ORIGINAL ARTICLE

Evaluation of MALDI-TOF mass spectrometry and Sepsityper Kit™ for the direct identification of organisms from sterile body fluids in a Canadian pediatric hospital

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M Tadros, A Petrich. Evaluation of MALDI-TOF mass spectrometry and Sepsityper Kit™ for the direct identification of organisms from sterile body fluids in a Canadian pediatric hospital. Can J Infect Dis Med Microbiol 2013;24(4):191-194.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) can be used to identify bacteria directly from positive blood and sterile fluid cultures. The authors evaluated a commercially available kit – the Sepsityper Kit (Bruker Daltonik, Germany) – and MALDI-TOF MS for the rapid identification of organisms from 80 flagged positive blood culture broths, of which 73 (91.2%) were blood culture specimens and seven (8.7%) were cerebrospinal fluid specimens, in comparison with conventional identification methods. Correct identification to the genus and species levels was obtained in 75 of 80 (93.8%) and 39 of 50 (78%) blood culture broths, respectively. Applying the blood culture analysis module, a newly developed software tool, improved the species identification of Gram-negative organisms from 94.7% to 100% and of Gram-positive organisms from 66.7% to 70%.

MALDI-TOF MS is a promising tool for the direct identification of organisms cultured from sterile sites.

Key Words: *Blood cultures; Blood culture analysis module; Direct identification; MALDI-TOF MS*

L'évaluation de la spectrométrie de masse à MALDI-TOF et du Sepsityper Kit^{MC} pour l'identification directe des organismes des liquides organiques stériles dans une population canadienne d'âge pédiatrique

La spectrométrie de masse à temps de vol par désorption-ionisation par impact laser assisté par matrice (MALDI-TOF MS) peut être utilisée pour identifier les bactéries directement dans le sang et les cultures positives de liquide stérile. Les auteurs ont évalué une trousse commerciale, la Sepsityper Kit (Bruker Daltonik, Allemagne), et la MALDI-TOF MS pour identifier rapidement des organismes dans 80 bouillons d'hémoculture signalés comme positifs, dont 73 (91,2 %) étaient des échantillons d'hémoculture et sept (8,7 %), des échantillons de liquide céphalorachidien, par rapport aux méthodes d'identification classiques. Les chercheurs ont obtenu la bonne identification du genre et de l'espèce dans 75 des 80 (93,8 %) et 39 des 50 (78 %) bouillons d'hémoculture, respectivement. La mise en application du module d'analyse d'hémoculture, un nouvel outil informatique, a fait passer l'identification des espèces d'organismes Gram négatif de 94,7 % à 100 % et des organismes Gram positif de 66,7 % à 70 %. La MALDI-TOF MS est un outil prometteur pour l'identification directe d'organismes cultivés à partir de foyers stériles.

Bloodstream and central nervous system infections are associated with high morbidity and mortality (1,2). With current diagnostic methods, final results are available often more than 72 h after sampling (3). Because outcome of septic patients has been shown to depend on the adequacy of early antimicrobial chemotherapy, crucial treatment decisions currently often rely on Gram-stain results from positive blood culture broths until identification can be determined following culture isolation (4). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) enables the rapid identification to the species level of Gram-positive and Gram-negative bacteria, as well as yeasts, by comparing the spectra obtained from measuring molecular masses of proteins and other bacterial components obtained from whole organism extracts (5). A crude bacterial load of approximately 5×10^3 colony forming units is necessary for reliable MALDI-TOF analysis, suggesting that bacterial identification may be performed directly on positive blood culture pellets (6). We evaluated the Sepsityper Kit (Bruker Daltonik, Germany) for the rapid identification of organisms from flagged positive blood culture broths. The Sepsityper Kit is a commercially available kit used for preparing cells directly from positive blood culture broths.

METHODS

Seventy-three blood culture specimens and seven cerebrospinal fluid (CSF) specimens submitted to the laboratory between February and May of 2011 were prospectively analyzed using the Bruker MALDI-TOF MS and Sepsityper Kit for the direct detection of organisms and compared with standard phenotypic identification methods. Blood was inoculated into BD BACTEC Standard 10/Aerobic F, BD BACTEC Standard Anaerobic/F or BD BACTEC Peds Plus/F (Becton Dickinson, USA) bottles at the bedside. CSF specimens received at the microbiology laboratory were inoculated into BD BACTEC Peds Plus/F bottles for enrichment. Blood culture broths were incubated in the BD BACTEC FX instrument (Becton Dickinson, USA). All BACTEC blood culture bottles are charcoal free.

