ORIGINAL ARTICLE

Efficacy of Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry for Rapid Bacterial Identification of Positive Blood Culture

Mohammed A. Jeraiby, MBBS, MD

Department of Biochemistry, Faculty of Medicine Jazan University
Microbiology Laboratory, Prince Mohammed bin Nasser Hospital
Jazan, Saudi Arabia

Correspondence
Dr. Mohammed A. Jeraiby
Department of Biochemistry
Faculty of Medicine, Jazan University
P.O. Box 114, Jazan 45142
Saudi Arabia
e-M: mmojer@jazanu.edu.sa
mojer2011@hotmail.com

Submission: 22 May 2020
Accepted: 05 Oct 2020

Citation
Jeraiby MA. Efficacy of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid bacterial identification of positive blood culture. JKAU Med Sci 2020; 27(2): 27-32. DOI:10.4197/Med.27-2.5

Abstract

The main objective of this study was to evaluate the identification of microorganisms from positive blood cultures by using the Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry. In this prospective study, positive blood cultures were collected between March and November 2019 at the Prince Mohammed bin Nasser Hospital in Jazan. Rapid identification by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry was obtained the same day from blood agar plates after a shortened incubation period of a maximum of three hours. Two hundred ninety-three (73.6%) isolated micro-organisms were correctly identified, of which 198 (67.57%) were Gram-positive and 95 (32.42%) Gram-negative. Incorrectly identified ones were obtained from young colonies where the Matrix-Assisted Laser Desorption/Ionization score was < 2 in 68 bottles. A polymicrobial-blood stream infection was observed in 11 (1.6%) cases only. Our data demonstrated a high rate of identification of microorganisms within three hours of subculturing of positive blood culture bottles.

Keywords

Rapid bacterial identification; Blood culture; Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

Introduction

Blood stream infections contribute considerably to mortality in Medical Care Units, and any delay in diagnosis and proper treatment increases the risk of death[1]. Microbiologists must identify blood cultures isolated correctly and as early as possible. Identifying pathogens quickly and accurately in the clinical laboratory is vital because this helps clinicians choose effective antimicrobial therapies and reduces the length of hospital stays[2]. Kumar *et al.*[2] showed that approximately 20% of patients with septic shock were initially given inadequate antimicrobial therapy, which was associated with a five-fold reduction in survival. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex LT, Bruker Bremen, Germany) enables the rapid identification of organisms after isolation from clinical specimens. We evaluated whether MALDI-TOF MS correctly identifies isolates from positive blood culture bottles subcultured on a blood agar plate after a 3-hour incubation period. It accurately identified bacterial and yeast species and appeared to be an

alternative time-saving conventional testing method with a positive impact on patient outcomes when implemented correctly[3-7].

Method

Patients: the blood cultures BACTEC™ Plus Anaerobic/F Culture Vials (Becton, Dickinson and Co., Sparks, MD USA) were taken from inpatients at the Prince Mohammed bin Nasser Hospital in Jazan, Saudi Arabia between March and November 2019 for a prospective study. The processing of MALDI-TOF MS was conducted at the Microbiology Laboratory at CHU Saint-Etienne, France. All blood positive cultures that were detected during the workday of the technicians involved in the study were included. Samples were limited to a single vial per puncture site per patient per day to increase the probability of detection. The inclusion criteria were all cases that presented clinical pictures of septicemia. The exclusion criteria were patient samples with no symptoms or signs of septicemia, samples with HIV, viral hepatitis, debilitating diseases and/or cancer.

