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Application of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in rapid identification and antimicrobial susceptibility testing of bacterial bloodstream infections

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Application of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in rapid identification and antimicrobial susceptibility testing of bacterial bloodstream infections

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Abstract

Background: Bloodstream infection (BSI) is a severe common clinical infection，without timely intervention, the infection may progress to sepsis and increase the risk of mortality in patients. MALDI- TOF MS can rapidly identify pathogens directly from positive blood cultures. However, few systematic evaluations have assessed MALDI- TOF MS combined with rapid antimicrobial susceptibility testing (RAST) for positive blood cultures. Evidence regarding identification accuracy among different pathogens, agreement between RAST and conventional susceptibility testing, and clinical value remains insufficient. This study compared the performance of direct MALDI- TOF MS identification with conventional methods, analyzed the concordance between RAST and routine susceptibility results, and explored the diagnostic value of this combined workflow for bacterial BSI, aiming to shorten turnaround time and support antimicrobial stewardship.

Methods: From January 2024 to August 2026, 428 positive blood cultures were collected from the Microbiology Laboratory of Guangzhou Red Cross Hospital. Pathogens were enriched via high- speed centrifugation sedimentation for direct MALDI- TOF MS identification. Monomicrobial positive broths underwent RAST following EUCAST guidelines, with susceptibility readouts at 4 h, 6 h and 8 h.

Subcultured colony MALDI- TOF MS identification and VITEK 2 Compact susceptibility results served as references to assess concordance.

Results: Among 428 isolates, 295 Gram- negative bacilli showed 91.5% identification concordance; 28 Gram- positive bacilli 78.6%; 105 Gram- positive cocci 82.9%. RAST analysis included 279 specimens. Categorical agreement (CA) rose with incubation time. For Enterobacterales, CA exceeded 90% for most antibiotics at 8 h. Gram- positive cocci yielded poor growth at 4 h precluding inhibition zone measurement, while concordance improved markedly at 8 h.

Conclusion: MALDI- TOF MS combined with RAST achieves rapid, reasonably accurate pathogen identification and preliminary susceptibility testing for positive blood cultures. The 8- hour susceptibility results have good clinical utility and can guide early antibiotic selection in BSI patients.

Keywords:bloodstream infection, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, conventional antimicrobial susceptibility testing, rapid antimicrobial susceptibility testing,bacterial，sepsis

1 Introduction

Bloodstream infection (BSI) is a common severe infectious disease in clinical practice. After invading the bloodstream, pathogens multiply rapidly and release toxins, which can trigger systemic inflammatory response syndrome, and even progress to sepsis, septic shock, and multiple organ dysfunction failure, characterized by acute onset, critical condition, and high mortality.[1]

Multiple clinical studies have indicated that early implementation of appropriate antimicrobial therapy can significantly improve the prognosis of patients with bloodstream infections, reduce the risk of complications, and markedly decrease patient mortality [2]. Currently, blood culture is still considered the "gold standard" for diagnosing bloodstream infections [3] . However, the traditional microbiological detection workflow includes multiple steps such as isolation and culture, purification,

identification, and antimicrobial susceptibility testing, and the turnaround time (TAT) of susceptibility reports usually takes 72–96 hours, which is difficult to meet the clinical demand for timely intervention in critically ill patients [4-5]. Therefore, improving experimental methods to shorten the reporting time of microbial identification and antimicrobial susceptibility testing has become an important challenge in clinical microbiology.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MA LDI-TOF MS) is a rapid, accurate, and high-throughput technique widely appli ed in microbial identification. It enables rapid pathogen identification directly from positive blood culture specimens, greatly shortening the identification time [6]. In addition, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) proposed the rapid antimicrobial susceptibility testing (RAST) meth od for positive blood cultures, which can directly use positive blood culture br oth to complete susceptibility testing in a short time, providing preliminary refe rence for clinical medication [7-10].

At present, systematic evaluations of the combined application of MALDI-TOF MS and RAST on positive blood culture specimens are still limited, especially studies on identification accuracy for different pathogen species, concordance between susceptibility results and conventional methods, and their clinical guiding value. This study aimed to investigate the efficacy of this combined techni que in the diagnosis of bacterial bloodstream infections by comparing the detec tion rates of rapid MALDI-TOF MS identification and conventional identificatio n, and analyzing the concordance between RAST and traditional antimicrobial s usceptibility testing results, to provide a scientific basis for shortening the clini cal diagnosis cycle and optimizing antimicrobial use strategies.

2 Materials and Methods

Study design

This is a diagnostic comparative study. Positive blood culture samples flagged positive by the automated blood culture system and showing monomicrobial growth

on Gram staining were tested in parallel using a rapid assay protocol and a conventional gold-standard protocol. In the rapid group, bacterial pellets were obtained from positive broths by centrifugation for direct species identification via MALDI-TOF MS. For isolates identified as RAST target organisms, rapid antimicrobial susceptibility testing was further performed using the EUCAST RAST disk diffusion method, with incubation at 35 °C and interpretation after 4–8 h. The conventional gold-standard group involved subculture on blood agar to obtain isolated single colonies, followed by MALDI-TOF MS identification and standard antimicrobial susceptibility testing on the VITEK 2 Compact system. Identification and AST results from the two protocols were compared to evaluate the performance of the rapid assay.

The study workflow is presented in Figure 1.

