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Short Communication

Evaluation of the Rapid Sepsityper protocol and specific MBT-Sepsityper module for the identification of bacteremia and fungemia using Bruker Biotyper MALDI-TOF MS

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Abstract The rapid identification method, the Rapid Sepsityper protocol with a specific MBT-Sepsityper module (Bruker Daltonics), based on the MALDI Biotyper platform, accurately identified 93.5% (116/124) of microorganisms at the species level in the 124 flagged blood culture samples from patients with monomicrobial bloodstream infections. Gram-negative bacilli (95.6%, 43/45) had a higher identification rate than Gram-positive cocci (93.3%, 70/75) and yeasts (75%, 3/4). The Rapid Sepsityper protocol displayed poor identification performance for polymicrobial samples.

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Introduction

Sepsis is a life-threatening medical emergency that affects more than 30 million people worldwide annually.[1] Early identification of the causative microorganism from patients with bloodstream infection remarkably improves patient survival.[1–3] Compared with traditional microbiological identification methods, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been reported to timely and accurately identify microorganisms in flagged blood culture bottles.[3–5] A new version of the Rapid Sepsityper Kit (Bruker Daltonics GmbH, Bremen, Germany) was designed to shorten the processing time and increase identification performance. This allowed the identification of microorganisms by MALDI-TOF MS directly from the positive blood cultures in 10 min without the requirement of the extraction step.[6–8]

Methods

We evaluated flagged blood culture bottles (BACTEC Plus Aerobic/F bottles or BACTEC Anaerobic Lytic/10 bottles, BACTEC FX system [Becton Dickinson Microbiology Systems, Sparks, MD, USA]) obtained from 124 patients with bloodstream infections at the China Medical University Hospital, between December 1, 2020, and April 30, 2021. Microbial identification was performed using two methods: the conventional MALDI TOF-MS Biotyper system (Bruker Daltonics GmbH, Bremen, Germany) using the sub-cultured growth colonies from the positive culture bottles (conventional method) and the Rapid Sepsityper protocol with a specific MBT-Sepsityper module (Rapid Sepsityper protocol) using the MALDI Biotyper platform (Bruker Daltonics GmbH).[8] A 1.0 ml sample collected from a flagged blood culture bottle was transferred to a 1.5 ml centrifuge tube before analysis using the MALDI TOF-MS Biotyper system and Biotyper 3.0 software (Bruker Daltonics GmbH).[8] For further processing of the Rapid Sepsityper kit, detailed procedures described by Buchan et al. were followed.[8] For each patient, only the first positive blood culture was included in the study.

With the conventional method, scores $\geq$2.000 indicated species-level identification. Scores ranging from 1.700 to 1.999 indicated genus-level identification. Scores $<$1.700 indicated no reliable identification.[4,5] With the Rapid Sepsityper protocol, identification scores $\geq$1.800 indicated species-level identification. Scores ranging from 1.600 to 1.799 indicated genus-level identification. Scores $<$1.600 indicated no reliable identification.[7,8] All isolates with discrepant species/genus identification results between the two methods were tested twice. Considering the polymicrobial bloodstream infections, if at least one of the isolates identified by the conventional method was correctly recognized by the Rapid Sepsityper protocol, the results between the two methods were considered concordant.

Results

The Rapid Sepsityper protocol enabled the accurate identification of 116 (93.5%) isolates at the species/genus level from the 124 flagged blood culture bottles compared with the conventional methods. Among the 116 isolates with concordant results, 100 (86.2%) were identified at the species level. Additionally, 11 (9.5%) were identified at the species level using the Rapid Sepsityper protocol and at the genus level using the conventional method (Table 1).

Gram-negative bacilli (95.6%, 43/45) had a higher identification rate than Gram-positive cocci (93.3%, 70/75) and yeasts (75%, 3/4). Table 2 shows the identification results of eight patients with polymicrobial bacteremia identified using the conventional method and the Rapid Sepsityper protocol. Among the eight flagged blood culture bottles from the eight patients, only one species/genus was identified among the top ten identification results using the Rapid Sepsityper protocol from seven bottles on the two species present and two species, including *Klebsiella aerogenes* (score value, 2.06) and *Enterococcus faecalis* (score value, 1.46), were found in one sample on the three species present. The identification results of these two methods were considered concordant, although the Rapid Sepsityper protocol failed to identify all polymicrobial samples.

