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Comparison of a Sepsityper® kit and in-house membrane filtration methods for rapidly diagnosing positive blood cultures via MALDI−TOF MS

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ABSTRACT

Background: Rapidly identifying pathogens and determining their antimicrobial susceptibilities using samples directly from flagged blood culture bottles pose significant challenges for clinical laboratories. Thus, a cost-effective and efficient sample-processing method is urgently needed to address this issue. To fulfill this need, we developed a novel protocol to rapidly identify pathogens and determine their antimicrobial susceptibilities using samples directly from blood culture bottles.

Methods: Samples were either processed by the Sepsityper kit or our in-house methods. In our approach, we processed the samples using either a nonionic surfactant (Triton X-100) or a NaOH-sodium dodecyl sulfate (SDS) solution, followed by membrane filtration (MF) and centrifugation. Subsequently, the samples were analyzed using MALDI-TOF mass spectrometry (MS) for identification and the Vitek® 2 for antimicrobial susceptibility determination.

Results: In this study, 122 clinical blood culture samples were analyzed, and our MF protocol displayed enhanced accuracy in identifying gram-positive organisms (n = 58) and gram-negative bacilli (n = 64) compared to the Sepsityper method. In particular, the Triton-MF and SDS-MF techniques outperformed Sepsityper in identifying gram-negative bacilli, with accuracy rates of 92.2 %, 85.9 %, and 78.1 %, respectively. Notably, both the Triton-MF and SDS-MF methods exhibited high categorical agreement (CA) for antimicrobial susceptibility testing (AST) for carbapenem against Enterobacterales, with CAs of 100 % and 98.7 %, respectively. Additionally, both methods exhibited a perfect CA and essential agreement of 100 % for *Enterococcus faecium* AST for vancomycin.

Conclusion: These findings strongly indicate that our MF methods have the potential to streamline the identification and AST of bacteria in positive blood cultures.

1. Introduction

Effective management of bacteremia requires timely identification of pathogens and determination of their susceptibility to antimicrobial agents.[1–3] Various methods, including genotypic, phenotypic, and imaging technologies, to improve the speed and accuracy of testing are being explored, but further investigations are needed to provide reliable and efficient results for clinicians and microbiologists.[4–6] MALDI-TOF mass spectrometry (MS) is a rapid and effective identification method for identifying isolates from clinical specimens. However, the use of commercial purification methods, such as Sepsityper®, the Vitek® MS Blood Culture Kit, and Rapid BACpro® II, for analyzing samples directly from flagged blood culture bottles remains complicated.[7–12]

Among the various extraction kits available for MALDI-TOF MS identification, Sepsityper is the most widely employed. Previous studies have shown that the Sepsityper workflow is a reliable method for antimicrobial susceptibility testing (AST) of samples directly from positive blood culture bottles, with a categorical agreement (CA) in AST ranging from 97.0 % to 99.1 %.[13–15] Nonetheless, the accuracy of species identification has varied considerably across different studies, ranging from

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67 % to 95.6 %.[16,17] Moreover, the implementation costs associated with these commercial kits may increase the overall expenses of integration into routine workflows.

Previously, we developed a saponin-based sample processing method for rapidly identifying bacterial pathogens through MALDI-TOF MS, with an identification rate of nearly 90 % at the genus level and over 70 % at the species level.[18,19] However, the saponin-based method involves a five-step hands-on process and takes 35 min, which limits its application in routine practice. Here, we developed an optimized sample processing protocol that uses Triton X-100 or NaOH-SDS to replace saponin and involves only four hands-on steps (Fig. 1). Overall, our methods outperformed Sepsityper in both species identification and AST of blood culture samples using MALDI-TOF MS and the Vitek 2 system, respectively, indicating that they are cost-effective and feasible for rapidly diagnosing bloodstream infections.

2. Materials and methods

2.1. Sample collection and conventional identification

Blood culture bottles (Bactec Plus Aerobic/F, Anaerobic/F, or Myco/F Lytic culture bottles; Becton and Dickinson, Sparks, MD, USA) were incubated at 35 °C in a BACTEC™ FX (Becton and Dickinson) automated machine until they were flagged positive. Subsequently, we conducted Gram staining of the blood culture, followed by subculturing on blood agar plates (Becton and Dickinson) and chocolate agar plates (Becton and Dickinson), excluding those specimens identified as yeast under microscopic examination. To identify the colonies that grew on the plates, we utilized MALDI-TOF MS with Bruker Biotyper® 3.1 software (Bruker Daltonics, Bremen, Germany) and a database of 6903 MSP entries. A spectral score ≥2.00 indicated identification at the species level, a score of 1.700–1.999 indicated identification at the genus level, and a score <1.70 indicated unreliable identification.

