ORIGINAL RESEARCH

Clinical Application of Matrix-Assisted Laser-Resolved Ionization Time-Of-Flight Mass Spectrometry for Rapid Detection of Blood-Borne Pathogens

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ABSTRACT

Objective • To evaluate the performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) in the identification of clinical pathogenic microorganisms.

Methods • Blood culture-positive specimens were collected from inpatients in our hospital from March to December 2022 and identified using VITEK 2XL (biochemical), VITEK MS (colony), VITEK MS (bacterial membrane) and VITEK MS (separating gel) methods, respectively, to compare the compliance rate and identification values of the four methods.

Results • A total of 280 strains were included in the analysis, including 155 (55.36%) Gram-negative and 125 (44.64%) Gram-positive strains. 279 (99.64%) of the 280 strains were identified by VITEK 2XL (biochemical), including 154 (99.35%) Gram-negative and 125 (100%) Gram-positive strains. VITEK MS (colony) identified 278 (99.29%) strains, including 153 (98.71%) Gram-negative and 125 (100%) Gram-positive. 261 (93.21%) strains were identified in VITEK MS (bacterial membrane), including 148 (95.48%) Gram-negative and 113 (90.40%) Gram-positive strains. VITEK MS (separating gel) identified 232 (82.86%) strains, including 136 (87.74%) Gram-negative and 96 (76.80%) Gram-positive strains.

Conclusion • MALDI-TOF MS findings are highly consistent with traditional culture identification methods in terms of identification accuracy, and the VITEK MS (bacterial membrane) and VITEK MS (separating gel) identification methods significantly reduce the turnaround time for identification in the laboratory. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Blood culture (BC) is the gold standard and tool used for the diagnosis of pathogens causing bloodstream infections (BSIs) in clinical microbiology. Traditional BC identification needs incubation of blood culture bottles, transformation culture, and biochemical identification. In most cases, accurate and reliable results can't be obtained within 18-24 hours after a blood culture is positive, and analysis may take longer for slow-growing bacteria or fungi during the culture.[1] Patients' chance of survival decreases by 7.6% for every hour of delay in the use of appropriate antibiotics, whereas patients treated with inappropriate antibiotics have a roughly fivefold reduction in survival.[2] Rapid and accurate identification of the pathogen can more quickly determine the appropriate treatment for BSI and improve patients' survival.[3]

Modern mass spectrometry technology has enhanced the understanding of whole biological systems through direct analysis of biological molecules such as microbial proteins, lipids, carbohydrates, and amino acids, and it has been applied in the field of life sciences.[4,5] Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), as a new technique, has been widely used in clinical microbial diagnosis in the last ten years. It is gradually replacing the traditional biochemical identification methods.[6,7] MALDI-TOF MS is a rapid mass spectrometry technique developed in the late 1980s with relatively high sensitivity to various sample types, which is a tool for rapid and accurate analysis in conventional laboratories. It has already been used to identify mycobacteria,[8,9] nocardia,[10]

yeasts,[11] mucorales,[12] and anaerobes[13] isolated from solid media of clinical specimens. MALDI-TOF MS is applicable to clinical microbiology laboratory, especially to routine identification of pure colonies cultured on solid medium and direct identification of microorganisms in blood culture. In this study, we evaluated the reliability and validity of MALDI-TOF MS as a routine diagnostic method for the identification of pathogens in clinical blood culture, as well as its role in rapid identification of pathogens.

MATERIALS AND METHODS

Strains

Blood culture positive specimens of inpatients from March to December 2022 were collected and identified. Inclusion criteria: The same patient was sent for multiple bottles of blood culture, and the first positive sample was obtained from the automatic blood culture instrument. Exclusion criteria: Routine Gram staining microscopy was performed on the positive blood culture bottle, if the results indicate no bacterial infection, or if the results indicate infection due to two or more types of pathogens.

Instruments and Reagents

BacT/Alert 3D 480 automatic blood culture system (BioMérieux, France), VITEK MS IVD 3.0 automatic rapid microbial mass spectrometry identification system (BioMérieux, France), VITEK MS original matching matrix solution (α-cyano-4-hydroxycinnamic acid), 70% formic acid (BioMérieux, France), separating gel coagulation tube (BD Biosciences, United States), VITEK automatic rapid microbial identification drug sensitivity system. *Escherichia coli* (ATCC 8739); aerobic and facultative anaerobic microorganism culture bottle, and anaerobic and facultative anaerobic microorganism resin culture bottle (BioMérieux, France). Colombia blood agar plate, eosin methylene blue agar plate, and chocolate agar medium (without antibiotics).

