Eur J Clin Microbiol Infect Dis (2017) 36:807–812
DOI 10.1007/s10096-016-2864-9

ORIGINAL ARTICLE

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) for rapid identification of micro-organisms in the routine clinical microbiology laboratory

C. Wattal[1] · J. K. Oberoi[1] · N. Goel[1] · R. Raveendran[1] · S. Khanna[1]

Received: 24 September 2016 / Accepted: 1 December 2016 / Published online: 16 December 2016

Abstract The study evaluates the utility of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) Vitek MS for identification of microorganisms in the routine clinical microbiology laboratory. From May 2013 to April 2014, microbial isolates recovered from various clinical samples were identified by Vitek MS. In case of failure to identify by Vitek MS, the isolate was identified using the Vitek 2 system (bioMerieux, France) and serotyping wherever applicable or otherwise by nucleic acid-mediated methods. All the moulds were identified by Lactophenol blue mounts, and mycobacterial isolates were identified by molecular identification systems including AccuProbe (bioMerieux, France) or GenoType Mycobacterium CM (Hain Lifescience, Germany). Out of the 12,003 isolates, the Vitek MS gave a good overall ID at the genus and or species level up to 97.7% for bacterial isolates, 92.8% for yeasts and 80% for filamentous fungi. Of the 26 mycobacteria tested, only 42.3% could be identified using the Saramis RUO (Research Use Only) database. VITEK MS could not identify 34 of the 35 yeast isolates identified as *C. haemulonii* by Vitek 2. Subsequently, 17 of these isolates were identified as *Candida auris* (not present in the Vitek MS database) by 18S rRNA sequencing. Using these strains, an in-house superspectrum of *C. auris* was created in the VITEK MS database. Use of MALDI-TOF MS allows a rapid identification of aerobic bacteria and yeasts in clinical practice. However, improved sample extraction protocols and database upgrades with inclusion of locally representative strains is required, especially for moulds.

✉ C. Wattal
chandwattal@gmail.com

[1] Sir Ganga Ram Hospital, New Delhi, Delhi 110060, India

Introduction

Rapid and accurate identification of pathogens is essential for appropriate antimicrobial therapy to improve patient outcomes. However, conventional biochemical methods take days, automated systems require at least 4–12 hours and molecular methods may still be relatively expensive, labor intensive and not suitable for large-scale routine identification. Recently, new mass spectrometry (MS) based technology, the matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has been introduced as a way to accurately identify bacteria and fungi in minutes. The technology is based on the analysis of highly abundant, mainly ribosomal, proteins in the mass range of 2,000 to 20,000 Daltons. A mass spectrum can be acquired from an unknown micro-organism, and its proteomic fingerprint is then compared to those in a reference spectral database to ascertain the likely genus and species. Compared to standard phenotypic identification, it requires reagents that are inexpensive and the technology as such seems as accurate as 16S rRNA sequencing [1].

The aim of the present study was to evaluate the performance of the MALDI-TOF Vitek MS for rapid identification of micro-organisms in real time in a routine-diagnostic microbiology laboratory.

Materials and methods

This study was performed at a 650-bed super-speciality hospital in New Rajinder Nagar, New Delhi. Non-repetitive aerobic microbial isolates recovered from various clinical samples including blood, body fluids, respiratory specimens, urine, pus and tissues received in the microbiology laboratory from May 2013 to April 2014 were identified by MALDI-

TOF MS (Vitek MS system, bioMerieux, France). The isolates were recovered from clinical samples using standard methodology [2].

Briefly, the primary media used for isolation were 5% sheep blood agar (bioMerieux, France), MacConkey agar (HiMedia, India), Hichrome agar (HiMedia, India), chocolate agar (bioMerieux, France), Lowenstein Jensen media (HiMedia, India), and Sabrauds Dextrose agar (HiMedia, India), wherever applicable.

Sample preparation for Vitek MS identification from microbial growth

Bacteria and yeasts [3, 4]

Briefly, freshly grown bacterial and yeast isolates were smeared as a thin film onto disposable MALDI target slides using a 1 μL disposable loop. The dried microbial film was then overlaid with 1.0 μL α-cyano-4-hydroxycinnamic acid (CHCA) MALDI-TOF MS matrix and air dried for 1–2 min at room temperature. The slide was then inserted into the Vitek MS system for data acquisition. For yeasts, the dried film was overlaid with 0.5-μL formic acid before addition of matrix.

Filamentous fungi

Extractions were performed according to the Inactivation/Extraction Protocol for Moulds and Vitek MS Identification (27 September 2013) (bioMerieux, Hazelwood, MO) [5].