2.2. Sepsityper® kit

The Sepsityper Kit® (Bruker Daltonik GmbH, Germany) was used according to the manufacturer's instructions for the rapid protocol. Briefly, 1 mL of positive blood culture was mixed with 200 µL of lysis buffer and centrifuged at 15,495×*g* for 2 min. The pellet was washed with washing buffer and centrifuged at 15,495×*g* for 1 min. The resulting pellet was used for direct identification and AST.[8,20] For identification with the standard Biotyper® module, a score ≥1.800 indicated species-level identification, a score ranging from 1.600 to 1.799 indicated genus-level identification, and a score <1.600 indicated unreliable identification.[10]

2.3. In-house methods

The in-house methods incorporated blood cell lysis and microfiltration for all specimens, as depicted in Fig. 1. In brief, 2.5 mL of positive blood culture was drawn and mixed with 0.5 mL of lysis buffer in a syringe. Two distinct lysis buffers were used to lyse blood cells: 1 % Triton X-100 (Sigma–Aldrich, St. Louis, MO, USA) in ddH_2O or NaOH-sodium dodecyl sulfate (0.2 N NaOH, Creative Life Sciences, Taiwan; 1 % SDS, Sigma–Aldrich) in ddH_2O.[21] Subsequently, the mixture was filtered through a sterile Minisart® syringe filter with a pore size of 3 µm (DTC SepsiFilt Kit, Taiwan) to remove blood cell debris. Afterward, 1.5 mL of the filtrate was centrifuged at 15,495×*g* at ambient temperature for 1 min. The pellet was then used for identification and AST. The interpretation of the MALDI-TOF MS results was based on conventional scoring criteria.

2.4. Antimicrobial susceptibility testing and analysis

We utilized the Vitek 2 AST-GN322 and GP-638 cards for gram-negative and gram-positive bacterial susceptibility testing, respectively. Samples processed by Sepsityper or our in-house methods were suspended in saline (0.85 % NaCl, w/v), adjusted to 0.5 McFarland on a Vitek 2 density meter, and used for AST by the Vitek 2 XL system (bioMérieux). The susceptibility of isolates determined later through conventional methods served as the reference standard for calculating discrepancies. CA refers to the level of agreement in interpretive results between the two methods according to Clinical and Laboratory Standards Institute (CLSI M100-S30) criteria. We did not evaluate essential agreement for the Vitek 2 system because it provided only limited or off-scale MIC values. VMEs represent the proportion of resistant isolates falsely classified as susceptible by direct methods, while MEs indicate the proportion of susceptible isolates falsely classified as resistant. MIC values used to define susceptible or resistant via the conventional method but determined as intermediate by the direct method, or vice versa, are referred to as mEs. Acceptable accuracy was defined as CA ≧90 %, VME and ME ≦ 3 %, and mE ≦ 10 %.[15]

3. Results

3.1. Comparison of the ability of Triton-MF, SDS-MF, and sepsityper to identify monomicrobial samples

A total of 140 positive blood cultures from specimens from 135 patients were evaluated at National Taiwan University Hospital (NTUH) between February 15 and April 18, 2022. According to the conventional identification results, 87.1 % (122/140) of the samples were monomicrobial, while 12.9 % (18/140) were polymicrobial. Among the