VITEK MS Mass Spectrometry Direct Identification after Separating Gel Coagulation Tube Pretreatment

(1) Draw 3.0 mL bacterial solution from the positive blood culture bottle into the separation rubber tube, and centrifuge at 1600xg for 10 min. Discard the supernatant, add 1.0 mL deionized water to resuspend the pellet, transfer to a 1.5 mL Ep centrifuge tube, centrifuge at 12000xg for 3 min, and discard the water and cells in the upper layer. Resuspend the pellet again in 1.0 mL of deionized water, centrifuge at 12000xg for 3 min, and discard the water and cells in the upper layer. Add 500 μl of absolute ethanol, mix well, centrifuge at 12000xg for 3 min, discard the supernatant, remove the ethanol substance at room temperature for 2 min, and take 1 μl of precipitate for spot target mass spectrometry identification.[14] (2) Take 1 μL of colony sample and smear it on 48-well slide, dry naturally, and add 0.5 μL of 70% formic acid dropwise, dry naturally. Then, add 1 μL of α-cyano-4-hydroxycinnamic acid matrix solution dropwise, and dry naturally. (3) After complete drying, load the target plate into the mass spectrometer for identification. (4) Compare the obtained mass spectrum with the spectrum in the reference database to identify the species, and record the VITEK MS (separating gel) identification result.

Identification of Biofilm and Colony after Culture

Mix the bacterial solution of the blood culture bottle with positive results, inoculate it on the culture dishes (blood plate and eosin methylene blue plate), and then place it in a 35°C incubator with CO_2 for culture. After incubation for 4h-6h, a layer of biofilm was formed, and VITEK MS mass spectrometry identification was directly carried out with the biofilm, then the VITEK MS (biofilm) result was recorded. Incubation was then continued for 18h-24h to form a single colony, which was then used for simultaneous VITEK MS mass spectrometry identification and VITEK 2XL (biochemical) identification, and the VITEK MS (colony) and VITEK 2XL (biochemical) results were recorded.

16S rRNA Identification

Individual colonies were identified by VITEK MS mass spectrometry and VITEK 2XL (biochemical), and when the results of the two methods were inconsistent, the type of strain was identified by 16S rRNA sequencing. The strains were submitted to Beijing Ruibiotech Co., Ltd. for sequencing. The obtained results were compared with the existing sequences in National Center for Biotechnology Information (NCBI) database and 16SrRNA sequencing was used as the final reference method.

RESULTS

Comparison of Identification Results of 280 Strains of Pathogenic Bacteria in Blood Culture

In this study, 298 positive blood culture specimens were collected, 12 bottles of mixed infection were excluded, 6 bottles of false positive were excluded, and 280 strains of pathogenic bacteria were included in the analysis. The identified pathogens were divided into two categories, including 155 strains of Gram-negative bacteria (55.36%) and 125 strains of Gram-positive bacteria (44.64%). Among 280 strains, the VITEK 2XL (biochemical) identification method identified a total of 279 (99.64%), including 154 (99.35%) Gram-negative strains and 125 (100%) Gram-positive strains. The VITEK MS (colony) identification method identified a total of 278 strains (99.29%), including 153 strains (98.71%) of Gram-negative bacteria and 125 strains (100%) of Gram-positive bacteria. The VITEK MS (bacterial membrane) identification method identified 261 strains (93.21%), including 148 strains (95.48%) of Gram-negative bacteria and 113 strains (90.40%) of Gram-positive bacteria. The VITEK MS (separating gel) identification method identified 232 (82.86%) strains, including 136 (87.74%) Gram-negative strains and 96 (76.80%) Gram-positive strains (See Table 1). Different protein peak maps were generated by direct mass spectrometry identification after pretreatment of gel separator, bacterial membrane mass

Figure 1. Results of Mass Spectrometric Identification of *Escherichia coli* A: Results of Direct Mass Spectrometric Identification After Gel-Enhanced Tube Pretreatment; B: Results of Bacterial Membrane Mass Spectrometric Identification After 4h-6h Culture; and, C: Results of Single Colony Mass Spectrometric Identification After 18h-24h Culture.

