ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2018, 56, 234–241
doi: 10.1093/mmy/myx042
Advance Access Publication Date: 12 July 2017
Original Article

Original Article

Rapid detection of fluconazole resistance in *Candida tropicalis* by MALDI-TOF MS

Saikat Paul, Pankaj Singh, Shamanth A S, Shivaprakash M. Rudramurthy, Arunaloke Chakrabarti and Anup K Ghosh*

Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh 160012, India

*To whom correspondence should be addressed. Dr. Anup K Ghosh, Associate Professor, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh 160012, India. Tel: +91 172 2755156; Fax: +91 172 2744401; E-mail: anupkg3@gmail.com

Received 7 December 2016; Revised 23 February 2017; Accepted 4 May 2017; Editorial Decision 3 March 2017

Abstract

With the changing epidemiology and emergence of antifungal resistance among *Candida* species, rapid antifungal susceptibility testing (AFST) is crucial for optimization of antifungal therapy. This study was conducted to standardize a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI -TOF MS) based AFST method (ms-AFST) for susceptibility of *Candida tropicalis* isolates. Clinical isolates of *C. tropicalis* were confirmed for fluconazole resistance by the CLSI (M27-A3) method. The incubation period and drug concentration were optimized to determine the minimal profile change concentration (MPCC) by MALDI-TOF MS. The data were analyzed first by direct visual observation of the spectra followed by composite correlation index (CCI) matrix analysis, virtual gel analysis, and cluster analysis for confirmation. Finally, the correlation between minimum inhibitory concentrations (MICs) and MPCCs was evaluated. A total of 15 fluconazole resistant (MICs ranging from 16 to 128 μg/ml) and 19 fluconazole susceptible *C. tropicalis* isolates (MIC $\leq$1 μg/ml) were included in this study. All *C. tropicalis* isolates had significant spectral changes after 4h incubation with fluconazole. Of 34 isolates, MPCCs and MICs were equivalent for 16 isolates, and the MPCC was one dilution lower than the respective MIC in the remaining 18 isolates. This finding was further supported by visual analysis, CCI matrix analysis, virtual gel and principal component analysis dendrogram analysis. The correlation between MPCC and MIC was significant ($P < .05$). Therefore, a MALDI-TOF MS based AFST assay may be used as a rapid screening technique for fluconazole resistance in *C. tropicalis*.

Key words: Antifungal susceptibility testing, matrix assisted laser desorption ionization-time of flight mass spectrometry, minimal profile change concentration, composite correlation index, principal component analysis, *Candida tropicalis*.

Introduction

Candida species are the commonest agents causing invasive fungal infections in humans. Previously, *Candida albicans* was considered as the predominant pathogen but non-*Candida albicans Candida* (NCAC) species have emerged as the predominant pathogens in Asian countries.[1,2] In India, *Candida tropicalis* is the commonest agent followed by *C. albicans*, *C. krusei, C. parapsilosis, and C. glabrata* in candidemia patients.[1] The changing epidemiology of *Candida* infection is partly ascribed to the misuse and overuse of antifungal drugs especially fluconazole.[2,3] Several studies from India have reported emergence of azole resistance in so-called susceptible *C. albicans* and *C. tropicalis*.[1]

Both Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antibiotic Susceptibility Testing (EUCAST) approved broth microdilution (BMD) methods for antifungal susceptibility testing for *Candida* species.[4–6] The determination of minimum inhibitory concentrations (MICs) by these methods are time consuming and may vary among different settings.[7,8] Other methods including E-test, Sensititre, and Vitek-2 are also available for antifungal susceptibility testing (AFST) but may not correlate well with the reference standard methods.[9] *In vivo* models were also developed for determining MICs but fail to correlate with *in vitro* methods.[10] A simple, rapid, and cost-effective method like matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) based antifungal susceptibility testing (ms-AFST) may be used to validate the conventional AFST methods.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry is an emerging technique in clinical microbiology for rapid identification of clinical pathogens.[11,12] However, with the emergence of drug resistance in microbes, AFST is crucial for optimization of therapy. Several studies reported the use of MALDI-TOF MS based antimicrobial susceptibility testing in bacteria.[13–15] But the clinical application of this technique for routine AFST is not validated currently. A study by De Carolis et al. reported the use of a ms-AFST for identification of caspofungin-resistant isolates of *Candida* and *Aspergillus* by a composite correlation index (CCI) approach.[16] Similar studies also reported the detection of fluconazole and triazole resistance in *Candida* by ms-AFST.[17,18] More studies are required to validate and improve this novel technique. In this study, we evaluated the ms-AFST method for identification of fluconazole resistance among *C. tropicalis* clinical resistant isolates.