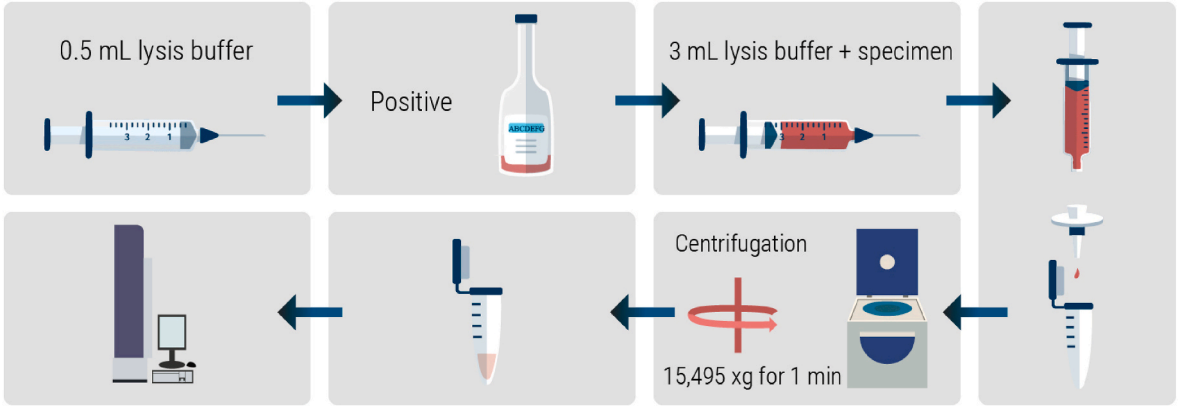

Fig. 1. Schematic representation of the procedure employed for the in-house methods for the direct identification of bacterial pathogens in positive blood culture bottle samples by MALDI-TOF MS and Vitek 2.

monomicrobial samples, 52.5 % (64/122) of the microbes were identified as gram-negative bacteria, and 47.5 % (58/122) of the microbes were identified as gram-positive bacteria by MALDI-TOF MS Biotyper® 3.1 (Table 1). According to the rapid diagnosis results, 69.7 % of the Sepsityper-processed samples were identified at the genus level (score ≥1.6), and 60.7 % were identified at the species level (score ≥1.8). In contrast, the overall identification rates for our Triton-MF- and SDS-MF-processed samples were 74.6 % and 72.1 %, respectively, at the genus level (score ≥1.7) and 63.9 % and 57.4 %, respectively, at the species level (score ≥2.0).

Regarding the conclusive outcomes for the 64 g-negative bacilli, the identification rates for Sepsityper, Triton-MF, and SDS-MF were 78.1 % (50/64), 92.2 % (59/64), and 85.9 % (55/64) at the genus level, respectively. Furthermore, the species-level identification rates were 73.4 %, 89.1 %, and 71.9 %, respectively. Notably, no misidentification was detected with the Triton-MF and SDS-MF methods, while Sepsityper misidentified *Elizabethkingia meningoseptica* as *Elizabethkingia anopheles* in one sample (Table 1).

Regarding the samples with gram-positive pathogens, the identification percentages at the genus level were 55.2 % (32/58) with Triton-MF, 56.9 % (33/58) with SDS-MF, and 60.3 % (35/58) with Sepsityper. The species-level identification percentages for the three methods were 36.2 %, 41.4 %, and 46.6 %, respectively. The Sepsityper method resulted in four cases of misidentification, whereas no misidentification

Table 1
Comparison of the MALDI-TOF MS identification results for the monomicrobial samples processed by the in-house methods and the Sepsityper kit.

Organism(s)[a]	No. of isolates	No. of isolates (%)								
		In-house methods						Species[f]	Sepsityper kit	
		Triton-MF		NI[de]	SDS-MF					
		Species[b]	Genus[c]		Species[b]	Genus[c]	NI[de]		Genus[g]	NI[dh]
Gram-negative	64	57 (89.1)	2 (3.1)	5 (7.8)	46 (71.9)	9 (14.1)	9 (14.1)	47 (73.4)	3 (4.7)	14 (21.9)
Acinetobacter baumannii	2	2	0	0	0	1	1	2	0	0
Acinetobacter nosocomialis	1	1	0	0	0	0	1	0	0	1
Aeromonas hydrophila	1	1	0	0	1	0	0	1	0	0
Aeromonas veronii	1	1	0	0	1	0	0	1	0	0
Burkholderia cepacia complex	5	4	0	1	0	2	3	0	1	4
Bacteroides fragilis	2	1	1	0	0	0	2	0	1	1
Citrobacter koseri	1	1	0	0	1	0	0	1	0	0
Campylobacter jejuni	1	0	0	1	0	0	1	0	0	1
Enterobacter cloacae complex	2	2	0	0	2	0	0	2	0	0
Escherichia coli	22	21	0	1	21	1	0	20	0	2
Elizabethkingia anophelis	0	0	0	0	0	0	0	1[i]	0	0
Elizabethkingia meningoseptica	2	2	0	0	1	1	0	0	0	1
Klebsiella aerogenes	1	1	0	0	1	0	0	1	0	0
Klebsiella pneumoniae	15	14	1	0	13	2	0	13	0	2
Morganella morganii	1	1	0	0	1	0	0	1	0	0
Pseudomonas aeruginosa	2	1	0	1	1	0	1	1	0	1
Stenotrophomonas maltophilia	1	1	0	0	0	1	0	0	0	1
Serratia marcescens	2	1	0	1	1	1	0	1	1	0
Salmonella spp.	2	2	0	0	2	0	0	2	0	0
Gram-positive	58	21 (36.2)	11 (19.0)	26 (44.8)	24 (41.4)	9 (15.5)	25 (43.1)	27 (46.6)	8 (13.8)	23 (39.6)
Abiotrophia defectiva	1	0	0	1	0	0	1	0	0	1
Bacillus cereus	3	1	1	1	1	1	1	2	0	1
Enterococcus faecalis	6	5	1	0	6	0	0	3	0	3
Enterococcus faecium	7	5	1	1	6	1	0	4	1	2
Parvimonas micra	1	0	0	1	0	0	1	0	0	1
Staphylococcus aureus	13	3	0	10	4	1	8	10	2[j]	1
Staphylococcus capitis	6	2	0	4	1	1	4	3	0	3
Staphylococcus caprae	2	1	0	1	1	0	1	1	0	1
Staphylococcus epidermidis	10	2	3	5	4	2	4	1	3	5
Staphylococcus haemolyticus	1	1	0	0	0	1	0	0	0	1
Staphylococcus schweitzeri	0	0	0	0	0	0	0	1[k]	0	0
Streptococcus anginosus	1	0	0	1	0	0	1	1	0	0
Streptococcus dysgalactiae	2	0	2	0	0	1	1	0	0	2
Streptococcus gallolyticus	1	0	1	0	0	0	1	0	0	1
Streptococcus mitis	1	0	0	1	0	0	1	0	0	0
Streptococcus oralis	2	1	1	0	1	1	0	0	0	1
Streptococcus pneumoniae	0	0	0	0	0	0	0	1[l]	1[m]	0
Streptococcus salivarius	1	0	1	0	0	0	1	0	1	0
Total	122	78 (63.9)	13 (10.7)	31 (25.4)	70 (57.4)	18 (14.8)	34 (27.9)	74 (60.7)	11 (9.0)	37 (30.3)

