Research Article

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Rapid pathogen identification in peritoneal dialysis effluent by MALDI-TOF MS following blood culture enrichment

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Abstract

Objectives: Rapid pathogen identification in peritoneal dialysis effluent (PDE) is crucial for managing peritoneal dialysis-related peritonitis (PDRP). This study evaluated a modified Sepsityper Kit protocol with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for direct identification from positive PDE cultures.

Methods: A total of 143 positive PDE culture bottles were prospectively analyzed between August 2022 and November 2023. The standard Sepsityper Kit protocol was modified by incorporating additional centrifugation and washing steps. Following processing with the modified kit, the samples were directly identified by MALDI-TOF MS. The performance of this method was then compared with the reference identification method.

Results: Among the samples, 139 (97.2 %) were monomicrobial and 4 (2.8 %) polymicrobial. Compared to the reference method, the modified Sepsityper Kit achieved an overall agreement of 89.9 % (score ≥ 1.60) and 77.7 % (score ≥ 1.80). At the ≥ 1.60 cutoff, agreement rates were 95.2 % for Gram-negative bacteria, 87.8 % for Gram-positive bacteria, and 85.7 % for fungi.

Conclusions: MALDI-TOF MS is a reliable and rapid tool for the identification of pathogens in positive PDE blood culture bottles.

Keywords: peritoneal dialysis-related peritonitis; rapid identification; MALDI-TOF MS; Sepsityper Kit; pathogen

Introduction

Peritoneal dialysis serves as a vital renal replacement therapy for end-stage renal disease [1]. However, the efficacy and longevity of this treatment are frequently compromised by the occurrence of peritoneal dialysis-related peritonitis (PDRP), a prevalent and serious complication that remains a major cause of technique failure and patient morbidity [2]. The rapid and accurate identification of causative pathogens in peritoneal dialysis effluent (PDE) is therefore critical for guiding targeted antimicrobial therapy and improving clinical outcomes [3].

The diagnosis of PDRP currently relies on clinical features and laboratory tests [4]. Conventional PDE culture can identify the organisms causing peritonitis, but this process takes at least 48 h [5]. Although, polymerase chain reaction-based assays can require less time, these assays are often labor-intensive and expensive [6]. Previous studies have demonstrated that matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a reliable and accurate tool for identifying bacteria [7]. It has been widely used for the direct identification of colonies on solid plates and clinical specimens, such as blood, urine, and cerebrospinal fluid [8–10]. However, direct identification employing MALDI-TOF MS from positive PDE blood culture bottles is scarce.

In the literature, several MALDI-TOF MS-based preprocessing methods, such as differential centrifugation and

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washings, selective lysis of blood cells, the use of serum separator tubes, transient incubation, and commercial processing with the Sepsityper Kit, have been employed to accelerate pathogen identification [11]. Among these, the Sepsityper Kit has shown particular promise [12]. Therefore, the aim of this study was to compare the performance of the Sepsityper Kit-assisted MALDI-TOF MS with the reference identification method for identifying pathogens in PDE.

Materials and methods

Sample collection

A total of 143 positive PDE vials were prospectively collected between August 2022 and November 2023. All PDE samples were inoculated at the bedside into aerobic (BacT/Alert FA Plus) and anaerobic (BacT/Alert FN Plus) culture vials (bioMérieux, France) and promptly transported to the laboratory. The vials were incubated using the BacT/ALERT 3D automated system (bioMérieux, France). All bottles were cultured for up to five days or until flagged as positive by the system. Positive samples underwent Gram staining and were subcultured onto blood agar, chocolate agar, and MacConkey agar plates (Jiangmen Kailin, China). Simultaneously, each positive sample was processed in parallel using the Sepsityper Kit (Bruker Daltonik, Germany) for rapid microbial identification. This research protocol was approved by our hospital's Ethics Committee in compliance with the Declaration of Helsinki principles.