Methods

Yeast isolates and antifungal susceptibility testing (AFST)

The resistant and susceptible clinical isolates of *C. tropicalis* were obtained from National Culture Collection of Pathogenic Fungi (NCCPF), Chandigarh, India (www.nccpf.com). The isolates were revived on Sabouraud dextrose agar (SDA; Himedia, India) and were reconfirmed for azole resistance by disk diffusion method as per CLSI protocol M44-A2.[4] The result was further confirmed by the BMD method (M27-A3) according to standard CLSI guidelines. The cut-off MIC values for susceptible and resistant isolates were determined as per the M27-S3 interpretive guidelines of CLSI.[6] In brief, the cell count was adjusted to 1×10^6 to 5×10^6 cfu/ml spectrophotometrically at an optical density of 0.09–0.13 at 530nm. Fluconazole stock (12.8mg/ml) solution was used for preparing drug dilutions. Drug concentrations ranging from 128 to 0.125 μg/ml were used for susceptibility testing. A 50% inhibition in growth in comparison to a growth control was noted as the MIC after 24h incubation. *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality control strains.

Optimization of incubation period and drug concentration to determine the minimal profile change concentration (MPCC)

One each of resistant (NCCPF-420193) and susceptible isolates (NCCPF-420203) were used for initial standardization of MPCC by MALDI-TOF MS. In brief, fresh growth of *C. tropicalis* was inoculated in yeast extract peptone dextrose (YPD) broth (Himedia) and incubated overnight in a shaker at 37°C. One ml (1×10^6 cells/ml) from the overnight growth was then transferred into a 250-ml conical flask containing 30 ml YPD broth.[17] The cultures were exposed at concentrations of fluconazole ranging from 0.125 to 128 μg/ml. One untreated culture was used as control. The cultures were incubated at 37°C and harvested at 2, 4, 8, 12, 16, and 20h intervals (Fig. S1, S2, S3, S4). Cell pellets obtained by centrifugation at 13,000 rpm were then subjected to protein extraction followed by MALDI-TOF MS analysis. Spectra were captured and analyzed for each strain under different culture conditions, that is, incubation periods and drug concentrations.[16]

Sample preparation for MALDI-TOF MS analysis

Both on-plate and off-plate extraction methods were used for sample preparation of MALDI-TOF MS. The protein

was extracted from fungi according to the method described from our laboratory.[11]

MALDI-TOF MS measurement

A Microflex LT Biotyper instrument (Bruker Daltonics, Bremen, Germany) was used for MALDI-TOF MS analysis. Bruker recommended bacterial test standard (BTS 8255343) was used to calibrate the instrument. As the most distinct, clear and significant spectra lie in a mass range of 5000 to 10000 *m/z*, protein mass spectra of samples were acquired by Flex Control 3.4 software (Bruker Daltonics) in the above-mentioned region (Fig. S5). Laser frequency for every run was 60 Hz with a linear positive mode. The default settings of the MALDI-TOF MS instrument were as follows: lens; 8.5 kV, ion source 1; 20 kV and ion source 2; 18.1 kV. Flex control software automatically acquired the spectrum of each spot. Each spectrum was generated by 240 laser shots (40 laser shot steps at six randomly selected positions of a single spot). The quality of raw spectra was analyzed by Flex Analysis 3.0 software (Bruker Daltonics), and each spectrum generated was analyzed directly against reference spectra. Further data were analyzed by MALDI Biotyper 3 software (Bruker Daltonics MC, Italy).[11]

Minimal profile change concentration detection by MALDI-TOF MS

MPCC is the minimum drug (fluconazole) concentration at which the mass spectra profile/fingerprint alters. Visual inspection methods were used for the detection of MPCC by MALDI-TOF MS. The acquired spectra were subsequently uploaded to Flex Analysis software in MALDI TOF Biotyper and visually analyzed after smoothing and baseline-subtractions.[16] The visual inspection analysis of MPCC was applied to all isolates after initial standardization.

