Clinical Microbiology and Infection 29 (2023) 127–129

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Commentary

Matrix Assisted Laser Desorption Ionisation/Time Of Flight (MALDI/TOF) mass spectrometry is not done revolutionizing clinical microbiology diagnostic

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ARTICLE INFO

Article history:
Received 25 June 2022
Received in revised form
1 October 2022
Accepted 3 October 2022
Available online 8 October 2022

Editor: E.J. Kuipers

Keywords:
External quality controls
MALDI TOF mass spectrometry
Mass spectra quality
Reproducibility
Resistance prediction
Typing

The introduction of MALDI TOF mass spectrometry (MALDI TOF MS) in clinical microbiology at the end of 2010 has been a revolution for microbial identification [1]. Unlike the conventional uses of MALDI TOF MS in clinical chemistry laboratories, detecting and quantifying specific peaks of known proteins, the routine use of MALDI TOF MS in clinical microbiology is based on the comparison of mass spectra (intensity of m/z values corresponding to peptides in the low m/z region) to a database of mass spectra, allowing the attribution of matching scores without any a priori on the corresponding proteins. This technique is easy-to-use, fast, requires a low amount of cells, and is cost effective for labs identifying >100 bacteria species per day. Because it is a powerful tool, many authors have attempted to use MALDI TOF MS to obtain additional data (a) direct resistance detection (e.g., methicillin-resistant *Staphylococcus aureus*/methicillin-susceptible *Staphylococcus aureus* detection): [2] (b) virulence factor detection (e.g. delta toxin of *S. aureus*); [3]; (c) typing approaches for outbreak detection or multilocus sequence typing (e.g. *S. aureus* clones) [4]; (d) resistance mechanism detection with functional test (e.g. carbapenemase detection) [5]; and (e) resistance detection through growth/no growth detection (e.g. MALDI Biotyper antibiotic susceptibility test rapid assay [MTB-ASTRA® kit], direct-on-target microdroplet growth assay) [6]. However, the use of these protocols remains very scarce in routine practice. Whereas MALDI TOF MS is widely used in isolated colonies, the main additional use of MALDI TOF MS lies in the direct identification of positive blood cultures that reduces the delay in species identification. Despite the availability of two commercial kits (SepsisType kit and VITEK® MS Blood Culture Kit), many laboratories use 'homemade' protocols either for better performance or to reduce the number of manual steps for sample preparation [7]. Despite all applications previously described, MALDI TOF MS remains today almost exclusively used routinely for the identification of microorganisms because of the heterogeneous performance. Indeed, these applications are limited by their low practicability and reliability, partly attributable to the low accuracy (e.g. mass spectrometer calibration drift and mass peak shifts) and reproducibility (e.g. peak number, spectral resolution, non-quantitative peak intensities) of the generated spectra by the linear MALDI TOF technology. These applications are also limited by external factors, such as the protein size (over or below the m/z acquisition window) or level of expression (poorly expressed proteins can be not detected because their level of expression may be below the limit of detection of the instrument). Although they are totally embedded in identification algorithms, these varying parameters are poorly controlled for resistance and virulence detection algorithms, and they should be taken into account within these algorithms for better performance and possible use in routine practice.

Mass spectral quality (MSQ) depends on many criteria: (a) the instrument itself, including settings, service, age of laser, detector

DOI of original article: https://doi.org/10.1016/j.cmi.2022.05.017.
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https://doi.org/10.1016/j.cmi.2022.10.005

wear; (b) reagents, including the type of matrix, slides, and their cleaning; and (c) operators: skills and training. As described by Cuenod et al. [8], five important spectrum features are involved in MSQ: (a) the number of ribosomal marker peaks detected, (b) the median relative intensity of ribosomal marker peaks, (c) the sum of the intensity of all detected peaks, (d) a high measurement precision, and (e) the reproducibility of peaks between technical replicates. Based on these results, Cuenod et al. [9] published in this issue of the 'Clinical Microbiology and Infection' an international multicentre study with an external quality control based on 47 diverse bacterial strains analysed on 41 instruments (Maldi Biotyper® and VITEK MS®) used by 36 laboratories. Before any intervention, this study underlined a large heterogeneity of performance between the included instruments. At the species level, the worst instrument identified only 22.5% of strains, whereas the best identified 78.2%. This article described many 'good practice' factors associated with MSQ that could be rapidly applied in clinical microbiology platforms/labs: (a) use of steel target when possible, (b) slide cleaning using a 'methanol acetone' protocol, (c) subscription of service contract to have regular hardware service by the instrument manufacturer, (d) concentration/centralization of microorganism deposit on one or a limited number of workstations with laboratory technicians mastering MALDI TOF MS skills, (e) opening of the matrix tube for <7 days, and (f) for strain storage, systematic subculturing on agar plate before identification. Based on these results, a calibration of each instrument was conducted using proteins with known mass, and new measurements were performed with an external quality control including the same strain collection. Surprisingly, the error measurement was then lower for only 14 out of 36 laboratories and higher for 11 out of 36. To explore the impact of sample preparation on MSQ, Cuenod et al. [9] used the well-known 'formic acid overlay protocol' (FA) and different species-group-specific protocols. The FA protocol increased the performance only for the *Staphylococcus* group, and the dedicated *Staphylococcus* protocol had a negative effect by generating noisy spectra, thereby decreasing the global identification performance. Species-group-specific protocols had positive effects only for viridians streptococci. Finally, this article demonstrated a very large heterogeneity in terms of performance that could be responsible for the erroneous identification in routine practice without alarm/error for the final user. The performance level of each MALDI TOF mass spectrometer need to be monitored locally weekly using internal quality control (QC) [10] and twice a year using external QC strains. The service frequency needs to follow the manufacturer's recommendations. Moreover, this study suggested that calibration criteria used by each instrument manufacturer probably need to be improved: (a) reduction of acceptance intervals for error measurements notably and also for all setting parameters; (b) frequency increase in 'routine service' performed by laboratory technicians or service engineers; (c) reduction of life of some sensitive spared parts: laser? detectors?; and (d) use of well-defined proteins to calibrate instrument instead of *Escherichia coli* strains or mixed protein extracts (Bruker Bacterial Test Standard (BTS)).

