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Clinical evaluation of an in-house Xpert Lysate-based Method combined with MALDI-TOF MS for the rapid identification of positive blood cultures

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Abstract

Background In clinical practice, bloodstream infections (BSIs) can lead to a very high mortality rate. Rapid identification (ID) of the pathogenic bacteria in positive blood cultures (PBCs) can guide the clinical implementation of effective antibiotic treatment in advance, thereby helping to reduce the mortality rate.

Methods Using the Conventional Culture Method results as criteria, we evaluated the effectiveness of our newly developed in-house method, the Xpert Lysate-based Method, for rapidly identifying pathogens in PBCs across four hospitals.

Results A total of 629 monomicrobial PBCs were investigated. The Xpert Lysate-based Method correctly identified 96.18% of PBCs at the species level and 97.30% at the genus level. When the confidence scores of MALDI-TOF MS were ≥ 2.000, ≥ 1.700, and ≥ 1.500, the percentages of correctly identified PBCs at the species level were 67.47%, 94.24%, and 98.88%, respectively. When the score threshold of species-level ID was set to 1.500, the rates reached 98.25% for Gram-positives (GPs), 93.54% for Gram-negatives (GNs), 70.00% for anaerobes, and 94.74% for fungi, respectively. The median confidence score of ≥ 2.000 indicated high certainty in identifying common BSI pathogens. Additionally, one of the microbial species was correctly identified in 10 out of 11 PBCs with polymicrobial growth. The entire operation process took an average of 10 min of hands-on time and 15 to 20 min for time-to-result.

Conclusion The method of directly identifying microbial pellets using MALDI-TOF MS, extracted via the Xpert Lysate-based Method we developed, is non-inferior to the conventional Culture Method in terms of ID performance. Meanwhile, it is easy to operate and more time-efficient, making it suitable for routine workflow in the rapid etiological ID of PBCs.

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Keywords Bloodstream infection, Blood culture, MALDI-TOF MS, In-house, Time-saving workflow, Lysis

Introduction

Sepsis caused by bloodstream infections (BSIs) is a significant medical burden and a major cause of morbidity and mortality globally. Literature reports that sepsis causes 50 million cases and 10 million deaths annually, accounting for 20% of all global mortality [1]. Timely diagnosis is crucial for optimizing clinical outcomes, as the mortality rate increases with each hour of delayed effective antimicrobial therapy [2].

Recently, metagenomic next-generation sequencing (mNGS) of plasma cell-free DNA has emerged as a promising diagnostic technology for BSIs. However, the high rate of false positives in the culture-independent molecular ID of BSIs from whole blood remains a significant challenge to address [3]. Blood culture remains the "gold standard" for diagnosis, but it is limited by its long turnaround time (TAT), ranging from 1 to 7 days depending on the pathogen. Rapid identification (ID) technology of positive blood cultures (PBCs) is one of the effective methods to shorten the TAT time. 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 PBCs [4–6]. However, they are limited by high costs and restricted microorganism panels.

For clinical microbiology laboratories, the most widely used method remains the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This technique identifies pure microbial colonies grown on agar by matching protein spectral fingerprints to reference databases [7–9]. When a blood culture yields a positive alarm, an initial Gram stain is performed to determine the type of microorganisms present. The blood culture broth is then transferred to appropriate agar plates for subculturing, allowing microorganism colonies to form for ID by MALDI-TOF MS (the conventional Culture Method). For fast-growing pure isolates, direct ID is possible once scant growth is visible after 4 to 6 h of incubation. This approach is referred to as the short-term Culture Method [7, 10]. However, slow-growing isolates, especially anaerobes and fungi, typically require 24 to 72 h. Polymicrobial broth isolates need overnight or longer incubation to form discernible mature colonies for subsequent individual analysis of MALDI-TOF MS. Consequently, clinical microbiology laboratories need a faster method to identify pathogens and support clinicians in making timely treatment decisions for BSIs.

For the sample preparation for MALDI-TOF MS, directly isolating and concentrating microorganisms from blood culture broth offers a rapid alternative to subculturing. However, it is crucial to address the challenges posed by blood cell debris, hemoglobin, and nutrients in the broth, which can interfere with spectral peaks and impact the accuracy of ID. Several costly commercial protocols are currently available to address this issue, including the Sepsityper® kit (Bruker Daltonics, Billerica, USA) [10], the Vitek® MS blood culture kit (BioMérieux, Marcy-l'Étoile, France) [11], the FAST™ System using the FAST-PBC Prep™ cartridges (Qvella, Richmond Hill, Canada) [12], and the FASTinov® sample *prep* (FASTinov, Porto, Portugal) [13]. To reduce the financial burden, clinical microbiology laboratories have developed several in-house protocols that perform comparably to commercial protocols. These self-developed methods utilize separation gels tube or lysis buffers, such as 0.15 M NH_4Cl, 5.0% saponin, 0.2% Triton X-100, and 5.0% SDS, to remove blood cells and obtain pure microbial pellets through centrifugation [14–18]. For detailed performance indicators of these methods, refer to Supplementary File: Table S4.

In this prospective study, we developed a novel in-house lysis protocol combined with MALDI-TOF MS to enable the rapid ID of BSIs. We conducted parallel evaluations of its real-life microbiological application compared to the conventional Culture Method, a standard laboratory ID procedure, across four hospitals. The lysis buffer, composed of sodium hydroxide and isopropanol, was sourced from the Xpert® MTB/RIF Assay commercial kit (Cepheid, Sunnyvale, USA) [19]. Consequently, we designated this protocol as the "Xpert Lysate-based Method". Compared with current rapid methods, this method offers superior efficacy in terms of ID speed and accuracy (particularly for Gram-positives [GPs] accuracy).

Materials and methods

Clinical sample collection

This project was a multicenter study conducted in four hospitals in Zhejiang Province, China. It was organized by the Hubin branch of Zhejiang Provincial Hospital of Chinese Medicine (Hospital A) and included the Qiantang branch of Zhejiang Provincial Hospital of Chinese Medicine (Hospital B), Hangzhou First People's Hospital (Hospital C), and The Quzhou Affiliated Hospital of Wenzhou Medical University (Hospital D).