Software based analysis

The finding of the visual inspection method was further confirmed by three software-based analyses: CCI matrix analysis, virtual gel analysis, and cluster analysis. In brief, CCI matrix analysis was performed as follows. Flex control software was set in mass range of 5000 to 10000 *m/z* to achieve uniform spectra and to avoid the hindrance from unwanted spectra. Acquired spectra were subsequently analyzed by the tool in the MALDI Biotyper software to determine composite correlation index (CCI).[13] The CCI matrix was used to determine the relation between acquired spectra statistically. To evaluate CCI values, spectra were divided at distinct intervals, and all the intervals were compared with the whole data set. The correlation among all the intervals gives the CCI, which was used to calculate the difference between spectra. A CCI value of '1' represented complete correlation between the spectra, whereas a CCI value of '0' indicated no correlation between the spectra. The numerical values of CCI were automatically visualized as a 'heat-map' in CCI matrix window. The closeness of the spectra was determined by the color of the square in the heat-map.[16] For visual gel analysis, the spectra of both susceptible and resistance isolates acquired at different drug concentration and untreated control were analyzed by virtual gel analysis in MALDI Biotyper software. This virtual gel view represented all the peaks present in a spectral file and was used to determine the difference between different spectra of *C. tropicalis* strains used in the study. The cluster analysis was performed by making the principal component analysis (PCA) dendrograms. The PCA dendrogram represented the relation and closeness of each spectrum to one another.[15] Three independent biological replicates were used for each drug concentration tested while making the dendrogram.

Results

Strains

A total of 34 isolates were tested by disk diffusion method; 13 were found resistant, two susceptible dose dependent (SDD), and 19 susceptible against fluconazole. Susceptibility profiles of these 34 strains were further confirmed by the CLSI BMD method, and those 13 isolates were reconfirmed as resistant against fluconazole with MICs ranging from 16 μg/ml to 128 μg/ml. Two SDD isolates by the disc diffusion method were found to be resistant by the BMD method. Altogether, 15 resistant and 19 susceptible isolates were analyzed throughout the study (Table 1).

MPCC determination by MALDI TOF MS

In visual inspection analysis, both the resistant (NCCPF-420193) and susceptible isolates (NCCPF-420203) showed spectral changes after 4h when challenged with 128 μg/ml and 1 μg/ml fluconazole, respectively (Fig. 1). On comparing MPCC results with respective MICs, spectral changes were observed in both resistant and susceptible isolates in comparison to the untreated control. Of the 15 fluconazole resistant isolates, only MPCCs of four isolates were exactly the same as MICs, whereas MPCCs of 11 isolates were one level lower to respective MIC drug concentrations. In case of susceptible isolates, MPCCs for 11 out of 19 isolates matched with MICs, whereas eight isolates had spectral changes at one dilution lower than their corresponding MICs (Table 1).

Table 1. Antifungal disc diffusion and broth dilution susceptibility testing of *Candida tropicalis* resistant and susceptible strains.

NCCPF no.	Disc diffusion susceptibility testing		Broth dilution antifungal susceptibility testing		MPCC (μg/ml)
	Zone diameter (25 μg/ disc)	Susceptibility	MIC (μg/ml)	Susceptibility	
420182	No zone	R	16	R	8
420183	No zone	R	64	R	32
420184	No zone	R	32	R	16
420185	12 mm	R	32	R	16
420186	14 mm	R	16	R	16
420187	14 mm	R	32	R	16
420188	16 mm	SDD	16	R	8
420189	No zone	R	128	R	128
420190	14 mm	R	16	R	8
420201	14 mm	R	64	R	32
420191	17 mm	SDD	64	R	32
420192	No zone	R	16	R	16
420193	No zone	R	128	R	128
420194	No zone	R	32	R	16
420195	No zone	R	128	R	64
420196	25 mm	S	1	S	1
420198	31 mm	S	0.5	S	0.5
420197	28 mm	S	0.5	S	0.5
420199	30 mm	S	1	S	0.5
420200	27 mm	S	0.5	S	0.25
420202	20 mm	S	1	S	1
420203	32 mm	S	1	S	1
420204	30 mm	S	0.5	S	0.5
420205	24 mm	S	1	S	1
420206	27 mm	S	0.5	S	0.5
420207	21 mm	S	1	S	0.5
420208	20 mm	S	1	S	1
420209	19 mm	S	1	S	0.5
420210	26 mm	S	0.5	S	0.25
420211	22 mm	S	0.5	S	0.25
420212	28 mm	S	0.5	S	0.5
420213	22 mm	S	1	S	0.5
420214	25 mm	S	1	S	1
420215	26 mm	S	0.5	S	0.5

MIC, minimum inhibitory concentration; MPCC, minimal profile change concentration detection; R, resistant; S, susceptible; SDD = susceptible-dose dependent.