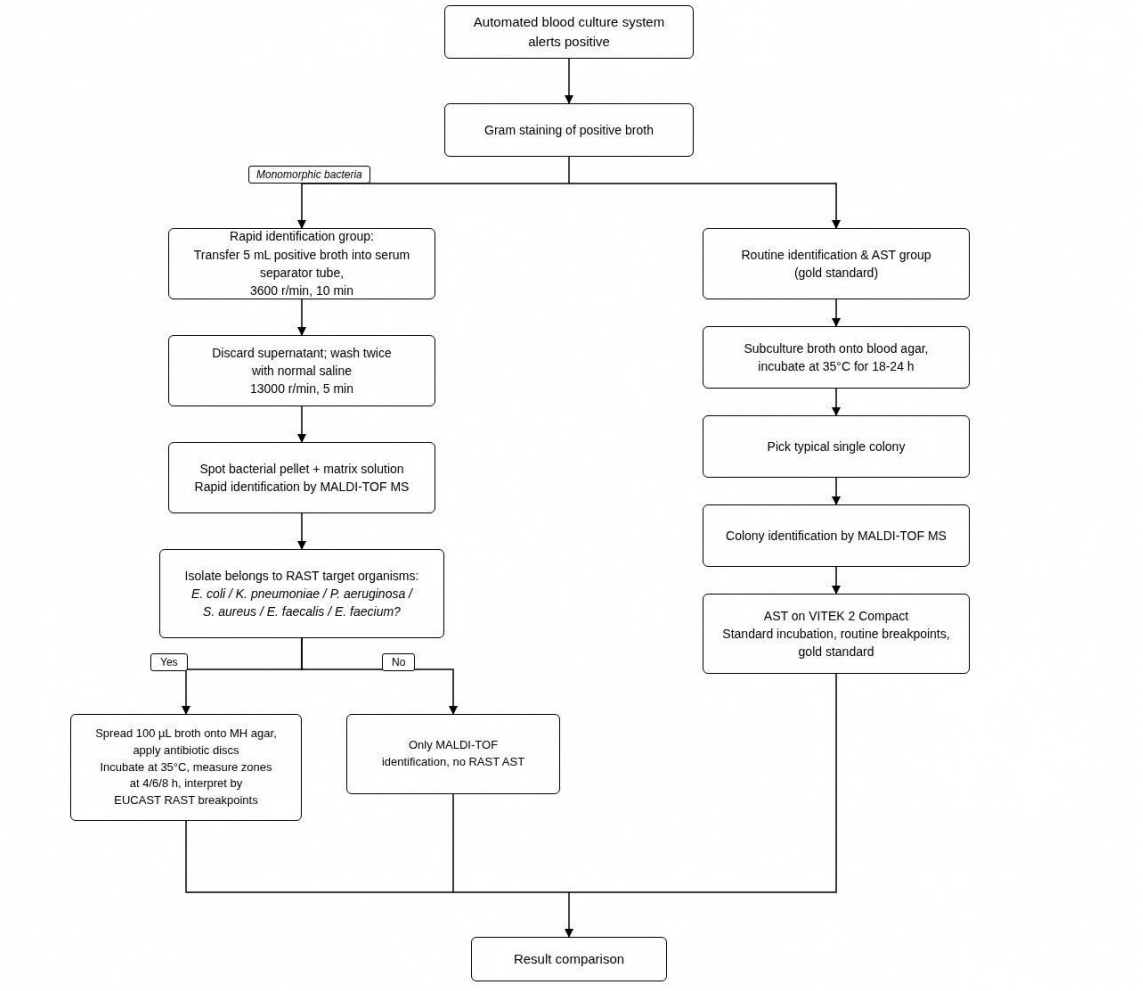

Figure 1. Flowchart of rapid identification and antimicrobial susceptibility testing versus conventional identification and susceptibility testing.

2.1 Specimen Source

A total of 428 positive blood culture specimens were collected from the Microbiology Laboratory of the Department of Clinical Laboratory, Guangzhou Red Cross Hospital, from January 2024 to August 2026. Repeated specimens from the same patient on the same day and specimens with mixed infections of two or more species were excluded.

2.2 Instruments and Reagents

Bactec FX automated blood culture system and matching blood culture bottles (BD, USA); vacuum blood collection tubes (BD, USA); Vitek MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (bioMérieux, France); VITEK 2 Compact automated microbial identification and susceptibility analysis system (bioMérieux, France); constant temperature incubator (Thermo Fisher Scientific); high-speed centrifuge (Thermo Fisher Scientific); MH agar plates (Jiangmen Kailin Biotechnology); antimicrobial susceptibility discs (Thermo Fisher Scientific). Quality control strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212.

2.3 Methods

2.3.1 Rapid identification of positive blood culture specimens

After blood culture bottles signaled positive, the broth was removed for Gram staining. When bacteria of a single morphology were observed under the microscope, 5 mL of positive culture was aspirated with a sterile syringe and injected into a vacuum blood collection tube with coagulation-promoting separation gel, centrifuged at 3600 r/min for 10 min, and the supernatant was discarded. The precipitate was resuspended in 1 mL of normal saline and transferred to a 1.5 mL centrifuge tube, centrifuged at 13000 r/min for 5 min, and the supernatant was discarded. The pellet was washed again with 1 mL of normal saline, centrifuged at 13000 r/min for 5 min, and the supernatant was discarded, retaining the bacterial pellet. A 1 μL aliquot of the pellet was spotted onto a metal target plate, mixed with 1 μL of matrix solution, and subjected to rapid

identification after the target plate was dried. Quality control strains: *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048.

