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ORIGINAL ARTICLE

Early identification of microorganisms in blood culture prior to the detection of a positive signal in the BACTEC FX system using matrix-assisted laser desorption/ ionization–time of flight mass spectrometry

Ming-Cheng Wang [a,b], Wei-Hung Lin [c], Jing-Jou Yan [d], Hsin-Yi Fang [e], Te-Hui Kuo [a], Chin-Chung Tseng [a], Jiunn-Jong Wu [f,g,*]

[a] *Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan*
[b] *Institute of Clinical Pharmacy and Pharmaceutical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[c] *Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[d] *Department of Pathology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[e] *Department of Biotechnology, Chia Nan University of Pharmacy and Science, Tainan, Taiwan*
[f] *Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[g] *Infectious Disease and Signaling Research Center, National Cheng Kung University, Tainan, Taiwan*

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KEYWORDS
Blood culture system;
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Background: Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) is a valuable method for rapid identification of blood stream infection (BSI) pathogens. Integration of MALDI-TOF MS and blood culture system can speed the identification of causative BSI microorganisms.

* Corresponding author. Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Number 1 University Road, Tainan 70101, Taiwan.
E-mail address: jjwu@mail.ncku.edu.tw (J.-J. Wu).

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Mass spectrometry;
Matrix-assisted laser desorption ionization—time of flight mass spectrometry;
Time to detection

Materials and methods: We investigated the minimal microorganism concentrations of common BSI pathogens required for positive blood culture using BACTEC FX and for positive identification using MALDI-TOF MS. The time to detection with positive BACTEC FX and minimal incubation time with positive MALDI-TOF MS identification were determined for earlier identification of common BSI pathogens.
Results: The minimal microorganism concentrations required for positive blood culture using BACTEC FX were $>10^7$—10^8 colony forming units/mL for most of the BSI pathogens. The minimal microorganism concentrations required for identification using MALDI-TOF MS were $> 10^7$ colony forming units/mL. Using simulated BSI models, one can obtain enough bacterial concentration from blood culture bottles for successful identification of five common Gram-positive and Gram-negative bacteria using MALDI-TOF MS 1.7—2.3 hours earlier than the usual time to detection in blood culture systems.
Conclusion: This study provides an approach to earlier identification of BSI pathogens prior to the detection of a positive signal in the blood culture system using MALDI-TOF MS, compared to current methods. It can speed the time for identification of BSI pathogens and may have benefits of earlier therapy choice and on patient outcome.

Introduction

Blood stream infections (BSIs) commonly result in critical illness and have been associated with high morbidity and mortality rates.[1,2] BSIs are more common in immunocompromised patients, or patients receiving surgery, developing multiorgan dysfunction, or requiring mechanical ventilation or renal replacement therapy.[3,4] Similar to treatment of acute myocardial infarction or stroke, administration of appropriate antibiotics in a timely manner is mandatory to improve the outcome.[5—7] Therefore, early identification of pathogenic microorganisms is important.

The standard method to detect infectious microorganisms in positive blood cultures involves overnight agar medium subcultures from the positive blood culture bottles in order to obtain a sufficient number of bacteria needed for species identification. Molecular diagnostic approaches have been applied in the first hours of a suspected infection. Polymerase chain reaction assay and sequencing of amplified product for species identification of the causative pathogen have provided increased detection sensitivity of BSI.[8—10] Matrix-assisted laser desorption/ionization—time of flight mass spectrometry (MALDI-TOF MS) has been recognized as a fast and reliable method for early microorganism identification, based on the characteristic protein profiles for each microorganism. Direct inoculation of microorganisms from positive blood culture bottles into the MALDI-TOF MS system has decreased the time needed to obtain identification.[11—13] The minimal bacterial concentrations of different BSI microorganisms required for blood culture bottles flagged as positive by automatic incubation systems and for identification using MALDI-TOF MS remain to be elucidated.[14—16]

The aims of this study were to investigate the minimal microorganism concentrations required for blood culture bottles flagged as positive using the BACTEC FX blood culture system and for identification of BSI pathogens using MALDI-TOF MS. The time to detection (TTD) with positive BACTEC FX and minimal incubation time with positive identification by MALDI-TOF MS were determined for earlier identification of five common BSI pathogens. They were *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus haemolyticus*, and *Staphylococcus aureus*.