The above results were further confirmed by CCI software-based analysis.[15] This CCI value was defined when the acquired spectra of isolates were similar to the observed spectra at maximal effective concentration (128 μg/ml for resistant and 4 μg/ml for susceptible) and dissimilar to the spectrum observed without any drug treatment (Fig. 2). A CCI value of 1 represented the highest correlation, whereas a CCI value of 0 indicated no correlation. In the heat-map, similar spectra were denoted as 'hot' (a contrast of yellow to dark red color) and dissimilar spectra were represented as 'cold' (a contrast of green to dark blue color). The software automatically generated the correlation values for different concentrations of fluconazole against two extreme concentrations of the drug. MPCCs were assigned to those CCI values at which the spectrum corresponded to a maximum fluconazole concentration vs. the spectrum observed without fluconazole. The CCI matrix and their corresponding values represented that a high correlation was found between MPCC and MIC within 1 drug dilution (Fig. 2). The virtual gel analysis showed similar band patterns below and above the MPCC. In resistant and susceptible isolates, the band patterns were different at drug concentrations 128 μg/ml and 1 μg/ml, respectively (Fig. 3). Similarly, PCA cluster analysis represented that high

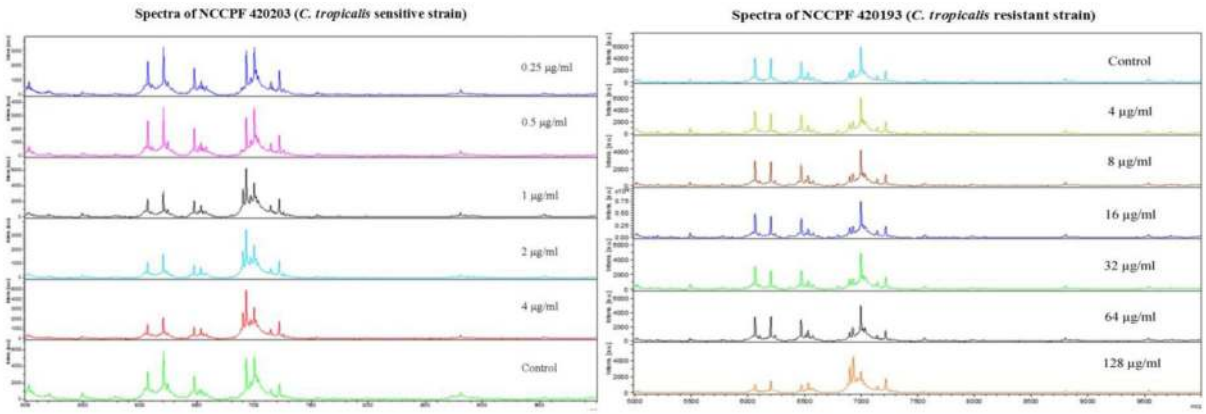

Figure 1. MALDI-TOF mass spectral profile analysis of captured spectra from *C. tropicalis* susceptible (NCCPF 420203; MIC 1 μg/ml) and resistant (NCCPF 420193; MIC 128 μg/ml) isolate at different concentrations of fluconazole. Alteration in the acquired peaks is visible at and above the isolate's minimum profile change concentration. This Figure is reproduced in color in the online version of *Medical Mycology*.

