentation was studied.

Ribosomal protein identification: Matrix-Assisted Laser Desorption Ionization - Time of Flight (MALDI TOF) Mass Spectrometry.[11-15]

Software

- MALDI Biotyper 3.1 (Bruker Daltonik GmbH, Germany)
- FlexAnalysis version 3.4 (Bruker Daltonik GmbH, Germany)

Preparation of the HCCA matrix solution

Among 250 µl standard solvent was added to a tube of HCCA. The HCCA was dissolved by vortexing at room temperature until the solution is clear.

Loading the bacterial colony to target plate

Actively grown bacterial cultures supplied by the Dept of Microbiology, at a tertiary care Public hospital were employed for this analysis. Smear of bacteria (single colony) as a thin film directly onto a spot on a MALDI target plate was done. The bacterial smear was overlaid with 1 µl of HCCA solution and allowed to dry at room temperature. 1 µl of bacterial standard was loaded (Bruker Daltonik GmbH, Germany) on to a separate well and 1 µl of matrix added to it. The target plate was allowed to dry at room temperature and then loaded into the instrument AUTOFLEX speed (Bruker Daltonik GmbH, Germany).

Analysis on MALDI-TOF MS instrument

Mass spectra were acquired in a linear positive ion extraction mode at a laser frequency of 200 Hz within a mass range from 2,000 to 20,000 Da. The ion source 1 voltage was 19.5 kV, ion source 2 voltages were maintained at 18.2 kV, lens voltage at 7kV and the extraction delay time was 240ns. The spectra were calibrated externally using the bacterial standard calibration mixture (Escherichia coli extracts including the additional proteins RNase A and myoglobin, supplied by Bruker Daltonik GmbH, Germany). The MALDI biotyper software .0 (Bruker Daltonik GmbH, Germany) was used to identify the isolates and visualize the mass spectra.

Data analysis and reporting

The strains showing ≥1.7 log value with strain in database were confirmed as the member of that genus and strains showing ≥2.0 log values were confirmed as the member of that species.

Mechanism and principle of MALDI-TOF MS machine

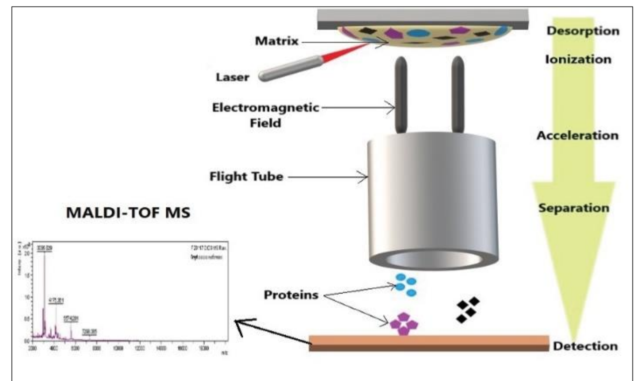

Figure 1: Components and mechanism of the MALDI-TOF MS machine.

The basic principle of mass spectrometry is the ionization of a neutral molecule and the subsequent accurate determination of the resulting primary ions and their decay products in high vacuum.

A typical mass spectrometer is composed of three components: an ion source, a mass analyser, and a detector (Figure 1).

MALDI-TOF MS technique requires mixing of microbial growth from a pure colony with a specified matrix solution, followed by smear preparation on target plate. The target plate is air dried and loaded inside the instrument. It is then exposed to a source of ionization. After ionization, ionized peptides and proteins travel towards detector in a vacuum tube (flight tube) and get separated based on their mass to charge ratio (m/z). A mass spectrum of the strain under study is generated, which was then compared with that of the other strains present in the reference database.

RESULTS

Specimen distribution

In this study a total of 50 yeast strains were subjected to identification by conventional as well as MALDI -TOF MS. Yeast strains (48) were isolated from different clinical specimens (Figure 2): Sputum:16, CSF:10, Blood:7, Urine:3, Gastric lavage:3, Skin:3, Tissue and grain:3, Bronchoalveolar lavage:1, Nail:1, Pleural fluid:1. Two control strains were also used (ATCC 90028: *Candida albicans* and ATCC 204092 *Cryptococcus neoformans*).