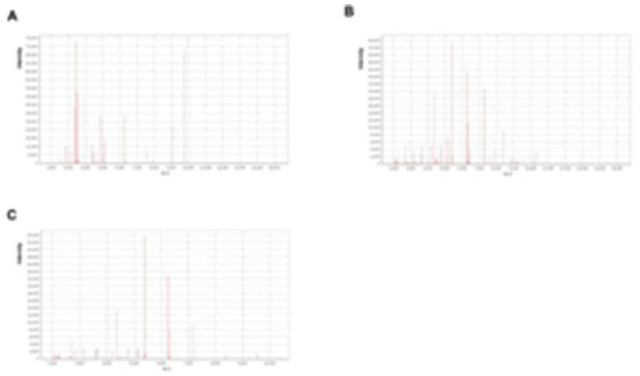
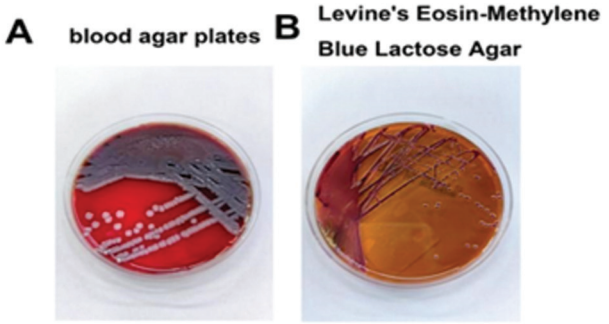


Figure 2. Single Colony Formed in Blood Plate and Eosin Plate After 18h-24h Culture of *Escherichia coli.*



spectrometry identification after 4h-6h of culture, and single colony mass spectrometry identification after 18h-24h of culture. The position, size, and shape of the peaks were different but the confidence of identification results was 99.9%. The mass spectrometry identification results of *Escherichia coli* are shown in Figure 1.

The individual colonies formed by *Escherichia coli* on blood and eosin methylene blue agar plates after 18h-24h of incubation can be seen in Figure 2.

Comparison of Identification Results of 155 Strains of Gram-Negative Bacteria

VITEK 2XL (biochemical) identified 154 (99.35%) of 155 Gram-negative bacteria; one strain of *Chryseobacterium indologenes* was not identified. VITEK MS (colony) identified 153 strains (98.71%), of which 147 strains were identified with confidence of 60%~99%, consistent with VITEK 2XL (biochemical) identification results; 6 strains of *Enterobacter cloacae complex* were identified as *Enterobacter cloacae/ Enterobacter asburiae* with 50% confidence, and they were identified as *Enterobacter cloacae complex* by VITEK 2XL (biochemical); 2 strains of *Chryseobacterium indologenes* were not identified.

VITEK MS (bacterial membrane) identified 148 strains (95.48%), of which 142 strains were identified with confidence of 60%~99%, consistent with VITEK 2XL (biochemical) identification results; 6 strains of *Enterobacter cloacae complex* were identified as *Enterobacter cloacae/Enterobacter asburiae* with a confidence level of 50%. 2 strains of *Escherichia coli*, 1 strain of *Pseudomonas aeruginosa*, 1 strain of *Acinetobacter baumannii*, 2 strains of *Chryseobacterium indologenes*, and 1 strain of *Bacteroides ovatus* were not identified.

136 strains (87.74%) were identified by VITEK MS (separating gel), among which 132 strains were identified with confidence of 60%~99%, consistent with VITEK 2XL (biochemical) identification results; 4 strains of *Enterobacter cloacae complex* were identified as *Enterobacter cloacae/ Enterobacter asburiae* with a confidence level of 50%. 6 strains of *Klebsiella pneumoniae*, 5 strains of *Escherichia coli*, 2 strains of *Enterobacter cloacae complex*, 1 strain of *Pseudomonas aeruginosa*, 2 strains of *Acinetobacter baumannii*, 2 strains of *Chryseobacterium indologenes*, and 1 strain of *Bacteroides ovatus* were not identified (See Table 2).

Comparison of Identification Results of 125 Gram-Positive Bacteria

Out of 125 Gram-positive bacteria, a total of 125 (100%) were identified using VITEK 2XL (biochemical) identification method. A total of 125 strains (100%) were identified by VITEK MS (colony), which was consistent with VITEK 2XL (biochemical) results. The confidence was 60%~99% with only one identification result.

