


RESEARCH ARTICLE

Evaluation of an optimized method to directly identify bacteria from positive blood cultures using MALDI-TOF mass spectrometry

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Abstract

Background: Although various methods have been developed to directly identify bacteria from positive blood cultures by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), the necessity of using commercial kits still leads to a high cost and long assay time. Moreover, few evaluations of these methods have been conducted. This study aimed to evaluate the feasibility of an optimized MALDI-TOF MS method for direct identification of bacteria in positive blood cultures.

Methods: A total of 829 non-repeated positive cultures were collected from July 2018 to August 2019, and direct identification was performed by an optimized MALDI-TOF MS method. The same positive blood cultures were sub-cultivated to obtain a single bacterial colony and identified by classical biochemical BD testing, which is the gold standard to compare the accuracy of direct identification of positive blood cultures by MALDI-TOF MS.

Results: After excluding 7 false-positive samples from the 829 positive blood cultures, the most accurate rate of direct identification by this optimized MALDI-TOF MS method was for gram-negative bacteria (91.5%), followed by gram-positive bacteria (88.3%), fungi (84.8%), anaerobic bacteria (80%), and other rare bacteria (66.67%).

Conclusion: Common bacteria in positive blood cultures can be identified directly within 1 hour by MALDI-TOF MS, and thus, this optimized method can be used as a primary identification method by clinicians. Routine implementation of this method may significantly increase the optimal utilization rate of antibiotics and decrease mortality in bacteremia patients.

KEYWORDS

bacteria, blood culture, identification, MALDI-TOF MS

Youhua Yuan and Junjie Wang contributed equally to this work.

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1 | INTRODUCTION

Bloodstream infection (BSI), one of the most common infections in hospitals, has attracted attention due to its high mortality and rapid change.¹ Blood culture is one of the most important diagnostic methods for BSI,² in which Gram staining serves as the first step in the identification of positive blood culture samples according to the traditional report flowchart, followed by isolation, phenotypic and biochemical identification, and drug susceptibility testing, taking a total of 24 or 48 hours to report results to clinicians. This traditional detection method for BSI is thus slow and increases patient mortality.³ In particular, in instances where pathogens are rare or difficult to grow, reporting results regarding these pathogens may take a long time. Therefore, it is critically important to develop methods to rapidly identify pathogens from positive blood culture.⁴

In 2009, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was first introduced for clinical microbial identification in Europe⁵ and in the United States.⁶ The principle is to identify bacteria according to their protein profiles, which are compared with standard profiles of known bacteria in a database.⁷ Because this method does not require conventional Gram staining and biochemical reactions, bacterial identification with pure colonies can be completed within a few minutes;⁸ thus, it is considered to be simple, fast, high-throughput, and cost-effective.⁹ In almost all studies, the accuracy of MS-based identification was better or equivalent to that of the commonly used biochemical identification methods, but with a short inspection cycle and a reduced cost.¹⁰

In recent years, the rapid development of MS technology has shortened the identification time of bacteria from positive blood culture to less than 2 hours when pure colonies are not obtained; thus, MS provides a timely and reliable basis for treating patients with BSI.¹¹ Because patient conditions are relatively critical and complicated, clinicians urgently need to know the identity of causal pathogens. However, the current MS identification from positive blood cultures still requires more time than desired, given the urgency of cases with BSI. Considering these limitations, it is necessary to develop a more rapid, direct identification method for bacterial identification based on MS from positive blood cultures in order to improve treatment strategies. Although various efforts have been made to directly identify bacteria from positive blood cultures by MS, the necessity of using commercial kits still leads to a high cost and long assay time.¹² Thus, the aim of this study was to overcome these shortcomings by exploring a simple, rapid, and inexpensive method for direct identification of bacteria from positive blood cultures. Moreover, the efficiency of this optimized method was evaluated by comparing its consistency rate with that of the routine biochemical method used for bacterial identification.

2 | MATERIALS AND METHODS

2.1 | Sample collection

A retrospective study was conducted to collect 829 bottles of positive blood culture that were sent for clinical examination by the

Henan Provincial People's Hospital (Zhengzhou, China) from July 2018 to August 2019. The inclusion criterion for the blood samples was as follows: if the BD automatic blood culture system issued a positive alarm, the positive bottle was removed from the BD automatic blood culture system, confirmed by microscopy after routine smear Gram staining, and included in the study. The exclusion criterion was as follows: if the positive culture was found to be bacteria-free by microscopy after routine Gram staining, the sample was excluded from the study. For patients from whom multiple blood cultures were obtained simultaneously, only the blood culture that was first reported to be positive was used for further experiments to avoid repetition.