Reference identification method

All solid media were incubated at 35 °C under 5 % CO_2 for 18–24 h in a Thermo Fisher Scientific incubator. Following incubation, bacterial colonies were harvested and subjected to identification through either (1) MALDI-TOF MS (Bruker Daltonics, Germany) or (2) biochemical profiling using the VITEK 2 system (bioMérieux, France). Discordant results between these two methods were resolved by molecular sequence-based identification targeting the partial 16S rRNA gene, *gyrB*gene, and the ITS region [13].

Sepsityper Kit protocol

The Sepsityper Kit was used following the manufacturer's instructions with some modifications. Prior to the Sepsityper protocol, 2 mL of broth was transferred into a 3 mL sterile tube and centrifuged at 8,000 rpm for 2 min. The supernatant was discarded, and 1 mL of deionized water was added. The mixture was vortexed thoroughly, centrifuged at 13,000 rpm for 2 min, and the supernatant was discarded again. Another 1 mL of deionized water was added, followed by 200 µL of the Sepsityper lysis reagent. The mixture was vortexed for 10 s and incubated at room temperature for 5 min before centrifugation at 13,000 rpm for 1 min. After discarding the supernatant, the pellet was resuspended in 1 mL of Sepsityper Washing Buffer and centrifuged again at 13,000 rpm for 1 min. The supernatant was discarded, and the pellet was resuspended in 1 mL of deionized water, followed by centrifugation at 13,000 rpm for 2 min. The pellet was then resuspended in 30 µL of 70 % formic acid (Sigma-Aldrich), vortexed for 5 s to ensure complete dispersion, and incubated at room temperature for 2 min. Next, 30 µL of acetonitrile (Sigma-Aldrich) was added, and the sample was centrifuged at 13,000 rpm for 2 min. Finally, 1 µL of the supernatant was spotted in duplicate onto a 96-spot polished steel target plate (Bruker Daltonics, Germany). Each spot was overlaid with 1 µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution and air-dried prior to MALDI-TOF analysis. Figure 1 summarized the modified Sepsityper Kit protocol employed in this study.

Analysis with MALDI-TOF MS

Mass spectra were automatically acquired and analyzed using the Bruker MALDI Biotyper Microflex LT/SH system (Bruker Daltonics, Bremen, Germany) equipped with the MBT-Sepsityper-RUO module. In accordance with the manufacturer's recommendations, identification thresholds were set at scores of ≥1.60 for reliable genus-level identification and ≥1.80 for species-level identification.

Results

Comparison of the identification results between the reference identification method and the Sepsityper Kit method

Among 143 positive PDE culture bottles analyzed, 139 (97.2 %) were monomicrobial and 4 (2.8 %) were polymicrobial, as determined by the reference identification method. The monomicrobial isolates comprised 90 (64.7 %) Gram-positive bacteria, 42 (30.2 %) Gram-negative bacteria, and 7 (5.0 %) fungi. The distribution of these 139 microbial species and their corresponding identification scores were summarized in Table 1. Compared to the reference method, the Sepsityper Kit method demonstrated an overall agreement rate of 89.9 % for isolates with a score ≥1.60 and 77.7 % for those with a score ≥1.80. When stratified by microbial group (Table 2), the agreement rates for Gram-negative bacteria were 95.2 %

