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Analysis of the Feasibility of Using MALDI-TOF MS Technology to Identify the Species and Drug Resistance of Pathogenic Bacteria of Sepsis

Chun Wang[1, a], Xiannian Zheng[1, b, *], Liang Chen[1, c], Weiqi Wang[1, d]

Department of Emergency Medicine, Wuhan Fifth Hospital, China

[a]80716463@qq.com, [b]zhengxiannian163@163.com, [c]1060456205@qq.com, [d]1044606240@qq.com

Abstract

Objective Rapid identification of pathogenic bacteria in the blood of septicemic patients and drug resistance analysis by matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI-TOF MSMALDI-TOF MS) drug resistance analysis. Methods in this study, The blood culture results of 100 patients with sepsis from January 2020 to January 2022 in Wuhan Fifth Hospital and the emergence of drug resistance were retrospectively analyzed, as well as the detection of pathogenic species and drug resistance based on the MALDI-TOF MS system for the isolated pathogens. Results 228 strains of pathogens were isolated and cultured, including 110 strains of Gram-positive bacteria, mainly Staphylococcus aureus and Streptococcus pneumoniae. 118 strains of gram-negative bacilli, mainly multidrug-resistant Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. The resistance rates of Staphylococcus aureus to cefuroxime, cefdizime, tazobactam, tegacyclin, ciprofloxacin and amikacin were 40.28%, 43.06%, 44.44%, 12.33%, 76.92% and 48.12% respectively. The resistance rates of multidrug resistant Acinetobacter baumannii to cefuroxime, tazobactam, cefoperazone, tegacyclin, ciprofloxacin, imipenem and meropenem were 88.24%, 36.46%, 15.13%, 11.76%, 95.54%, 55.63% and 55.63% respectively. The resistance rates of Escherichia coli to imipenem and meropenem were 16.6%. The resistance rates of Klebsiella pneumoniae to cefuroxime, tazobactam, cefoperazone, tegacyclin and amikacin were 49.80%, 55.17%, 16.24%, 14.30% and 38.42% respectively. The resistance rates of Pseudomonas aeruginosa to tazobactam, tegacyclin, imipenem and meropenem were 25.70%, 100%, 20% and 15% respectively. The incidence of drug resistance of other pathogens was not high (all less than 10%). A total of 228 strains of six pathogenic bacteria isolated and cultured were tested for their resistance to antibiotics by traditional K-B method and MALDI-TOF, and the results of the two groups were compared. The results of the two groups were subjected to independent sample t-test. There was no significant statistical difference in the resistance rates obtained by the two methods (P value was greater than 0.05). The ROC curve analysis of the two groups was carried out. The area under the ROC curve obtained by K-B method was 0.638, The area under the MALDI-TOF curve was 0.628, and there was no significant difference between the two (P value 0.403). Conclusion MALDI-TOF MS technique can effectively identify pathogenic bacteria and analyze drug resistance in sepsis patients with reliable results, which is expected to provide a reference for the treatment of sepsis patients.

Keywords

MALDI-TOF MS; Sepsis; Antibiotics selection; Drug resistance.

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1. INTRODUCTION

Infection caused by sepsis is one of the common causes of shock in patients clinically. Research results have shown that the mortality of septic shock is as high as 54% [1-3]. In order to improve the prognosis of these patients, early initiation of appropriate antibiotic treatment is the most important measure. Once sepsis is diagnosed, it needs to be treated empirically in time, and the use of antibiotics should be adjusted according to the subsequent drug sensitivity results [4]. The application of matrix assisted laser desorption ionization time of flight mass spectrometer (MALDI-TOF MS) technology in clinical microbial identification has greatly shortened the time required for identification of pathogenic bacteria compared with traditional blood culture technology [5-7]. With the emergence of gram-negative bacteria with multiple drug resistance, it is far from enough to determine the types of pathogens. Therefore, it is an urgent problem to find a method for early identification of antibiotic sensitivity in the treatment of sepsis. At present, MALDI-TOF MS technology is mainly used in microbial identification, drug development and monitoring, gene polymorphism research, gene mutation detection and other fields [8-12]. In this study, MALDI-TOF MS technology was used to identify pathogens and analyze the feasibility of this technology in identifying the distribution of pathogens and drug resistance analysis in septic patients.