Among the 43 Gram-negative bacilli isolates with accurate identification results (concordant), *Escherichia coli* (n = 17), *Klebsiella pneumoniae* (n = 10), and *Salmonella*

Table 1 Identification of microorganisms in flagged blood culture bottles using the MALDI Biotyper. Comparison of microbial identification results from 124 monomicrobial flagged blood culture bottles using two methods: the Rapid Sepsityper protocol and the conventional MALDI TOF-MS Biotyper system using the sub-cultured growth colonies from the positive culture bottles. Patterns of identification (I–VII) were defined based on the identity at the species or the genus level detected by the two methods.

Type	Rapid Sepsityper protocol		Conventional method		No. (%) of isolates
	Species-level (score value $\geq$ 1.800)	Genus level only (score value 1.600–1.799)	Species-level (score value $\geq$ 2.000)	Genus level only (score value 1.700–1.999)	
I	V	–	V	–	102 (82.3)
II	V	–	–	V	11 (8.9)
III	–	V	V		7 (5.6)
IV	–	V	–	V	0
V	–	–	V	–	2 (1.6)
VI	–	–	–	V	1 (0.8)
VII	–	–	–	–	1 (0.8)

Table 2 Comparison of identification results of eight patients with polymicrobial bacteremia obtained using the MALDI Biotyper with sub-cultured growth colonies (conventional method) and Rapid Sepsityper protocol with the MALDI Biotyper (Rapid Sepsityper protocol).

No.	Identification results (score value)	
	Rapid Sepsityper protocol (top 10 identification results including those obtained using the conventional methods)	Conventional method
1	*Salmonella* spp. (2.4)	*Salmonella* spp. (2.11) and *Pseudomonas aeruginosa* (2.4)
2	*Enterococcus faecium* (1.94)	*Enterobacter cloacae* (2.09) and *Enterococcus faecium* (2.55)
3	*Escherichia coli* (2.16)	*Klebsiella pneumoniae* (1.95) and *Escherichia coli* (2.05)
4	*Klebsiella pneumoniae* (2.25)	*Klebsiella pneumoniae* (2.23) and *Pseudomonas aeruginosa* (2.26)
5	*Staphylococcus capitis* (1.89)	*Acinetobacter baumannii/calcoaceticus* complex (2.13) and *Staphylococcus capitis* (2.21)
6	*Klebsiella aerogenes* (2.06) *Enterococcus faecalis* (1.46)	*Enterobacter cloacae* (2.09), *Klebsiella aerogenes* (2.24), and *Enterococcus faecalis* (2.48)
7	*Bacteroides fragilis* (1.95)	*Bacteroides fragilis* (2.23) and *Clostridium tertium* (2.19)
8	*Enterococcus faecalis* (2.53)	*Enterococcus faecalis* (2.26) and *Escherichia coli* (2.08)

spp. (n = 4) were most commonly identified. This was followed by *Enterobacter bugandensis* (n = 3), *Pseudomonas aeruginosa* (n = 2), *K. aerogenes, Enterobacter cloacae, Serratia marcescens, Providencia rettgeri, Pseudomonas luteola, Campylobacter jejuni,* and *Bacteroides fragilis*. Among the 70 Gram-positive cocci, *Staphylococcus aureus* (n = 48), *Staphylococcus epidermidis* (n = 6), *Staphylococcus capitis* (n = 5), and *Enterococcus hirae* (n = 4) were the most commonly identified. This was followed by *Enterococcus faecium* (n = 3), *Staphylococcus hominis* (n = 2), and one each of *Staphylococcus warneri* and *Corynebacterium striatum*. The three *Candida* isolates included *Candida albicans*, *C. tropicalis*, and *Candida parapsilosis*.

Table 3 summarizes the eight discrepant identification results in flagged blood culture bottles using the Rapid Sepsityper protocol with the MALDI Bruker Biotyper compared with those obtained using the MALDI Biotyper with sub-cultured growth colonies (conventional method). In addition to the four isolates without reports of bacterial species or genera, discrepant identification results were found mainly for *Streptococcus* and *Staphylococcus* species. The identification results of the four discrepant isolates (two *S. epidermidis* and one each of *Streptococcus mitis* and *Streptococcus agalactiae*) using the conventional method (but not Rapid Sepsityper protocol) were identical to those obtained using a biochemical identification system. The identification time from the reporting of a positive Gram stain in flagged blood culture bottles to the entry of a final result of microbial identification in the laboratory information management system using the Rapid Sepsityper protocol and the conventional method for reporting causative microorganisms at species/genus levels was approximately 30 min and 28 h and 13 min, respectively.