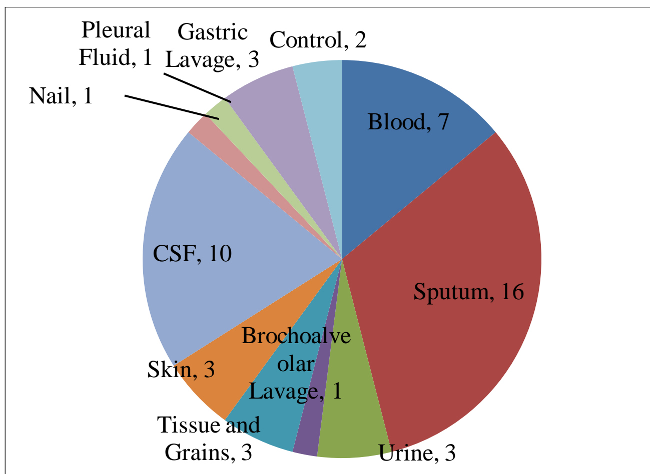

Figure 2: Specimen wise distribution of yeast strains tested.

Conventional identification

Conventional identification was based on the staining and culture methods described in the methodology. Urease positive capsulated yeasts were identified as *Cryptococcus neoformans. Rhodotorula spp* isolated by the pigment and urease positivity. Germ tube positive yeasts were taken as *Candida albicans* and all other were classified as Non-albicans Candida. All *Candida spp* were subjected to Dalmau techniques and identified as per their characteristic morphology defined in methodology (Table 1).

MALDI-TOF MS Identification

All the strains were subjected for two runs of MALDI-TOF MS to check the reproducibility of the results. Interpretations of the score was as done as follows:

Table 2: The comparison between conventional identification and MALDI-TOF MS of yeast strains isolated from various sources (N=50) on the first and second run of the MALDI-TOF MS bio typing system.

	Total sample Size	Run 1			Run 2		
		Fully Concordant[a]	Partially Concordant[b]	NRI[c]	Fully Concordant[a]	Partially Concordant[b]	NRI[c]
Controls	2	2	0	0	2	0	0
Urine	3	3	0	0	2	1	0
Sputum	16	13	2	1	10	4	2
CSF	10	8	2	0	1	5	4
Gastric lavage	3	3	0	0	2	1	0
Blood	7	1	1	5	1	1	5
Tissue and Grains	3	2	0	1	2	0	1
Skin scraping	3	2	1	0	2	1	0
Pleural fluid	1	1	0	0		1	0
Nail	1	1	0	0	1	0	0
Bronchoalveolar lavage	1	0	1	0	0	1	0

a) Concordant at both Genus and Species Level
b) Concordant up to Genus level only
c) Not reliable result

Table 3: Correlation between Conventional and MALDI-TOF MS Identification for different strains of yeasts (values as per single best result).

Identification as conventional method	Total samples	Fully Concordanta	Partially Concordantb	NRIc
Candida albicans	19	17 (89.47%)	1 (5.2%)	1 (5.2%)
Candida tropicalis	3	1 (33.33%)	2 (66.66%)	0
Candida parapsiliosis	7	7 (100%)	0	0
Non-Albicans Candida	8	0	1 (12.5%)	7 (87.5%)
Trichosporon	2	2 (100%)	0	0
Cryptococcus neoformans	8	7 (87.5%)	1 (12.5%)	0
Rhodotorula	1	0	1	0

a) Match at both Genus and Species Level
b) Match up to Genus level only
c) Not reliable result

- The strains with more than 2.0 score value indicates reliable Genus as well as species level identification.
- The strains with score value ranging from 1.7-1.99 indicate only genus level identification.
- The strains with score value less than 1.7 could not been identified by MALDI TOF MS and hence indicated as not reliable identification.

All the three yeast strains (100%) isolated from urine had more than 2.0 score value on MALDI-TOF MS analysis - showing reliable Genus as well as species level identification. Out of the 16 yeast strains isolated from sputum, thirteen strains (81%) had more than 2.0 score value while two strains were having score value ranging from 1.7-1.99 on the first run and 4 on the second run, which indicates only genus level identification. There were 1 and 2 strains respectively in the first and second run which gave score value less than 1.7 and could not be identified by MALDI TOF MS.

From 10 yeast strains isolated from CSF, eight strains (80%) had a reliable Genus as well as species level identification. There were two strains (20%) which had a score value of 1.7-1.99 on the first run, but this changed to 5 strains on the second run. An observation of 4 strains on the second run with no reliable information was also made.

All the three yeast strains (100%) isolated from Gastric lavage had more than 2.0 score value on at least 1 run. Out of Seven yeast strains isolated from blood, only one strain (14.2%) had a reliable Genus as well as species level identification on both runs. One more strain (14.2%) had only genus level identification. The remaining five strains (71.6%) gave score value less than 1.7 and could not been identified by MALDI TOF MS. From the three yeast strains isolated from tissue, two strains (67%) had a reliable Genus as well as species level identification, while one strain (33%) gave score value less than 1.7 could not been identified by MALDI TOF MS.