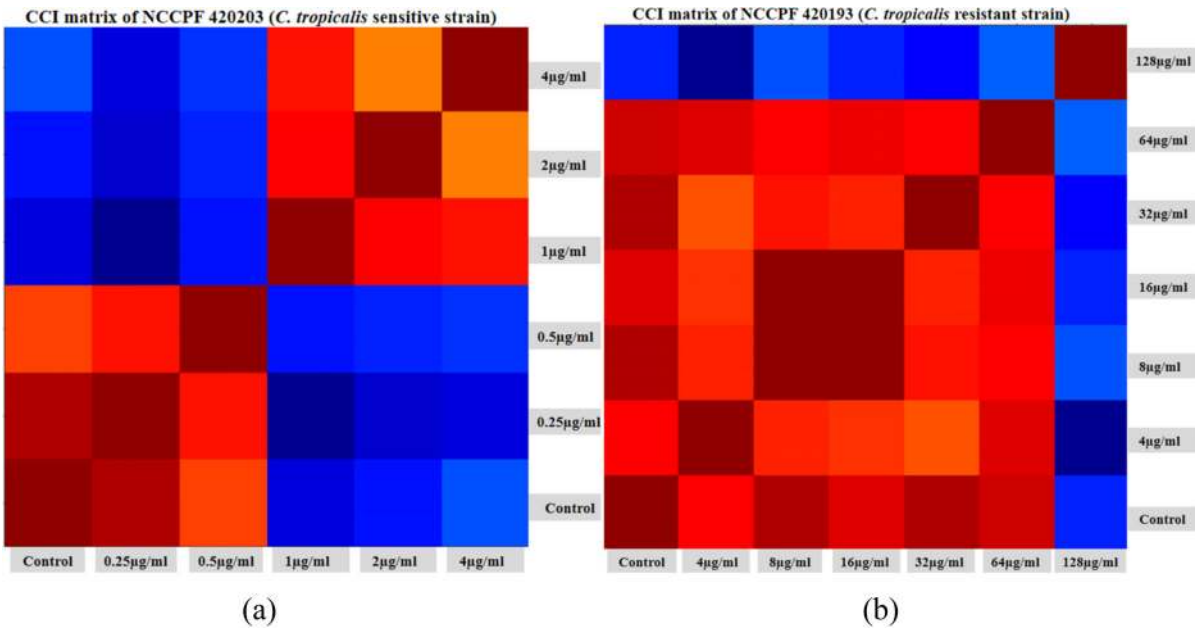

Figure 2. Composite correlation index (CCI) matrix of representatives mass spectra obtained from corresponding *C. tropicalis* cells after exposure to serial fluconazole concentrations (including no drug treatment) ranging from 0.25 to 128 μg/ml. Comparison of spectra at stipulated drug concentration numerical correlation index was derived, and visualized as CCI matrix and translated into heat map. Closely related spectra are represented as hot colors (yellow to deep red), whereas distantly related spectra are represented as cold colors (green to deep blue). Spectra obtained below MPCC showed higher similarity with the null drug treatment. Spectra obtained at and above MPCC shower significant similarity with the extreme drug concentration. (a) *C. tropicalis* susceptible isolate (NCCPF 420203; MPCC 1 μg/ml), (b) *C. tropicalis* resistant isolate (NCCPF 420193; MPCC 128 μg/ml). This Figure is reproduced in color in the online version of *Medical Mycology*.

correlation was found between MPCC and MIC (Fig. 4). Thus, the results obtained from virtual gel analysis and PCA confirmed the visual inspection method findings.

The values of MPCC and MIC of each isolate were used to compare the correlation between two methods. The MPCC and MIC comparison of 34 strains clearly revealed the significant correlation ranged 90–100% ($P < .05$), depending on whether spectrum altered at MIC drug concentration or one level lower drug concentration with respect to MIC (Fig. 5).

Discussion

MALDI-TOF MS-based antimicrobial susceptibility testing is a novel approach and has been established for bacterial strains in a clinical setting.[19–21] However, unlike bacteria, very few studies have evaluated the accuracy of ms-AFST in clinical laboratories. The resistant and susceptible *C. tropicalis* strains confirmed by BMD method were included for standardization of ms-AFST in this study. After standardization of optimum exposure times of antifungals,

