ORIGINAL ARTICLE

Improving Septicemia Diagnostics: A Comparative Analysis of Direct and Post-Culture MALDI-TOF MS* Methods for Bacterial Identification

Amélioration du diagnostic de la septicémie: Analyse comparative des méthodes d'identification bactérienne par MALDI-TOF MS* directement et après culture

Fouad Assi[1,2]; Lamia Melalka[1]; Brahim Jabri[3,4]; Yassine Sekhsokh[1,5]; Mimoun Zouhdi[1,2]

1. Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco
2. Central Laboratory of Bacteriology, Serology and Hygiene - Ibn Sina University Hospital of Rabat, Morocco
3. Higher Institute of Nursing Professions and Technics of Health, Rabat, Morocco
4. Research Laboratory in Oral Biology and Biotechnology, Faculty of Dental Medicine, Mohammed V University in Rabat, Morocco
5. Mohammed V Military Training Hospital, Rabat, Morocco

Abstract

Introduction: Bloodstream infections are serious conditions requiring precise bacterial identification for effective treatment. Traditional culture-based methods, while reliable, are time-consuming. The direct identification method by MALDI-TOF MS promises rapid and accurate identification directly from positive blood cultures.
Aim: To evaluate and compare the direct MALDI-TOF MS identification method for positive blood culture samples with the post-culture MALDI-TOF MS method, which is currently recognized as the gold standard in bacteriological identification.
Methods: during the study period, 324 positive blood culture samples received at the Central Laboratory of Bacteriology, Serology, and Hygiene of the IBN SINA Hospital Center in Rabat were included in the study. Each sample was processed for microorganism identification by MALDI-TOF MS using both direct and post-culture methods.
Results: The direct identification method by MALDI-TOF MS showed a lower overall identification success rate (64.8%) compared to the post-culture method (100%). However, it allowed for bacterial identification in less than one hour without the need for a sub-culturing step, highlighting the technique's potential to enhance the diagnostic process.
Conclusion: The direct identification method by MALDI-TOF MS has the potential to improve the speed of bacterial identification in positive blood cultures compared to the current gold standard of identification after culture. Despite its limitations, the direct method offers an opportunity to improve diagnosis and patient management, especially when combined with the standard method.

Key words: MALDI-TOF MS, Blood culture, Bacteria, Bloodstream infections, Septicemia.

Résumé

Introduction: Les infections sanguines nécessitent une identification rapide et précise pour une prise en charge efficace. Bien que les méthodes de culture soient fiables, elles sont chronophages. La méthode d'identification directe par MALDI-TOF MS offre une alternative prometteuse pour une identification rapide à partir des hémocultures positives.
Objectif: Évaluer la méthode directe par MALDI-TOF MS comparée à la méthode après culture, reconnue comme référence.
Méthodes: 324 échantillons d'hémocultures positives ont été analysés au Laboratoire Central de Bactériologie, de Sérologie et d'Hygiène du Centre Hospitalier IBN SINA de Rabat, utilisant les deux méthodes.
Résultats: La méthode directe a correctement identifié 64,8 % des hémocultures positives, contre 100 % pour la méthode après culture. Cependant, elle a permis une identification bactérienne en moins d'une heure, sans nécessiter d'étape de culture, démontrant son potentiel à accélérer le diagnostic.
Conclusion: La méthode directe par MALDI-TOF MS pourrait améliorer la rapidité d'identification bactérienne à partir des hémocultures positives par rapport à la norme actuelle. Malgré ses limites, cette méthode offre une opportunité significative pour améliorer le diagnostic et la gestion des patients, surtout lorsqu'elle est combinée avec la méthode standard.

Mots clés: MALDI-TOF MS, Hémoculture, Bactéries, Infections sanguines, Septicémie.

Correspondance
Fouad Assi
Central Laboratory of Bacteriology, Serology and Hygiene - Ibn Sina University Hospital of Rabat, Morocco
Email: fouad_assi@um5.ac.ma

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Introduction

Bloodstream infections (BSIs), also known as bacteremia and septicemia, are a significant global cause of morbidity and mortality (1). They are associated with prolonged hospital stays, high costs of care, and numerous clinical challenges, including healthcare-associated infections (HAIs), notably with the emergence of antibiotic-resistant microorganisms (2,3).

The traditional gold standard in microbial diagnostics of BSIs is blood culture with post-culture identification, a highly accurate technique but time-consuming (4). This method leads to delays in making critical therapeutic decisions and the use of broad-spectrum empirical treatments that are suboptimal or unnecessary in 25% to 50% of patients (5).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is one of the newest technological advancements in microbiological diagnostics (6). MALDI-TOF MS is a rapid, accurate, and inexpensive method to identify microorganisms based on the analysis of their protein spectra. This technology has significantly reduced the time required to identify pathogens, thereby improving the overall management of patients and treatment plans (7).