2.3.2 RAST for positive blood cultures

Referring to EUCAST RAST guidelines for positive blood cultures, for specimens identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Enterococcus faecalis* by rapid MALDI-TOF MS, 100 μL of broth was directly aspirated from the blood culture bottle, evenly spread on a 90 mm MH agar plate, and corresponding antimicrobial discs were applied. Plates were incubated at 35°C, and inhibition zone diameters were measured after 4, 6, and 8 hours of incubation, respectively. Susceptibility results were interpreted according to EUCAST RAST breakpoints. Quality control strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcusfaecalis* ATCC 29212.

2.3.3 Conventional identification and susceptibility testing of positive blood culture specimens

When the automated blood culture system signaled positive, the positive culture bottle was removed, Gram staining was performed on the sample, and the sample was subcultured onto blood agar plates and incubated at 35°C for 18–24 hours. After colony formation, well-isolated colonies with typical morphology were selected for microbial identification by MALDI-TOF MS. According to identification results, antimicrobial susceptibility testing was performed using the VITEK 2 Compact automated microbial analysis system, with results interpreted according to CLSI M100, which served as the gold standard. Quality control strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcusfaecalis* ATCC 29212.

2.3.4 Comparison of susceptibility results

The concordance between RAST and conventional susceptibility results was compared with the following criteria: ① **Categorial agreement (CA)**: both conventional and RAST results were resistant (or susceptible); ② **Errors**: including three types:Very major error (VME): false susceptible, where conventional result was resistant but RAST result was susceptible;Major error (ME): false resistant, where conventional result was susceptible but RAST result was resistant;Minor error (mE): where conventional result was intermediate and RAST result was susceptible or resistant.

ATU results were not included in the calculation of agreement rates.

3 Results

3.1 Comparison of Rapid MALDI-TOF MS Identification and Conventional Identification Results

Among 428 single-species positive specimens, there were 295 strains of Gram-negative bacilli, with a 91.5% (270/295) concordance rate between direct identification and post-culture colony MALDI-TOF MS identification. Among them, *Escherichia coli*, the most frequently isolated species, had a concordance rate of 94.3% (151/160). There were 28 strains of Gram-positive bacilli, with a concordance rate of 78.6% (22/28); *Corynebacterium striatum* was the most frequently isolated, with a concordance rate of 82.4% (14/17). There were 105 strains of Gram-positive cocci, with a concordance rate of 82.9% (87/105); *Staphylococcus aureus* was the most frequently isolated, with a concordance rate of 85.7% (18/21) (Table 1).

TABLE1 Comparison of Vitek MS rapid identification and conventional identification results [n (%)]

Species	n	Vitek MS rapid identification	Conventional identification

Species	n	Vitek MS rapid identification	Conventional identification
Gram- negative bacilli	295	270 (91.5)	295 (100.00)
Escherichia coli	160	151 (94.3)	160 (100.00)
Klebsiella pneumoniae	65	60 (92.3)	65 (100.00)
Acinetobacter baumannii	21	18 (85.7)	21 (100.00)
Pseudomonas aeruginosa	18	16 (88.9)	18 (100.00)
Proteus mirabilis	12	11 (91.7)	12 (100.00)
Serratia marcescens	8	6 (75.00)	8 (100.00)
Stenotrophomonas maltophilia	5	4 (80.00)	5 (100.00)
Bacteroidesfragilis	4	3 (75.00)	4 (100.00)
Fusobacterium nucleatum	2	1 (50.00)	2 (100.00)
Gram- positive bacilli	28	22 (78.6)	28 (100.00)
Corynebacterium striatum	17	14 (82.4)	17 (100.00)
Corynebacteriumjeikeium	7	5 (71.42)	7 (100.00)
Cutibacterium acnes	4	3 (75.00)	4 (100.00)
Gram- positive cocci	105	87 (82.9)	105 (100.00)
Staphylococcus aureus	21	18 (85.7)	21 (100.00)
Staphylococcus epidermidis	21	16 (76.2)	21 (100.00)
Enterococcusfaecalis	20	17 (85.0)	20 (100.00)
Enterococcusfaecium	16	15 (87.5)	16 (100.00)
Staphylococcus hominis	12	9 (60.00)	12 (100.00)
Staphylococcus haemolyticus	10	8 (80.0)	10 (100.00)
Streptococcus agalactiae	5	4 (80.0)	5 (100.00)

3.2 Concordance Between RAST and Conventional Antimicrobial Susceptibility Testing Results

A total of 279 positive blood culture specimens were included in RAST analysis, including 15 strains of *Pseudomonas aeruginosa*, 62 strains of *Klebsiella pneumoniae*, 146 strains of *Escherichia coli*, 20 strains of *Staphylococcus aureus*, 16 strains of *Enterococcus faecium*, and 20 strains of *Enterococcus faecalis*. The concordance between RAST and conventional results was compared at 4, 6, and 8 hours of incubation. The overall categorical agreement (CA) gradually increased with prolonged incubation time.