Together, these measures could be associated with MSQ improvements that would enable the generalization of more MALDI TOF MS applications in routine. Based on the use of reproducible spectra with high MSQ, a recent study by Weis et al. [11] has described a machine-learning model using MALDI TOF spectra to predict resistance directly on the spectra used for identification. This artificial intelligence (AI) technology had an area under the receiver operating characteristics curve of 0.8, 0.74, and 0.74 for the prediction of oxacillin resistance for *S. aureus,* and ceftriaxone resistance for *E. coli* and *Klebsiella pneumoniae*, respectively, when using database built from local spectra/resistance data. However, the use of spectra/resistance data from another external laboratory could decrease, as well as increase, the performance level. Thus, these results suggested the need for very large multicentre databases completed with local data continuously collected during "routine" use. Moreover, whole-genome sequencing could also be used in the future to predict ribosomal protein masses associated with virulent factors [12], and probably with specific resistances or successful lineages.

As clearly demonstrated by Cuenod et al. [9], MSQ must be improved to increase the reproducibility between clinical laboratories. To this aim, four areas of improvement can be easily implemented: (a) about the instrument, service, as well as settings, needs to be more robust and more rigorous. Similar to proteomic laboratories, MALDI TOF MS calibration perhaps needs to be performed with well-defined proteins spanning the variety of microorganism identification; (b) about the method, the systematic (or not) deposit of FA needs to be arbitrated for routine use, as well as the use of reusable steel-made targets versus single use plastic targets; (c) about the deposit, a wide variety of tools can be used in routine practice. The manual deposit could be performed with loop, toothpick, commercialized pen (PickMe®), which could be responsible for the large heterogeneity of MSQ. Automatic deposit on targets could also be used. Mainly based on pipet tip (e.g. Colibri®, Galaxy®, and IdentifiA®), these instruments increase identification performance probably because of the hypothetic increase in precision and reproducibility of the deposit step [13]; and (d) about databases, links between microorganism spectra and resistance profiles need to be implemented by manufacturers. The enrichment of databases must be continuous, including local customer spectra/resistance data to increase the performance level following local epidemiologic data. An AI using learning machine protocol could be continuously used to maintain the performance levels for resistance prediction, as well as virulent factor detection or strong lineages relatedness suggesting outbreak and/or transmission. Moreover, this AI needs to differentiate mass spectra/resistance profiles of strains isolated from identical or different patients and 'in fine' to detect outbreaks early. In the future, these data could be integrated in hospital stewardship software [9].

In conclusion, the MALDI TOF MS era in clinical microbiology is not over and will probably bring new data based on routinely acquired spectra. Lots of improvements for sample preparation probably need to be integrated, as well as many software/AI updates. Moreover, other proteomic methods are emerging, such as Raman and non-targeted and targeted MS using bottom-up proteomics. Notably, liquid chromatography coupled to electrospray ionization MS can simultaneously identify, type, and characterize both the resistance and virulence profiles of microorganism(s) [14]. Requiring less than 30 to 60 minutes, this approach could be used either on colonies or positive blood culture bottles with cost efficiency and fully automated instrument [15]. However, instruments, as well as sample preparation, also require improvements to be easy and reliable enough for routine use. Thus, whereas genomic/genetic approaches (whole-genome sequencing and next generation sequencing, high multiplexed syndromic tests) are commonly pictured as 'the future of routine microbiology', proteomic-based methods on cultivated colonies or directly on primary samples (positive blood culture and urine) are not done revolutionizing clinical microbiology diagnostic.

Author contribution

OD has written the commentary, TC corrected and improved it, JPR and FV improved it.

Transparency declaration

OD received a research grant from bioMérieux, BD and Copan. OD received travel grants for congress from Pfizer, Eumedica, bioMérieux and Copan. The other authors declare that they have no conflicts of interest.

Funding

This commentary did not required external funding.

Acknowledgements

The authors would like to thank Hélène Boyer (*Hospices Civils de Lyon*) for help in manuscript preparation.

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