Microbiological procedures: bottles received at the Microbiology Laboratory of the Prince Mohammed bin Nasser Hospital were incubated in automated BACTEC™ 9240 blood culture instruments (Becton, Dickinson and Co., Sparks, MD USA) at 37°C. Positive blood culture bottles detected by the instrument were processed in the laboratory using Gram staining and were subcultured on blood agar plates, which were incubated under aerobic and anaerobic conditions until growth was noticed. The Granada agar plate (GAP; Biomedics SL, Madrid, Spain) incubated under appropriate conditions were read after three hours of incubation. Colonies observed on agar plates within three hours were identified using MALDI-TOF MS and MALDi Biotyper v3.0 software (Bruker Daltonik GmbH, Bremen, Germany) combined with the 3573-spectrum database. Briefly, colonies observed on agar plates were picked up using tips, smeared onto the target, and dried at room temperature. Then, they recovered with 1.2 uL of HCCA α-cyano-4-hydroxycinnamic acid-based matrix (Bruker Daltonik GmbH, Bremen, Germany) and dried at room temperature prior to MALDI-TOF MS analysis.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification after short incubation-period: MALDI Biotyper scores of ≥ 2 were used to identify organisms at the species level. Scores under 2 were rejected.

Data of the patients was collected according to the ethical approval at KAUH; which was obtained from KAUH Ethical and Technical Committee (No. 1316-13).

Ethical considerations were followed in agreement with the Declaration of Helsinki throughout this study and it was approved by the Research Committee / Jazan Hospital IRB (H-10-Z-068), Kingdom of Saudi Arabia (Reference No. 2005).

All patients provided written informed consent and were assured about the confidentiality of their personal information.

MedCalc Statistical Software version 13.1.2 (MedCalc Software, Ostend, Belgium) was used to conduct statistical analyses.

Results

In this study, the BACTEC™ 9240 instrument identified as positive blood culture bottles from 8,390 patients. Of 670 clinical isolates, 272 (40.6%) could not be tested because the laboratory was not open around the clock. The remaining 398 (59.4%) were tested using MALDI-TOF MS on the same day after three hours of incubation. Of these, 293 (73.6%) had a positive monomicrobial score of ≥ 2, of which 198 (67.57%) were correctly identified as Gram-positive and 95 (32.42%) as Gram-negative species (Table 1). Direct identification showed that the dominant microorganisms were *Staphylococcus aureus* (76, or 25.9%), *Staphylococcus epidermidis* (73, or 24.9%), and *Escherichia coli* (64, or 21.8%). Young colonies with MALDI scores of < 2 occurred in 68 bottles. Of these, 20 (29.4%) were incorrectly identified as *Staphylococcus epidermidis* (Table 1).

Of the 398 bottles tested, 37 (9.29%) showed no colonies after three hours of incubation. These were identified after overnight incubation under appropriate conditions according to manufacturer recommendations. Species in these bottles included *Escherichia coli* (8), *Staphylococcus aureus* (4), *Corynebacterium amycolatum* (4), *Staphylococcus epidermidis* (4), *Candida albicans* (3), *Staphylococcus warneri* (3), *Staphylococcus capitis*, *Streptococcus pneumoniae*, *Peptoniphilus harei*, *Candida krusei*,

Table 1. All pathogens detected in positive blood culture bottles which were identified after a three-hour incubation of subculture agars with a score of ≥ 2 or <2

Micro-organism Identification			
Score ≥ 2	**No.**	**Score < 2**	**No.**
Staphylococcus aureus	76	*Staphylococcus epidermidis*	20
Staphylococcus epidermidis	73	*Staphylococcus aureus*	8
Escherichia coli	64	*Gemella spp*	4
Klebsiella pneumoniae	21	*Streptococcus pneumoniae*	4
Streptococcus pneumoniae	11	*Lactobacillus kefiri*	4
Enterococcus faecalis	10	*Abiotrophia defectiva*	4
Staphylococcus hominis	4	*Enterococcus faecium*	3
Enterobacter cloacae complex	4	*Escherichia coli*	3
Staphylococcus capitis	3	*Staphylococcus capitis*	3
Staphylococcus haemolyticus	3	*Enterococcus faecalis*	2
Clostridium difficile	3	*Streptococcus mutans*	2
Serratia marcescens	3	*Staphylococcus equorum*	2
Streptococcus gallolyticus	2	*Streptococcus constellatus*	2
Streptococcus parasanguinis	2	*Streptococcus gallolyticus*	1
Enterococcus faecium	2	*Klebsiella pneumoniae*	1
Enterococcus gallinarum	2	*Staphylococcus massiliensis*	1
Listeria monocytogenes	2	*Propionibacterium*	1
Propionibacterium	2	*Haemophilus influenza*	1
Enterobacter ludwigii	2	*Corynebacterium amycolatum*	1
Staphylococcus caprae	1	*Staphylococcus lugdunensis*	1
Staphylococcus warneri	1		
Streptococcus mitis/oralis	1		
Proteus mirabilis	1		