Conventional identification methods

Gram stains were performed on positive blood culture broths before subculture on sheep blood, horse blood, chocolate and MacConkey agars (Oxoid, Canada), as appropriate, and incubated at 37°C in CO_2 for 18 h to 24 h, and anaerobically for 48 h. Isolates were then identified using the Phoenix automated microbiology system (BD Diagnostic Systems, USA). Gram-positive cocci resembling staphylococci on Gram stain were subjected to the tube coagulase test (rabbit plasma, BD BBL; Becton Dickinson, USA), and staphylococcal isolates were tested using the staphylococcal latex agglutination test (Pastorex; Bio-Rad, USA). Gram-positive cocci arranged in pairs or chains were subcultured on blood agar with optochin disk, bile esculin agar and chocolate agar, and further biochemical tests were selected according to growth on the different plates based on standard protocols (7). For Gram-negative bacilli, subculture on CHROM Orientation agar (BD BBL; Becton Dickinson, USA), blood and MacConkey agars was performed. When identification using the Phoenix system was inconclusive or suspected to be incorrect, phenotypic identification of isolates was performed using API (bioMérieux, France). For cases in which all available phenotypic tests were inconclusive, amplification of the 16S DNA gene was performed followed by sequencing, or the specimen was sent to the provincial Public Health Laboratory (Ontario) for identification (8,9).

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TABLE 1
MALDI-TOF MS identification of Gram-negative bacteremia compared with standard phenotypic identification

Organism, samples (n)	Not identified, n	Correctly identified but low score (1.7 to 1.5), n	Identified to genus level using Biotyper 2.0*	Identified to genus level using Blood Culture Update v. 3.1.0.4*	Identified to species level using Biotyper 2.0	Identified to species level using Blood Culture Update v. 3.1.0.4
Escherichia coli (5)	0	0	5 (100)	5 (100)	5 (100)	5 (100)
Klebsiella pneumoniae (3)	0	0	3 (100)	3 (100)	3 (100)	3 (100)
Klebsiella oxytoca (1)	0	0	1 (100)	1 (100)	1 (100)	1 (100)
Enterobacter cloacae (2)	0	0	2 (100)	2 (100)	2 (100)	2 (100)
Salmonella paratyphi A (1)	0	0	1 (100)	1 (100)	1 (100)	1 (100)
Salmonella Emek (1)	0	0	1 (100)	1 (100)	1 (100)	1 (100)
Serratia marcescens (2)	0	0	2 (100)	2 (100)	2 (100)	2 (100)
Pseudomonas aeruginosa (2)	0	0	2 (100)	2 (100)	2 (100)	2 (100)
Pseudomonas species (1)	0	0	0†	1 (100)	0	1 (100)
Acinetobacter lwoffi (1)	0	0	1 (100)	1 (100)	1 (100)	1 (100)
Total (19)	0	0	18 (94.7)	19 (100)	18 (94.7)	19 (100)

*Data presented as n (%) unless otherwise specified. *Bruker Daltonik, Germany; †One specimen of* Pseudomonas *species was incorrectly identified as* Pasteurella bettayae, *with a score of 2.2 using Biotyper 2.0. It was correctly identified using Biotyper 3.1 software with BloodCulture Update v. 3.1.0.4. Definitive identification was performed by amplification and sequencing of 16S ribosomal DNA gene. MALDI-TOF MS Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry*

TABLE 2
MALDI-TOF MS identification of Gram-positive organisms in bacteremia/cerebrospinal fluid specimens compared with standard phenotypic identification

Organism, samples (n)	Not identified	Correctly identified but low score (<1.7)	Identified to genus level using Biotyper 2.0*	Identified to genus level using Blood Culture Update v. 3.1.0.4*	Identified to species level using Biotyper 2.0	Identified to species level using Blood Culture Update v. 3.1.0.4
Viridans group streptococci (13)	1 (7.7)	4 (30.7)	12 (92.3)	12 (92.3)	5 (38.4)†	7 (54)‡
Streptococcus pneumoniae (2)	0 (0)	0 (0)	2 (100)	1 (50)	2 (100)	1 (50)
Staphylococcus aureus (9)	0 (0)	0 (0)	9 (100)	9 (100)	9 (100)	9 (100)
Coagulase-negative staphylococci (30)	1 (3.3)	9 (30)	29 (96.7)§	29 (96.7)	–	–
Micrococcus species (5)	2 (40)	0 (0)	3 (60)	3 (60)	3 (60)§	3 (60)
Enterococcus fecalis (1)	0 (0)	0 (0)	1 (100)	1 (100)	1(100)	1 (100)
Total (60)	4 (6.7)	13 (21.7)	56 (93.3)	55 (91.7)	20 (66.7)	21 (70)