Two strains (67%) isolated from skin scrapings had a reliable Genus as well as species level identification while one strain (33%) had a genus level identification only.

Both the strains isolated from pleural fluid and Nail had score more than 2.0 score value on at least one run, indicating a reliable Genus as well as species level identification. While the single strain isolated from Bronchoalveolar lavage had score value ranging from 1.7-1.99 indicating only genus level identification (Table 2).

As shown in Table 3, there were 19 samples of *Candida albicans*, of which 17 (89.47%) showed concordance at Genus as well as species level on at least one run. *Candida parapsiliosis* and Trichosporon had 100% concordance with the conventional identification, while 7 out of 8 *Cryptococcus neoformans* had Genus as well as species level concordance. The single strain of Rhototorula had a score ranging from 1.7-1.99 which indicates only genus level identification on one run only. Non-albicans Candida had the least reliable results on MALDI-TOF MS typing with 7 out of 8 strains (87.5%) having no reliable information and only 1 with Genus level identification in concordance with the conventional identification (Table 3).

Comparison between conventional and MALDI-TOF MS

Out of 50 strains tested, both the methods gave concordant identification in 42(84%) cases while 8 (16%) strains were identified as nonalbicans candida by conventional gave no reliable information on MALDI-TOF MS (Figure 3).

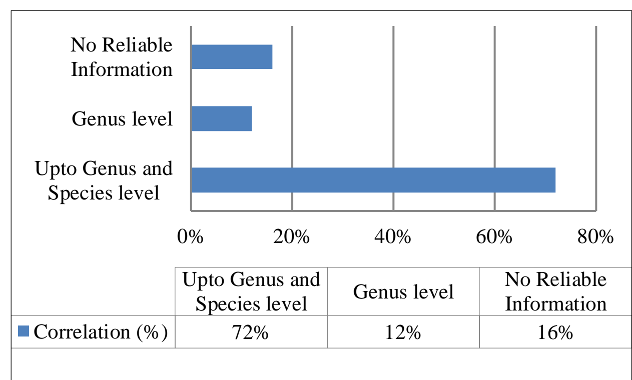

Figure 3: Correlation between conventional and MALDI-TOF MS identification for yeasts.

DISCUSSION

Thus, in the current study, we have attempted to correlate the use of MALDI-TOF MS with conventional phenotypic methods for the identification of clinical yeast isolates. We have examined the performance and reproducibility of MALDI-TOF MS and evaluated its turnaround time in relation to the conventional and morphological identification methods. Conventional identification of yeasts depends on the colour, type of growth, germ tube production, Dalmau morphology and various biochemical characters which are usually studied by assimilation and fermentation reactions (Table 1). Assimilation and fermentation studies though helpful in further speciation of yeasts are cumbersome and need lot of technical expertise and resources.

This study clearly shows the conventional methods correlated well (100%) with MALDI TOF MS for genus level identification excluding Non-albicans candida (Table 3 and Figure 3). There was also 100% concordance for identification of Candida albicans, tropicalis, parapsilosis and Cryptococcus neoformans up to species level. Representative spectra of each species were characteristic as shown in Figure 4 a-d and hence can be useful in the rapid identification of such species.