Blood culture bottles (BacT/Alert FA/FN/PF Plus; BioMérieux Inc., Durham, USA) were incubated in the BacT/ALERT® 3D system (BioMérieux, Marcy-l'Étoile,

France). To avoid duplication, only the first PBC from each individual was used when multiple PBCs occurred simultaneously. PBCs that could not be subcultured were excluded. From June 2023 to February 2024, a total of 631 clinical PBCs were collected from inpatient and outpatient (emergency) departments. This included 620 monomicrobial PBCs (191 from Hospital A, 159 from Hospital B, 85 from Hospital C, and 185 from Hospital D) and 11 polymicrobial PBCs (3 from Hospital A, 2 from Hospital C, and 6 from Hospital D).

Mock-inoculated blood culture

To increase the isolation rates of pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Acinetobacter baumannii* from PBCs, simulations were conducted using three vials for each pathogen. Nine strains were isolated from different clinical sputum specimens. Firstly, the bacterial cells were standardized in 0.90% NaCl to 0.5 MacFarland (1.5×10^8 colony-forming units [CFU]/mL) using a DensiCHEK Plus instrument (BioMérieux, Marcy-l'Étoile, France), then diluted to 1×10^3 CFU/mL. Next, 1 mL of the 1×10^3 dilution was added to 9 mL of blood from healthy donors to achieve a final concentration of 1×10^2 CFU/mL. Subsequently, 10 mL of this bacterial suspension was injected into an aerobic FA bottle to create a positive simulation with 25 CFU/mL (1000 CFU/bottle). The mixture was thoroughly homogenized and loaded into the BacT/ALERT® 3D system. To ensure no contamination, 1 mL of sterile saline and 9 mL of fresh blood were injected into a FA bottle as the negative control and cultured for 5 days.

The conventional Culture Method

Gram staining microscopy guided the subculturing of blood culture broth onto suitable agar plates under optimal conditions. Blood agar was used for Gram-negative bacilli, Gram-positive cocci, or rod-shaped bacilli. Suspected *H. influenzae* (small Gram-negative bacilli) was cultured on nutrient-rich chocolate agar. Sabouraud dextrose agar was used for fungal spores or hyphae. Agar plates were sourced from BIO-KONT (Wenzhou, China). For suspected anaerobes, inoculation was performed using GENbag anaer (BioMérieux, Marcy-l'Étoile, France). Subcultures were incubated at 35 °C for 15 to 48 h for bacteria and at 28 °C for 24 to 72 h or longer for fungi, yielding mature colonies for MALDI-TOF MS (as illustrated in the upper part of Fig. 1).

The Xpert Lysate-based Method

Firstly, the blood culture broth was homogenized. After disinfecting the top of the culture bottle, 1 mL of broth was extracted using a syringe and transferred to a 1.5 mL Eppendorf centrifuge tube. Then, 60 µL of the sample preparation reagent from the Xpert® MTB/RIF commercial kit was added to the tube. The mixture was inverted 5 to 7 times to ensure thorough mixing. The lysate was then centrifuged at 15,000 rpm for 1 min, and the supernatant was discarded. The microbial pellet was washed with 1 mL of pure water, followed by another centrifugation at 15,000 rpm for 1 min. This washing step was repeated once, with as much supernatant as possible removed using a micropipette. Finally, the pellet was resuspended in 20 µL of 100% formic acid, and 1 µL of the microbial suspension was evenly applied onto the the MSP 96 target polished steel plate (Bruker Daltonics,

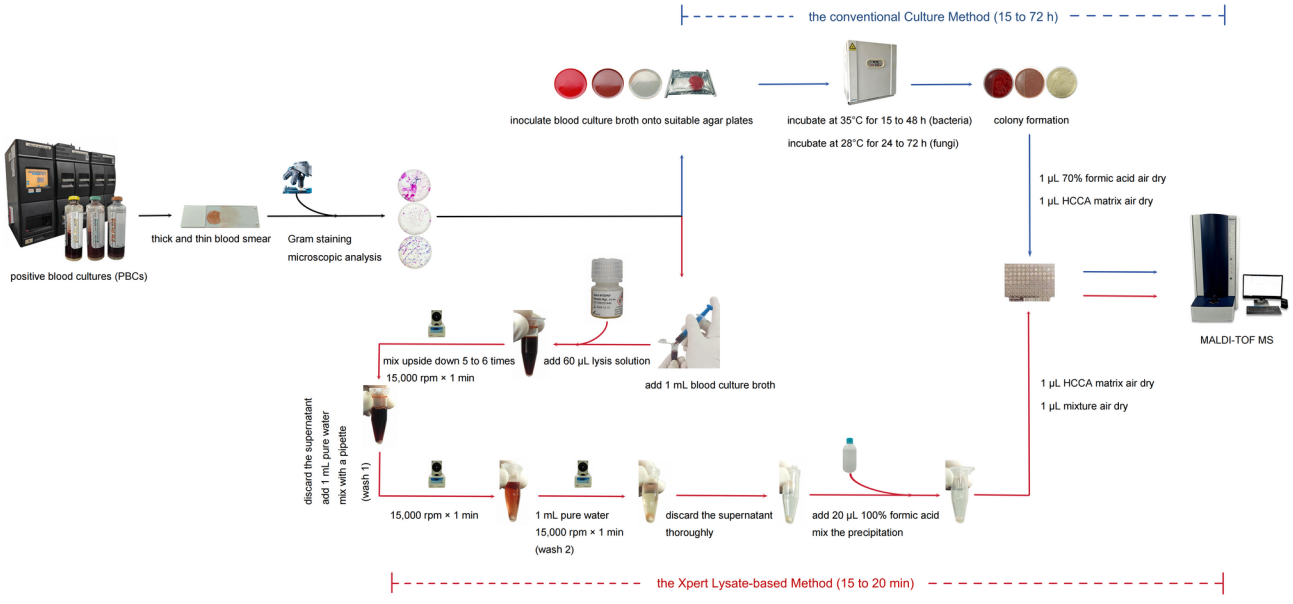

Fig. 1 Operation diagram of the Xpert Lysate-based Method and the conventional Culture Method

Bremen, Germany) for ID by MALDI-TOF MS (as illustrated in the lower part of Fig. 1).