A total of 113 strains (90.40%) were identified by VITEK MS (bacterial membrane), which was consistent with VITEK 2XL (biochemical) results. The confidence was 60%~99% with only one identification result; 2 strains of *Staphylococcus hominis*, 2 strains of *Staphylococcus capitis*, 1 strain of *Enterococcus faecium*, 4 strains of *Streptococcus pharyngis*, 1 strain of *Streptococcus intermedius*, and 2 strains of *Corynebacterium striatum* were not identified. A total of 96 strains (76.80%) were identified by VITEK MS (separating gel), which was consistent with the VITEK 2XL (biochemical) results. The confidence was 60%~99% with only one identification result; 5 strains of *Staphylococcus aureus*, 6 strains of *Staphylococcus hominis*, 4 strains of *Staphylococcus epidermidis*, 2 strains of *Staphylococcus capitis*, 2 strains of *Staphylococcus haemolyticus,* 4 strains of *Enterococcus faecium*, 2 strains of *Enterococcus faecalis*, 2 strains of *Streptococcus anginosus*, and 1 strain of *Corynebacterium striatum* were not identified (See Table 3).

16S rRNA Identification

Two *Chryseobacterium* strains were rechecked by 16S rRNA sequencing, and the results showed that one strain was *Chryseobacterium indologenes* and the other was *Chryseobacterium gleum*. They were identified as *Chryseobacterium indologenes* by the VITEK 2XL (biochemical) identification, and were not identified by the VITEK MS (colony) identification.

Table 1. Comparison of Identification Results of 280 Strains [n (%)]

Pathogens	Total number of strains	VITEK 2XL (biochemical)		VITEK MS (colony)		VITEK MS (bacterial membrane)		VITEK MS (separating gel)	
		Number of identified strains (n)	Identification rate (%)	Number of identified strains (n)	Identification rate (%)	Number of identified strains (n)	Identification rate (%)	Number of identified strains (n)	Identification rate (%)
Gram-negative bacteria	155	154	99.35	153	98.71	148	95.48	136	87.74
Gram-positive bacteria	125	125	100	125	100	113	90.40	96	76.80
Total	280	279	99.64	278	99.29	261	93.21	232	82.86

Table 2. Comparison of Identification Results of 155 Gram-Negative Bacteria [n (%)]

Pathogens	Total number	VITEK 2XL (biochemical)		VITEK MS (colony)		VITEK MS (bacterial membrane)		VITEK MS (separating gel)	
		Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate (%)
Klebsiella pneumoniae	58	58	100	58	100	58	100	52	89.66
Escherichia coli	56	56	100	56	100	54	96.43	51	91.07
Proteus mirabilis	8	8	100	8	100	8	100	8	100
Enterobacter cloacae complex	6	6	100	6	100[a]	6	100[a]	4	66.67[a]
Klebsiella aerogenes	4	4	100	4	100	4	100	4	100
Klebsiella oxytoca	2	2	100	2	100	2	100	2	100
Serratia marcescens	2	2	100	2	100	2	100	2	100
Pseudomonas aeruginosa	10	10	100	10	100	9	90	9	90
Acinetobacter baumannii	6	6	100	6	100	5	83.33	4	66.67
Chryseobacterium indologenes/gleum	2	1	50	0	0	0	0	0	0
Bacteroides ovatus	1	1	100	1	100	0	0	0	0

[a]The identification results of mass spectrometry were *Enterobacter cloacae*/*Enterobacter asburiae* with confidence of 50%

Table 3. Comparison of Identification Results of 125 Gram Positive Bacteria [n(%)]

Pathogens	Total number	VITEK 2XL (biochemical)		VITEK MS (colony)		VITEK MS (bacterial membrane)		VITEK MS (separating gel)	
		Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate (%)	Identification number (n)	Identification rate(%)
Staphylococcus aureus	38	38	100	38	100	38	100	33	86.84
Staphylococcus hominis	16	16	100	16	100	14	87.5	10	62.5
Staphylococcus epidermidis	15	15	100	15	100	15	100	11	73.33
Staphylococcus capitis	12	12	100	12	100	10	83.33	10	83.33
Staphylococcus haemolyticus	8	8	100	8	100	8	100	6	75
Enterococcus faecium	21	21	100	21	100	20	95.24	17	80.95
Enterococcus faecalis	8	8	100	8	100	8	100	6	75
Streptococcus anginosus	4	4	100	4	100	0	0	2	50
Streptococcus intermedius	1	1	100	1	100	0	0	1	100
Corynebacterium striatum	2	2	100	2	100	0	0	0	0