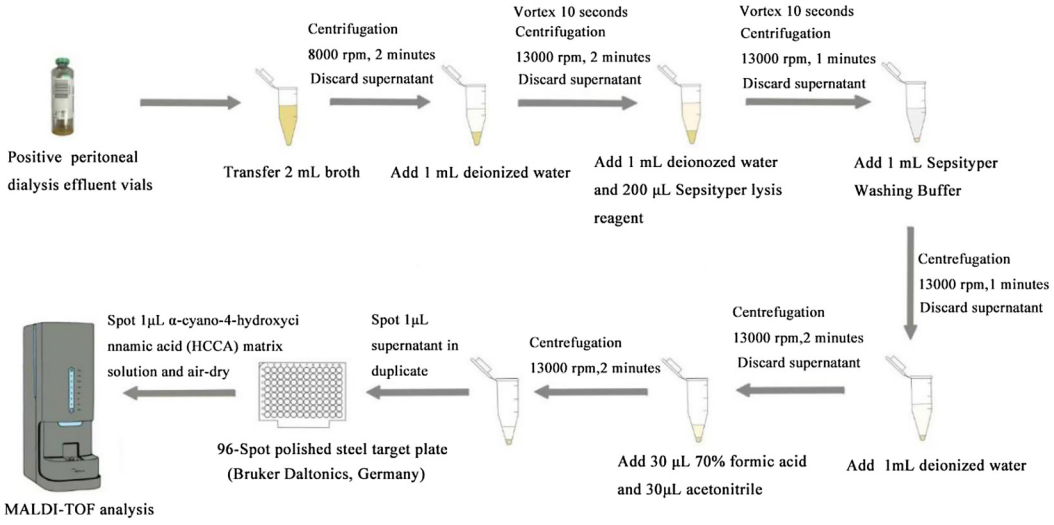

Figure 1: Overview of the modified Sepsityper Kit protocol for rapid identification from positive blood cultures.

(score ≥1.60) and 88.1 % (score ≥1.80). The corresponding rates for Gram-positive bacteria were 87.8 and 73.3 %, while those for fungi were 85.7 and 71.4 %.

Discordant microorganism identification by the reference method and the Sepsityper Kit method

As summarized in Table 3, five discordant identification results were observed between the two methods. These discrepancies primarily involved species discrimination within the genera of *Staphylococcus*, *Streptococcus*, and *Acinetobacter*.

Identification scores of four mixed microorganisms by the Sepsityper Kit method

In this study, only four polymicrobial samples were identified. Among them, three samples were correctly identified for only one microbial species, while the remaining sample was accurately identified for both bacterial species, as detailed in Table 4.

Discussion

Early identification of pathogens in PDRP is crucial for clinicians to initiate targeted antibiotic therapy. In this study, after enrichment with a blood culture system, the PDE samples were centrifuged and washed to reduce host protein interference before being processed with the Sepsityper Kit for direct identification by MALDI-TOF MS. Compared to the reference method, the Sepsityper Kit demonstrated high concordance, with an overall agreement rate of 89.9 % for isolates with a score ≥1.60 and 77.7 % for those with a score ≥1.80.

Current sample preparation methods for direct microbial identification from body fluids via MALDI-TOF MS include various in-house protocols and commercial systems, such as the Sepsityper® Kit (Bruker Daltonics, Bremen, Germany), VITEK® MS blood culture kit (bioMérieux), and the rapid BACpro® II kit (Nittobo Medical Co., Tokyo, Japan) [14]. Among these, the Sepsityper Kit has emerged as the predominant commercial solution for processing positive blood culture samples [12]. A meta-analysis indicated that the Sepsityper Kit enabled reliable species-level identification in 80 % of 3,320 positive blood culture bottles, with Gram-negative bacteria consistently showing higher identification rates (90 %) compared to Gram-positive bacteria (76 %) or yeast (66 %) [15]. In this study, we modified the standard Sepsityper protocol by incorporating additional centrifugation and washing steps to address challenges specific to PDE, including high protein concentration, elevated salt and glucose levels, and interference from high-abundance host proteins (e.g., albumin and IgG) that can obscure microbial protein signatures in mass spectrometry [16, 17]. Compared to the reference identification method, our modified Sepsityper Kit protocol demonstrated significantly improved performance. The overall agreement rates for Gram-negative bacteria were 95.2 % (score ≥1.60) and 88.1 % (score ≥1.80). The corresponding rates for Gram-positive bacteria were 87.8 and 73.3 %, and those for fungi

Table 1: MALDI Biotyper bacterial identification scores obtained from monomicrobial dialysis effluent blood cultures with the Sepsityper Kit.