Among Gram-negative bacilli, the CA of most antimicrobial agents for *Escherichia coli* and *Klebsiella pneumoniae* exceeded 90% at 8 hours; the CA of piperacillin/tazobactam and meropenem was relatively lower. For *Klebsiella pneumoniae* at 8 hours, the CA of meropenem, amikacin, gentamicin, and tobramycin was 95. 16%, 95. 16%, 96.77%, and 96.77%, respectively. For *Escherichia coli* at 8 hours, the CA of meropenem reached 97.26%, while a few minor errors remained for ciprofloxacin and tobramycin. Only 15 strains of *Pseudomonas aeruginosa* were included with a limited sample size, and results of some antimicrobial agents fluctuated greatly; the CA of ceftazidime reached 100.00% at 8 hours.

The overall CA of Gram-positive cocci was lower than that of Gram-negative bacilli, with a higher proportion of indeterminate (ATU) results. Growth was extremely weak at 4 hours, making inhibition zone diameters unmeasurable. For *Staphylococcus aureus* at 8 hours, the CA of cefoxitin (MRSA screening) was 95.00%, and that of gentamicin was 85.00%; clindamycin still had a high proportion of ATU. For *Enterococcus faecium* at 8 hours, the CA of ampicillin reached 100.00%, and that of high-level gentamicin resistance screening was 93.75%; vancomycin had an extremely high proportion of ATU at all time points with limited reference value; the CA of linezolid was 97.75% at 8 hours. Susceptibility results of *Enterococcus faecalis* were generally poor: the CA of ampicillin was less than 60% at all time points with a

high proportion of ATU; the CA of high-level gentamicin resistance screening reached 100.00% at 8 hours; the proportion of ATU for vancomycin was as high as 75%–80%

For *Pseudomonas aeruginosa*, one major error (ME) for piperacillin/tazobactam was observed at 6 h and another at 8 h. For *Klebsiella pneumoniae,* one ME for cefotaxime occurred at each of 4 h, 6 h and 8 h; one ME for amikacin was detected at 4 h, and one very major error (VME) for ciprofloxacin was noted at 4 h. For *Escherichia coli,* five MEs were found for ceftazidime; four VMEs and four MEs for amikacin; two MEs and two VMEs for gentamicin.(Tables 2–7).

TABLE 2 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Pseudomonas aeruginosa* [n (%)]

Antimicrobial agent	Time（h）	CA(n)		CA	ATU	VME	ME	mE
		S	R					
Piperacillin/tazobactam	6	10	2	12（80.00）	2(13.33)	0	1(6.67)	0
	8	11	2	13（86.67）	1(6.67)	0	1(6.67)	0
Imipenem	6	11	1	12（80.00）	3(20.0)	0	0	0
	8	12	1	13（86.67）	2(13.33)	0	0	0
Ceftazidime	6	8	6	14（93.33）	1(6.67)	0	0	0
	8	9	6	15（100.00）	0	0	0	0
Meropenem	6	10	2	12（80.00）	3(20.0)	0	0	0
	8	12	2	14（93.33）	1(6.67)	0	0	0
Ciprofloxacin	6	8	4	12（80.00）	3(20.0	0	0	0
	8	8	5	13（86.67）	2(13.33)	0	0	0
Gentamicin	6	8	5	13（86.67）	2(13.33)	0	0	0
	8	8	6	14（93.33）	1(6.67)	0	0	0
Tobramycin	6	8	5	13（86.67）	1(6.67)	0	0	1(6.67)
	8	8	6	14（93.33）	0	0	0	1(6.67)

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error

TABLE 3 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Klebsiella pneumoniae* [n (%)]

Antimicrobial agent	Time（h）	CA(n)		CA	ATU	VME	ME	mE
		S	R					
Piperacillin/tazobactam	4	43	9	52（83.87）	10（16.13）	0	0	0
	6	43	12	55（88.71）	7（11.29）	0	0	0
	8	43	14	57（91.94）	5（8.06）	0	0	0
Ceftazidime	4	38	14	52（83.87）	10（16.13）	0	0	0
	6	39	15	54（87.10）	8（12.90）	0	0	0
	8	39	15	54（87.10）	8（12.90）	0	0	0
Cefotaxime	4	37	14	51（82.23）	10（16.13）	0	1（1.61）	0
	6	37	15	52（83.87）	9（14.52）	0	1（1.61）	0
	8	37	15	52（83.87）	9（14.52）	0	1（1.61）	0
Meropenem	4	50	6	56（90.32）	6（9.68）	0	0	0
	6	51	7	58（93.55）	4（6.45）	0	0	0
	8	51	8	59（95.16）	3（4.84）	0	0	0
Ciprofloxacin	4	32	17	49（79.31）	12（19.35）	1（1.61）	0	0
	6	33	20	53（85.49）	9（14.52）	0	0	0
	8	33	21	54（87.10）	8（12.90）	0	0	0
Amikacin	4	54	4	58（93.55）	3（4.84）	0	1（1.61）	0
	6	55	4	59（95.16）	3（4.84）	0	0	0
	8	55	4	59（95.16）	3（4.84）	0	0	0
Gentamicin	4	52	6	58（93.55）	4（6.45）	0	0	0
	6	52	8	60（96.77）	2（3.23）	0	0	0

	8	52	8	60（96.77）	2（3.23）	0	0	0
Tobramycin	4	53	5	58（93.55）	4（6.45）	0	0	0
	6	53	6	59（95.16）	3（4.84）	0	0	0
	8	53	7	60（96.77）	2（3.23）	0	0	0

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error.