MALDI-TOF MS

In the conventional Culture Method, 1 μL 70% formic acid (70% v/v) was applied to the steel target plate. A single colony was picked and spread evenly using a toothpick. For the Xpert Lysate-based Method, a 1 μL microbial suspension was spotted. After air drying, each spot was covered with 1 μL HCCA matrix (Bruker Daltonics, Bremen, Germany) at a concentration of 5 mg/mL, prepared in a solvent comprising 50.0% acetonitrile, 47.5% ultrapure water, and 2.5% trifluoroacetic acid. MALDI-TOF MS analysis was performed using the MicroFlex LT System and its proprietary software (Bruker Daltonics, Bremen, Germany). Mass spectra were automatically collected within the m/z range of 2,000 to 20,000 and analyzed using MALDI Biotyper RTC software. Each spot's similarity score [log(score)] was calculated by matching the protein spectra with the reference library spectra. If the result indicated "no peaks found", the spectrum of the target spot was manually acquired using flexControl software, and the corresponding ID was determined using MALDI Biotyper OC software. The strain with the highest score (best-matched ID) was recorded as the ID result. If the score was below 1.500 and the top two results did not correspond to the same species, the strain was still classified as no ID result.

Statistical analyses

The conventional Culture Method was used as the reference method for ID. A Pearson Chi-square test was used to compare the ID rates across four hospitals. A Pearson Chi-square test was used to compare the ID rates between aerobic and anaerobic PBCs. A paired t-test was used to compare the mean confidence scores between two methods. Statistical analyses used SPSS 20. Differences with $P\text{-}value < 0.05$ were considered statistically significant.

Result

Clinical sample characteristics

Among the entire set of 631 clinical PBCs investigated in this study, 620 were monomicrobial and 11 were polymicrobial. Of these, 591 (93.66%) were peripheral blood samples, and 40 (6.34%) were catheter-derived blood samples. This included 353 (55.95%) aerobic cultures, 273 (43.26%) anaerobic cultures, and 5 (0.79%) pediatric cultures. The clinical departments with the highest number of PBCs were the Intensive Care Unit (26.78%, 169), Emergency Dept. (13.47%, 85), Hematology Dept. (11.41%, 72), Medical Oncology (9.83%, 62), and Nephrology Dept. (4.75%, 30) (as illustrated in Supplementary File: Fig. S1).

Based on the results of the conventional Culture Method, a total of 79 distinct microorganisms were identified from the clinical monomicrobial PBCs across the four hospitals. The five most frequently isolated microorganisms were *Escherichia coli* (21.61%, 134), *Klebsiella pneumoniae* (14.35%, 89), *Staphylococcus epidermidis* (9.03%, 56), *Staphylococcus capitis* (6.45%, 40), and *Staphylococcus aureus* (5.32%, 33) (as illustrated in Supplementary File: Fig. S2).

ID rate without score threshold

The conventional Culture Method results served as the standard. Without a score threshold for species-level ID, the ID rates of clinical monomicrobial PBCs using the Xpert Lysate-based Method were 97.91% (187/191) in Hospital A, 98.11% (156/159) in Hospital B, 92.94% (79/85) in Hospital C, and 95.14% (176/185) in Hospital D. These differences were not statistically significant ($\chi^2 = 6.460$, $P\text{-}value = 0.091$). Aerobic PBCs had a higher ID rate (96.83%, 336/347) compared to anaerobic PBCs (95.90%, 257/268), but this difference was also not statistically significant ($\chi^2 = 0.383$, $P\text{-}value = 0.536$).

Among the entire set of 629 monomicrobial PBCs investigated, 620 were clinical and 9 were simulated. Without applying a score threshold, the overall ID rates of species-level reached 96.18% (605 PBCs) and genus-level reached 97.30% (612 PBCs) using the Xpert Lysate-based Method. Of the 605 PBCs, 601 (99.34%) were identified automatically by MALDI Biotyper, while four PBCs (0.66%) required manual IDs: *Micrococcus luteus* (score = 2.056), *K. pneumoniae* (score = 1.721), *Pseudomonas aeruginosa* (score = 1.604), and *Bacteroides vulgatus* (score = 1.605). The highest species-level ID rate was observed for GPs at 98.82% (252/255), followed by fungi at 95.45% (21/22), Gram-negatives (GNs) at 94.88% (315/332), and anaerobes at 85.00% (17/20). A total of 24 (3.82%) PBCs were incorrectly identified. Seven (1.11%) PBCs, including three isolates of *Enterobacter cloacae*, were misidentified as different species within the same genus. Additionally, 17 (2.70%) PBCs, including seven isolates of *P. aeruginosa*, had no ID results. Refer to Table 1 for details.

ID rate limited by score threshold

In this analysis, 67 monomicrobial PBCs were excluded due to the absence of confidence score for the Xpert Lysate-based Method, as indicated by being marked "missing" in the "MS score" column in Supplementary File: Table S1. A total of 562 monomicrobial PBCs were included, comprising 538 correctly identified PBCs with MALDI-TOF MS scores, and 24 PBCs that failed to be identified, as illustrated in Table 1.