2.2 | Routine biochemical identification

Positive cultures were simultaneously transferred onto a blood agar plate, chocolate agar plate, and MacConkey agar plate (Autobio Diagnostics Ltd), and pure colonies were obtained for routine biochemical identification. The Phoenix 100 automatic biochemical identification system (BD Diagnostics) was used for routine biochemical identification.

2.3 | Statistical software

FlexControl 3.0, Biotype 3.0, and ChinProTool 3.0 (Bruker Company) were used to output credit scores of isolates for statistical analyses. Prism 7.0 (GraphPad) software was used to plot the results. Statistical analyses were performed with SPSS 20.0 software (IBM Corporation). Two-tailed *P* values <.05 were considered statistically significant.

2.4 | Microbe identification by MALDI-TOF MS

2.4.1 | MS pre-treatment

Through a preliminary experiment, the experimental group was compared by several different specimen-processing methods, and the standard experimental procedure was established after optimization and improvement: 3.0 mL of blood was taken from the positive culture flask and transferred to a 3.5-mL tube containing plasma separation gel, which was centrifuged at 3000g for 10 minutes. After discarding the supernatant, 1.0 mL deionized water was added to resuspend the precipitate and then transferred into a new 1.5-mL Eppendorf tube to repeat the centrifugation. The supernatant was discarded, and the upper liquid was carefully removed to retain the white bacterial membrane. Next, 1 μ L of the white bacterial membrane was subjected to MS.

2.4.2 | MALDI-TOF MS analysis

After drying the bacterial pellet on a MALDI-TOF MS target plate (Bruker Daltonics), 1 μ L of alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was placed onto each spot and air-dried.

Triplicate spots were generated for every sample. MALDI-TOF MS analysis was performed by the Microflex LT system (Bruker Daltonics) with MALDI BIOTYPER 3.3 (Bruker Daltonics) software. Analysis results were presented as an average score of three repeated values for every sample. According to the manufacturer's instructions, a score <1.7 indicates no reliable identification, a score between 1.7 and 1.999 indicates identification to the genus level, and a score ≥ 2 indicates identification to the species level.

3 | RESULTS

3.1 | Distribution of pathogens

Seven false-positive blood bottles were excluded, and from the positive cultures, 822 strains of bacteria and fungi were isolated, including 325 strains of gram-positive bacteria (39.5%), 402 strains of gram-negative bacteria (48.9%), 33 strains of fungi (4.01%), 20 strains of anaerobic bacteria (2.4%), and 42 strains of rare bacteria (5.1%). All the samples were identified using Microflex LT/SH MS with a total consistency rate of 88.7%; the highest identification consistency rate was of gram-negative bacteria, followed by that of gram-positive bacteria, yeast, and rare bacteria, in that order. There was a significant difference between the identification consistency rates of the different species of bacteria and fungi ($P = .002$; Figure 1). Additionally, among the 822 strains of bacteria, the MS score for 713 strains (86.7%) was higher than 1.7, and 485 strains (59%) had a score of more than 2.0 (Table S1).