TABLE 4 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Escherichia coli* [n (%)]

Antimicrobial agent	**Time（h）**	**CA(n)**		**CA**	**ATU**	**VME**	**ME**	**mE**
		S	**R**					
Piperacillin/tazobactam	4	95	19	114（78.08）	32（21.92）	0	0	0
	6	104	20	124（84.93）	22（15.07）	0	0	0
	8	121	20	141（96.58）	5（3.42）	0	0	0
Ceftazidime	4	82	31	113（77.40）	30（20.55）	0	3（2.05）	0
	6	95	33	128（87.67）	16（11.27）	0	2（1.37）	0
	8	97	33	130（89.04）	16（11.27）	0	0	0
Cefotaxime	4	86	29	115（78.77）	30（20.55）	0	1（0.69）	0
	6	99	30	129（88.36）	17（11.64）	0	0	0
	8	101	30	131（89.73）	15（10.27）	0	0	0
Meropenem	4	102	14	116（79.45）	29（19.86）	0	0	1（0.69）
	6	121	14	135（92.47）	11（7.53）	0	0	0
	8	128	14	142（97.26）	4（2.74）	0	0	0
Ciprofloxacin	4	74	32	106（72.60）	35（23.97）	0	1（0.69）	4（2.74）

	6	82	36	118（80.82）	25 （17.12）	0	0	3 （2.05）
	8	91	36	127（86.99）	15 （10.27）	0	0	3 （2.05）
Amikacin	4	105	12	117（80.14）	24 （16.44）	3 （2.05）	2（1.37）	0
	6	111	14	125（85.61）	19 （13.14）	1 （0.69）	1 （0.69）	0
	8	115	15	130（89.04）	15 （10.27）	0	1 （0.69）	0
Gentamicin	4	97	15	112（76.71）	30 （20.55）	2 （1.37）	2 （1.37）	0
	6	104	18	122（83.56）	24 （16.44）	0	0	0
	8	107	20	127（86.99）	19 （13.01）	0	0	0
Tobramycin	4	102	13	115（78.77）	31 （21.23）	0	0	0
	6	109	14	123（84.25）	23 （15.75）	0	0	0
	8	113	15	128（87.67）	18 （12.33）	0	0	0

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error.

TABLE 5 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Staphylococcus aureus* [n(%)]

Antimicrobial agent	**Time（h）**	**CA(n)**		**CA**	**ATU**	**VME**	**ME**	**mE**
		S	**R**					
Cefoxitin (screening)	4	U	U	U	U	U	U	0
	6	14	3	17（85.00）	0	0	3（15.00）	0
	8	16	3	19（95.00）	0	0	1（5.00）	0
Gentamicin	4	12	4	16（80.00）	4（20.00）	0	0	0
	6	13	4	17（85.00）	3（15.00）	0	0	0
	8	13	4	17（85.00）	3（15.00）	0	0	0

Clindamycin	4	U	-	U	U	U	U	U
	6	13	-	13（65.00）	7（35.00）	0	0	0
	8	14	-	16（80.00）	6（20.00）	0	0	0

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error; -, no interpretation standard; U, unmeasurable.

TABLE 6 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Enterococcusfaecium* [n (%)]

Antimicrobial agent	**Time（h）**	**CA(n)**		**CA**	**ATU**	**VME**	**ME**	**mE**
		S	**R**					
Ampicillin	4	U	U	U	U	U	U	U
	6	6	9	15（93.75）	1（6.25）	0	0	0
	8	6	10	6（100.00）	0	0	0	0
Gentamicin (high-level aminoglycoside resistance screening)	4	U	U	U	U	U	U	U
	6	8	7	15（93.75）	1（6.25）	0	0	0
	8	8	7	15（93.75）	1（6.25）	0	0	0
Vancomycin	4	-	U	U	U	U	U	U
	6	-	3	3（18.75）	13（81.25）	0	0	0
	8	-	3	3（18.75）	13（81.25）	0	0	0
Linezolid	4	-	-	-	-	-	-	-
	6	13	0	13（81.25）	3 （18.75）	0	0	0
	8	15	0	15（97.75）	1（6.25）	0	0	0

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error; -, no interpretation standard; U, unmeasurable.

TABLE 7 Concordance of RAST and conventional antimicrobial susceptibility testing results for *Enterococcus faecalis* [n (%)]

Antimicrobial agent	Time (h)	CA(n)		CA	ATU	VME	ME	mE
		S	R					
Ampicillin	4	U	-	U	U	U	U	U
	6	10	-	10（50.00）	10（50.00）	0	0	0
	8	11	-	11（55.00）	9（45.00）	0	0	0
Gentamicin (high-level aminoglycoside resistance screening)	4	U	U	U	U	U	U	U
	6	8	11	19（95.00）	1（5.00）	0	0	0
	8	8	12	20（100.00）	0	0	0	0
Vancomycin	4	-	U	U	U	U	U	U
	6	-	5	5（25.00）	15（75.00）	0	0	0
	8	-	5	5（25.00）	15（75.00）	0	0	0
Linezolid	4	U	U	U	U	U	U	U
	6	17	0	17（85.00）	3（15.00）	0	0	0
	8	20	0	20（10.00）	0	0	0	0

Note: CA, categorical agreement; ATU, indeterminate result; VME, very major error (false susceptible); ME, major error (false resistant); mE, minor error; -, no interpretation standard; U, unmeasurable.