Mycobacteria/Nocardia [6]

Briefly, a 1-μL loop full of bacterial biomass cultured on Lowenstein Jensen media was collected in a 1.5-mL screw-top microcentrifuge tube containing 500 μL of 70% ethanol and 200 μL of 0.5-mm silica beads (MoBio Laboratories, Inc.). All tubes were vortexed for 15 min in a horizontal position using a Vortex-Genie 2 with a 24-tube adaptor at maximum speed. The tubes were then kept at room temperature for 10 min to complete inactivation of the mycobacteria. The liquid contents (excluding beads) were then transferred to a new 1.5-mL snap-top microcentrifuge tube and centrifuged at 10,000 *g* for 2 min. The supernatant was decanted and the pellet was resuspended and kept for 2–5 min in formic acid, after which 10 μL of 98% acetonitrile was added to each tube. Following mixing by vortexing for 20 s and subsequent centrifugation at 10,000 *g* for 2 min, the supernatant was deposited on the VITEK MS slide and overlaid with 1 μL CHCA matrix.

MALDI-TOF MS acquisition

Data were processed automatically by the instrument software, and the new spectra were compared with those in the reference IVD approved database. As recommended by the manufacturer, the *Escherichia coli* ATCC 8739 strain was used as a calibrator and internal ID control and inoculated on the calibration spots of each acquisition group. In case no identification was achieved, the whole procedure was repeated with a fresh colony. For mycobacterial isolates, the results were read additionally in the Saramis RUO database.

Other identification methods used in the study

The other identification system used was the Vitek 2 system (for bacteria and yeasts) using the GP, GN, YST card (bioMerieux, France) as applicable, serotyping (for Salmonella and Shigella spp.) or otherwise by nucleic acid-mediated methods. Such methods were applied when Vitek MS failed to generate identification results. All the moulds and mycobacterial isolates were also identified by Lactophenol blue mounts and AccuProbe (bioMerieux, France) and GenoType Mycobacterium CM (Hain Lifescience GmbH, Nehren, Germany) molecular identification systems, respectively. These methods are used widely and are considered standard of care in clinical practice. We used all methods according to their manufacturer's instructions.

Results

MALDI-TOF MS identification performance

A total of 12,003 microbial isolates were tested using Vitek MS, which included 5,350 enterobacteriaceae, 2763 Gram-negative bacteria other than enterobacteriaceae, 1100 Enterococci, 179 Streptococci and related species, 1297 Staphylococci and related species, 20 gram positive aerobic bacilli, 1248 yeasts, 20 filamentous fungi and 26 mycobacteria. The Vitek MS could provide ID to the species level for 95.8% (n = 11509) isolates, and an ID only to the genus level was obtained for 1.11% (n = 134) of isolates (mostly *Salmonella and Enterobacter cloacae*). No ID could be provided in 2.93% (n = 352) of isolates and eight *Shigella flexneri* isolates were expectedly misidentified as *E. coli*. Such non-lactose fermenting organisms were subsequently identified using Vitek-2. Eight isolates belonging to four species were not yet included in the database of the Vitek MS system (2 *Brucella melitensis*, 3 *Burkholderia pseudomallei*, 1 *Nocardia cyriacigeogrica*- identified by 16S rRNA, 1 *Mycobacterium abscessus* and 1 *Mycobacterium chelonae*).

Vitek MS gave a good overall ID to the genus and or species level up to 97.7% for bacterial isolates (97.3%

enterobacteriaceae, 98.1% of gram negative bacteria other than enterobactericeae, 98.5% Enterococci and 89.9% Streptococci and related species, 98.3% Staphylococci and related species and 95% gram positive aerobic bacilli) as shown in Table 1. Vitek MS results correlated completely with gram reaction of the isolates.

Out of the 1,248 fungal isolates tested, 1175 (92.6%) could be identified by Vitek MS, the identification rates being 92.8% and 80% for yeasts and moulds, respectively (Table 1). For 34 of the 35 yeast isolates identified as *C. haemulonii* by Vitek 2, no identification was obtained using VITEK MS. Subsequently, 17 of these isolates were identified as *C. auris* (a novel species, not present in the Vitek MS database) by 18S rRNA sequencing, and using these strains, a new superspectrum of *C. auris* was created in VITEK MS using the RUO module. Out of a total of 26 mycobacterial isolates tested, only 11 (42.3%) could be identified by Vitek MS, using the Saramis RUO database (Table 1).