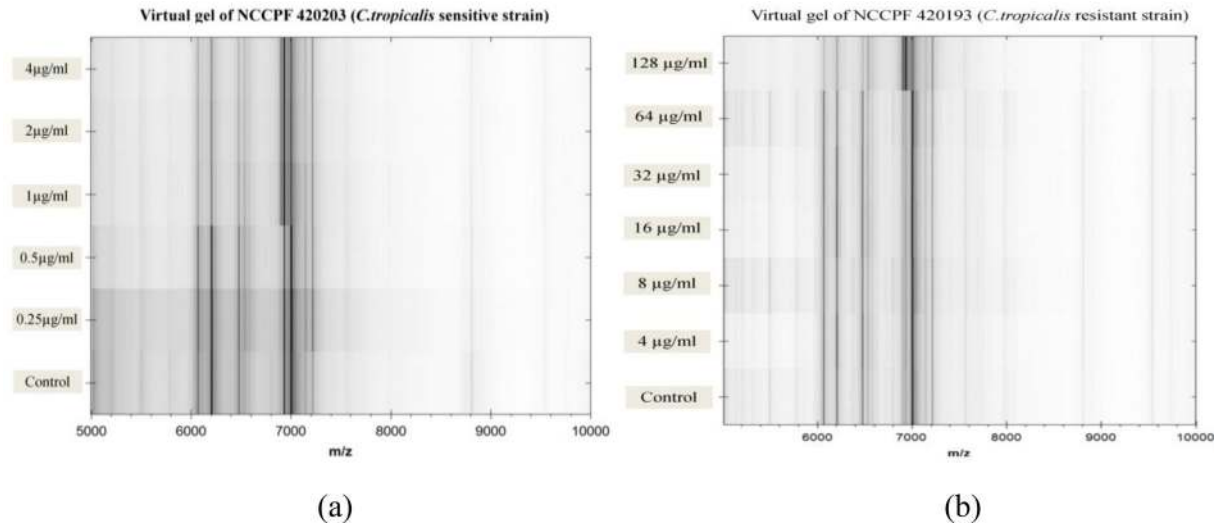

Figure 3. Virtual gel analysis by special tool in MALDI Biotyper 3 revealing changes in band pattern at MIC levels of *C. tropicalis* exposed to increasing concentration of fluconazole. X axis represents the m/z values and y axis represents running spectra acquired at different drug concentration of fluconazole of (a) *C. tropicalis* susceptible (NCCPF 420203; MIC 1 μg/ml) and (b) resistant (NCCPF 420193; MIC 128 μg/ml) isolate. This Figure is reproduced in color in the online version of *Medical Mycology*.

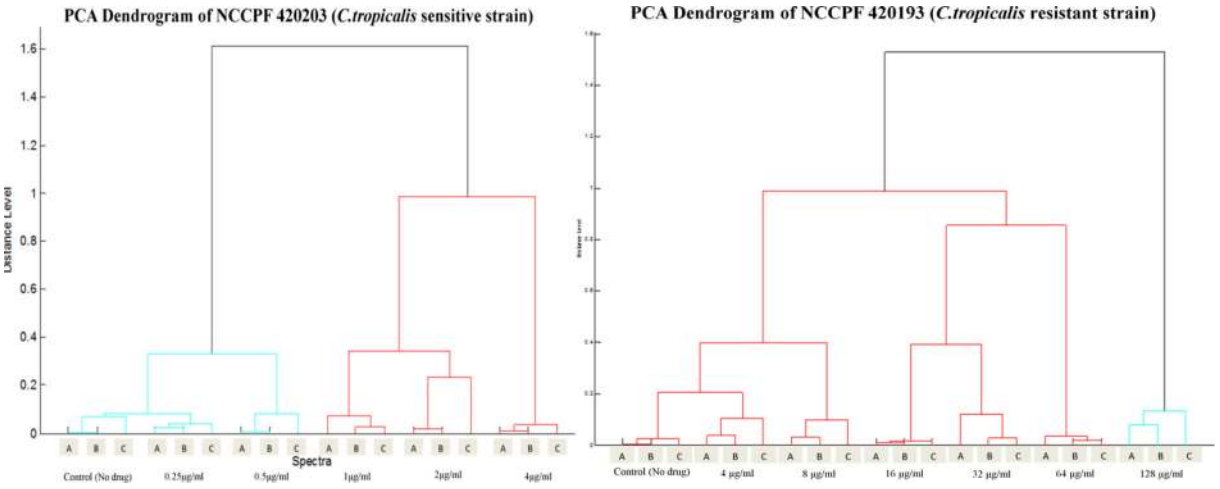

Figure 4. Principal component analysis of spectra derived from three biological replicates at each different concentration depicts separate clustering according to the isolate's MPCC. Distance is relative units. This Figure is reproduced in color in the online version of *Medical Mycology*.

ms-AFST accurately detected the resistant isolates within 5h. A high level of correlation ($P < .05$) was observed between the values of MPCC and MIC for all isolates suggesting the accuracy of ms-AFST comparable to BMD methods.