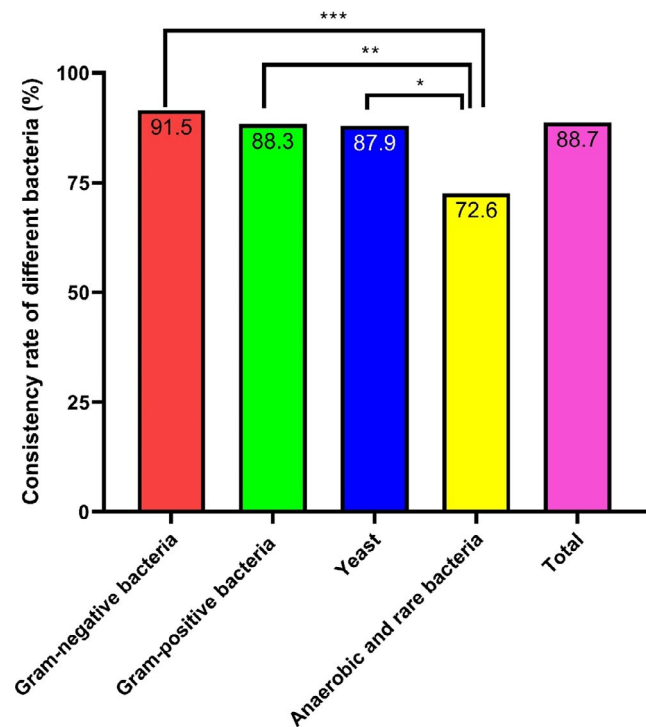


FIGURE 1 Consistency rates of the pathogens identified by MALDI-TOF MS. Gram-negative bacteria had the highest identification consistency rates. * $P < .05$, ** $P < .01$, and *** $P < .001$

3.2 | Gram-negative bacteria

Overall, 402 isolates were identified as gram-negative bacteria. Among them, 91.5% (368/402) were identified consistently. There was a significant difference in consistency rates among different species of gram-negative bacteria ($P < .01$). For example, the identification consistency rate was 100% for *Pseudomonas aeruginosa*, *Aeromonas hydrophaga*, *Klebsiella onionensis*, and *Baumannii/acinetobacter acetate complex*, followed by that for *Klebsiella pneumoniae* and *Escherichia coli* (96%; Figure 2). Additionally, among the 402 strains of gram-negative bacteria, the MS score for 371 strains (92.3%) was identified as at the genus level with a score >1.7; 267 strains (66.4%) were identified as species with a score >2.0 (Table S2).

3.3 | Gram-positive bacteria

Three hundred and twenty-five isolates were identified as gram-positive bacteria. A total of 88.3% (287/325) of the isolates were consistently identified using this optimized method. Among them, the highest consistency rate of identification was of *Staphylococcus haemolyticus*, *Staphylococcus cephalococcus*, *Staphylococcus goat*, and *Staphylococcus intermediate*, which was 100%, followed by that of *Staphylococcus epidermidis*, *Staphylococcus humanus*, and *Staphylococcus aureus*, which was above 90%. Therefore, there was a significant difference in the consistency rates among different species of gram-positive bacteria ($P < .01$; Figure 3). Additionally, among the 325 strains of gram-positive bacteria, the MS score for

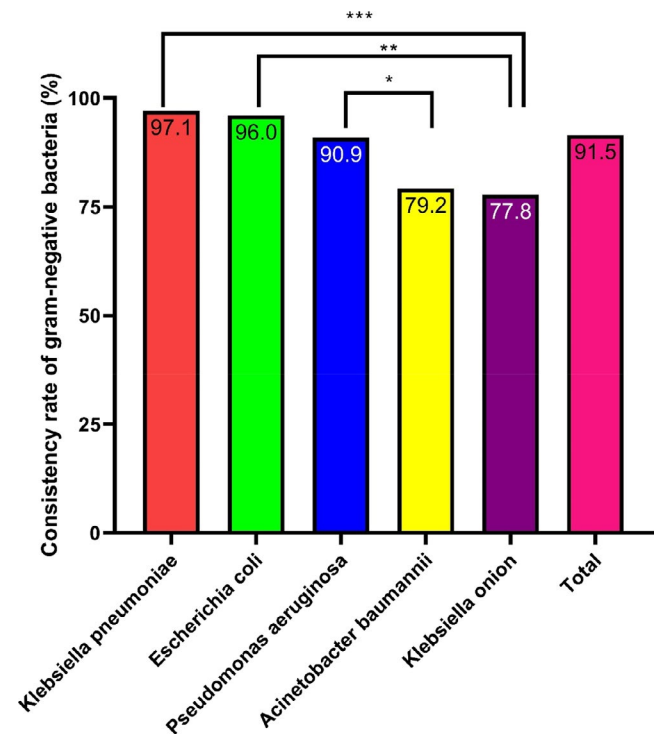


FIGURE 2 Consistency rates of the gram-negative bacteria identified by MALDI-TOF MS *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* had high identification consistency rates. * $P < .05$, ** $P < .01$, and *** $P < .001$

279 strains (85.8%) was higher than 1.7, and 189 strains (58.2%) had a score of more than 2.0 (Table S3).