As shown in Table 2, when the score thresholds for species-level ID were set at 2.000, 1.700, and 1.500, the

Table 1 Identification (ID) rate for 629 monomicrobial positive blood cultures (PBCs) using the the Xpert Lysate-based Method

microorganisms	no. of strains	correct ID no. (%)		failed ID			
		species level	genus level	the conventional Culture Method result	the Xpert Lysate-based Method result	frequency	MALDI-TOF MS score
total	629	605 (96.18)	612 (97.30)				
Gram-negatives	332	315 (94.88)	322 (96.99)	*Enterobacter cloacae*	*Enterobacter bugandensis*	×2	2.185; 1.976
				Enterobacter cloacae	*Enterobacter asburiae*	×1	1.945
				Enterobacter asburiae	*Enterobacter cloacae*	×1	1.724
				Serratia ureilytica	*Serratia marcescens*	×1	1.956
				Pseudomonas aeruginosa	No result	×7	
				Acinetobacter baumannii	*Acinetobacter nosocomialis*	×1[a]	1.813
				Acinetobacter nosocomialis	*Acinetobacter seifertii*	×1	1.718
				Stenotrophomonas maltophilia	No result	×2	
				Haemophilus influenzae	No result	×1[a]	
Gram-positives	255	252 (98.82)	252 (98.82)	*Staphylococcus epidermidis*	No result	×1	
				Rothia mucilaginosa	No result	×1	
				Granulicatella adiacens	No result	×1	
anaerobes	20	17 (85.00)	17 (85.00)	*Bacteroides fragilis*	No result	×2	
				Clostridium paraputrificum	No result	×1	
fungi	22	21 (95.45)	21 (95.45)	*Talaromyces marneffei*	No result	×1	

[a] A simulated strain

overall ID rates of monomicrobial PBCs using the Xpert Lysate-based Method were 64.59% (363/562), 90.21% (507/562), and 94.66% (532/562), respectively. In addition, for GPs, the rates were 64.19% (147/229), 93.89% (215/229), and 98.25% (225/229), respectively. For GNs, the rates were 68.03% (200/294), 90.48% (266/294), and 93.54% (275/294), respectively. For anaerobes, the rates were 45.00% (9/20), 50.00% (10/20), and 70.00% (14/20), respectively. For fungi, the rates were 36.84% (7/19), 84.21% (16/19), and 94.74% (18/19), respectively.

Of the 538 PBCs with correct species-level ID, 363 PBCs (67.47%) had scores ≥ 2.000, 507 PBCs (94.24%) had scores ≥ 1.700, and 532 PBCs (98.88%) had scores ≥ 1.500. Only six PBCs (1.12%) had scores < 1.500, with one strain each of *Corynebacterium afermentans*, *Stenotrophomonas maltophilia*, *Aeromonas caviae*, *Bacteroides fragilis*, *Eggerthella lenta*, and *Bacteroides pyogenes*. When a species-level ID threshold of 1.500 was determined as the most suitable for judgment, the ID rates of the top five isolates were: *E. coli* (100.00%, 117/117), *K. pneumoniae* (100.00%, 82/82), *S. epidermidis* (97.96%, 48/49), *S. capitis* (100.00%, 35/35), and *S. aureus* (100.00%, 32/32). Additionally, for other common BSI pathogens, the rates were: *Enterococcus faecium* (100.00%, 27/27), *P. aeruginosa* (53.33%, 8/15), *E. cloacae* (78.57%, 11/14), *Enterococcus faecalis* (100.00%, 12/12), *A. baumannii* (87.50%, 7/8), *Corynebacterium striatum* (100.00%, 7/7), *Serratia marcescens* (100.00%, 6/6), *S. pneumoniae* (100.00%, 6/6), *Candida albicans* (100.00%, 6/6), and *Proteus mirabilis* (100.00%, 5/5). Refer to Table 2 for details.

Comparison of confidence scores between the two methods

In this analysis, 135 correctly identified monomicrobial PBCs were excluded due to the absence of analytical factors, as indicated by being marked "missing" or "≥ 2.000" in the "MS score" column in Supplementary File: Table S1. A final inclusion of 470 PBCs with recorded precise confidence scores for either method was utilized for comparison.

The overall mean ± standard deviation (SD) value of confidence scores using the Xpert Lysate-based Method was 2.066 ± 0.210, which was lower than that for the conventional Culture Method at 2.245 ± 0.178, with a statistical significance level of *P-value* < 0.001. This decreasing trend was also observed in the top five isolates, with *P-values* of < 0.001 for *E. coli*, *K. pneumoniae*, *S. epidermidis*, and *S. aureus*, and a *P-value* of 0.012 for *S. capitis*. Refer to Supplementary File: Table S2 for details.

Comparison of confidence scores for various microorganisms

In this analysis, 82 correctly identified monomicrobial PBCs were excluded due to the absence of a precise confidence score for the Xpert Lysate-based Method, as indicated by being marked as "missing" or "≥ 2.000" in the

Table 2 Identification (ID) performance of the Xpert Lysate-based Method under different species-level score thresholds of MALDI-TOF MS