2. MATERIALS AND METHODS

2.1. General information

Collect the blood of 100 patients with sepsis in our hospital from January 2020 to January 2022 and conduct blood culture. There are 57 males and 43 females, with an average age of 44.1 ± 9.0 years. Among them, there are 48 cases of pulmonary infection, 25 cases of urinary system infection, 16 cases of biliary tract infection, 5 cases of abdominal cavity infection, and 6 cases of open wound infection. The culture process is in the Bactec FX blood culture system (Becton, Dickinson, Heidelberg, Germany). All positive blood cultures were Gram stained. The pathogens found after staining were identified by MALDI-TOF MS with MALDI Sepsi typer kit (Bruker Daltonik, product No. 34036769, Germany). This study has been informed by the patient and approved by the Ethics Committee of our hospital (project ethics No. KT201705).

2.2. Methods

MALDI-TOF MS identification of bacteria and yeasts: use sterilized disposable toothpicks to pick out the monoclonal colonies growing on the blood plate or Sabao weak agar plate for 18-24 hours and smear them on the target plate, add 1uL CHCA matrix to cover the target (yeast is added with 0.5uL formic acid first), and identify the dried spots by mass spectrometry.

MALDI-TOF MS analysis: The spectrum used in Microflex LT desktop mass spectrometer (Bruker Daltonik) measurement was obtained in a linear positive mode at 60Hz laser frequency. According to the following parameters, an optimization method is established for the low quality range. The optimal parameters are: the maximum laser frequency in the acquisition range of 100~1000 Da; The accelerating voltage is 18.98 kV; IS2 voltage is 17.09 kV; In instrument calibration and analysis, WHONET 5.5 software was used for drug sensitivity results statistics. Before identification of pathogenic bacteria by MALDI-TOF MS, Escherichia coli ATCC 8739 was used as the standard strain reference.

2.3. Inclusion and exclusion criteria

Inclusive criteria: ① The clinical diagnosis of sepsis; ② The result of blood culture was positive; ③ There are drug sensitivity results; Exclusion criteria: ① The diagnosis of sepsis was inconformity; ② Blood culture results were negative; ③ No drug sensitivity results; ④ Sample contamination.

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2.4. Statistical methods

SPSS 20.0 was used for statistical analysis of the collected data. The measurement data conforming to the normal distribution were expressed as mean and standard deviation. The difference between groups was tested by independent sample t test; The counting data were expressed by percentage and number of cases. The ROC curve was drawn by R software 3.4.4 to compare the difference between the drug resistance rates detected by the two groups of test methods. The abscissa (X axis) of ROC curve is specific, also known as false positive rate (false positive rate). The closer the X axis is to zero, the higher the accuracy rate is; Longitudinal mapping (Y-axis) is sensitivity, also known as true positive rate. The larger the Y-axis is, the higher the accuracy is. The accuracy of the detection method is expressed by the area under the curve (AUC). The higher the AUC value, the higher the accuracy of the detection method. The difference was statistically significant with $P<0.05$.

3. RESULTS

3.1. Type and composition of pathogenic bacteria in blood culture of septic patients

Among 100 patients, there are 57 males and 43 females. 228 strains of pathogenic bacteria were isolated and cultured, including 110 strains of gram-positive bacteria and 118 strains of gram-negative bacteria. The specific distribution is as follows (Table 1).

Table 1. Species and composition ratio of pathogens in blood culture identified by MALDI-TOF MS

Bacterial name	Number	Composition ratio (%)	Total [n (%)]
G+ bacteria			110 (48.2)
S.aureus	78	70.9	
S.pneumoniae	32	29.2	
Gram-negative bacilli			118 (51.8)
MRAB	34	28.8	
E.coli	36	30.5	
K.pneumoniae	29	24.6	
P. Aeruginosa	19	16.1	
Total	228	100	228 (100)

This table describes the species, number and composition ratio of the detected strains.

3.2. Drug resistance of pathogens

The resistance of the pathogenic bacteria identified in the samples included in this study to various antibacterial drugs is as follows (Table 2).

3.3. Paper diffusion method (K-B method) test results to verify the MALDI-TOF test results of bacterial resistance

228 strains of six pathogenic bacteria isolated and cultured were tested for their antibiotic resistance rate by traditional K-B method and MALDI-TOF method respectively, and the results of drug resistance rate of the two groups were compared. The results of the two groups were tested by independent sample t test, and there was no significant statistical difference in the drug resistance rate (p values were greater than 0.05). The results of the two groups were

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analyzed by ROC curve. The area under the ROC curve obtained by K-B method was 0.638, and the area under the MALDI-TOF curve was 0.628, There is no significant statistical difference between the two methods (p value is 0.403). See Table 3 for the comparison results of drug resistance of six pathogens detected by the two methods and Figure 1 for the ROC curve.