[a]Identification by a conventional protocol.
[b]The MALDI-TOF MS scores were >2.0.
[c]The MALDI-TOF MS scores were 1.7–1.99.
[d]NI, not identified.
[e]The MALDI-TOF MS scores were <1.7.
[f]The MALDI-TOF MS scores were >1.8.
[g]The MALDI-TOF MS scores were 1.6–1.79.
[h]The MALDI-TOF MS scores were <1.6.
[i]*E. meningoseptica* was misidentified as *E. anophelis*.
[j]*S. epidermidis* was misidentified as *S. aureus*.
[k]*S. aureus* was misidentified as *S. schweitzeri*.
[l]*S. oralis* was misidentified as *S. pneumoniae*.
[m]*S. mitis* was misidentified as *S. pneumoniae*.

was observed with the Triton-MF and SDS-MF methods. Specifically, one specimen initially identified as *Staphylococcus schweitzeri* by Sepsityper was later determined to be *Staphylococcus aureus* through conventional methods, while two other specimens initially identified as *Streptococcus pneumoniae* were subsequently identified as *Streptococcus oralis* and *Streptococcus mitis*. Furthermore, a specimen initially identified as *S. aureus* by the Sepsityper method was later determined to be *Staphylococcus epidermidis* (Table 1).

Comparison of antimicrobial susceptibility determination among the Triton-MF, SDS-MF, and Sepsityper methods.

We further performed AST for the samples derived from blood cultures. Only samples that met the specifications of the Vitek 2 system and were correctly identified at the species level were subjected to susceptibility testing for CLSI-recommended antibiotics based on the identification results. Thus, 36 Triton-MF samples, 32 SDS-MF samples, and 28 Sepsityper samples with gram-negative bacteria were eligible for AST analysis, resulting in 544, 497, and 438 sets of minimal inhibitory concentration (MIC) data, respectively (Table 2). The percentages of the samples with very major errors (VMEs), major errors (MEs), and minor errors (mEs) are summarized in Table S1. The CAs were 96.3 %, 97.0 %, and 93.8 % and the essential agreement (EA) were 96.9 %, 98.0 %, 94.1 % for the Triton-MF, SDS-MF, and Sepsityper methods, respectively. The VME percentages were 6.1 %, 5.4 %, and 8.0 %, respectively, while the ME percentages were 0.8 %, 0.3 %, and 3.3 %, respectively. The mEs accounted for 1.8 %, 1.6 %, and 2.3 % of the results, respectively.