3.4 | Yeasts

Yeasts were isolated from 33 positive blood cultures. Among them, 87.9% (29/33) were identified consistently. Moreover, 25 strains were identified as at the genus level with a score >1.7, and 7 strains were identified as species with a score >2.0. Both *Candida parapsilosis* and *Candida glabrata* had an identification consistency rate of 100%, followed by *Candida albicans* and *Candida tropicalis* with an identification consistency rate of 80%. There was a significant difference in consistency rates among different species of yeast ($P < .001$), as shown in Figure 4 and Table S4.

3.5 | Anaerobic bacteria and rare bacteria

Sixty-two anaerobic and rare bacteria were isolated in this study; 72.6% (45/62) of the isolates were consistently identified. Moreover, there was a significant difference in the consistency rates among different species of anaerobic bacteria and rare bacteria ($P < .001$; Figure 5). Further, 39 strains were identified as at the genus level with a score >1.7, and 23 strains were identified as species with a score >2.0, as shown in Table S5.

3.6 | Aerobic and anaerobic blood culture bottles

After excluding seven false-positive blood bottles (four aerobic vials and three anaerobic vials), 822 positive vials were obtained from the

829 positive culture vials; these samples included 549 aerobic vials and 273 anaerobic vials. Among these samples, there was a significant difference in the consistency rates among different types of blood bottles ($P < .01$; Figure 6).

4 | DISCUSSION

After the evaluation of identification consistency rates of MALDI-TOF MS with 822 positive blood samples, we found that by utilizing this optimized identification method, the exact identity of the pathogen responsible for BSI can be obtained within 1 hour, which provides time to administer rapid and accurate treatment to patients with BSI. Compared with traditional phenotypic identification methods, the advantage of this optimized method is that it enables direct and rapid identification of microbes in positive blood culture bottles; thus, it saves about 48 hours, which is typically required for isolation, culturing, and identification.¹⁰ Moreover, compared with other MALDI-TOF MS methods,^{11,12} this optimized method requires less time to rapidly and directly identify common bacteria from positive blood culture. Additionally, this method is simple, accurate, and cost-effective. Further, microbiological laboratory staff can select the appropriate kits for routine drug susceptibility testing based on the identification of bacteria by this method. It is tempting to speculate that routine implementation of this method will significantly increase the optimal utilization rate of antibiotics and further decrease mortality in bacteremia patients.

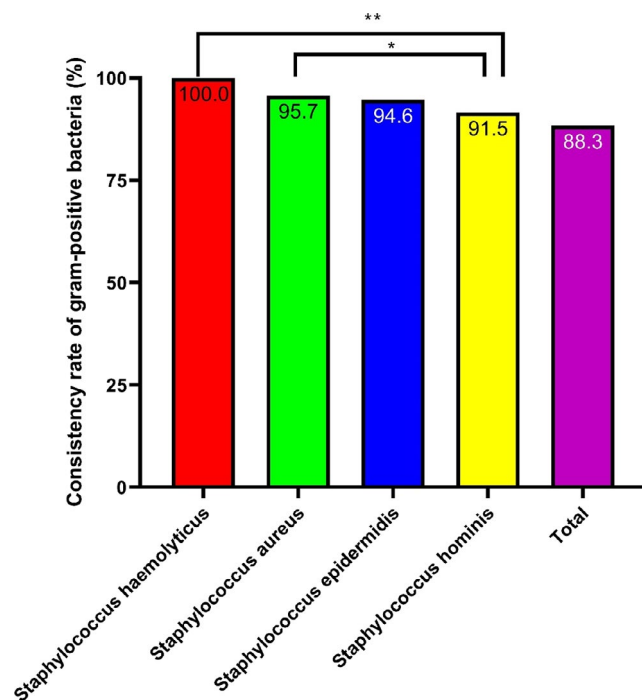


FIGURE 3 Consistency rates of the gram-positive bacteria identified by MALDI-TOF MS *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* had high identification consistency rates. * $P < .05$, ** $P < .01$, and *** $P < .001$