Acinetobacter ursingii, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus pettenkoferi*, *Streptococcus gordonii*, *Lactobacillus rhamnosus*, and *Lactobacillus fusarium*. In 11 bottles, a polymicrobial-blood stream infection with two microorganisms was identified.

Discussion

To guide appropriate treatment for patients with septicemia, it is necessary to identify microorganisms in a short period of time. In addition, it is important to avoid blood culture contamination, which leads to unnecessary antimicrobial treatment and a prolonged hospital stay[8]. In total, this study had a rate of success of 73.6% after a single run of MALDI-TOF MS within three hours of subculture. The causative microorganisms were successfully identified and validated by a microbiologist without the need for additional phenotypic galleries. This was in line with a previous study reported by Zabbe *et al*.[9] in 2015, in which 77% of positive blood bottles were correctly identified using MALDT-TOF MS after three hours of incubation. Other studies have reported the correct identification of isolates from positive blood bottles using MALDT-TOF MS after five hours of incubation of subculture plates[10,11]. We excluded 11 bottles yielding a co-infection in which two microorganisms were identified. This is often due to contaminant pathogens when more than one organism is present.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has changed the process of bacterial identification, as it allows a quicker delivery of more precise results than conventional methods[12,13]. In many publications, MALDI-TOF MS technology has been shown to be superior to conventional methods for acceptable identification at the genus and species levels for isolated causative microorganisms[14-17]. However, it has limitations with the identification of certain bacteria, like alpha-hemolytic streptococci (*i.e.,* the *Streptococcus mitis* group and *Streptococcus pneuomniae*) and *Listeria spp*, which are regularly misidentified by mass spectrometry[18-20]. In our agreement percentages, *Staphylococcus aureus* and *Staphylococcus epidermidis* were most seen after the first day of incubation.

In conclusion, the identification of isolated microorganisms from positive blood culture bottles after a short-term incubation of subcultured blood agar plates is possible by using MALDI-TOF MS. In

this study, 73.6% of bacteria were correctly identified at the species level after three hours of incubation of subcultured agar plates.

Conflict of Interest

The author declared that there is no conflict of interest that is related to this study and this article.

Disclosure

The author did not receive any type of commercial support either in the form of compensation or financial support for this case report. The author has no financial interest in any of the products, devices, or drugs mentioned in this article.

Ethical Approval

The study was approved by the Ethics Committee of the KAUH in Jeddah, Kingdom of Saudi Arabia, also known as the Institutional Review Board of Hospitals.

References

[1] Retamar P, Portillo MM, López-Prieto MD, Rodríguez-López F, de Cueto M, García MV, Gómez MJ, Del Arco A, Muñoz A, Sánchez-Porto A, Torres-Tortosa M, Martín-Aspas A, Arroyo A, García-Figueras C, Acosta F, Corzo JE, León-Ruiz L, Escobar-Lara T, Rodríguez-Baño J; SAEI/SAMPAC Bacteremia Group. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. Antimicrob Agents Chemother 2012; 56(1): 472-478.

[2] Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, Dodek P, Wood G, Kumar A, Simon D, Peters C, Ahsan M, Chateau D; Cooperative Antimicrobial Therapy of Septic Shock Database Research Group. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 2009; 136(5): 1237-1248.

[3] Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010; 48(2): 444-447.

[4] Marklein G, Josten M, Klanke U, Müller E, Horré R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol 2009; 47(9): 2912-2917.

[5] Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA, Peterson LE, Musser JM. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med 2013; 137(9): 1247-1254.

[6] Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective evaluation of a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. J Clin Microbiol 2012; 50(10): 3301-3308.