Regarding clinically significant gram-negative Enterobacterales, 29, 27, and 25 (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, *Serratia marcescens*, *Morganella morganii*, and *Klebsiella aerogenes*) samples were available for comparison of Triton-MF, SDS-MF, and Sepsityper, respectively (Table 3). The AST results for the Triton-MF and SDS-MF samples exhibited CAs of 97.7 % and 96.3 %, respectively, for cephalosporins (cefotaxime, ceftazidime, and cefepime). Only one *E. coli* isolate showed a VME for cefotaxime and a mE for ceftazidime. In contrast, the AST results for Sepsityper samples demonstrated a lower CA of 90.7 %, with one VME, two MEs, and four mEs. In addition to the aforementioned *E. coli* with errors via the MF methods, the Sepsityper method exhibited an additional ME for a *Klebsiella pneumoniae* strain for cefotaxime and ceftazidime. Regarding carbapenem susceptibility, Triton-MF and SDS-MF showed 100 % and 98.7 % CA, respectively, while the Sepsityper method demonstrated a CA of 97.3 %, with two MEs observed for one sample with *K. pneumoniae* and one sample with *E. cloacae* complex. Regarding the carbapenem-resistant Enterobacteriaceae isolates, this study included two carbapenem-resistant *K. pneumoniae* isolates, each of which showed 100 % CA across the three methods tested.

On the other hand, due to the relatively lower identification rates of gram-positive bacteria in samples using these three methods (ranging from 36.2 % to 46.6 %, Table 1), only 16, 17, and 4 Triton-MF, SDS-MF, and Sepsityper samples, respectively, were eligible for AST analysis, generating 155, 173, and 48 sets of MIC data, respectively. The CAs were 91.0 %, 91.3 %, and 91.7 %, and the EAs were 87.7 %, 88.4 %, and 81.3 %, respectively, with VME percentages of 3.8 %, 5.3 %, and 37.5 % and ME percentages of 2.1 %, 1.9 %, and 0 %, respectively (Table 2). The detailed discrepancies in the AST results are summarized in Table S1.

Given the prevalence and threat of vancomycin-resistant enterococci (VREs), we focused particularly on the AST results for Enterococcus species. However, none of the Sepsityper-processed samples with enterococci were eligible for AST analysis. Only 4 and 5 samples with *Enterococcus faecium* and *Enterococcus faecalis*, respectively, processed by the MF methods were analyzed. As shown in Table 2, both MF methods demonstrated remarkable accuracy. Triton-MF achieved 95.9 % CA for *E. faecalis* and 97.2 % CA for *E. faecium*, while SDS-MF achieved 95.9 % CA for *E. faecalis* and 100 % CA for *E. faecium*. The mE percentages were 4.1 % and 4.1 % for samples containing *E. faecalis* processed by the Triton-MF and SDS-MF methods, respectively. However, MRSA isolate was not included in this study.

In conclusion, the analysis of AST data revealed that the Triton-MF and SDS-MF methods exhibited greater accuracy for identifying gram-negative bacteria and *Enterococcus* species, highlighting their potential for use in clinical diagnostics.

4. Discussion

The rapid and accurate diagnosis of pathogenic bacteria in the blood of patients with bacteremia and sepsis, along with determination of their antibiotic susceptibility, has long been an unmet clinical need. Our study demonstrated that the simple and cost-effective Triton-MF method can significantly improve bacterial identification and AST using MALDI TOF MS and the Vitek 2, respectively, for samples derived directly from positive blood culture bottles. Therefore, this method will become an effective tool in clinical laboratories, providing physicians with valuable information for the timely management of patients with bacteremia and sepsis during the critical first 24 h.[22]

In this study, we observed that Sepsityper and our methods all showed better performance for detecting gram-negative bacteria than for detecting gram-positive bacteria. This finding is consistent with a meta-analysis of 21 reports that showed an 80 % species-level identification rate using Sepsityper for 3320 positive blood culture bottle samples, with the identification rate (90 %) greater for gram-negative bacteria than for gram-positive bacteria (76 %) and yeast (66 %).[23] Another report also supported this trend, showing a better performance for the detection of gram-negative (>90.0 %) than gram-positive bacteria (64.0 %).[23] However, our research revealed a slightly lower performance for Sepsityper, with a 71.9 % identification rate for gram-negative bacteria and 43.1 % for gram-positive bacteria. Several factors might hamper the performance of MALDI-TOF MS identification of specimens from blood culture bottles, including poor pellet quality, reduced biomass after extraction, the presence of thick bacterial cell walls or capsules, the presence of clot-forming or cluster-forming isolates, and the presence of residual blood proteins, which we also observed in our current and previous studies.[13,21]