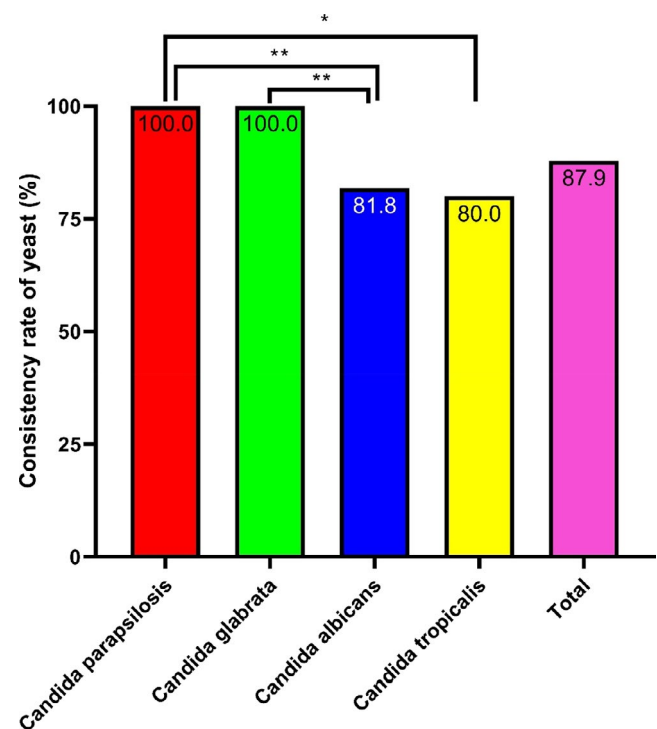


FIGURE 4 Consistency rates of the yeast identified by MALDI-TOF MS *Candida parapsilosis* and *Candida glabrata* had high identification consistency rates. * $P < .05$, ** $P < .01$, *** $P < .001$

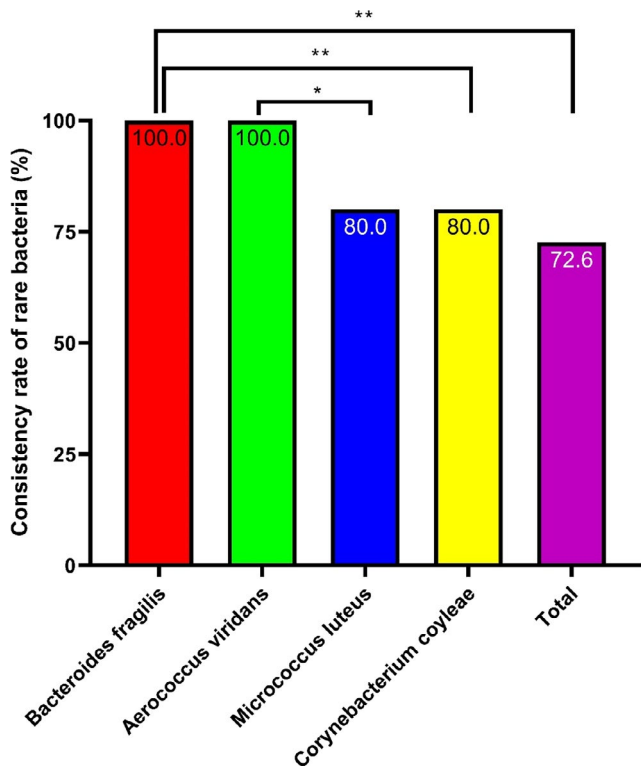


FIGURE 5 Consistency rates of the rare bacteria identified by MALDI-TOF MS *Bacteroides fragilis* and *Aerococcus viridans* had high identification consistency rates. * $P < .05$, ** $P < .01$, and *** $P < .001$

In this study, we found that gram-negative bacteria had the highest percentage of credibility scores (greater than 2), followed by gram-positive bacteria, fungi, anaerobic bacteria, and rare bacteria. These findings were consistent with previous reports, which found that the accuracy of MS for bacterial identification was higher for gram-negative bacteria than for gram-positive bacteria.^{11,12} Additionally, the accuracy of MS was high in the identification of common bacteria, including common gram-positive bacteria, such as *S aureus*, *Staphylococcus epidermis*, *Corynebacterium striatum*, *Enterococcus faecium*, and *Enterococcus faecalis*, as well as common gram-negative bacteria, such as *E coli*, *K pneumoniae*, *P aeruginosa*, and *Acinetobacter baumannii*. We also found that MS showed a high consistency rate in the identification of fungi, such as *C parapsilosis* and *C glabrata*; anaerobic bacteria, such as *Bacteroides fragilis*; and rare bacteria, such as *Garcinia micrococcus*. The low accuracy of identification of *Streptococcus* by MS may be related to high similarity between different species in the genus *Streptococcus*, such as *Streptococcus mitis*, *Streptococcus sanguis*, and *Streptococcus oralis*.¹³ For *Staphylococcus*, the main purpose is to distinguish *S aureus* from coagulase-negative *Staphylococcus*.