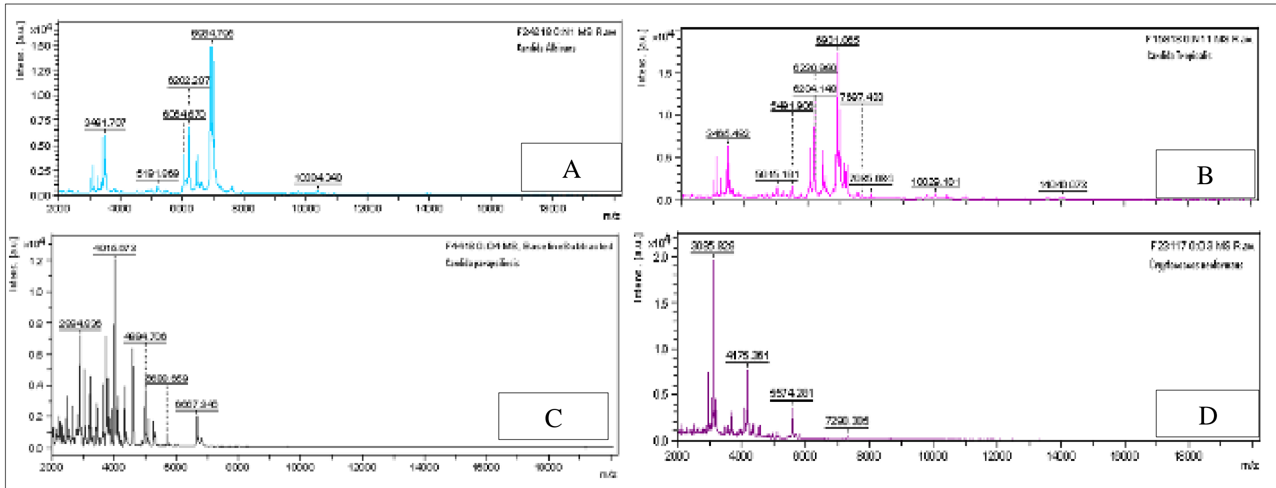

Figure 4: Representative MALDI-TOF MS spectra of fungal strains indicating the protein profile (2-20KD. A) *Candida albicans* B) *Candida tropicalis* C) *Candida parapsiliosis* D) *Cryptococcus neoformans*.

In case of the identification of Rhodotorula and Trichosporon, identification matched up to genus level, however species level identification by conventional fermentation and assimilation was not attempted. Out of 50 strains tested, 8 (16%) strains which were identified as Non-albicans candida by conventional methods gave no reliable information on MALDI-TOF MS. Non-albicans candida is evolving into a major challenge clinically and we are witnessing newer species especially from blood specimens. There is a need to strengthen our MALDI TOF MS database by studying more and more such strains so that in future we get reliable identification from our database itself.

Two runs of MALDI-TOF MS were carried out for each strain which showed 88% reproducibility. The first run of MALDI was comparable with the conventional methods.

Authors found variable reproducibility on the second run, specifically for the results of CSF and sputum samples, which were poorly reproducible on the second run. 6 strains (12%) which comprised of Candida, Trichosporon and Rhodotroula (1 each) and 3 strains of *Cryptococcus neoformans* did not show reproducible results on the second run; This may be due to variability in the sample source or may also be due to contamination. Thus, the single best result of the two runs was taken into consideration while tabulating results in comparison with conventional techniques.

In the study conducted by Panda et al, with similar objectives to this study, MALDI-TOF MS was used for the identification of clinical fungal isolates (yeasts and filamentous fungi) and similarly correlated to conventional methods.[2] Using an identical methodology to this study, they found the correlation between MALDI TOF MS and conventional identification for 125 fungal isolates included in their study to be 87.2% at the species level and 90.4% at the genus level. Their correlation of Yeast identification was 100% both at the genus and species levels whereas, in mould identification only 10.81% isolates had correct identification up to the genus level, 56.7% isolates had correct identification both at the genus and species levels, whereas 32.42% isolates were deemed No Reliable Identification (NRI). In this study, the MALDI TOF MS results revealed correlation of Yeast identification of 100% at genus level excluding Non-albicans candida, though species level identification had a lower correlation.

MALDI-TOF mass spectrometry yielded 96.3% and 84.5% accurate species level identification for 138 common and 103 archived strains of yeast tested in the study conducted by N. Dhiman et al.[16] They found the average time of MALDI-TOF MS to be appreciably faster than normal methods, with an average hands on time per specimen to be 5.1 min and the average specimen turnaround time to be 0.64 hours as versus the average hands on time of 4.4 min and specimen turnaround time of 2.5 hours for germ tube methods. They also found the average cost of MALDI-TOF MS to be $0.50 as versus the conventional cost of $0.80-$1 per sample. Our study supports the above findings and had similar turnaround times for both conventional as well as MALDI-TOF MS method.

In the study conducted by Spanu et al, 17 the reliability of the MALDI Biotyper system in species-level identification of yeasts directly from blood culture bottles was carried out. Identification results were concordant with those of the conventional culture-based method for 95.9% of *Candida albicans* and 86.5% of non-albicans. The whole procedure was characterized as fast and economical (calculated time of 30 minutes (median) and costs of less than 1 USD per an isolate).