Our rapid diagnosis method requires a smaller volume (2.5 mL) of blood culture samples, while other commercial kits typically need at least 3 mL of culture broth.[20] Despite using less sample, our Triton-MF method outperformed the Sepsityper method in both pathogen identification and AST. In addition, we included filters in the MF workflow, eliminating the need for deionized water for the repeated washing-centrifugation processes required to improve the identification of bacteria in the precipitate by MALDI-TOF MS. According to the manufacturer's instructions for Sepsityper, a centrifugal washing step is carried out after adding the lysis buffer, and the pellets can be used directly for identification. By incorporating lysis buffer and filters into the MF workflow, we streamlined the procedure, reducing the need for washing, centrifugation, and personnel hands-on time. Overall, our MF workflow efficiently extracts bacteria while maintaining bacterial viability for AST, and the remaining blood culture broth can be further investigated in other experiments.

The results of this study show that the MF method is less accurate in identifying gram-positive bacteria, particularly *S. aureus*, which is one of the most important gram-positive pathogens. To improve the accuracy of gram-positive isolate identification, previous studies employed centrifugation and membrane filtration technology (CMFT), which combines vacuum filtration with differential centrifugation.[20] Compared to the Sepsityper protocol, the new CMFT protocol exhibited significantly improved performance for gram-positive isolates. Using a score threshold of >2.0, the CMFT approach resulted in the successful identification of 51 isolates (76.1 %), while it 66 isolates (98.5 %) were identified with a score threshold of >1.7.[24] In contrast, Sepsityper identified only 29 isolates (43.3 %) and 51 isolates (76.1 %) using the respective score thresholds.[24] However, notably, this method requires filter membrane cleaning, which is impractical in clinical laboratories. In our study, the percentage of gram-positive bacterial genera identified

Table 2
Comparison of Vitek 2 antimicrobial susceptibility testing results for the monomicrobial samples processed by the in-house methods and the Sepsityper kit.