*Data presented as n (%) unless otherwise specified. *Bruker Daltonik, Germany; †Seven specimens were misidentified as* Streptococcus pneumoniae *using Biotyper 2.0; ‡Five specimens were misidentified as* Streptococcus pneumoniae *using Biotyper 3.1 software with Blood Culture Update v. 3.1.0.4. Conventional identification does not allow identification to the species level; §Conventional identification limited to catalase test and bacitracin disk. MALDI-TOF MS Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry*

MALDI Sepsityper Kit

The kit was used according to the manufacturer's instructions. Briefly, 200 µL of lysis buffer was added to 1 mL of positive blood culture fluid in a reaction tube. The tube was vortexed for 10 s before centrifugation at 13,000 rpm for 1 min. The supernatant was then discarded, and the pellet was suspended in 1 mL of washing buffer and recentrifuged at 13,000 rpm for 1 min. The supernatant was discarded once more, the pellet was resuspended in 300 µL of deionized water and 900 µL of ethanol was added.

Ethanol/formic acid extraction

The suspension obtained following sample preparation as described above was centrifuged at 13,000 rpm for 2 min, and the supernatant discarded. The pellet was centrifuged for an additional 2 min before removal of residual ethanol. Sequentially, 50 µL each of formic acid (70% v/v) and 100% acetonitrile was added to the pellet (or less depending on pellet size) and thoroughly mixed after each reagent was added. The resuspension was centrifuged again at 13,000 rpm for an additional 2 min, and 1 µL of the supernatant was spotted onto the steel target plate. Analysis was performed following air-drying of 1 µL alpha-cyano-4-hydroxycinnamic acid matrix solution placed onto the dried sample spot in duplicate.

MS fingerprinting

Mass spectra were generated using the Microflex LT MS system operated by the MALDI-Biotyper automation control (Bruker Daltonik, Germany). The degree of spectral concordance is expressed as a logarithmic identification score and interpreted by Biotyper software, version 2.0 (Bruker Daltonik). Scores ≥2.300 indicated species identification with a high level of confidence, ≥2.000 indicated species identification, 1.700 to 1.999 indicated genus identification, and <1.700 indicated no identification (10,11). Recently, the manufacturer has developed a new software tool, the MALDI Biotyper 3.1 software with Blood Culture Update v. 3.1.0.4 for the analysis of blood culture spectra that uses a lower cut-off value (Bruker Daltonik, personal communication). The spectra were reanalyzed using this newly developed blood culture analysis module and concordance of each software version was compared with conventional methods. This expanded database also includes updates such as increased spectra for analysis.

RESULTS

Eighty positive blood culture broths were identified by the BACTEC FX automated blood culture system, of which 73 (91.2%) were blood culture specimens and seven (8.7%) were CSF specimens. Seventy specimens (87.5%) were monomicrobial and 10 (12.5%) were poly-microbial. Correct identification to the genus and species level was obtained in 75 of 80 (93.8%) and 39 of 50 (78%) blood culture broths, respectively. Gram-negative organisms (Table 1) were identified correctly to the genus level in 18 of 19 (94.7%) and to the species level in 18 of 19 (94.7%) specimens. Gram-positive organisms (Table 2) were identified correctly to the genus level in 56 of 60 (93.3%) and to the species

TABLE 3
Results of identification of polymicrobial specimens (n=10) using MALDI-TOF MS and standard methods

Specimen	Method of identification	
	MALDI-TOF MS, score	Conventional
CSF	*Staphylococcus warneri*, 1.7	CNST, 2 types
Blood (autopsy)	*Streptococcus salivarius*, 1.7	Viridans group streptococci, CNST
Blood (autopsy)	*Escherichia coli*, 2.0	*E coli*, viridans group streptococci
Blood (autopsy)	*E coli*, 2.3	*E coli*, viridans group streptococci
Blood	*Staphylococcus hominis*, 2.1	CNST, 2 types
Blood	*Staphylococcus epidermidis*, 1.9	CNST, 2 types
Blood	*S epidermidis*, 1.46	CNST 2 types
Blood	*Streptococcus pneumoniae*, 2.15	Viridans group streptococci, 2 types
Blood	*Klebsiella pneumoniae*, 2.2	*K pneumoniae*, *Enterobacter cloacae*, *Staphylococcus aureus*
Blood	*Streptococcus parasanguinis*, 1.35	Viridans group streptococci, *Neisseria* species, *Stomatococcus* species

CNST Coagulase-negative staphylococci; CSF Cerebrospinal fluid; MALDI-TOF MS Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

level in 20 of 30 (66.7%) specimens. There were more Gram-negative organisms correctly identified to the species level than Gram-positive organisms (95% CI 0.01 to 0.9; P=0.03). Applying the blood culture analysis module, 100% of Gram-negative organisms were correctly identified to the species level, and 91.7% and 70% of Gram-positive organisms were identified to genus and species levels, respectively.