Discussion

The introduction of MALDI-TOF MS technology in clinical microbiology laboratories can reduce the time required for identification while improving the accuracy of microbe identification. Our study prospectively analyzed the routine use of Vitek MS in a clinical microbiology laboratory over a period of one year. In our hands, overall 95.8% of isolates were correctly identified to the species level, with high identification rates (97.7%) for bacteria and yeasts (92.8%), consistent with that reported in literature [3, 7, 8]. Failure or wrong identification in routine workflow could be due to poor quality deposits (too many peaks, like in mucoid organisms, e.g. in *Klebsiella spp*.) or too little peaks in case of thick cell walls where prior extraction is necessary, especially in moulds and mycobacteria, lack of representative spectra in the instrument database, alteration of protein expression in response to variations in environmental stimuli (culture conditions), failure of internal calibration and controls (seen when much older colonies, i.e. around 10–13 day old of calibrator *E. coli* strain were used) and closely related species expressing similar proteins can lead to additional errors.

The misidentification of Shigella isolates as *E. coli* in our study because of close genetic relatedness has also been reported previously [3, 7]. The manufacturers have already accepted these anomalies of the technology. Salmonella typing using MALDI-TOF MS also poses a challenge for clinical laboratories as a large number of reproducible peaks are required for subspecies identification [9]. In our study, out of a total of 145 isolates of Salmonella, 76 were identified as Salmonella group by VITEK MS but serotyping needs to be done for final identification.

An in-house superspectrum for *C. auris* was created in the RUO module using genetically identified strains, thereby indicating the utility of Vitek MS technology to identify novel microbial species by creating separate databases. Girard et al. [10] recently also created a novel *C. auris* superspectrum in the Vitek MS database using 12 reference strains and could successfully identify 46 of the 50 *C. auris* isolates recovered from clinical specimens obtained from three different countries using this expanded Vitek MS database, proving that Vitek MS could be efficiently used for identifying this novel species.

Among the moulds, VITEK MS in our hands identified 80% of isolates which remains comparable to data in other reports [4]. There are relatively few studies involving the use of MALDI-TOF MS for identification of filamentous fungi, probably because of difficulties related to phenotypic heterogeneity and lack of standardization in protein extraction protocols. Panda et al. [11], in a recent study on 37 isolates of filamentous fungi, found that only 68% of the isolates could be identified by MALDI-TOF MS although after modification of extraction protocols the rates increased to 87%. Improvement of extraction protocols along with a continuous expansion of the reference database library is therefore necessary to further improve the reliability of identification.

We have tried a few protocol modifications to identify monomorphic organisms directly from positive blood culture broths using Vitek MS. This could achieve 70.7% correct identification within 2 hours of a blood culture bottle turning positive [12].

MALDI-TOF MS has recently been adapted for identification of mycobacteria, and the majority of the work has been done using the Bruker Daltonics Flex system. In our study, the ID rates of mycobacterial species using the VITEK MS Saramis database were found to be very low (42.8%). Higher ID rates ranging from 82 to 95% [6, 13] have been reported recently using the Saramis RUO database; whereas in contrast during a study using the IVD database, out of the 102 mycobacterial isolates, none could be identified [14]. In our study the IVD database resulted in similar results but the Saramis RUO database could identify at least 42.8% isolates as mentioned above. Many of these issues are expected to be sorted out by the new V3 version of IVD database. In the meanwhile, we are trying to modify the extraction procedure for mycobacteria and the trial runs are encouraging. The modified procedure improved identification rate to >80% (unpublished data) using the Saramis RUO database. Since the number of isolates tested are small the study is still being continued. Also it would be interesting to observe the sensitivity of the V3 version in Vitek MS IVD in identifying mycobacteria in due course of time.

The main limitation of our study was that all discrepancies between identification by Vitek MS and alternative methods could not be resolved since sequencing was limited due to

Table 1 MALDI-TOF MS identification of bacterial, fungal and mycobacterial isolates