While the MALDI-TOF technique has surpassed the limitations of traditional identification techniques based on biochemical characteristics in terms of accuracy and speed, it generally requires a culture step on a solid nutrient medium (8). To bypass the subculture step, various protocols have been proposed, including commercial kits such as the Sepsityper® (9). These solutions differ in terms of performance and costs but can reduce the identification time by an additional 24 hours, which is a valuable time-saving measure in the management of sepsis (10).

The objective of this study is to propose a simple in-house MALDI-TOF direct technique based on the combination of centrifugation in a tube with a separator gel and a series of differential centrifugations. This technique will be compared to the conventional post-culture MALDI-TOF identification technique to evaluate its performance and discuss its possible routine implementation to quickly transmit preliminary results of bacterial identifications to clinicians.

Methods

Study design

This prospective study was conducted at the Central Laboratory of Bacteriology, Serology and Hygiene of the IBN SINA Hospital Center in Rabat, from March 1, 2023 to March 1, 2024. During this period, 324 blood cultures were selected for additional analysis using the Direct in-house method, after being detected as positive by the automated incubation system BACTEC-FX ™ (Becton Dickinson, USA) and confirmed to be monomicrobial by Gram staining (11).

Post culture conventional identification

Consists of the inoculation of positive blood cultures on culture media (Chocolate agar, CLED and Chapman salt agar) for a period of 18-24 h at 36 ± 1◦ C, then the identification from the isolated colonies was performed by MALDI-TOF MS following the manufacturer's instructions (12).

Direct In-house method

To obtain a concentrated and pure pellet of bacteria directly from selected blood cultures; several steps were followed:

- Centrifugation of 3.5 ml of the positive blood culture using a sterile tube with Separator Gel (Vacutest®) at 4000 rpm for 5 min, the aim of this step is to sediment the cells and other debris at the bottom of the tube and concentrate the bacteria on the surface of the gel.
- The bacteria are collected from the surface of the gel, re-suspended in 3ml of distilled water for washing and re-centrifuged at low speed (1000 rpm for 1 minute) to eliminate any other cells persisting in the bacterial suspension.
- The supernatant is collected and recentrifuged this time at high speed (13,000 rpm for 5 min), and then the supernatant is eliminated.
- The pellet obtained is washed a second time with distilled water then subjected to another centrifugation at high speed (13,000 rpm for 1 min), after which the supernatant is delicately removed and the pellet is collected.
- Using a 1 µL inoculation loop a small amount of the obtained pellet is smeared onto an empty position on the MALDI target plate. The materiel is overlayed with 1 µL of 70% formic acid and dried at room temperature.
- Within 30 min after drying, material is Overlayed with 1.0 µL of alpha-Cyano-4-hydroxycinnamic acid (HCCA) matrix solution and dried at room temperature (13).

MALDI-TOF processing

Protein analysis of the bacterial pellet was done using mass spectrometer MALDI Microflex LT (BrukerDaltoniK GmbH, Bremen, Germany) with FLexControl v. 3.0 software (Bruker DaltoniK GmbH, Bremen, Germany).

For each identification series, a BTS (standard bacterial test) quality control was carried out according to the manufacturer's instructions.

The interpretation of the MALDI-TOF identification results was done by applying the scores proposed by the manufacturer as follows: a score greater than 2 indicated species identification, a score in the range 1.7–1.99 indicated genus identification, and a score <1.7 indicated no identification (14).

Ethical Considerations

The study adhered to the ethical guidelines outlined in the Helsinki Declaration and did not require formal ethics approval or informed consent. It used anonymized

blood culture samples intended for disposal, focusing on comparing two diagnostic methods without patient intervention or sharing results with treating physicians. The research aimed to improve diagnostic methods while ensuring patient anonymity.

Results

The post-culture method, considered the gold standard technique in our comparison, successfully identified all bacteria from all 324 blood cultures included in this study; versus 210 blood cultures bacteria identified by the direct MALDI-TOF method. Bacteria were classified into Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB).

For the Culture Method:
- GNB Identification Rate: 100% (77.53% at the species level)
- GPB Identification Rate: 100% (51.91% at the species level)

For the Direct Method:
- GNB Identification Rate: 88.76% (48.31% at the species level)
- GPB Identification Rate: 55.74% (12.34% at the species level)

The table 1, summarizes the bacteria identified by applying each of the methods described previously (direct MALDI-TOF and Post-culture MALDI-TOF) on the bacteria isolated from the selected blood cultures, according to the scores of the identification levels and the type of Gram staining of the bacteria identified.

