Enferm Infecc Microbiol Clin. 2019;37(5):287–289

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Editorial

Rapid identification of microorganisms directly from positive blood cultures by MALDI-TOF MS

Identificación rápida de microorganismos directamente de hemocultivos positivos por MALDI-TOF MS

Marina Oviaño

Servicio de Microbiología, Complejo Hospitalario Universitario A Coruña, La Coruña, Spain

Bacteraemia and sepsis are among the leading causes of morbidity and mortality in hospitalized patients.[1] Empirical antimicrobial treatment is administered until the pathogen is identified and the antimicrobial susceptibility is established. Adequate therapy improves patient outcome, whereas delayed antimicrobial treatment increases the mortality by 8% for each hour that therapy is delayed.[2] As well as having a direct impact on patient care, optimal antimicrobial treatment also leads to savings in laboratory, pharmaceutical and patient care costs and potentially reduces the use of inappropriate antimicrobials, which in turn reduces the selection of multi-drug resistant microorganisms.[3]

Different methods have been used to identify microorganisms isolated from blood cultures. Biochemical differentiation of microorganisms directly from positive blood cultures, which is the most frequently used method, requires at least 6 h to provide results.[4] Molecular methods are generally faster than phenotypic approaches because they are culture independent. The most commonly used methods are based on hybridization and microarray assays involving non-amplified nucleic acid detection. The commercial solutions PNA-FISH and Quick-FISH (AdvanDx, Woburn, MA, USA) yield a time to result of between 1.5 and 3 h, with high sensitivity and specificity.[5] FilmArray (Idaho Technology, USA), a multiplex PCR-based system designed to detect 24 microorganisms and 3 antibiotic resistance targets, has a sensitivity of around 90% and provides results in 1 h.[6] However, the main limitation of these methods is the high cost, as they are more expensive than traditional methods, especially since the introduction of MALDI-TOF MS.

Nonetheless, the routine use of MALDI-TOF MS in clinical practice has revolutionized microbiology laboratories, saving the turnaround time of microbiological reports from days to hours and even surpassing the speed of molecular techniques.[7] Despite the initial high investment in the equipment, the low cost per test is estimated to yield savings in microbial identification of around 50% annually.[8] These savings, together with the simplicity of the procedures and the high level of automation, have made this technology almost irreplaceable. New capacities of the technique are reported frequently, ranging from identification of microorganisms grown on solid culture media, to direct identification from the clinical sample and finally to detection of antimicrobial resistance.

Direct MALDI-TOF MS-based identification of bacteria from blood cultures is more complex than identification from subcultures, due to the need for efficient separation of bacteria from the blood cells, as well as the presence of human proteins, activated charcoal and different resins used to neutralize the antibiotics that are present in blood culture bottles.[9] Furthermore, sample processing protocols have not yet been standardized. To date, only one commercial kit is available: the MALDI Sepsityper IVD Kit (Bruker Daltonik, GmbH, Germany).[10] This is an *in vitro* (IVD) diagnostic kit with European Conformity (CE) marking. The kit contains all reagents and consumables required for isolation of bacteria in 50 positive blood culture samples. The price per sample is 10 euros and the turnaround time is 20 min. The main advantage of the use of the Sepsityper kit to identify blood culture isolates is the broader identification capacity relative to molecular methods, which may further improve clinical management and greatly reduce the time to result.[11] Numerous in-house methods have also been developed with different lysis reagents, such as SDS and saponin, to reduce the cost per test relative to that of commercial methods. In most cases, there is no statistically significant differences between the results obtained with the different methods, except for identification of yeasts, for which the Sepsityper kit seems to be less accurate than the in-house method developed by Bidart et al.[12] In summary, the main advantages of the commercial kits are that they are standardized, with certified quality, and are equipped with ready-to-use solutions, whereas the main inconvenient is the high cost.