4 Discussion

Bloodstream infections have an acute onset and rapid progression, with approximately 50 million cases and 10 million deaths worldwide each year [11]. Mortality increases significantly with every 1-hour delay in effective antimicrobial therapy [12]. Therefore, early and accurate pathogenic detection and susceptibility results are crucial for patient diagnosis and treatment. Early initiation of effective antimicrobial therapy can significantly improve patient prognosis and reduce the risk of severe complications such as sepsis and septic shock.

Traditional methods are too slow: blood culture is the gold standard for diagnosing bloodstream infections, but subculture takes 15–72 hours, and even longer for slow-growing or anaerobic bacteria. Commercial rapid detection products have high costs and limited pathogen panels. Therefore, a low-cost, easy-to-operate, broad-coverage protocol that yields identification results within 1 hour has become an urgent need for laboratories. This study used high-speed centrifugation for pretreatment of positive blood culture specimens, combined with direct MALDI-TOF MS identification and EUCAST RAST, to evaluate the clinical application value of this combined protocol in the rapid diagnosis of bacterial bloodstream infections.

The pretreatment method of positive blood culture specimens directly determines the success rate and accuracy of direct MALDI-TOF MS identification [13]. In this study, bacteria in blood culture bottles were enriched by high-speed centrifugation using coagulation-promoting separation gel tubes. The results showed that the direct mass spectrometry identification concordance rate was high for Gram-negative bacilli, reaching 91.5%, while relatively lower for Gram-positive bacilli and Gram-positive cocci, at 78.6% and 82.9%, respectively. The possible reasons are as follows: on the one hand, Gram-positive bacteria have a dense cell wall structure, and bacterial protein extraction efficiency after centrifugation and washing is weaker than that of Gram-negative bacilli; on the other hand, opportunistic pathogens such as *Corynebacterium* and some coagulase-negative staphylococci have low bacterial loads, resulting in less protein extraction and easy identification failure.

Tsuchida S et al. [14] proposed a three-step lysis-filtration protocol (ammonium chloride lysis, 0.45 μm membrane filtration, centrifugation collection), with an identification rate as high as 99.2%, but the procedure is relatively cumbersome. Advanced molecular diagnostic methods, such as the BioFire FilmArray BCID2 panel (bioMérieux, Marcy-l'Étoile, France) and the Accelerate Pheno™ system (Accelerate Diagnostics Inc., Tucson, USA), have facilitated rapid and highly sensitive detection of pathogens from positive blood cultures [15-17]. However, they are limited by high

costs and restricted microorganism panels. The pretreatment method used in this study achieves favorable identification efficacy without special reagents, with simple operation steps and low cost, which is suitable for ordinary clinical microbiology laboratories.

This study also observed that the direct identification concordance rate of the most common clinical bloodstream pathogens, such as *Escherichia coli* and *Klebsiella pneumoniae*, was at a high level, enabling rapid and accurate identification of *Enterobacterales*, which account for the highest proportion of clinical cases, and has good practical value. Previous studies have shown that the incidence of anaerobic bloodstream infections is approximately 2.2% [18]. Missing anaerobic blood cultures will lead to missed diagnosis and inappropriate treatment. The method in this study can directly identify anaerobic bacteria, with a 75% identification success rate for *Bacteroides fragilis*, which is crucial for laboratories without anaerobic culture conditions. It can not only greatly shorten the identification time, but also avoid missed detection of anaerobic bacteria. D'Inzeo T et al. [19] showed that the correct identification rate of direct MALDI-TOF MS for anaerobic bacteria at the species level was 52.7% in a study on direct identification of anaerobic bacteria from positive blood cultures. When only single-culture specimens were analyzed, identification rates varied significantly among different genera: *Bacteroides*/Parabacteroides had the highest identification rate, reaching 97. 1%. Khankan N et al. [20] confirmed that rapid identification of Gram-negative anaerobic bacteria directly from positive blood culture bottles by MALDI-TOF MS can significantly shorten identification time from 45.3 hours to 15.0 hours and accelerate antibiotic de-escalation therapy.

RAST, which does not rely on isolated and purified single colonies, can directly perform disc diffusion susceptibility testing using positive blood culture broth, greatly shortening susceptibility reporting time, and has become a research hotspot in rapid phenotypic susceptibility testing of bloodstream infections in recent years [21-22]. The results of this study indicated that the RAST categorical agreement rate for

Gram-negative bacilli gradually increased with prolonged incubation time, and the overall CA reached 91.30% at 8 hours, which was consistent with most domestic and international studies on RAST [23-25].

Among Gram-negative bacilli, the CA of most antimicrobial agents for *Escherichia coli* and *Klebsiella pneumoniae* exceeded 90% at 8 hours. For *Escherichia coli*, very major categorical errors occurred with amikacin, which may be related to blurred inhibition zone edges, manual interpretation errors, and differences between RAST breakpoints and conventional susceptibility breakpoints. The number of *Pseudomonas aeruginosa* strains included was small, and results of some drugs fluctuated greatly. The detection efficacy of RAST for this species still needs to be further verified with expanded samples.

Compared with Gram-negative bacilli, the overall RAST concordance rate of Gram-positive cocci in this study was lower, and the proportion of indeterminate ATU results was higher. The cefoxitin MRSA screening results for *Staphylococcus aureus* improved significantly with prolonged incubation time, achieving good concordance at 8 hours, but there were still many ATU results for clindamycin. Among *Enterococcus* species, high-level gentamicin resistance screening showed good concordance at 8 hours, while there were a large number of ATU results for ampicillin and vancomycin. The possible reasons are that Gram-positive cocci grow slower than *Enterobacterales*, and inhibition zone outlines are unclear after short incubation, with large interference factors in manual interpretation.