microorganisms	no. of strains	538 monomicrobial positive blood cultures (PBCs) correctly identified by the Xpert Lysate-based Method no. (%)			
		score ≥ 2.000	score ≥ 1.700	score ≥ 1.500	score < 1.500
Total	**562**	**363 (64.59)**	**507 (90.21)**	**532 (94.66)**	**6 (1.07)**
Gram-positives	**229**	**147 (64.19)**	**215 (93.89)**	**225 (98.25)**	**1 (0.44)**
Staphylococcus	**144**	**90 (62.50)**	**139 (96.53)**	**143 (99.31)**	
Staphylococcus epidermidis	49	14 (28.57)	47 (95.92)	48 (97.96)	
Staphylococcus capitis	35	24 (68.57)	33 (94.29)	35 (100.00)	
Staphylococcus aureus	32	30 (93.75)	32 (100.00)	32 (100.00)	
Staphylococcus hominis	22	19 (86.36)	22 (100.00)	22 (100.00)	
Staphylococcus cohnii	2		1 (50.00)	2 (100.00)	
Staphylococcus haemolyticus	2	2 (100.00)	2 (100.00)	2 (100.00)	
Staphylococcus equorum	1	1 (100.0)	1 (100.00)	1 (100.00)	
Staphylococcus caprae	1		1 (100.00)	1 (100.00)	
Enterococcus	**42**	**34 (80.95)**	**39 (92.86)**	**42 (100.00)**	
Enterococcus faecium	27	20 (74.07)	24 (88.89)	27 (100.00)	
Enterococcus faecalis	12	12 (100.00)	12 (100.00)	12 (100.00)	
Enterococcus casseliflavus	3	2 (66.67)	3 (100.00)	3 (100.00)	
Streptococcus	**18**	**12 (66.67)**	**18 (100.00)**	**18 (100.00)**	
Streptococcus pneumoniae	6[a]	3 (50.00)	6 (100.00)	6 (100.00)	
Streptococcus agalactiae	5	5 (100.00)	5 (100.00)	5 (100.00)	
Streptococcus oralis	4	3 (75.00)	4 (100.00)	4 (100.00)	
Streptococcus pyogenes	1		1 (100.00)	1 (100.00)	
Streptococcus dysgalactiae	1		1 (100.00)	1 (100.00)	
Streptococcus anginosus	1	1 (100.00)	1 (100.00)	1 (100.00)	
Other Gram-positives	**25**	**11 (44.00)**	**19 (76.00)**	**22 (88.00)**	**1 (4.00)**
Corynebacterium striatum	7	1 (14.29)	5 (71.43)	7 (100.00)	
Micrococcus luteus	4	4 (100.00)	4 (100.00)	4 (100.00)	
Bacillus subtilis	3		3 (100.00)	3 (100.00)	
Listeria monocytogenes	2	2 (100.00)	2 (100.00)	2 (100.00)	
Brevibacterium casei	2	2 (100.00)	2 (100.00)	2 (100.00)	
Rothia mucilaginosa	1				
Bacillus cereus	1	1 (100.00)	1 (100.00)	1 (100.00)	
Corynebacterium afermentans	1				1 (100.00)
Corynebacterium tuberculostearicum	1	1 (100.00)	1 (100.00)	1 (100.00)	
Corynebacterium aurimucosum	1			1 (100.00)	
Gordonia sputi	1		1 (100.00)	1 (100.00)	
Granulicatella adiacens	1				
Gram-negatives	**294**	**200 (68.03)**	**266 (90.48)**	**275 (93.54)**	**2 (0.68)**
Enterobacterales	**252**	**196 (77.78)**	**243 (96.43)**	**247 (98.02)**	
Escherichia coli	117	101 (86.32)	114 (97.44)	117 (100.00)	
Klebsiella pneumoniae	82	63 (76.83)	82 (100.00)	82 (100.00)	
Enterobacter cloacae	14	9 (64.29)	11 (78.57)	11 (78.57)	
Serratia marcescens	6	2 (33.33)	6 (100.00)	6 (100.00)	
Klebsiella aerogenes	5	5 (100.00)	5 (100.00)	5 (100.00)	
Proteus mirabilis	5	3 (60.00)	4 (80.00)	5 (100.00)	
Citrobacter freundii	3	2 (66.67)	3 (100.00)	3 (100.00)	
Salmonella spp.	3	1 (33.33)	3 (100.00)	3 (100.00)	
Citrobacter koseri	3	2 (66.67)	3 (100.00)	3 (100.00)	
Morganella morganii	2	2 (100.00)	2 (100.00)	2 (100.00)	
Proteus vulgaris	2	1 (50.00)	2 (100.00)	2 (100.00)	
Enterobacter kobei	2	2 (100.00)	2 (100.00)	2 (100.00)	
Serratia ureilytica	2		1 (50.00)	1 (50.00)	
Raoultella ornithinolytica	1	1 (100.00)	1 (100.00)	1 (100.00)	

Table 2 (continued)

microorganisms	no. of strains	538 monomicrobial positive blood cultures (PBCs) correctly identified by the Xpert Lysate-based Method no. (%)			
		score ≥ 2.000	score ≥ 1.700	score ≥ 1.500	score < 1.500
Enterobacter bugandensis	1		1 (100.00)	1 (100.00)	
Enterobacter asburiae	2		1 (50.00)	1 (50.00)	
Klebsiella oxytoca	1	1 (100.00)	1 (100.00)	1 (100.00)	
Proteus hauseri	1	1 (100.00)	1 (100.00)	1 (100.00)	
Non-fermenting bacteria	**36**	**4 (11.11)**	**20 (55.56)**	**24 (66.67)**	**1 (2.78)**
Pseudomonas aeruginosa	15	2 (13.33)	6 (40.00)	8 (53.33)	
Acinetobacter baumannii	8[a]		7 (87.50)	7 (87.50)	
Stenotrophomonas maltophilia	5		2 (40.00)	2 (40.00)	1 (20.00)
Acinetobacter nosocomialis	3	1 (33.33)	1 (33.33)	2 (66.67)	
Burkholderia cepacia	2		2 (100.00)	2 (100.00)	
Moraxella osloensis	1	1 (100.00)	1 (100.00)	1 (100.00)	
Rhizobium radiobacter	1		1 (100.00)	1 (100.00)	
Acinetobacter parvus	1			1 (100.00)	
Other Gram-negatives	**6**		**3 (50.00)**	**4 (66.67)**	**1 (16.67)**
Haemophilus influenzae	3[a]		1 (33.33)	2 (66.67)	
Aeromonas caviae	2		1 (50.00)	1 (50.00)	1 (50.00)
Aeromonas veronii	1		1 (100.00)	1 (100.00)	
Anaerobes	**20**	**9 (45.00)**	**10 (50.00)**	**14 (70.00)**	**3 (15.00)**
Bacteroides fragilis	5	2 (40.00)	2 (40.00)	2 (40.00)	1 (20.00)
Clostridium perfringens	3	2 (66.67)	3 (100.00)	3 (100.00)	
Eggerthella lenta	3	2 (66.67)	2 (66.67)	2 (66.67)	1 (33.33)
Actinomyces neuii	2	1 (50.00)	1 (50.00)	2 (100.00)	
Clostridium ramosum	1	1 (100.00)	1 (100.00)	1 (100.00)	
Lactobacillus paracasei	1	1 (100.00)	1 (100.00)	1 (100.00)	
Veillonella parvula	1			1 (100.00)	
Bacteroides vulgatus	1			1 (100.00)	
Bifidobacterium longum	1			1 (100.00)	
Bacteroides pyogenes	1				1 (100.00)
Clostridium paraputrificum	1				
Fungi	**19**	**7 (36.84)**	**16 (84.21)**	**18 (94.74)**	
Candida albicans	6	1 (16.67)	6 (100.00)	6 (100.00)	
Candida tropicalis	5	2 (40.00)	5 (100.00)	5 (100.00)	
Candida parapsilosis	2	1 (50.00)	1 (50.00)	2 (100.00)	
Cryptococcus neoformans	2	1 (50.00)	2 (100.00)	2 (100.00)	
Candida auris	2	1 (50.00)	1 (50.00)	2 (100.00)	
Pichia norvegensis	1	1 (100.00)	1 (100.00)	1 (100.00)	
Talaromyces marneffei	1				