Discussion

A recent report by Watanabe et al. demonstrated that the direct identification method using the Rapid Sepsityper protocol with the MALDI Bruker Biotyper accurately

Table 3 Comparison of the eight incorrect identification results from the monomicrobial flagged blood culture bottles obtained using the Rapid Sepsityper protocol with the MALDI Biotyper and the conventional MALDI Biotyper with sub-cultured growth colonies from the flagged blood culture bottles.

Rapid Sepsityper protocol		Conventional method	
Identification results	Score value	Identification results	Score value
No organism identification possible	1.55	*Candida parapsilosis*	1.74
No peaks found	—	*Pseudoxanthomonas kaohsiungensis*	2.34
No peaks found	—	*Bacteroides fragilis*	2.21
No organism identification possible	1.49	No peaks found	—
Streptococcus pneumoniae	1.92	*Streptococcus mitis* group	2.32
Streptococcus pyogenes	1.62	*Streptococcus agalactiae*	2.02
Lactobacillus kalixensis	1.62	*Staphylococcus epidermidis*	2.00
Staphylococcus lugdunensis	2.07	*Staphylococcus epidermidis*	2.04

identified 89.4% (203/227) of infected samples, and Gram-negative bacilli (95.2%) had a higher identification rate than Gram-positive cocci (84.4%).[7] Non-acceptable identification was high among the Streptococcus species. Using a similar protocol, the identification rates for Gram-positive cocci were higher in this study than those reported by Watanabe et al. and other studies[6—8]; the smaller sample size in this study might have contributed to this difference.

Although BioFire FilmArray Blood Culture Identification (BCID) panel-2 (BCID-2, BioFire Diagnostics, bioMe'rieux, Salt Lake City, UT, USA) has been useful for the rapid identification of bacterial and fungal bloodstream pathogens and their antibiotic resistance determinants from positively flagged blood cultures, the number of detectable microbial targets is limited to 23 bacteria and seven fungi.[9] Among the 116 isolates correctly identified by the Rapid Sepsityper protocol in this study, 12 isolates are not listed as the target organisms in the BCID-2 panel. These include four isolates of *S. capitis*, two *E. hirae*, and one of each of *S. hominis*, *S. warneri*, *C. striatum*, *P. rettgeri*, *C. jejuni*, and *P. luteola*. If the eight aforementioned coagulase-negative staphylococci are excluded, four (3.4%) isolates were identified using the Rapid Sepsityper protocol using the MALDI Bruker Biotyper but were not present in the BCID-2 panel.

Buchan et al. demonstrated that the mean identification time of the MALDI Biotyper/Sepsityper was 23—83 h earlier than that of routine biochemical identification methods (defined as the time from reporting of a positive Gram stain from the flagged blood culture bottles to the entry of the final result in the laboratory information management system) for Gram-positive isolates. It was 34—51 h earlier for Gram-negative isolates, provided that all identifications were completed within 20 min of using the MALDI Biotyper/Sepsityper.[8] Our results are in accordance with their findings.[8]

In this study, the Rapid Sepsityper protocol displayed poor identification performance for polymicrobial samples. In a study by Ponderand et al., there were four polymicrobial blood cultures; only one of the two species present was accurately identified using Rapid Sepsityper® protocol without any warning, highlighting that the blood culture may be polymicrobial.[10]

There are several limitations in this study. First, the sample size of the flagged blood cultures was small, with a small number of *Candida* species isolated. Second, the failure of the Rapid Sepsityper protocol to identify polymicrobial bloodstream infection needs further evaluation to improve its performance. Finally, this study was conducted at a tertiary referral center. Therefore, samples containing various rare pathogens may not reflect the actual performance of the Rapid Sepsityper protocol in clinically relevant situations.

In conclusion, the favorable performance associated with a reduced turnaround time of the rapid identification method may help implement a rapid detection strategy for bloodstream infections in the routine workflow of clinical microbiology laboratories. A study on the application of the Rapid Sepsityper protocol with a specific MBT-Sepsityper module in the MALDI-TOF MS-based machine learning model for faster detection and prediction of methicillin-resistant *S. aureus*,[11] vancomycin-resistant enterococci, and other multidrug-resistant organisms recovered from various clinical specimens is ongoing in our clinical microbiology laboratory.

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Declaration of competing interest

The authors declare no conflicts of interest.

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