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LETTER TO THE EDITOR

Direct Identification of Pathogens in Blood Cultures by MALDI-TOF

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The aim of this study was to validate an in-house methodology for direct bacterial identification of positive blood culture bottles using Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF) methodology and to attest its clinical benefit in reducing diagnostic time of bacteremia detection in patients admitted to hospitals in Rio de Janeiro.

Primary bloodstream infections were detected using the BACTEC FX® (Becton Dickinson) automated equipment with the following bottles: BD BACTEC® Plus Aerobic / F and BD BACTEC Plus + ® Anaerobic / F. For identification by MALDI-TOF Microflex® MS (Bruker Daltonik) analyzer Maldi FlexControl software Version 3.0 was used together with Maldi Biotyper Version 3.0.

Sample preparation for MALDI-TOF analysis:

After a positive blood culture was detected by BD BACTEC FX ®, a Gram stain was performed to identify the morphology of the possible pathogen, and the formic acid protein extraction procedure was performed for direct identification.

To obtain samples for MALDI-TOF identification, 6 mL of culture medium obtained from a positive blood culture vial were transferred to a Vacuette ® serum gel tube. Two mL of saponin were added in order to obtain the complete lysis of erythrocytes. Tubes were centrifuged at 3000 rpm for 10 minutes and supernatant was removed. The pellet was resuspended in 2 mL of sterile water, vortexed and the entire volume was transferred to a 2 ml microtube. Finally, the formic acid extraction procedure was performed and 2 µl of the supernatant was used to obtain protein identification spectrum.

Matrix Preparation:

HCCA Matrix (α-cyano-4-hydroxycinnamic acid) was prepared following the manufacturer's instructions (Bruker Daltonik).

Spotting MALDI-TOF Target Plates:

Samples left at room temperature and 2 µl of supernatant were inoculated on the steel target plate (Bruker Daltonik). Spots were left to air dry and 1 µl of HCCA matrix solution was added to each spot. Identification was performed by MALDI-TOF Microflex ® MS (Bruker Daltonik) analyzer used together with Maldi FlexControl software Version 3.0 and Maldi Biotyper Version 3.0.

To confirm results obtained by MALDI-TOF, we used the automated WalkAway Microscan ® 96 SI (Siemens) identification equipment with identification and susceptibility testing (NUC-55, PC-33 and RY-ID).

For the identification of *Streptococcus pneumoniae,* the optochin disc (Oxoid) was used as additional confirmation.

MALDI-TOF generates results with reliable identification and interpretation at genus level with scores between 2.0 and 2.29 and for genus and species level, with scores between 2.30 and 3.0 (14). Our interpretation was based on several recently published studies, where authors used scores > 2.0 as a reliable mark for interpretation at genus and species levels in samples obtained directly from positive blood cultures. Other publications accept lower scores and the new Bruker Maldi Biotyper software version 3.1 has a special module to address blood culture protocol evaluations based on the following score interpretations:

- 2.0 – 3.00: Highly probable identification at genus and species level
- 1.8 – 1.999: Safe identification at genus level and probable at species level
- 1.6 – 1.799: Probable identification at genus level

In our in-house developed protocol we were able to identify and confirm scores higher than 1.8; hence, this was considered our cutoff value for acceptable identifications. We were able to identify the following pathogens directly from positive blood culture bottles: *Escherichia coli* (n=30), *Klebsiella pneumoniae* (n=35), *Citrobacter freundii* (n=10), *Serratia marcescens* (n=12), *Haemophilus influenzae* (n=2), *Streptococcus pneumoniae* (n=2), *Staphylococcus aureus* (n=11), *Pseudomonas aeruginosa* (n=8), *Burkholderia cepacia* (n=1), *Enterococcus faecalis* (n=5), *Staphylococcus hominis* (n=5), *Staphylococcus epidermidis* (n=6), *Aeromonas hydrophila* (n=1), *Candida tropicalis* (n=2), *Candida albicans* (n=3) and *Enterobacter cloacae* (n=5). The following bacteria were identified, but were not considered contaminants: *Bacillus pumilus* (n=1) and *Staphylococcus pettenkoferi* (n=1).

Table 1 lists microorganisms identified with scores higher than 1.8, respectively.

Traditional microbiological methods to identify infectious agents are based on phenotypic methods using biochemical tests

Tabela 1 - Microorganisms identified with scores higher than 1.8, respectively.

Microorganism (n)	Score 1.8 a 2.0	Score ≥ 2.0
Aeromonas hydrophila (1)	0	1
Bacteroides thetaiotaomicron (1)	1	0
Burkholderia cepacia (5)	1	5
Candida albicans (20)	11	9
Candida parapsilosis (7)	2	5
Candida pelliculosa (1)	0	1
Candida tropicalis (2)	0	2
Citrobacter freundii (10)	1	9
Enterobacter cloacae (5)	0	5
Enterococcus faecalis (15)	1	15
Escherichia coli (30)	2	28
Haemophilus influenzae (4)	2	2
Klebsiella oxytoca (1)	0	1
Klebsiella pneumoniae (35)	4	31
Pseudomonas aeruginosa (8)	3	5
Serratia marcescens (12)	1	11
Staphylococcus aureus (11)	0	11
Staphylococcus cohnii (3)	3	0
Staphylococcus epidermidis (17)	9	8
Staphylococcus haemolyticus (4)	2	2
Staphylococcus hominis (17)	7	10
Streptococcus massiliensis (1)	0	1
Streptococcus peroris (1)	1	0
Streptococcus pneumoniae (2)	0	2

and/or automated systems that take from 12 to 24 hours to identify microorganisms. For some situations, identification procedures can take days to be concluded and, once results are obtained, the clinical impact can be lost and correct antimicrobial therapy can be delayed. Such consequences can be critical to patients, because turnaround time is of extreme importance in cases such as septic shock – when a two- hours- delay in treatment or inadequate treatment can double mortality rates. Many studies were developed during the last years demonstrating the effectiveness of MALDI-TOF methodology for rapid identification of bacterial species. Many protocols were developed to identify pathogens directly from automated blood cultures as soon as they were detected as positive. Some studies suggest that using MALDI-TOF technique directly from automated blood cultures could reduce bacterial identification turnaround time by? 34 hours (10).

We conclude that MALDI-TOF is a methodology of unprecedented use in clinical microbiology, as well as an easy to perform and low-cost method, which fits into the clinical microbiology laboratory as an additional tool to identify microorganisms. For patients with suspected sepsis, empirical broad spectrum antimicrobial therapy should be used until the diagnosis is concluded. Applying MALDI-TOF directly to positive blood culture bottles for rapid identification of pathogens leads to reductions in turnaround time and anticipation of antimicrobial therapy based solely on this identification, leading to a better direction in antimicrobial therapy, in less time, with fewer adverse effects and lower costs than a broad-spectrum empiric therapy. Hence, evolution, prognosis and clinical outcome are more likely to succeed.

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