[a] Including three simulated strains

"MS score" column in Supplementary File: Table S1. A final inclusion of 523 PBCs was selected for analysis.

As illustrated in Fig. 2A, among the frequently isolated GPs, *S. epidermidis* had the lowest median score at 1.910 (interquartile range [IQR] 1.846–2.033), while *S. aureus* had the highest median score at 2.198 (IQR 2.102–2.285). Both *S. capitis* and *Staphylococcus hominis* had median scores ≥ 2.000, at 2.066 (IQR 1.970–2.166) and 2.144 (IQR 2.030–2.256), respectively. *E. faecium* and *E. faecalis* had similar median scores of 2.183 (IQR 1.937–2.287) and 2.190 (IQR 2.112–2.350). As illustrated in Fig. 2B, among the frequently isolated GNs, *P. aeruginosa* had the lowest median score at 1.865 (IQR 1.646–2.060). *E. coli*, *K. pneumoniae*, and *E. cloacae* had median scores of 2.175 (IQR 2.058–2.258), 2.138 (IQR 2.019–2.252), and 2.206 (IQR 2.057–2.245), respectively—all ≥ 2.000. Additionally, the median score for *Candida spp.* was relativly lower, at 1.892 (IQR 1.744–2.018).

ID performance of polymicrobial PBCs

Of the 11 polymicrobial PBCs, 9 contained 2 microbial species, and 2 contained 3 microbial species. Using the Xpert Lysate-based Method, no spectral peak was detected in one PBC. However, in the remaining 10

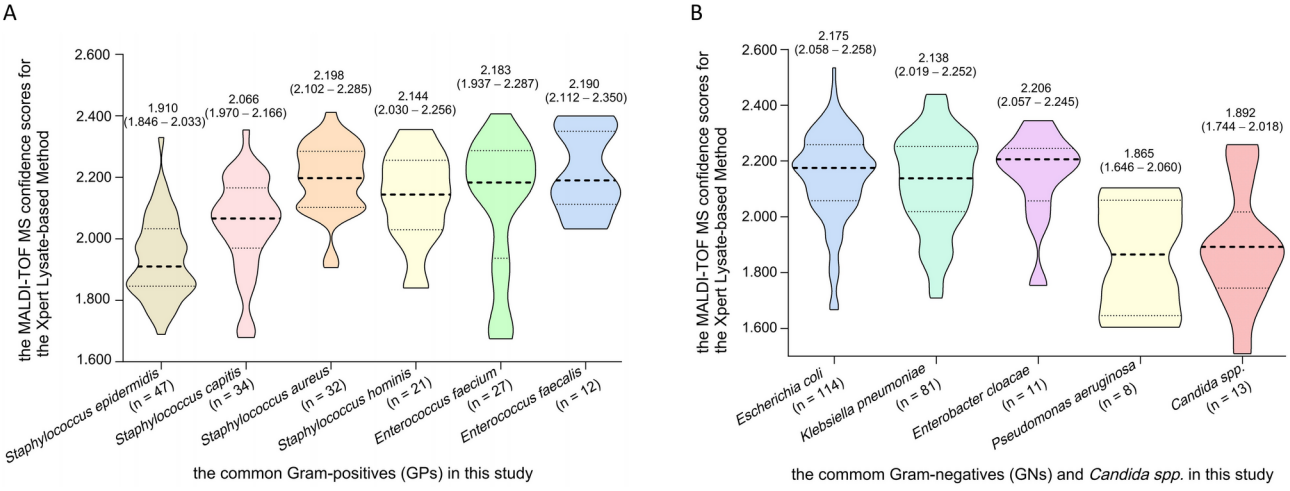

Fig. 2 Distribution of confidence scores of MALDI-TOF MS for the most frequent isolates identified by the Xpert Lysate-based Method. Quartiles (Median, 25th and 75th percentile). **A**, For Gram-positives. **B**, For Gram-negatives and *Candida spp*

PBCs, one of the microbial species was correctly identified, with a mean confidence score of 1.985. Refer to Supplementary File: Table S3 for details.

Discussion

Optimal species-level score threshold

The Bruker MALDI-TOF MS system, used for detecting pure microbial colonies from agar plates, categorizes results based on confidence scores into three groups: unreliable ID (< 1.700), genus-level ID (≥ 1.700 and < 2.000), or species-level ID (≥ 2.000). However, some studies have indicated that the score threshold for directly identifying PBCs could be lowered to 1.600 or 1.400, provided the top-ranking IDs were consistent [20, 21]. This adjustment could improve species-level ID rates without causing any misidentification. In this study, given the high concordance and absence of error IDs in the 1.500 to 2.000 range, the threshold for valid species-level ID using the Xpert Lysate-based Method could be dropped to 1.500. At this score threshold, a ID rate of 94.66% was achieved, effectively covering most PBCs. Additionally, despite the mean confidence scores for the common pathogens causing BSIs—*E. coli*, *S. aureus*, *K. pneumoniae*, *S. capitis*, *E. faecium*, *S. hominis*, *E. cloacae*, and *E. faecalis* [22, 23]—being lower with the Xpert Lysate-based Method compared to the conventional Culture Method, the scores still ≥ 2.000 for reliable species-level ID. These lower scores were due to the high background of non-specific proteins in the lysate and relatively lower analyte concentrations, resulting in poor spectral peaks. However, this did not significantly affect the accuracy of the final ID.