Reference method (no. of isolates)	Sepsityper Kit method (score)		
	<1.60	≥1.60	≥1.80
Gram-positive bacteria (90)	11	79	66
Staphylococcus aureus (10)	0	10	10
Staphylococcus epidermidis (16)	4	12	8
Staphylococcus haemolyticus (11)	2	9	7
Staphylococcus warneri (6)	0	6	5
Staphylococcus caprae (3)	0	3	3
Staphylococcus capitis (3)	0	3	3
Staphylococcus hominis (2)	0	2	2
Staphylococcus cohnii (2)	0	2	2
Enterococcus faecalis (6)	0	6	5
Enterococcus faecium (4)	0	4	3
Streptococcus oralis (4)	2	2	1
Streptococcus gordonii (8)	0	8	7
Streptococcus anginosus (2)	1	1	1
Streptococcus salivarius (6)	1	5	4
Streptococcus vestibularis (1)	0	1	1
Streptococcus mitis (3)	1	2	1
Corynebacterium striatum (2)	0	2	2
Corynebacterium jeikeium (1)	0	1	1
Gram-negative bacteria (42)	2	40	37
Escherichia coli (14)	0	14	14
Klebsiella pneumoniae (12)	2	10	8
Klebsiella oxytoca (2)	0	2	2
Pseudomonas aeruginosa (4)	0	4	4
Acinetobacter baumannii (4)	0	4	3
Enterobacter cloacae (1)	0	1	1
Alcaligenes faecalis (1)	0	1	1
Morganella morganii (1)	0	1	1
Proteus mirabilis (2)	0	2	2
Citrobacter freundii (1)	0	1	1
Fungi (7)	1	6	5
Candida parapsilosis (3)	1	2	1
Candida albicans (2)	0	2	2
Candida glabrata (1)	0	1	1
Candida tropicalis (1)	0	1	1
Total (139)	14	125	108

Table 2: Numbers and percentages of concordant results by two methods.

Group of microorganism	Reference method (no. of isolates)	Sepsityper Kit method (score)	
		≥1.60	≥1.80
Gram-positive bacteria	90	79(87.8)	66(73.3)
Gram-negative bacteria	42	40(95.2)	37(88.1)
Fungi	7	6(85.7)	5(71.4)
Total	139	125(89.9)	108(77.7)

Table 3: Discordant microorganism between two methods in identification results.

Sepsityper Kit method (score)	Reference method
S. aureus 1.82	*S. epidermidis*
S. haemolyticus 1.65	*S. epidermidis*
A. baumannii/Acinetobacter calcoaceticus 1.67	*A. baumannii*
S. mitis/S. oralis 1.62	*S. oralis*
Streptococcus pneumoniae 1.75	*S. mitis*

Table 4: MALDI Biotyper scores for identifying mixed infections in four positive dialysis effluent blood culture bottles.

Reference method	Sepsityper Kit method (score)
E. coli + *S. aureus*	*E. coli* (2.32)
	S. aureus (1.96)
E. coli + *E. faecalis*	*E. coli* (2.08)
E. coli + *E. faecalis*	*E. coli* (1.92)
E. faecalis + *S. haemolyticus*	*S. haemolyticus* (2.12)

were 85.7 and 71.4 %. These metrics are notably superior to those reported in prior studies. For instance, identification rates for Gram-negative bacilli and Gram-positive cocci in CSF samples were documented at 81.0 and 9.1 %, respectively, using a washing/centrifugation protocol [18]. Another study utilizing an in-house extraction method for ascitic fluid cultures reported rates of 86.0 % for Gram-negative and 73.0 % for Gram-positive organisms [19]. Furthermore, Noll et al. reported 81 % species-level identification concordance with conventional culture when applying the Sepsityper Kit to 37 synovial fluid samples enriched in the BacT/Alert system [20]. Although factors such as geographical origin, pathogen distribution, and sample size can influence the efficacy of the Sepsityper Kit protocol [21], the enhanced performance of our method can be attributed to three key modifications: (1) broth subculture to increase bacterial load, (2) the incorporation of additional centrifugation and washing steps, and (3) optimization of spectral cutoff thresholds.