In a study reported in the current issue of this journal, Martín-Pujol et al.[13] compared three MALDI-TOF MS-based procedures for identifying pathogens isolated from blood cultures. Two methods were directly applied to the positive blood culture isolates: the Sepsityper kit (Bruker Daltonik) and a non-commercial saponin

E-mail address: marina.oviano.garcia@sergas.es

method.[14] The third method involved identification after a short incubation subculture and was developed by Idevevich et al. to reduce the hands-on time and the cost of processing.[15] The recommended incubation time after subculture is 2 h for identification of Gram-negative microorganisms and 6 h for Gram-positive microorganisms. In the study reported by Martín-Pujol et al.,[13] the cultures were incubated for 3.5 h. The direct method with the Sepsityper kit performed significantly better than the direct in-house method with saponin. However, in comparison with the short incubation method, both of the other methods produced very similar results. Martín-Pujol et al. reported the advantages and disadvantages of both methods.[13] The Sepsityper method is very rapid, but requires a longer processing time and is more expensive. Although the short incubation method provides results much later, it requires less processing and can easily be incorporated into the clinical laboratory routine.

The MALDI Biotyper system obtains the spectrum of the unknown microorganism and transforms this into a peak list. The associated software then calculates a logarithmic score of between 0 and 3. This score quantifies the similarity between the peak list of the target organism and the reference peak list in the database using a mathematical algorithm. High values of this score represent high similarity with respective database entries. The species identification is interpreted according to the score. Thus, identification scores of >2.3 are required for reliable ('highly probable') identification to the species and genus level, score values between 2.0 and 2.3 represent probable identification to species level, scores between 1.7 and 2.0 represent a reliable genus level, and scores of <1.7 are regarded as unreliable. As the scores are logarithmic values, small differences in the scores represent large differences in the peak profile. However, in order to improve diagnostic sensitivity, some authors have proposed the use of lower cut-off values. This may be possible because the number of correct identifications increases when the confidence score thresholds are lowered, whereas the risk of misidentification and major errors is marginal. In this issue, Martín-Pujol et al.[13] used the threshold proposed by Kohlman et al., who considered that correct identification is indicated by scores $\geq$1.5 for Gram-positive microorganisms and $\geq$1.7 for other microorganisms.[16] In addition, the first three identifications must be identical. Application of these new scores increased the percentage of correct identifications without loss of specificity, resulting in one more indicator that the current scores could be lowered.

The MBT Compass Sepsityper IVD Module (Bruker Daltonik) has recently been launched in the market, thus facilitating the automated incorporation of these modifications in interpreting the identification scores. It supports result management, taking into account the complexity of a sample originated from blood cultures. The software defines a specific algorithm of acquisition, using a smaller peak-picking mass range to exclude blood specific peaks. In addition, identification results are reported with adapted score thresholds with a reliable identification to the species level for scores $\geq$1.8.[17]

According to Martín-Pujol et al.[13] the direct methods did not produce significant differences in the scores obtained for either Gram-positive or Gram-negative microorganisms. However, a recently published meta-analysis revealed that the accuracy of MALDI-TOF MS for the direct identification of Gram-positive bacteria using the Sepsityper kit (Bruker Daltonik) must be improved, as the overall rates of correct identification ranged from 0.17 to 0.98 with a cumulative effect of 0.72, while for Gram-negative microorganisms, the rates ranged from 0.66 to 1.00 with a cumulative effect of 0.92.[18] This is of particular concern as the management of bacteraemia is quite different depending on whether it is due to *Staphylococcus aureus* or to coagulase-negative staphylococci.

In recent years different studies have evaluated the clinical impact of the MALDI-TOF MS-based identification of microorganisms from blood cultures.[19] The most important clinical benefits of such identification reported in these studies are the earlier administration of correct antimicrobial therapy, reduced morbidity and mortality, reduction in hospital stays and reduced overall costs per hospitalized patient. Any of the methods reported by Martín-Pujol et al.[13] could be easily incorporated into the workflow of clinical microbiology laboratories, with faster time-to-result than conventional methods and with the ease of handling of particular interest. In the near future, in addition to direct identification from blood cultures, MALDI-TOF MS is also expected to be used for detecting bacterial determinants and for typing purposes in the control of outbreaks.[20]

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