Table 2. Statistics of Drug Resistance of Pathogens [n (%)]

antibiotic	S.aureus(n=78) Drug resistance rate	MRAB (n=34) Drug resistance rate	S.pneumoniae (n=32) Drug resistance rate	E.coli (n=36) Drug resistance rate	K.pneumoniae (n=29) Drug resistance rate	P. Aeruginosa (n=19) Drug resistance rate
Cefuroxime	31(40.28%)	30(88.24%)	3(9.38%)	3(8.33%)	14(49.80%)	2(9.2%)
Cefdizine	34(43.06%)	-	2(6.25%)	2(5.56%)	-	-
Tazobactam	35(44.44%)	12(36.46%)	2(6.25%)	3(8.33%)	16(55.17%)	5(25.70%)
cefoperazone	7(8.97%)	5(15.13%)	1(3.13%)	2(6%)	5(16.24%)	-
Tegacyclin	10(12.33%)	4(11.76%)	1(3.13%)	3(8.33%)	4(14.30%)	19(100%)
ciprofloxacin	60(76.92%)	32(95.54%)	1(3.13%)	14(39.46%)	-	-
Amikacin	38(48.12%)	-	2(6.25%)	3(8.33%)	11(38.42%)	1(5.60%)
Imipenem	-	19(55.63%)	-	6(16.6%)	2(7.45%)	4(20%)
Meropenem	-	19(55.63%)	-	6(16.6%)	2(7.45%)	3(15%)

Note: "-" indicates no drug sensitivity. This table describes the resistance rate of different strains to different antibiotics

Table 3. Comparison of drug resistance results of pathogens detected by K-B method and MALDI-TOF

	S.aureus(n=78)		MRAB(n=34)		S.pneumoniae(n=32)		E.coli(n=36)		K.pneumoniae(n=29)		P. Aeruginosa(n=19)	
	Drug resistance rate(%)		Drug resistance rate(%)		Drug resistance rate(%)		Drug resistance rate(%)		Drug resistance rate(%)		Drug resistance rate(%)	
	MALDI-TOF	K-B method	MALDI-TOF	K-B method	MALDI-TOF	K-B method	MALDI-TOF	K-B method	MALDI-TOF	K-B method	MALDI-TOF	K-B method
Cefuroxime	40.28	40.28	88.24	88.24	9.38	15.63	8.33	5.56	49.80	44.83	9.20	9.20
Cefdizine	43.06	44.44	-	-	6.25	6.25	5.56	5.56	-	-	-	-
Tazobactam	44.44	44.44	36.46	38.24	6.25	6.25	8.33	8.33	55.17	55.17	25.70	31.58
cefoperazone	8.97	12.33	15.13	15.13	3.13	3.13	6	6	16.24	13.79	-	-
Tegacyclin	12.33	12.33	11.76	14.71	3.13	3.13	8.33	8.33	14.30	17，24	100	100
ciprofloxacin	76.92	76.92	95.54	95.54	3.13	3.13	39.46	44.44	-	-	-	-
Amikacin	48.12	48.12	-	-	6.25	6.25	8.33	8.33	38.42	38.42	5.60	5.60
Imipenem	-	-	55.63	55.63	-	-	16.67	16.67	7.45	7.45	20	20
Meropenem	-	-	55.63	55.63	-	-	16.67	16.67	7.45	7.45	15	15
T value	-0.056		-0.039		-0.471		-0.045		0.734		-0.048	
P value	0.956		0.935		0.576		0.806		0.866		0.993	

This table compares the identification results of two different identification methods, and the results indicate that there is no significant difference between KB method and MALDI-TOF method.

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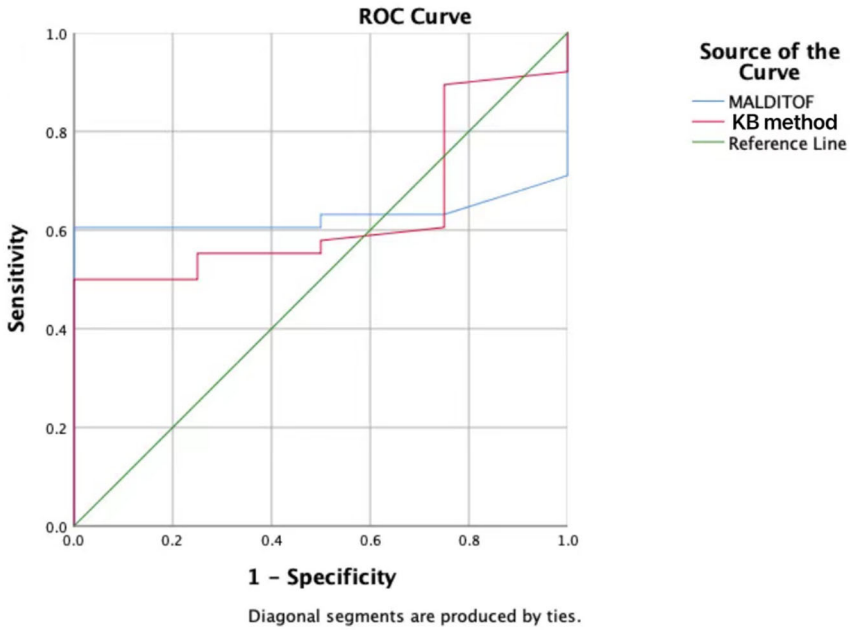