Thirty-nine organisms had scores ≥2 (48.8%), 23 (28.8%) had scores between 1.7 and 1.99, 10 (12.5%) had scores of between 1.69 and 1.5, and seven (8.8%) had scores <1.5.

In each polymicrobial specimen, only one organism was successfully identified (Table 3). One candidemia specimen was correctly identified as *Candida parapsilosis*, with a score of 1.98.

DISCUSSION

MALDI-TOF MS has significant potential for identifying organisms quickly and reliably from sterile body sites. Reported concordance rates between MALDI-TOF MS and conventional phenotypic methods for the direct identification of bacteria in non-charcoal-containing blood culture vials vary between 64.8% and 97% at the genus level, and 31.8% and 91.1% at the species level (10). Our study showed similar results, with overall concordance of 93.8% and 78% for genus and species levels, respectively. We also found some superiority in identification of Gram-negative organisms over Gram-positive organisms, which was also reported by Sogawa et al (11), Kok et al (12) and Vlek et al (13). Juiz et al (14) reported better scores using the Sepsityper Kit for extraction of Gram-positive bacteria compared with an in-house extraction method.

Despite the reliability and short turnaround time for identification of organisms directly from specimens, our study revealed a number of shortcomings of MALDI-TOF MS. First, identification of viridans group streptococci to the species level occurred in only 38.4% of the specimens that contained this organism; seven isolates were misidentified as *Streptococcus pneumoniae* (Table 4). Applying the blood culture analysis module, five viridans group streptococci isolates were misidentified as *S pneumoniae*. Misidentification of members of viridans group streptococci as *S pneumoniae* by MALDI-TOF MS was also reported by Kok et al (12). Biochemical tests, such as optochin susceptibility and bile solubility, can be used to differentiate *Streptococcus oralis* and

TABLE 4
MALDI-TOF MS* identification using BioTyper* 2.0 and scores isolates identified as viridans group streptococci using conventional identification methods

MALDI-TOF MS Identification	Score
Streptococcus pneumoniae	2.1
S pneumoniae	1.9
S pneumoniae	1.7
S pneumoniae	2.15
S pneumoniae	1.67
S pneumoniae	1.96
S pneumoniae	1.68
Streptococcus oralis	1.38
Streptococcus parasanguinis	1.35
S parasanguinis	1.7
Streptococcus salivarius	1.7
Streptococcus peroris	2.1
No reliable identification	na

**Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS); Bruker Daltonik, Germany. na Not applicable*

Streptococcus mitis from *S pneumoniae* isolates. Some authors have used a combination of MALDI-TOF and rapid antigen testing to achieve rapid identification of *S pneumoniae* (15). Also, Warnero et al (16) found that it was possible to distinguish different species of the Mitis group streptococci by close analysis of their mass peak profiles. The technology is also not able to discriminate between *Escherichia coli* and *Shigella* species (17); however, this may be of less clinical relevance in our study because isolation of *Shigella* species from blood cultures and sterile sites is exceedingly rare. Third, in our study, one organism was misidentified as *Pasteurella bettayae*, with a score of 2.2; final genotypic identification proved the organism was a *Pseudomonas* species. This error was probably due to lack of reference spectra for variants of some species in the MALDI-Biotyper 2.0 application software. This organism was correctly identified as a *Pseudomonas* species on reanalysis of its spectra using the MALDI Biotyper 3.1 software with Blood Culture Update v. 3.1.0.4. Finally, MALDI-TOF MS was only able to identify one organism in each polymicrobial specimen, which was also reported by Ferroni et al (15). However, polymicrobial bacteremia and sterile body site infections are relatively uncommon. The company is also currently working on software to analyze mixed specimens, which may assist in identifications in the future (Bruker Daltonik, personal communication).

There are several limitations to our study. The number of specimens included in the study was only a fraction of the number of actual specimens processed during the study period. This was due to the fact that the specimens were only processed on weekdays when the author was present at the microbiology laboratory. The main strength of our study is that it represents one of the first studies for direct identification of organisms from sterile body sites in a pediatric Canadian patient population.

CONCLUSIONS

MALDI-TOF MS is a promising tool for the direct identification of organisms from sterile body sites; its reliability and rapid turnaround time can likely improve patients' outcomes. Software library development will likely overcome most of the identification limitations.

ACKNOWLEDGEMENTS: The authors thank all members of the microbiology department at The Hospital for Sick Children, Toronto (Ontario) for their assistance in conducting this project. The authors also thank Bruker Daltonik for providing the Sepsityper Kit free of charge.

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