Hands-on time and time-to-result

With just 10 min of hands-on time, the Xpert Lysate-based Method integrates seamlessly into the routine workflow of microbiology laboratories. This flexibility allows laboratories to treat PBCs promptly or in batches based on internal sample volume and staffing models. Additionally, as shown in Fig. 3, this method's 20-minute time-to-result is much shorter than the the conventional Culture Method (15 to 72 h) and the short-term Culture Method (4 to 6 h) [7, 10]. The Culture Method often delays the diagnosis of slow-growing microorganisms in BSIs due to its lengthy culture cycle. In contrast, the Xpert Lysate-based Method effectively improved the ID speed, aiding in the accurate selection of clinical antibiotics. It also outperforms blood culture-dependent molecular assays like BioFire FilmArray (60 min) [5] and the Accelerate Pheno™ system (90 min) [4], both in ID speed and the variety of strains identified.

Usability and stability

The Xpert Lysate-based Method is remarkably convenient, requiring no specialized or hard-to-obtain equipment—only a pre-treated lysis buffer. It is easily implementable in routine clinical microbiology laboratories equipped with MALDI-TOF MS and the Xpert® MTB/RIF kit. The WHO-recommended tuberculosis rapid diagnostic technology Xpert® MTB/RIF has achieved widespread adoption globally, particularly in developing countries with high tuberculosis incidence rates [24, 25]. However, in such low- and middle-income countries, high costs and stringent laboratory requirements often limit hospitals' ability to access molecular diagnostic techniques for rapid pathogen detection in BSIs. This study provides a practical solution. While the lysis buffer is not sold separately, laboratories without the Xpert® MTB/RIF kit can prepare it using the provided formula. In our comparative study, a self-made lysis buffer (5% sodium hydroxide and 10% isopropanol) proved equally effective as a commercially available reagent in

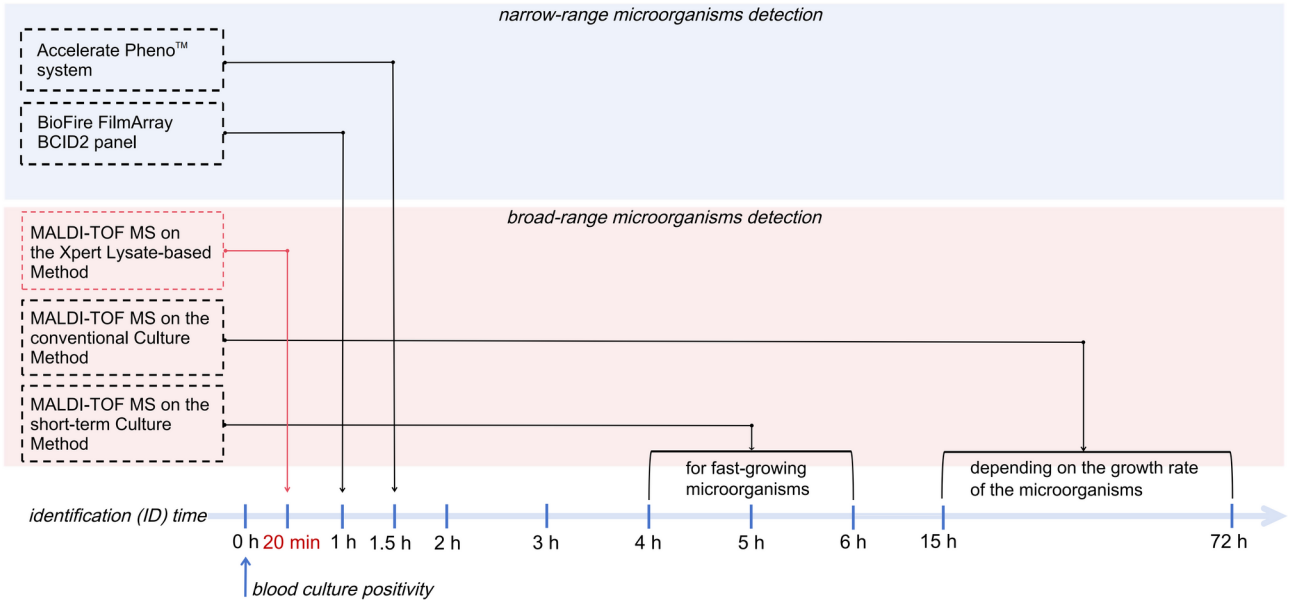

Fig. 3 Comparison of time-to-result for identifying positive blood cultures (PBCs): the Xpert Lysate-based Method vs. the Culture Method vs. molecular methods

identifying microorganisms from 48 clinical monomicrobial PBCs. There was no significant difference in the mean confidence scores between the two reagents, as detailed in Supplementary File: Table S5 and Fig. S3, further validating the efficiency of the self-made buffer.

The microbial pellets extracted from blood culture broths were nearly all automatically identified by the MALDI Biotyper system in this study, demonstrating its ease of use. Additionally, there were no statistical differences in the ID rates between the participating hospitals, indicating its stable performance across different operators and environments.