CONCLUSION

In conclusion, this research, though short term, revealed the utility of MALDI TOF MS for the identification of fungi. We found this technique to be extremely useful and reliable. However, until a strong database for fungi and a standardized technique is established, in most of diagnostic laboratories, the conventional level of fungal diagnosis will remain the mainstay. Conventional species level diagnosis is cumbersome, complicated and sometimes still unable to reach to the species level diagnosis. The standardization also needs a lot of patience and technical expertise. Newer species of Candida especially Non-albicans from blood specimens need special attention. MALDI TOF MS seems to be the answer for this, provided we first standardize the MALDI technique for all fungi and strengthen our database by carrying out larger studies on various fungal isolates from different specimens. MALDI-TOF MS is not an alternate to DNA sequencing-based methods or conventional methods but rather a complimentary tool. It is more viable than DNA sequencing considering its easy processing, short analysis time and simple reference spectra. MALDI TOF MS also holds a future for direct identification of slow growing isolates from clinical specimens which will hopefully revolutionize the normal clinical lab routine, easing and hastening the diagnosis of fungal infections and thus lessening the impact and burden of fungal infections on the Indian Healthcare system.

ACKNOWLEDGEMENTS

Authors would like to thank scientist-in-charge of the National Centre for Microbial Resource, Pune, Dr Yogesh S. Shouche for giving us the permission to use the Bruker Daltonik GmbH MALDI-TOF MS instrument and the technical staff of Microbiological Cell Culture (MCC) and the National Council for Microbial Research, especially Mr. Aabeejjeet N. Pansare who provided the expertise and resources that made this research possible.

Authors thank the mycology division of Grant Government Medical College, especially Mr Sudhakar Kamble for assistance with the conventional techniques of identification and Mr Santosh Khadkikar for help with the graphic design.

Funding: Funding was supported by the Indian Council of Medical Research (ICMR), grant number 2018-01194
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of this tertiary care public institute in Mumbai, IEC/Pharm/ICMR/125/Mar/2018.

REFERENCES

1. Verma S, Madhu R. The Great Indian Epidemic of Superficial Dermatophytosis: An Appraisal. Indian J Dermatol. 2017;62(3):227-36.
2. Panda A, Ghosh AK, Mirdha BR, Xess I, Paul S, Samantaray JC, et al. MALDI-TOF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. J Microbiol Methods. 2015;109:93-105.
3. González de Molina FJ, León C, Ruiz-Santana S, Saavedra P. Assessment of candidemia-attributable mortality in critically ill patients using propensity score matching analysis. Critical Care. 2012;16(3):R105.
4. Guery BP, Arendrup MC, Auzinger G. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: part I. Epidemiology and diagnosis. Intensive Care Med. 2009;35(1):55-62.
5. Chalupová J, Raus M, Sedlářová M, Sebela M. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnol Adv. 2014 Jan-Feb;32(1):230-41.
6. Cassagne C, Ranque S, Normand AC, Fourquet P, Thiebault S, Planard C, et al. Mould Routine Identification in the Clinical Laboratory by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry. PLoS ONE 2011;6(12):e28425.
7. Baron EJ, Finegold, SM. Baily and Scott’s Diagnostic Microbiology. 8th Edition. St. Louis, C.V. Mosby Company; 1990:7.
8. Koneman’s colour atlas and Textbook of Diagnostic Microbiology Practical. 6th Ed.1997.
9. Murray PR. Manual of Clinical Microbiology. 8th Ed. Washington, D.C: ASM Press.
10. Sutton DA, Fothergill AW, Rinaldi MG. Guide to Clinically Significant Fungi. Williams and Wilkins, 1998.
11. Fenselau C, Demirev PA. Characterization of intact microorganisms by MALDI mass spectrometry. Mass Spectrom Re. 2001;20:157-71.
12. Rahi P, Sharma, OP, Shouche YS. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) Based Microbial Identifications: Challenges and Scopes for Microbial Ecologists. Frontiers Microbiol. 2016;7:1359.
13. Ryzhov V, Fenselau C. Characterization of the protein subset desorbed by MALDI from whole bacterial cells. Anal Chem. 2001;73:746-50.
14. Suarez S, Ferroni A, Lotz A, Jolley KA, Guérin P, Leto J, et al. Ribosomal proteins as biomarkers for bacterial identification by mass spectrometry in the clinical microbiology laboratory. J Microbiol Methods. 2013;94(3):390-6.
15. Croxatto A, Prod’hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev. 2012;36:380-407.
16. Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL. Performance and cost analysis of matrix-assisted laser desorption ionization-time of

flight mass spectrometry for routine identification of yeast. J Clin Microbiol. 2011 Apr;49(4):1614-6.
17. Spanu T, Posteraro B, Fiori B. Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of Candida Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories. J Clini Microbiol. 2012;50(1):176-9.

Cite this article as: Fonseca M, Menon S, Rahi P, Patekar P, Chowdhary AS. MALDI-TOF MS: the proteomic approach, the future of fungal identification in India. Int J Res Med Sci 2020;8:2575-82.