In routine clinical settings, BMD, disk diffusion, and E-test are the most common methods used for AFST.[22] Despite the progress in the conventional AFST, these methods remain very laborious and time-consuming because of dependence on fungal growth in relation to antifungal concentration.[7] In addition, the interpretation of BMD results is visual and subjective, causing variation in the reading among different clinical laboratories.[7] This poses an urgent need of simple alternatives of currently available AFST methods. The ms-AFST may provide a suitable alternative for conventional AFST. At least the changes at a molecular (proteomic) level may be used to screen resistant isolates, and it may also reduce the AFST time to a few hours.[17] Few studies also reported the accuracy of ms-AFST comparable to new species-specific clinical break points and epidemiological cut off values.[10] Also, ms-AFST may be further automated in the future by MALDI-TOF MS vendors.

The epidemiology of invasive candidiasis has changed with the emergence of NCAC. In addition, the emergence of drug resistance is also a growing concern.[1,2] These issues increase the importance of AFST in a clinical setting for proper management of invasive fungal infections. In case of bacteria, multiple ms-AFST methods have been developed for the identification of resistant isolates.[19–21,23] However,

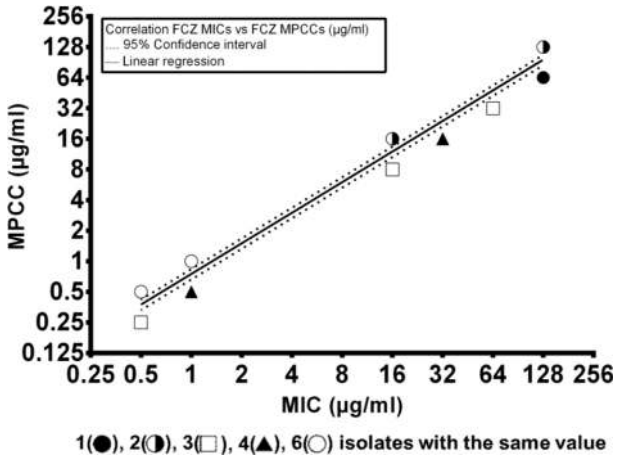

Figure 5. Correlation between MIC and MPCC (regression line). Detailed description of the isolates used for generating regression line is described in Table 1.

very few studies were published regarding its clinical application for fungi. On comparing MPCC and MIC, our findings were similar to the earlier study of De Carolis et al. where all 34 strains showed 100% concordance with ±1 dilutions value ($P < .05$).[16] The limited number of resistant isolates may be the reason of high correlation between these two techniques, which may fluctuate with addition of more isolates. However, for maximal acceptance of discrepancy agreement by CLSI guidelines, a cutoff value of two dilutions could be used.[24]

Other than fluconazole, resistance to caspofungin was reported in *Candida* clinical isolates.[16] However, the markers for most of the emerging resistant isolates are unknown. In India, *C. tropicalis* has emerged as the predominant candidemia isolate but unlike *C. albicans*, data regarding resistance patterns or mechanisms are missing for this species. This study suggests minimal drug-induced alterations in protein expression for development of resistance. The mass spectrum can be exploited as rapid and sensitive markers for the identification of resistant isolates of *C. tropicalis*.

This study suggests that ms-AFST can be used to implement AFST in routine clinical practice. However, as we have evaluated only one *Candida* species and one drug, and it will be necessary to test multiple isolates of other species and other antifungal drugs before implementing this technique in clinical laboratories. At least the finding of our study indicates that ms-AFST may be used as a rapid screening method for antifungal resistance.[16,17] It would be of interest to study for detection of multiple antifungal resistances simultaneously in *Candida* species.

In conclusion, ms-AFST is a rapid method compared to conventional AFST. It may be successfully combined with fungal identification by MALDI-TOF MS technology.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Acknowledgments

We duly acknowledge the Department of Medical Microbiology, PGIMER, Chandigarh for conducting this study.