Figure 1. The ROC curves of the two methods indicated that there was no significant difference between the accuracy of MALDI-TOF and the traditional KB method.

4. DISCUSSION

MALDI-TOF MS technology can complete the identification of microbial species and genus level in a short time by detecting the peptide/protein fingerprint of microorganisms, comparing with the microbial database after software processing, and analyzing the comparison results. The mechanism of drug resistance detection includes the following aspects: ① directly find the characteristic white between sensitive strains and drug resistant strains to distinguish sensitive and drug resistant strains; ② Detection by hydrolysis β- The lactamase activity indirectly reflects whether the bacteria to be tested are resistant to antibiotics according to the changes in the molecular weight of the hydrolysate or decarboxylation product of the antibacterial drugs detected and the original strain; ③ The resistance of strains to aminoglycoside antibiotics was determined by directly detecting the activity of methyltransferase that causes methylation of 16S rRNA; ④ The difference of protein expression in cell membrane and periplasmic space and the change of lipopolysaccharide structure were detected to distinguish sensitivity and drug resistance; ⑤ The mutation genes causing drug resistance were detected by primer extension; ⑥ Using isotope labeling method to label proteins, under the condition of the existence of antibacterial drugs, drug resistant strains will continue to grow and newly synthesize labeled proteins, while sensitive strains will not. Sensitive and drug resistant strains can be distinguished according to the changes in the mass spectrum peak of labeled proteins [13-22].

In this study, MALDI-TOF MS technology was used to early identify the types of pathogenic bacteria in the blood of septic patients and antimicrobial susceptibility results. The results showed that 228 strains of pathogenic bacteria were isolated and cultured from all experimental samples, including 110 strains of gram-positive bacteria, mainly Staphylococcus aureus and Streptococcus pneumoniae. 118 strains of Gram negative bacilli were mainly multidrug resistant Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. Among them, Staphylococcus aureus and multi drug resistant Acinetobacter baumannii were the pathogens with high drug resistance rate. Previous studies have shown that [23], MALDI-TOF MS technology can accurately identify microbial species, and the speed is greatly improved compared with traditional methods. In particular, Staphylococcus aureus, multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa and other

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bacteria prone to drug resistance are important factors that lead to increased treatment difficulty and even death of septic patients in recent years.

In this study, while MALDI-TOF MS technology was used to identify the types of pathogens and drug resistance in the blood of septic patients, the traditional paper diffusion method was also used to conduct a comparative analysis of the same samples. Compared with the traditional paper diffusion method, the results showed that there was no statistical difference between the two methods in the identification results of bacterial species and drug resistance rate, which also verified that the identification results of MALDI-TOF MS technology were reliable, and it was expected to adjust the use of clinical antibiotics according to the identification results. It has been reported that compared with the traditional paper diffusion method, MALDI-TOF MS technology can significantly shorten the time. According to the results of drug resistance analysis, targeted antibiotic treatment can be selected as soon as possible. Although the initial cost of mass spectrometer is relatively high [24-27]. However, compared with standard biochemical or molecular genetic techniques, the cost of identifying pathogens is still low. The use of MALDI-TOF MS has shortened the diagnostic process by about 24 hours [28].

To sum up, MALDI-TOF MS can effectively identify the types and drug resistance of pathogenic bacteria in sepsis patients, which is expected to provide a reference for the treatment of clinical sepsis. However, the sample size in this study is less, and subsequent studies will expand the sample size to include more strains, thus making the research results more objective and accurate.

5. CONCLUSIONS

This study used MS, a novel assay to analyze pathogenic bacteria and drug resistance in patients with sepsis, and verified the reliability of the results, providing new ideas for the diagnosis and treatment of patients with sepsis

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