MS is more accurate in the differential diagnosis of two kinds of similar bacteria. Rapid and accurate identification of bacteria from positive blood culture flasks is the greatest advantage of MS. In our study, the results for identification at the species and genus levels could be obtained from a positive blood culture flask within 1 hour, which is in sharp contrast to the present routine laboratory analysis that can only report the results of Gram staining, and requires an additional 24–48 hours for species identification.¹⁴ It is well known that an identification method that saves time is critical for BSI, especially

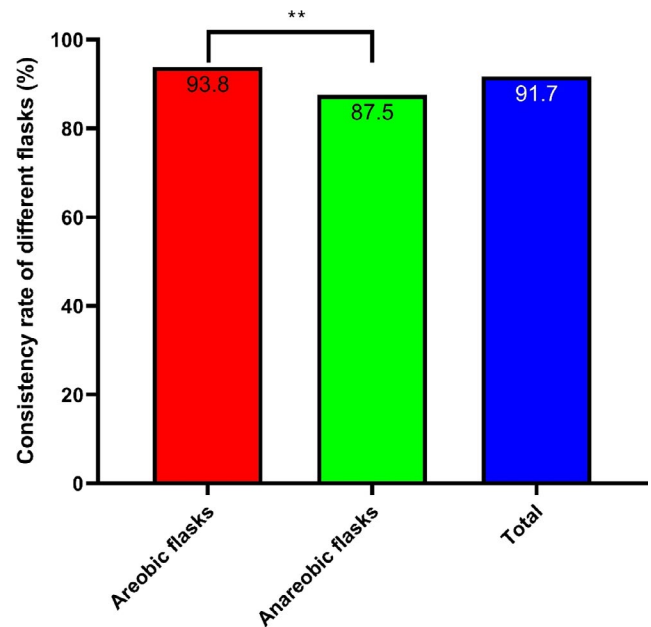


FIGURE 6 Consistency rates of bacteria from the blood culture flasks identified by MALDI-TOF MS. Aerobic blood flasks had high identification consistency rates. * $P < .05$, ** $P < .01$, and *** $P < .001$

for patients in need of quick treatment. Therefore, early identification of the causative pathogen may lead to a better, more efficient, and less expensive antibiotic treatment.¹⁵ If this technology is adapted and combined with drug resistance detection data in hospitals for the diagnosis and treatment of BSI, anti-infection treatment plans can be more accurately selected, which can enable curing of pathogenic infections and prevent the overuse of broad-spectrum antibiotics.¹⁶

It should be noted that there are some limitations in this study. For example, due to the number of specimens, the results of the first identification of some bacteria may be a bit far-fetched. However, the results of this study support the accuracy and rapidity of MS for direct identification of bacteria in positive blood culture flasks. Sample processing procedures, blood culture bottle types, and differences in the distribution of common strains may lead to differences in the results from different studies.^{17,18} Despite these limitations, MALDI-TOF MS has been proven to be a rapid and reliable method for the identification of pathogens directly from blood culture bottles. The technique is inexpensive, the identification can be completed within 1 hour, and specialized personnel is not required.¹⁹

In conclusion, this optimized method can be used to rapidly, simply, accurately, and cost-effectively identify common bacteria in positive blood cultures to meet the clinical demand for rapid diagnosis of common BSI pathogens. To carry out reasonable antibiotic treatment in early clinical stages and improve the survival rate of BSI patients, this method should be promoted.

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AUTHORS' CONTRIBUTIONS

YY and JW contributed to the writing of the manuscript; JW, SG, and ZY analyzed and interpreted the clinical data; BM, BW, YL, and NJ analyzed the antimicrobial susceptibility results; YY, SW, QZ, and JZ performed the statistical analyses; and all authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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