Table 1. Identified bacteria from positive blood cultures by the Post culture and Direct MALDI-TOF methods.

	Poste culture MALDI-TOF method			Direct MALDI-TOF method		
Identified Bacteria	**≥2.0 Species ID**	**1.7-1.99 Genus ID**	**<1.7 No ID**	**≥2.0 Species ID**	**1.7-1.99 Genus ID**	**<1.7 No ID**
Staphylococcus aureus	26	19	0	8	21	16
Staphylococcus haemolyticus	31	34	0	6	27	32
Staphylococcus hominis	34	29	0	5	29	29
Staphylococcus epidermidis	20	22	0	7	16	19
Staphylococcus capitis	2	1	0	1	1	1
Staphylococcus saprophyticus	1	1	0	0	1	1
Enterococcus faecalis	6	5	0	2	5	4
Enterococcus faecium	2	2	0	0	2	2
TOTAL GPB	**122 (51.91%)**	**113 (48.09%)**	**0 (0%)**	**29 (12.34%)**	**102 (43.40%)**	**104 (44.26%)**
Klebsiella pneumoniae	17	6	0	8	12	3
Klebsiella oxytoca	4	1	0	2	2	1
Pseudomonas aeruginosa	8	3	0	6	4	1
Escherichia coli	12	3	0	8	5	2
Acinetobacter baumannii	17	4	0	11	8	2
Enterobacter cloacae	11	3	0	8	5	1
TOTAL GNB	**69(77.53%)**	**20 (22.47%)**	**0(0%)**	**43(48.31%)**	**36 (40.45%)**	**10 (11.24%)**
Total Bacteria (324)	**191 (59.0%)**	**133 (41.0%)**	**0 (0%)**	**72 (22.2%)**	**138 (42.6%)**	**114 (35.2%)**

Discussion

The culture method demonstrates strong overall performance, particularly for GNB, with a high species-level identification rate. Its balanced identification of GPB at both species and genus levels indicates its reliability. However, the necessity for pure colonies and a 24-hour incubation period means that it is not suitable for urgent cases where immediate identification is needed.

The direct method identified fewer GNB (79) compared to the culture method (89). The species-level identification rate was lower (48.31%), and the genus-level identification rate was 88.76%. Additionally, 11.24% of GNB were not identified.

The direct method showed significant limitations for GPB identification. Only 131 out of 235 GPB were identified, with a very low species-level identification rate (12.34%). The genus-level identification rate was higher (55.74%), but a substantial portion (44.26%) of GPB was not identified.

The lower identification performance, and the high rate of unidentified GPB are consistent with the results of other similar studies and can be attributed to the rigidity of the GPB bacterial cell wall, which, despite the partial extraction on the MALDI target step, did not lead to a significant improvement in the identification rate. Some studies explain this difficulty in identifying GPB by their attachment to red blood cells (9,15). Generally, better purification of the bacterial pellet and the elimination of other elements present in blood could improve the performance of this direct method. The use of a filtration step, lysing solutions (such as sodium dodecyl sulfate or saponin) and short-term incubation are potential solutions that have demonstrated their effectiveness (16,17).

The comparative analysis between the culture method and direct method reveals key insights into their performance and applicability in clinical settings. Overall, the culture method, as the gold standard, outperforms the direct method in both GNB and GPB identification; it is essential for detailed and definitive diagnoses but requires 24 hours for pure colony growth and is not suitable for urgent cases where immediate results are needed. The direct method's higher rate of unidentified

bacteria and restriction to identifying only monomicrobial positive blood cultures is a significant drawback but is balanced by its speed with rapid results within an hour, making it suitable for emergency cases where immediate identification is necessary to guide initial treatment decisions and reduce false empirical treatments. By providing quick preliminary results, the direct method can help bridge the gap until more definitive results from the culture method are available.

Conclusion

The culture method remains the gold standard for bacterial identification due to its high accuracy and comprehensive identification capabilities, especially for GNB and GPB. However, its requirement for pure colonies and a 24-hour incubation period limits its use in urgent situations. The direct method, despite its lower performance, offers valuable rapid identification that can be critical in emergency settings. Its ability to provide quick results can reduce the reliance on empirical treatments and allow for more informed initial clinical decisions. Combining both methods can optimize patient outcomes, with the direct method providing immediate insights and the culture method offering definitive, detailed identification.

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