Organism	Total isolates tested	Correct ID to species	Correct ID to genus only	No ID	Mis-ID
GNB	**8113**	**7783**	**134**	**188**	**8**
Enterobactericiae	**5350**	**5091**	**115**	**136**	**8**
Citrobacter freundii	*19*	17		2	
Citrobacter koseri	*2*	2			
Citrobacter weria	*1*	1			
Citrobacter youngae	*1*	1			
E. cloacae	*35*		*33*	2	
E. coli	*2381*	2342		39	
Enterobacter aerogenes	*18*	*18*			
K.oxytoca	*7*	7			
K.pneumoniae	*2390*	2312		78	
Morganella morganii	*48*	47		1	
P. mirabilis	*211*	208		3	
P. vulgaris/P. penneri	*6*		*6*		
Pantoea agglomerans	*2*			2	
Provendncia rettgeri	*7*	7			
Provendncia stuartii	*3*	*3*			
Salmonella paratyphi A	*18*	*18*			
Salmonella group	*76*		*76*		
Salmonella typhi	*51*	*44*		7	
Serratia marcescens	*66*	*64*		2	
Shigella flexneri	*8*				8
Other GNB	**2763**	**2692**	**19**	**52**	
A. baumanii complex	*1346*	1331		15	
A. johnsonii	*1*	*1*			
A. junii	*2*	*2*			
A. lowffii	*5*	5			
Achromobacter xyloxidans/denitrificans	*4*		*4*		
Actinobacillis ureae	*2*	2			
Aeromonas hydrophila//caviae	*6*		*6*		
Brevindimonas diminuta	*4*	*4*			
Brucella melitensis[a]	*2*			2	
Burkholderia cepacia	*32*	*32*			
Burkholderia psuedomallei[a]	*3*	*0*	*0*	3	
Burkholderia vietnamensis	*2*	*2*			
Chryseobacterium indologenes	*6*	*6*			
Comamonas testosteroni	*3*	*2*		1	
Delftia acidovorans	*2*	*2*			
Elizabethkingia meningoseptica	*7*	*6*		1	
Eikenella corrodens	*2*	*2*			
H. influenzae	*24*	*24*			
Moraxella lacunata	*5*	*5*			
Myroides spp	*10*		*9*	1	
Neisseria cineria	*1*	*1*			
Neisseria meningitis	*2*	*2*			
Neisseria subflava	*2*	*2*			
Pseudomonas aeruginosa	*1155*	*1129*		26	
P. flouresens	*1*			1	
P. putida	*38*	*37*		1	
P. stutzeri	*4*	*4*			
Ralstonia pickettii	*3*	*3*			
Shewanella putrefaciens	*8*	*8*			
Stenotrophomonas maltophilia	*79*	*78*		1	
Vibrio cholerae	*1*	*1*			
Vibrio fluvalis	*1*	*1*			
GPC	**2576**	**2521**		**55**	
Enterococcus	**1100**	**1084**		**16**	
E. avium	*7*	*6*		1	
E.casseliflavus	*3*	*3*			
E. faecalis	*335*	*330*		5	
E. faecium	*746*	736		10	
E. gallinarum	*6*	6			
E. raffinosis	*3*	3			
Streptococcus & related species	**179**	**161**		**18**	
Aerococcus	1	1			
Lactococcus lactis ssp lactis	1	1			
Strept.agalactiae	25	22		3	
Strept.anginosis	23	21		2	
Strept. constellatus	4	4			
	2	2			

Table 1 (continued)

Organism	Total isolates tested	Correct ID to species	Correct ID to genus only	No ID	Mis-ID
Streptococcus dysgalactiae ssp dysgalactiae					
Streptococcus gallolyticus ssp gallolyticus	21	21			
Streptococcus infantarius ssp infantarius	3	3			
Strept. mitis/oralis	44	40		4	
Strept. ovis	1			1	
Strept. parasanguinis	1	1			
Strept. pneumoniae	24	23		1	
Strept. pyogenes	27	20		7	
Streptococcus salivarius ssp salivarius	2	2			
Staphylococci & related species	**1297**	**1276**		**21**	
Micrococcus luteus/lylae	3	3			
S. aureus	471	463		8	
S. capitis	17	17			
Staphylococcus cohnii ssp cohnii	7	7			
S. haemolyticus	231	229		2	
Staphylococcus hominis ssp hominis	150	148		2	
S. epidermidis	409	402		7	
S. saprophyticus	6	5		1	
S. warneri	3	2		1	
GPB	**20**	**19**		**1**	
Corynebacterium striatum	17	17			
Bacillus cereus	2	2			
Nocardia cyriacigeogrica[a]	1			1	
Yeasts	**1248**	**1159**		**89**	
C. albicans	383	369		14	
C. catenluata	12	12			
C. famata	13	9		4	
C. glabrata	215	213		2	
C. haemulonii	35	1		34	
C. guilliermondii	5	5			
C. kefyr	8	7		1	
C. krusei	26	24		2	
C. lipolytica	2	1		1	
C. lusitaniae	5	5			
C. parapsilosis	72	60		12	
C. pelliculosa	17	14		3	
C. rugosa	15	15			
C. tropicalis	370	358		12	
Cryptococcus neoformans	6	6			
Kodemea Ohmeri	2	2			
Saccharomyces cerevisae	1	1			
Stephanoascus ciferrii	1	1			
Trichosporon asahii	60	56		4	
Moulds	**20**	**16**		**4**	
A. fumigatus	9	8		1	
A. flavus	7	6		1	
A. oryzae	2	2			
A. terreus	1	0		1	
Fusarium solanii	1	0		1	
Mycobacteria	**26**	**11**		**15**	
Mycobacterium tuberculosis	22	10		12	
Mycobacterium intracellulare[a]	2	1		1	
Mycobacterium abscesses	*1*			1	
Mycobacterium chelonae[a]	*1*			1	
Total	**12003**	**11509**	**134**	**352**	**8**