Organism(s)	In-house methods														Sepsityper kit			
	Triton-MF						SDS-MF											
	N	CA (%)	EA (%)	VME (%)	ME (%)	mE (%)	N	CA (%)	EA (%)	VME (%)	ME (%)	mE (%)	N	CA (%)	EA (%)	VME (%)	ME (%)	mE (%)
Gram-negative	36	524/544 (96.3)	527/544 (96.9)	7/114 (6.1)	3/374 (0.8)	10/544 (1.8)	32	482/497 (97.0)	487/497 (98.0)	6/111 (5.4)	1/335 (0.3)	8/497 (1.6)	28	411/438 (93.8)	412/438 (94.1)	6/75 (8.0)	11/329 (3.3)	10/438 (2.3)
A. baumannii	2	19/19 (100)	19/19 (100)	0/3 (0)	0/11 (0)	0/19 (0)	1	10/10 (100)	10/10 (100)	0/2 (0)	0/6 (0)	0/10 (0)	2	19/19 (100)	18/19 (94.7)	0/3 (0)	0/11 (0)	0/19 (0)
A. hydrophila	1	9/10 (90.0)	9/10 (90.0)	1/1 (100)	0/9 (0)	0/10 (0)	1	9/10 (90.0)	9/10 (90.0)	1/1 (100)	0/9 (0)	0/10 (0)	1	9/10 (90.0)	9/10 (90.0)	1/1 (100)	0/9 (0)	0/10 (0)
B. cepacia complex	1	3/4 (75.0)	3/4 (75.0)	0/0 (0)	1/4 (25.0)	0/4 (0)	0	0	0	0	0	0	0	0	0	0	0	0
E. cloacae complex	2	32/32 (100)	32/32 (100)	0/13 (0)	0/15 (0)	0/32 (0)	2	32/32 (100)	32/32 (100)	0/13 (0)	0/15 (0)	0/32 (0)	2	30/32 (93.8)	30/32 (93.8)	0/13 (0)	1/15 (6.7)	1/32 (3.1)
E. coli	15	245/255 (96.1)	245/255 (96.1)	5/30 (16.7)	2/207 (1.0)	3/255 (1.2)	13	214/221 (96.8)	214/221 (96.8)	4/28 (14.3)	1/177 (0.6)	2/221 (0.9)	13	213/221 (96.4)	213/221 (96.4)	5/29 (17.2)	1/177 (0.6)	2/221 (0.9)
E. meningoseptica	2	17/20 (85.0)	17/20 (85.0)	1/20 (5.0)	0/0 (0)	2/20 (10.0)	2	19/20 (95.0)	19/20 (95.0)	0/20 (0)	0/0 (0)	1/20 (5.0)	0	0	0	0	0	0
K. aerogenes	1	16/16 (100)	16/16 (100)	0/5 (0)	0/10 (0)	0/16 (0)	1	15/16 (93.8)	16/16 (100)	0/5 (0)	0/10 (0)	1/16 (6.3)	1	16/16 (100)	16/16 (100)	0/5 (0)	0/10 (0)	0/16 (0)
K. pneumoniae	8	135/136 (99.3)	136/136 (100)	0/30 (0)	0/94 (0)	2/136 (1.5)	8	135/136 (99.3)	136/136 (100)	0/30 (0)	0/94 (0)	2/136 (1.5)	7	107/119 (89.9)	108/119 (90.8)	0/20	9/89 (10.1)	4/119 (3.4)
M. morganii	1	14/14 (100)	14/14 (100)	0/2 (0)	0/0 (0)	0/14 (0)	1	14/14 (100)	14/14 (100)	0/2 (0)	0/0 (0)	0/14 (0)	1	14/14 (100)	14/14 (100)	0/2 (0)	0/0 (0)	0/14 (0)
P. aeruginosa	1	7/10 (70.0)	9/10 (90.0)	0/0 (0)	0/6 (0)	3/10 (30.0)	1	8/10 (80.0)	9/10 (90.0)	0/0 (0)	0/6 (0)	2/10 (20.0)	1	7/10 (70.0)	7/10 (70.0)	0/0 (0)	0/6 (0)	3/10 (30.0)
S. marcescens	2	28/28 (100)	27/28 (96.4)	0/10 (0)	0/18 (0)	0/28 (0)	2	27/28 (96.4)	28/28 (100)	1/10 (10)	0/18 (0)	0/28 (0)	1	14/14 (100)	28/28 (100)	0/2 (0)	0/12 (0)	0/14 (0)
Gram-positive	16	141/155 (91.0)	128/146 (87.7)	2/52 (3.8)	2/94 (2.1)	10/155 (4.5)	17	158/173 (91.3)	145/164 (88.4)	3/57 (5.3)	2/106 (1.9)	10/174 (5.7)	4	44/48 (91.7)	39/48 (81.3)	3/8 (37.5)	0/38 (0)	1/48 (2.1)
S. aureus	0	0	0	0	0	0	1	12/12 (100)	12/12 (100)	0/0 (0)	0/12 (0)	0/12 (0)	3	35/36 (97.2)	32/36 (88.9)	0/2 (0)	0/33 (0)	1/36 (2.8)
Staphylococcus spp.	4	38/48 (79.2)	38/48 (79.2)	2/16 (12.5)	2/31 (6.5)	6/48 (12.5)	5	48/60 (80.0)	49/60 (81.7)	2/22 (9.1)	2/36 (5.6)	8/60 (13.3)	1	9/12 (75.0)	7/12 (58.3)	3/6 (50.0)	0/5 (0)	0/12 (0)
Streptococcus spp.	3	21/22 (95.5)	14/22 (63.6)	0/4 (0)	0/16 (0)	1/22 (4.5)	2	15/16 (93.8)	9/16 (56.3)	1/3 (33.3)	0/11 (0)	0/16 (0)	0	0	0	0	0	0
E. faecalis	5	47/49 (95.9)	44/44 (100)	0/11 (0)	0/35 (0)	2/49 (4.1)	5	47/49 (95.9)	43/44 (97.7)	0/11 (0)	0/35 (0)	2/49 (4.1)	0	0	0	0	0	0
E. faecium	4	35/36 (97.2)	32/32 (100)	0/21 (0)	0/12 (0)	1/36 (2.8)	4	36/36 (100)	32/32 (100)	0/21 (0)	0/12 (0)	0/36 (0)	0	0	0	0	0	0

[a]N, no. of isolates; CA, categorical agreement; EA, essential agreement; VME, very major error; ME, major error; mE, minor error.

Table 3
Comparison of Vitek 2 antimicrobial susceptibility testing results for cephalosporins and carbapenems using in-house methods and the Sepsityper kit for gram-negative *Enterobacterales*.