[7] Vlek AL, Bonten MJ, Boel CH. Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One 2012; 7(3): e32589.

[8] Murray PR, Masur H. Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Crit Care Med 2012; 40(12): 3277-3282.

[9] Zabbe JB, Zanardo L, Mégraud F, Bessède E. MALDI-TOF mass spectrometry for early identification of bacteria grown in blood culture bottles. J Microbiol Methods 2015; 115: 45-46.

[10] Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K. Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. Clin Microbiol Infect 2014; 20(10): 1001-1006.

[11] Verroken A, Defourny L, Lechgar L, Magnette A, Delmée M, Glupczynski Y. Reducing time to identification of positive blood cultures with MALDI-TOF MS analysis after a 5-h subculture. Eur J Clin Microbiol Infect Dis 2015; 34(2): 405-413.

[12] Carbonnelle E, Beretti JL, Cottyn S, Quesne G, Berche P, Nassif X, Ferroni A. Rapid identification of Staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2007; 45(7): 2156-2161.

[13] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009; 49(4): 543-551.

[14] Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr AM. Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin Lab 2009; 55(7-8): 289-296.

[15] Bizzini A, Jaton K, Romo D, Bille J, Prod'hom G, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. J Clin Microbiol 2011; 49(2): 693-696.

[16] Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, Iwen P, Dunn J, Hall G, Wilson D, Lasala P, Kostrzewa M, Harmsen D. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification

of nonfermenting bacteria. J Clin Microbiol 2008; 46(6): 1946-1954.

[17] Biswas S, Rolain JM. Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. J Microbiol Methods 2013; 92(1): 14-24.

[18] La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One 2009; 4(11): e8041.

[19] van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010; 48(3): 900-907.

[20] Steensels D, Verhaegen J, Lagrou K. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of bacteria and yeasts in a clinical microbiological laboratory: a review. Acta Clin Belg 2011; 66(4): 267-273.

فعالية مصفوفة بمساعدة الليزر الامتزاز / التأين وقت الرحلة الطيف الكتلي للالسريع البكتيرية تحديد إيجابي ثقافة الدم

محمد جريبي

مختبر الأحياء الدقيقة بمستشفى الأمير محمد بن ناصر
قسم الكيمياء الحيوية
كلية الطب، جامعة جازان
جازان ـ المملكة العربية السعودية

المستخلص.

المستخلص. كان الهدف الرئيسي من هذه الدراسة هو تقييم التعرف على الكائنات الحية الدقيقة من مزرعة الدم الإيجابية عن طريق استخدام جهاز مصفوفة الليزر بمساعدة التأين بالليزر في زمن الطيف الكتلي المصفوفة بمساعدة الليزر الامتصاص / التأين وقت الطيران الكتلي. أجرينا دراسة مستقبلية بين مارس ونوفمبر ٢٠١٩ في مستشفى الأمير محمد بن ناصر في جازان حيث تم التعرف على مزرعة دم إيجابية. تم الحصول على تحديد سريع بواسطة جهاز مالدي توف الكائنات الدقيقة في نفس اليوم من أطباق أجار فترة حضانة لأطباق أجار الدم تقل عن ٣ ساعات بعد المصفوفة بمساعدة الليزر الامتصاص / التأين وقت الطيران الكتلي. تم عزل الكائنات الحية الدقيقة بعدد مائتان وثلاثة وتسعون (٧٣,٦٪) بشكل صحيح، بما في ذلك ١٩٨ إيجابية الغرام (٦٧,٥٧٪) و ٩٥ (٣٢,٤٢٪) سلبية الغرام. ٦٨ عزلة لم يتم التعرف عليها بشكل صحيح حيث تم الحصول عليها من المستعمرات الشابة مع درجة كانت أقل من ٢. وقد لوحظ وجود عدة كائنات دقيقة في إحدى عشرة حالة (١,٦٪) فقط. أظهرت بياناتنا ارتفاع معدل تحديد الكائنات الحية الدقيقة في غضون ساعات قليلة من حضانة أطباق الآجار من القوارير الإيجابية لمزارع الدم.