Meanwhile, this study observed that 4 hours of incubation was too short for bacterial proliferation, and inhibition zones were not fully formed, making inhibition zone diameters unmeasurable; results improved at 6 hours; after 8 hours of incubation, inhibition zones became clearer, and the proportion of errors and indeterminate results decreased significantly, with the best overall efficacy. This suggests that 8-hour RAST results should be preferentially referenced in clinical practice, and 6-hour results can only be used as a preliminary reference and cannot be directly used as the

basis for final reports. Yıldız S S et al. [26] evaluated EUCAST rapid susceptibility testing for positive bottles of the Autobio BC60 blood culture system, comparing concordance rates of Gram-negative bacilli and Gram-positive cocci at 4, 8, and 20 hours, confirming that positive cocci are difficult to interpret at early stages due to slow growth, and 8-hour results have the best overall efficacy, providing evidence for clinical preference of 8-hour RAST, which is consistent with this study.

4.1 Limitations

This study has several limitations. First, the sample size of some rare species and *Pseudomonas aeruginosa* strains is small, resulting in limited statistical power. Second, RAST is only applicable to positive blood cultures with single pure species, and cannot detect mixed infections or fastidious slow-growing bacteria, limiting its application scenarios. Third, inhibition zone boundaries are blurred at early time points (4 and 6 hours), and result interpretation depends on the subjective experience of laboratory personnel, so manual bias is inevitable. Fourth, details of specimen pretreatment, centrifugation parameters, and washing times can interfere with mass spectrometry identification and RAST results, and results may vary between laboratories. Future studies can conduct multicenter research to expand sample size and further optimize the bacterial enrichment pretreatment process; introduce an automated image recognition and interpretation system to reduce subjective errors caused by manual zone reading; and combine resistance gene molecular detection to achieve complementation of phenotypic and genotypic results, further improving the diagnostic capability of rapid detection of bloodstream infections.Fifth, this study only assessed the diagnostic reliability of rapid identification and antimicrobial susceptibility testing and did not directly investigate patient outcomes in real clinical practice. Our subsequent work will focus on exploring how reduced TAT affects real-world patient prognosis.

5 Conclusion

High-speed centrifugation pretreatment combined with MALDI-TOF MS can directly complete rapid pathogen identification from positive blood culture specimens; 8-hour incubation of RAST can obtain clinically acceptable susceptibility reference results.

The combined mode can significantly shorten the turnaround time of pathogenic reports for bacterial bloodstream infections, and provide an important laboratory basis for early adjustment of empirical anti-infective regimens in clinical practice. The technique relies on existing mass spectrometry and disc diffusion platforms, and is easy to promote in clinical microbiology laboratories at all levels. However, this technique is a rapid preliminary test result and cannot completely replace standardized susceptibility testing after traditional isolation and culture. The final anti-infective regimen still needs to be comprehensively judged in combination with conventional susceptibility reports, patient clinical symptoms, imaging findings, and other laboratory indicators [27-29].

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Data availability：The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Guangzhou Red Cross Hospital (Approval No: 2026-308-01).The study utilized residual blood specimens remaining after routine clinical laboratory testing, with no additional samples collected specifically for research purposes. All samples and associated data were anonymized prior to analysis. Given the retrospective nature of the study, the use of leftover clinicalblood specimens , the minimal risk to participants, and the impracticability of obtaining individual consent, the requirement for informed consent was waived by the Ethics Committee.

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Competing interests：The authors declare no competing interests.

6 References

[1] Al Mohajer M, Darouiche R O. Sepsis Syndrome, Bloodstream Infections, and Device-Related Infections[J]. Medical Clinics of North America, 2012, 96(6): 1203-1223.

[2] Rotundo S, Russo A, Morena R, et al. Early targeted therapy guided by rapid phenotypic antimicrobial susceptibility testing in critically ill patients with gram-negative bacterial bloodstream infections: a retrospective cohort study[J]. JAC-Antimicrobial Resistance, 2026, 8(Supplement_3).

[3] Ye H, Su F, Cui X, et al. Evaluation of different blood culture bottles for the diagnosis of bloodstream infections in patients with HIV[J]. Infectious Diseases and Therapy, 2023, 12(11): 2611-2620.

[4] Iyer V, Castro D, Malla B, et al. Culture-independent identification of bloodstream infections from whole blood: prospective evaluation in specimens of known infection status[J]. Journal of Clinical Microbiology, 2024, 62(3): e0149823.

[5] Nielsen M E, Søgaard K K, Karst S M, et al. Application of rapid Nanopore metagenomic cell-free DNA sequencing to diagnose bloodstream infections: a prospective observational study[J]. Microbiology Spectrum, 2025, 13(5):

e0329524.

[6] D'Inzeo T, Fiori B, Liotti F M, et al. Direct Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Testing from Positive Blood Cultures for Rapid Identification of Bloodstream Infection-Causing Anaerobic Bacteria[J]. Journal of Clinical Microbiology, 2021, 59(7): e0052121.

[7] Inglis T J J, Ekelund O. Rapid antimicrobial susceptibility tests for sepsis; the road ahead[J]. Journal of Medical Microbiology, 2019, 68(7): 973-977.