Funding

This work was supported by Indian Council of Medical Research (ICMR), New Delhi, India.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Chakrabarti A, Chatterjee SS, Rao KL et al. Recent experience with fungaemia: change in species distribution and azole resistance. *Scand J Infect Dis*. 2009; 41: 275–284.
2. Chakrabarti A, Mohan B, Shrivastava SK et al. Change in distribution & antifungal susceptibility of *Candida* species isolated from candidaemia cases in a tertiary care centre during 1996–2000. *Indian J Med Res*. 2002; 116: 5–12.
3. Chakrabarti A, Sood P, Rudramurthy SM et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India. *Intensive Care Med*. 2015; 41: 285–295.
4. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*; *Approved Guideline*, 2nd ed. CLSI M44–A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2009.
5. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; 4th informational supplement. CSLI M27–S4. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
6. *Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*, 3rd ed. Approved Standard M27–A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
7. Lass-Florl C, Mayr A, Perkhofer S et al. Activities of antifungal agents against yeasts and filamentous fungi: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. *Antimicrob. Agents Chemother*. 2008; 52: 3637–3641.
8. Revankar SG, Kirkpatrick WR, McAtee RK et al. Interpretation of trailing endpoints in antifungal susceptibility testing by the National Committee for Clinical Laboratory Standards method. *J Clin Microbiol*. 1998; 36: 153–156.
9. Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A et al. Comparison of the Vitek 2 antifungal susceptibility system with the clinical and laboratory standards institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) Broth Microdilution Reference Methods and with the Sensititre YeastOne and Etest techniques for in vitro detection of antifungal resistance in yeast isolates. *J Clin Microbiol*. 2010; 48: 1782–1786.
10. Pfaller MA, Diekema DJ, Sheehan DJ. Interpretive breakpoints for fluconazole and *Candida* revisited: a blueprint for the future of antifungal susceptibility testing. *Clin Microbiol Rev*. 2006; 19: 435–447.
11. Ghosh AK, Paul S, Sood P et al. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections. *Clin Microbiol Infect*. 2015; 21: 372–378.
12. Panda A, Ghosh AK, Mirdha BR 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.

13. Sparbier K, Lange C, Jung J et al. MALDI biotyper-based rapid resistance detection by stable-isotope labeling. *J Clin Microbiol*. 2013; 51: 3741–3748.
14. Sparbier K, Schubert S, Weller U et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against beta-lactam antibiotics. *J Clin Microbiol*. 2012; 50: 927–937.
15. Vella A, De Carolis E, Vaccaro L et al. Rapid antifungal susceptibility testing by matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis. *J Clin Microbiol*. 2013; 51: 2964–2969.
16. De Carolis E, Vella A, Florio AR et al. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for caspofungin susceptibility testing of Candida and Aspergillus species. *J Clin Microbiol*. 2012; 50: 2479–2483.
17. Marinach C, Alanio A, Palous Met al. MALDI-TOF MS-based drug susceptibility testing of pathogens: the example of *Candida albicans* and fluconazole. *Proteomics*. 2009; 9: 4627–4631.
18. Saracli MA, Fothergill AW, Sutton DA et al. Detection of triazole resistance among *Candida* species by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). *Med Mycol*. 2015; 53: 736–742.
19. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. *J Clin Microbiol*. 2011; 49: 3321–3324.
20. Griffin PM, Price GR, Schooneveldt JM et al. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry to identify vancomycin-resistant enterococci and investigate the epidemiology of an outbreak. *J Clin Microbiol*. 2012; 50: 2918–2931.
21. Majcherczyk PA, McKenna T, Moreillon P et al. The discriminatory power of MALDI-TOF mass spectrometry to differentiate between isogenic teicoplanin-susceptible and teicoplanin-resistant strains of methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett*. 2006; 255: 233–239.
22. Menezes EA, Vasconcelos Junior AA, Angelo MR et al. Correlation between microdilution, Etest, and disk diffusion methods for antifungal susceptibility testing of fluconazole against *Candida* sp. blood isolates. *Rev Soc Bras Med Trop*. 2013; 46: 106–107.
23. Su KY, Yan BS, Chiu HC et al. Rapid sputum multiplex detection of the *M. tuberculosis* complex (MTBC) and resistance mutations for eight antibiotics by nucleotide MALDI-TOF MS. *Sci Rep*. 2017; 7: 41486.
24. Espinel-Ingroff A, Barchiesi F, Cuenca-Estrella M et al. International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of *Candida* spp. to fluconazole, itraconazole, posaconazole, and voriconazole. *J Clin Microbiol*. 2005; 43: 3884–3889.