Organism(s)	Antibiotic[a]	In-house method[b]								Sepsityper kit[b]			
		Triton-MF				SDS-MF							
		N	VME	ME	mE	N	VME	ME	mE	N	VME	ME	mE
E. coli	CTX	15	1	0	0	13	1	0	0	13	1	0	0
	CAZ	15	0	0	1	13	0	0	1	13	0	0	1
	FEP	15	0	0	0	13	0	0	0	13	0	0	0
	ETP	15	0	0	0	13	0	0	0	13	0	0	0
	IPM	15	0	0	0	13	0	0	0	13	0	0	0
	MEM	15	0	0	0	13	0	0	0	13	0	0	0
K. pneumoniae	CTX	8	0	0	0	8	0	0	0	7	0	1	1
	CAZ	8	0	0	0	8	0	0	0	7	0	1	1
	FEP	8	0	0	0	8	0	0	0	7	0	0	0
	ETP	8	0	0	0	8	0	0	0	7	0	0	0
	IPM	8	0	0	0	8	0	0	0	7	0	0	0
	MEM	8	0	0	0	8	0	0	0	7	0	1	0
E. cloacae complex	CTX	2	0	0	0	2	0	0	0	2	0	0	0
	CAZ	2	0	0	0	2	0	0	0	2	0	0	0
	FEP	2	0	0	0	2	0	0	0	2	0	0	1
	ETP	2	0	0	0	2	0	0	0	2	0	0	0
	IPM	2	0	0	0	2	0	0	0	2	0	1	0
	MEM	2	0	0	0	2	0	0	0	2	0	0	0
S. marcescens	CTX	2	0	0	0	2	0	0	0	1	0	0	0
	CAZ	2	0	0	0	2	0	0	0	1	0	0	0
	FEP	2	0	0	0	2	0	0	0	1	0	0	0
	ETP	2	0	0	0	2	0	0	0	1	0	0	0
	IPM	0	0	0	0	0	0	0	0	0	0	0	0
	MEM	2	0	0	0	2	0	0	0	1	0	0	0
M. morganii	CTX	1	0	0	0	1	0	0	0	1	0	0	0
	CAZ	1	0	0	0	1	0	0	0	1	0	0	0
	FEP	1	0	0	0	1	0	0	0	1	0	0	0
	ETP	1	0	0	0	1	0	0	0	1	0	0	0
	IPM	0	0	0	0	0	0	0	0	0	0	0	0
	MEM	1	0	0	0	1	0	0	0	1	0	0	0
K. aerogenes	CTX	1	0	0	0	1	0	0	0	1	0	0	0
	CAZ	1	0	0	0	1	0	0	0	1	0	0	0
	FEP	1	0	0	0	1	0	0	0	1	0	0	0
	ETP	1	0	0	0	1	0	0	0	1	0	0	0
	IPM	1	0	0	0	1	0	0	1	1	0	0	0
	MEM	1	0	0	0	1	0	0	0	1	0	0	0

[a] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem.
[b] VME, very major error; ME, major error; mE, minor error.

by the MF approach ranged between 36.2 % and 41.4 %, indicating the need for improvement. Future investigations might include bead beating of gram-positive cocci or the use of filters with larger membrane pore sizes.[25]

Our study has two significant limitations. First, the 3 μm pore size of the filter membrane prevents yeast cells, which typically range from 5 to 30 μm in size, from passing through, leading to their exclusion from the filtrate. Consequently, our method cannot identify yeast. Second, our analysis included only 140 blood culture samples acquired from a single hospital utilizing one blood culture system, potentially introducing bias into our findings owing to variations between systems and inadequate species diversity.

This study has developed a cost-effective sample processing workflow utilizing Triton X-100 or NaOH-SDS solutions combined with MF for the rapid identification and AST of pathogens from flagged blood culture bottles. Compared to the commercially available Sepsityper kit, the MF approach demonstrated potential advantages in identification accuracy for both Gram-positive and Gram-negative bacteria, with notably higher accuracy rates for Gram-negative bacilli when using the Triton-MF and SDS-MF methods. Moreover, both MF techniques showed high concordance in AST results, particularly for carbapenem susceptibility in *Enterobacteriaceae* and vancomycin susceptibility in *Enterococci*. These findings suggest that the MF approach holds clinical potential for the rapid identification and AST of pathogens, providing a viable enhancement to existing diagnostic workflows.

CRediT authorship contribution statement

Lee Tai-Fen: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wan Tsai-Wen:** Writing – review & editing, Validation, Investigation, Funding acquisition, Formal analysis, Data curation. **Hsu Wei-Yu:** Visualization, Funding acquisition, Formal analysis, Data curation. **Chen Xiang-Jun:** Validation, Software, Resources. **Chiu Hao-Chieh:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Huang Yu-Tsung:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

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Declaration of competing interest

All authors report no conflicts of interest.

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Appendix A. Supplementary data

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