This study identified five discordant identification results between the reference method and the Sepsityper Kit method. Specifically, two isolates confirmed as *Staphylococcus epidermidis* by the reference method were misidentified by the Sepsityper Kit as *Staphylococcus aureus* and *Staphylococcus haemolyticus*, respectively. Although our laboratory strictly adhered to the manufacturer's protocol, including the use of trifluoroacetic acid (TFA) protocol and

maintained rigorous microplate cleanliness, these misidentifications may be attributed to multiple factors. Potential reasons include target plates contamination, insufficient bacterial load, background spectral interference, high similarity of ribosomal proteins among closely related species, and limitations inherent in the current reference database, which is consistent with reports from previous studies [6, 22]. It is crucial to note that such misidentification in clinical practice may lead to erroneous pathogen reporting and subsequent inappropriate antimicrobial therapy, posing potential risks for treatment failure and the amplification of antimicrobial resistance. However, the direct MALDI-TOF MS identification from positive PDE samples employed in this study has not been clinically validated and should be considered for research purposes only. In this context, Gram staining morphology provides essential preliminary guidance, as the microscopic morphology of *S. aureus* differs noticeably from that of *coagulase-negative staphylococci* [23]. Furthermore, our findings align with known challenges in MALDI-TOF MS identification, particularly for closely related species/groups such as the *Acinetobacter baumannii/Acinetobacter calcoaceticus complex*, the *Streptococcus mitis/Streptococcus oralis group*, and *Streptococcus pneumoniae*. These difficulties primarily stem from high 16S rRNA gene sequence homology among these organisms [24]. Recent investigations into the low identification rates and suboptimal spectral quality for *S. pneumoniae* using the Sepsityper Kit suggest that the efficacy of the lysis buffer may be a critical factor, potentially compounded by intrinsic characteristics of different serotypes [25]; however, this conclusion warrants further validation with larger datasets.

Consistent with previous reports highlighting the challenge of polymicrobial infection identification for MALDI-TOF MS systems [26, 27], our study observed that among four polymicrobial samples, only one was accurately identified for both bacterial species, while the other three were correctly identified for a single species. This limited identification efficiency may be attributed to several factors, including unequal bacterial loads, background protein interference, and inherent limitations in the algorithmic discrimination of overlapping spectral profiles [14]. Although recent methodological advancements, such as the MALDI Biotyper MSP identification MIXED method, have shown promise in improving the identification of polymicrobial samples by enhancing the deconvolution of complex spectral data [28], its application in this study under specific experimental conditions did not yield satisfactory results, thereby suggesting that its efficacy may be influenced by sample-specific factors or require further protocol optimization.

This study has several limitations. First, the relatively small sample size, particularly the limited number of polymicrobial infections (only 4 cases) and fungal isolates (n=7), may affect the generalizability of the findings to broader clinical settings [29]. Second, the single-center design could limit the applicability of our results across regions with differing microbial epidemiology. Therefore, validation through future multicenter studies is necessary. Third, although the method demonstrates a significant advantage in rapid pathogen identification, our study lacks direct evidence linking the reduced time-to-identification to tangible improvements in clinical outcomes, such as optimized antibiotic stewardship or reduced hospitalization duration. Consequently, the practical clinical utility of this approach warrants further investigation in studies that incorporate analyses of clinical endpoints. Finally, the absence of standardized protocols for sample processing in direct bacterial identification complicates cross-study comparisons of method performance, as the efficacy of such in-house methods is often highly operator-dependent [30].

In summary, our study demonstrated that MALDI-TOF MS provides a convenient, rapid, and accurate approach for the direct identification of positive PDE samples.

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Author contributions: Qing-Nian Wu: obtained ethics approval, collected the data, and wrote the original draft of the manuscript. Zhi-Ying Deng, Rui-hang Huang, Wei-Da Liang, and Ping Chen: developed the original study protocol and supervised the investigation and data collection. Chun–Lin Liu and Liu Liu: reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are available upon request.

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