Comparison to other MS-based rapid methods

Despite the challenges in making standard comparisons among the MS-based commercial kits or in-house methods for rapid ID of PBCs, as mentioned in the introduction—refer to Supplementary File: Table S4 for details—due to varying assay conditions, the Xpert Lysate-based Method still demonstrated a distinct advantage. Under the same condition of a species-level score threshold of at least low confidence, the ID rates of GNs were approximately 90% or higher for all listed methods, except for 5% saponin lysis. However, the previously reported ID rates of GPs ranged from 71 to 85%, significantly lower than those for GNs [7, 10–18]. This discrepancy may be due to the increased peptidoglycan in the cell wall, which makes GPs more resistant to lysis [14, 26]. The lysis buffer used in the Xpert Lysate-based Method enhanced species-level ID accuracy to over 90% for both GPs and GNs, with the former showing higher accuracy at 93.89%. Coagulase-negative staphylococci (CoNS) were the primary contaminants in false-positive blood cultures [27]. Rapid and reliable ID of CoNS is crucial for determining BSIs and preventing unnecessary antibiotic treatments. The lysis buffer, initially designed to liquefy viscous sputum, exhibited strong solubility. It was speculated that it could affect the bacterial cell structure while destroying erythrocytes, thereby assisting the subsequent process of formic acid dissolving the cell wall and releasing proteins. In terms of time-to-result or hands-on time, most rapid methods based on lysis and/or centrifugation, combined with MALDI-TOF MS, require less than 30 min for PBCs' ID. The initial volume of blood culture broth used for extraction of the microbial pellet ranges from 1 to 8 mL.

Failed ID analysis

For monomicrobial PBCs, *P. aeruginosa* was the most common isolate that failed to yield ID results using the Xpert Lysate-based Method ($n=7$). Additionally, compared to other common BSI pathogens, *P. aeruginosa* had the lowest median confidence score at 1.865. This may be attributed to the bacteria's weaker resistance to the lysis buffer and its inability to preserve cell integrity, leading to reduced bacterial protein concentrations in the pellets. For such "fragile" bacteria, a recommended ratio of 1 mL blood culture broth to 30 µL lysis buffer was employed. Our experiments revealed that this ratio notably enhanced *P. aeruginosa* ID rates and confidence scores (as illustrated in Supplementary File: Table S6). For the other isolates with no ID results, the sample size was too small ($n<3$) to determine whether the cause was operational error or a limitation of the method.

The Xpert Lysate-based Method demonstrated no genus-level ID errors. The misidentified strains in this study were all incorrectly identified as other species within the same genus. This limitation was due to MS spectrum libraries' inability to distinguish closely related strains, a challenge that also occured in pure colony ID [28].

Filamentous fungi are more resistant to lysis than bacteria and *Candida spp.* due to their cell walls having abundant chitin content, and their protein fingerprints may vary across different growth cycles [29]. Additionally, the fungal loads observed in PBCs are relatively low [26], and their hyphal clusters are also challenging to aspirate from culture bottles with regular syringes. As a result, the only filamentous fungal strain detected in this study, *Talaromyces marneffei*, was not identified as expected.

In this study, one microbial species was correctly identified by the Xpert Lysate-based Method in 10 out of 11 PBCs with polymicrobial growth. Similar results were observed in 14 out of 16 cases using the serum separator gel tube [23] and in 20 out of 23 cases using the saponin lysis method [15]. Due to the inherent difficulty of mixture spectra matching, only highly abundant species had sufficiently defined spectral peaks. Notably, polymicrobial species with similar Gram stain characteristics and morphology might be misinterpreted as a single microorganism. Consequently, maintaining the standard practice of agar plate inoculation is crucial. It not only facilitates antibiotic susceptibility testing but also confirms the ID results of rapid methods, thereby minimizing the risk of overlooking polymicrobial infections.

Limitations and biases

This study has a drawback. Some PBCs that signaled positivity during the night (without a distinction made in recording) were not processed until the next day. This delay led to increased bacterial loads during extraction, exceeding the blood culture positivity threshold concentrations, with a median value of 5×10^8 CFU/mL [20]. Nevertheless, the real-time processing results of daytime PBCs showed that microbial pellets extracted from 1 mL blood culture broth, after a series of lysis, washing, and centrifugation, still met the detection limit for MALDI-TOF MS (1×10^5 CFU/spot) [11].

MALDI-TOF MS can detect specific spectral peaks generated by proteins associated with the expression of resistance genes (e.g., mecA in methicillin-resistant *S. aureus* or KPC in carbapenem-resistant *Enterobacterales*) [30, 31]. Whether the Xpert Lysate-based Method can rapidly identify BSI pathogens and simultaneously provide potential drug-resistant phenotypes for clinicians remains to be further explored.

Conclusion

This multicenter study suggested that the Xpert Lysate-based Method could serve as a promising pretreatment when combined with MALDI-TOF MS to shorten the TAT of PBCs and facilitate the rapid ID of BSIs. If available, this protocol can be seamlessly integrated into the daily workflow of clinical microbiology laboratories.

Abbreviations

BSIs	Bloodstream infections
mNGS	Metagenomic next-generation sequencing
TAT	Turnaround time
ID	Identification
PBCs	Positive blood cultures
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
CFU	Colony-forming unit
GNs	Gram-negatives
GPs	Gram-positives
SD	Standard deviation
IQR	Interquartile range
CoNS	Coagulase-negative staphylococci

Supplementary Information

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Supplementary File 1

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Not applicable.

Author contributions

The work presented here was carried out in collaboration among all authors. JM C conceived and designed the study. YL P and Q C conducted this study and drafted this manuscript. XL S and HY W analyzed the data. SH L, BW Z, and ZJ W performed experiments. J L revised the manuscript. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of the lead hospital, The First Affiliated Hospital of Zhejiang Chinese Medical University (IRB Approval No. 2024-KLS-601-01). In accordance with Article 39 (1) of *Ethical Review Approaches for Biomedical Research Involving Humans (2016)* in China, the IRB of the lead hospital, The First Affiliated Hospital of Zhejiang Chinese Medical University, waived the requirement for informed consent. The research utilized secondary biological specimens and anonymous clinical data, ensuring no involvement of personal privacy or commercial interests. All procedures adhered to the principles of the *Declaration of Helsinki*.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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