[a] Not included in the database

economic constraints. However, isolates were tested in comparison with the Vitek 2 ID system and other methods, as listed, which are widely used and are considered standard of care in clinical practice.

Conclusions

We found that MALDI-TOF MS could accurately identify bacteria and yeasts to both the genus and species levels for

gram positive and gram negative bacteria as well as commonly encountered yeasts which favors its potential application for almost all aerobic bacterial species and yeasts commonly encountered in clinical practice. The ability to modify or add entries to the MALDI-TOF MS database as demonstrated by creation of super-spectra for *Candida auris* can help to further improve identification. However, results for moulds and mycobacteria were not encouraging, hence improved sample extraction protocols and database upgrades with inclusion of locally representative strains is warranted.

Compliance with ethical standards

Ethical approval Ethics approval was obtained from the IRB of the hospital via their letter No.EC/02/14/626. The Committee waived the requirement for informed consent due to the nature of the study.

Funding This work was compiled out of our routine work.

Conflict of interest None to declare

Reference

1. Stevenson LG, Drake SK, Murray PR (2010) Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 8:444–447
2. Isenberg HD (2007) Clinical microbiology procedures handbook, 2nd edn. AM press, Washington DC
3. Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E (2012) Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J Clin Microbiol 50(8):2568–76
4. Iriart X, Lavergne RA, Fillaux J, Valentin A, Magnaval JF, Berry A, Cassaing S (2012) Routine identification of medical fungi by the new Vitek MS matrix-assisted laser desorption ionization-time of flight system with a new time-effective strategy. J Clin Microbiol 50(6):2107–10
5. McMullen AR, Wallace MA, Pincus DH, Wilkey K, Burnham CA (2016) Evaluation of the vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of clinically relevant filamentous fungi. J Clin Microbiol 54(8): 2068–73
6. Mather CA, Rivera SF, Butler-Wu SM (2014) Comparison of the bruker biotyper and vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry systems for identification of mycobacteria using simplified protein extraction protocols. J Clin Microbiol 52(1):130–8
7. Zhou C, Hu B, Zhang X, Huang S, Shan Y, Ye X (2014) The value of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in identifying clinically relevant bacteria: a comparison with automated microbiology system. J Thorac Dis 6(5):545–52
8. Westblade LF, Jennemann R, Branda JA, Bythrow M, Ferraro MJ, Garner OB, Ginocchio CC, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Rychert JA, Sercia L, Burnham CA (2013) Multicenter study evaluating the Vitek MS system for identification of medically important yeasts. J Clin Microbiol 51(7):2267–72
9. Dieckmann R, Helmuth R, Erhard M et al (2008) Rapid classification and identification of salmonellae at the species and subspecies levels by whole-cell matrix assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 74:7767–78
10. Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, Colombo AL, Hagen F, Meis JF, Chowdhary A (2016) Dentification and typing of the emerging pathogen Candida auris by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses. doi:10.1111/myc.12519
11. Panda A, Ghosh AK, Mirdha BR, Xess I, Paul S, Samantaray JC, Srinivasan A, Khalil S, Rastogi N, Dabas Y (2015) MALDI-TOF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. J Microbiol Methods 109:93–105
12. Wattal C, Oberoi JK (2016) Microbial identification and automated antibiotic susceptibility testing directly from positive blood cultures using MALDI-TOF MS and VITEK 2. Eur J Clin Microbiol Infect Dis 35:75–82
13. Machen A, Kobayashi M, Connelly MR, Wang YF (2013) Comparison of heat inactivation and cell disruption protocols for identification of mycobacteria from solid culture media by use of Vitek matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51(12):4226–9
14. Chen JH, Yam WC, Ngan AH, Fung AM, Woo WL, Yan MK, Choi GK, Ho PL, Cheng VC, Yuen KY (2013) Advantages of using matrix-assisted laser desorption ionization-time of flight mass spectrometry as a rapid diagnostic tool for identification of yeasts and mycobacteria in the clinical microbiological laboratory. J Clin Microbiol 51(12):3981–7