[8] Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles[J]. Journal of Antimicrobial Chemotherapy, 2019, 75(4): 968-978.

[9] Åkerlund A, Jonasson E, Matuschek E, et al.RAST Study Group. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories. J Antimicrob Chemother. 2020 Nov 1;75(11):3230-3238.

[10]Ekwall-Larson A, Fröding I, Mert B, et al. Analytical Performance and Potential Clinical Utility of EUCAST Rapid Antimicrobial Susceptibility Testing in Blood Cultures after Four Hours of Incubation[J]. Microbiology Spectrum, 2023, 11(2): e0500122.

[11]Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet. 2020 Jan 18;395(10219):200-211. [4] Faron ML, Buchan BW, Ledeboer NA. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for use with positive blood cultures: methodology, performance, and optimization[J]. J Clin Microbiol, 2017, 55(12): 3328-3338.

[12]Kim TH, Kang J, Jang H. Blood culture-free ultra-rapid antimicrobial susceptibility testing. Nature.2024;632:893–902. 10. 1038/s41586-024-07725-1.

[13]Dai Y, Xu X, Xue Y, et al. Evaluation of a Rapid and Simplified Protocol for Direct Identification of Microorganisms From Positive Blood Cultures by Using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)[J]. Frontiers in Cellular and Infection Microbiology, 2021, 11:

632679.

[14]Tsuchida S, Murata S, Miyabe A, et al. An improved in-house lysis-filtration protocol for bacterial identification from positive blood culture bottles with high identification rates by MALDI-TOF MS[J]. Journal of Microbiological Methods, 2018, 148: 40-45.

[15]Ullberg M, Özenci V. Identification and antimicrobial susceptibility testing of Gram-positive and Gram-negative bacteria from positive blood cultures using the accelerate pheno™ system. Eur J Clin Microbiol Infect Dis. 2020;39:139–49

[16]Caméléna F, Péan de Ponfilly G, Pailhoriès H. Multicenter evaluation of the filmarray blood culture identification 2 panel for pathogen detection in bloodstream infections. Microbiol Spectr. 2023;11:e0254722

[17]Bauer MJ, Peri AM, Lüftinger L. Optimized method for bacterial nucleic acid extraction from positive blood culture broth for Whole-Genome sequencing, resistance phenotype prediction, and downstream molecular applications. J Clin Microbiol. 2022;60:e0101222.

[18]Amoabeng P, Gassiep I, Vangaveti V, et al. The utility of anaerobic blood culture[J]. Pathology, 2019, 51(S1): S67.

[19]D'Inzeo T, Fiori B, Liotti F M, et al. Direct Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Testing from Positive Blood Cultures for Rapid Identification of Bloodstream Infection-Causing Anaerobic Bacteria[J]. Journal of Clinical Microbiology, 2021, 59(7): e0052121.

[20]Khankan N, Rust T, Pallotta A, et al. Impact of rapid pathogen identification of gram-negative anaerobic bacteria from blood cultures on antimicrobial stewardship[J]. Microbiology Spectrum, 2025, 13(12): e0238325.

[21]Cherkaoui A, Schorderet D, Azam N, et al. Fully Automated EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) from Positive Blood Cultures: Diagnostic Accuracy and Implementation[J]. Journal of Clinical Microbiology, 2022, 60(10): e0089822.

[22]Strubbe G, Messiaen A S, Vandendriessche S, et al. EUCAST rapid antimicrobial susceptibility testing (RAST) compared to conventional susceptibility testing:

implementation and potential added value in a tertiary hospital in Belgium[J]. Acta Clinica Belgica, 2023, 78(5): 385-391.

[23]Erdoğan G, Karakoç A, Yücel M, et al. Evaluation of EUCAST Direct Rapid Antimicrobial Susceptibility Test Method in Blood Culture Bottles with Positive Signal[J]. Mikrobiyoloji Bulteni, 2021, 55(4): 626-634.

[24]Tayşi M R, Çiçek Şentürk G, Çalışkan E, et al. Implementation of the EUCAST rapid antimicrobial susceptibility test (RAST) directly from positive blood culture bottles without the advanced identification systems[J]. Journal of Antimicrobial Chemotherapy, 2022, 77(4): 1020-1026.

[25]Liang B, Li X, Zheng Q, et al. Evaluation of three rapid antimicrobial susceptibility testing methods directly from positive blood cultures integrated with rapid pathogen identification[J]. BMC Microbiology, 2026, 26(1).

[26]Yıldız S S, Tavukcu E, Ölmez M E, et al. Evaluation of EUCAST rapid antibiotic susceptibility testing for positive blood cultures in the Autobio BC60 blood culture system[J]. Diagnostic Microbiology and Infectious Disease, 2025, 113(2): 116937.

[27]Åkerlund A, Jonasson E, Matuschek E, et al. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures:validation in 55 European laboratories[J]. University of Southern Denmark Research Portal (University of Southern Denmark), 2020.

[28]Strubbe G, Van Honacker E, Vandendriessche S, et al. Validation of EUCAST rapid antimicrobial susceptibility testing directly from positive blood cultures in a non-automated lab setting[J]. Acta Clinica Belgica, 2024, 79(5): 319-326.

[29]Özgen Top Ö, Çiftçi B, Büyükkörük M, et al. Impact of EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) on optimal antimicrobial therapy in gram-negative bloodstream infections[J]. Infectious Diseases Now, 2024, 54(8): 105007.