DISCUSSION

Bloodstream infection is characterized by acute onset and high mortality. 58.3% of patients with BSI in the intensive care unit were caused by Gram-negative bacteria, while 32.8% by Gram-positive bacteria, 7.8% by fungi, and 1.2% by obligate anaerobes.[14] Mortality in patients with sepsis-related hypotension increases by 7.6% for every hour of delay in the use of appropriate antibiotics.[2] Therefore, rapid identification of pathogens is essential for clinical treatment of BSI and reduction of mortality. Blood culture is the gold standard for identifying the causative agent of BSI. Traditional techniques have the advantage of providing accurate species identification and susceptibility results, however, traditional blood culture identification takes a long time, requiring 24-48 hours. MALDI-TOF MS mass spectrometry has made a breakthrough in the identification of clinical pathogenic bacteria, and can identify species by comparing the protein spectrum of pathogenic bacteria with the standard spectrum of known pathogenic bacteria in the database.[15] MALDI-TOF MS is a high-throughput and efficient system for microbial identification, which can improve the reliability of microbial identification and reduce operational complexity.[15-17] A systematic review and meta-analysis showed that compared to traditional phenotypic methods, MALDI-TOF MS was associated with 22.86 hour reduction in microbial identification time, 5.07 hour reduction in effective antibiotic treatment time, 23% reduction in mortality, 0.73 day reduction in hospital stay, and $4140 saving in hospitalization costs.[16] Thus, MALDI-TOF MS is a cost-effective system for BSI patients.

The results of this study showed that MALDI-TOF MS could provide accurate results quickly and timely when detecting the positive strains of clinical blood culture. The total accuracy was basically the same as that of traditional identification methods. VITEK MS (colony) and VITEK 2XL (biochemical) identification methods were highly consistent, and the accuracy reached 99.28%. VITEK MS identification was insufficient for identifying *Chryseobacterium*. *Bacteroides ovatus*, *Streptococcus anginosus*, *Streptococcus intermedius*, and *Corynebacterium striatum* did not grow obvious bacterial membrane, so they were not identified by mass spectrometry.

MALDI-TOF MS is widely used for routine identification of microbial pathogens and is gradually replacing the existing identification methods, including biochemical and 16S rRNA gene sequencing, leading to rapid clinical diagnosis.[17] Our results showed that MALDI-TOF MS significantly reduces intra-laboratory identification turnaround time. Early and definitive microbial identification provided to physicians is extremely important for patient recovery, enabling physicians to select appropriate targeted drug therapy earlier in the

clinical treatment process, thereby exerting a positive impact on patient care. Although MALDI-TOF MS is highly consistent with VITEK 2XL (biochemical) in terms of its accuracy, not all organisms can be accurately identified and the method cannot distinguish between organisms with highly conserved ribosomal proteins, such as *Escherichia coli* and *Shigella* species. When the identification result shows *Shigella* species, it should be confirmed by serological test in combination with the patient's clinical condition, medical history, and specimen source. When constructing the VITEK MS database, two or three bacterial strains were too similar and could not be distinguished, e.g., *Enterobacter cloacae* and *Enterobacter asburiae*, so the confidence was only 50%. Similarly, the identification of *Streptococcus mitis* and *Streptococcus oralis* was also associated with 50% confidence. In our data, 6 strains of *Escherichia cloacae complex* were identified as *Escherichia cloacae/Escherichia asburiae* by mass spectrometry. For slow-growing pathogenic bacteria, such as *Corynebacterium striatum*, *Streptococcus viridans*, anaerobic bacteria, etc., since no obvious bacterial membrane was grown in 4h-6h, the culture time could be extended for further study. If the organism to be tested is not included in the VITEK MS database, it is recommended to use other methods for identification. The results of this study are basically consistent with those of previous studies.[15,18]

CONCLUSION

In summary, the findings of MALDI-TOF MS is highly consistent with traditional clinical pathogenic microorganism culture identification methods in terms of identification accuracy. Further, the use of MALDI-TOF MS significantly shortens the identification turnaround time in the laboratory, helping the clinicians to select appropriate treatment methods at the earliest. Thus, MALDI-TOF MS is not only a promising tool for the clinical identification of pathogenic microorganisms in bloodstream but is also expected to play a more critical role in promoting the development of clinical microbiology.

AUTHOR DISCLOSURE STATEMENT

The authors declare no competing financial interests.

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