Materials and methods

Ethics statement

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital (NCKUH), Tainan, Taiwan (No. A-ER-101-391), and adhered to the Declaration of Helsinki. Informed consent was obtained from all healthy adult volunteers.

Determination of the minimal microorganism concentrations required for positive blood culture by the BACTEC FX system

From April 2013 to June 2013, we studied over 108 microorganisms, representing the pathogens most frequently responsible for bacteremia in our institute. These isolates were positively detected using the BACTEC FX automatic blood culture detection system (Becton Dickinson, Sparks, MD, USA) at the Microbiology Laboratory, Department of Pathology, National Cheng-Kung University Medical Center, Tainan, Taiwan. The blood culture bottles were processed by microbiologists when the laboratory was open (08.00—17.00, Monday—Sunday).

Blood culture bottles placed in the instruments were processed and tested according to the manufacturer's instructions. The bottles were incubated at 35 °C with rocking agitation for a total of 5 days. The FX unit tested each bottle every 10 minutes. All bottles were analyzed in the microbiology laboratory over the course of 5 days by the BACTEC FX system. Bottles flagged as positive were removed from the data units and processed.[17] The bacterial concentration was determined by a quantitative plate

count method: 0.1 mL of serial 10-fold dilutions was spread on the blood agar plate, and the colonies were counted on plates containing 30—300 colonies following overnight incubation at 35°C.

Determination of the minimal microorganism concentrations required for identification of microorganisms using MALDI-TOF MS

We inoculated 5 mL human blood of healthy adult volunteers with each strain of 18 common BSI microorganisms at NCKUH at a count of 5×10^8 colony forming units (CFU)/mL. We performed sequential dilutions in order to achieve aliquots of each microorganism at the following bacterial counts: 1×10^7 CFU/mL, 1×10^6 CFU/mL, 5×10^5 CFU/mL, 1×10^5 CFU/mL, 5×10^4 CFU/mL, and 1×10^4 CFU/mL. Three 100-μL aliquots of each dilution were plated onto blood agar plates. These plates were incubated at 37 °C in an aerobic atmosphere, and colonies were counted manually. The mean of three aliquots of each dilution were considered to be the final count for that aliquot.

The blood culture bottles were incubated in a BACTEC FX blood culture system. When a blood culture is flagged as positive by the BACTEC FX system, indicating microorganism growth, a Gram stain is performed to confirm the presence of the Gram-positive or Gram-negative bacteria. A 200 μL aliquot of lysis buffer (5% Saponin) was added to 1 mL of positive blood culture fluid in a reaction tube. The tube was vortexed for 10 seconds prior to centrifugation at 13,000*g* for 1 minute. The supernatant was then discarded, the pellet suspended with 1 mL of washing buffer, and recentrifuged at 13,000*g* for another minute. The supernatant was discarded once more, and the pellet resuspended in 300 μL of deionized water, and 900 μl of ethanol was added.

The suspension obtained following the above sample preparation was centrifuged at 13,000*g* for 2 minutes, and the supernatant was discarded. The pellet was centrifuged at 13,000*g* for another 2 minutes prior to the removal of the residual ethanol. Fifty microliters of formic acid (70% v/v) and 50 μL of 100% acetonitrile were added to the pellet, and mixed thoroughly after each reagent was added. The suspension was centrifuged again at 13,000*g* for another 2 minutes, and 1 μL of the supernatant was spotted onto the steel target plate. Analysis was performed following air-drying of 1 μL α-cyano-4-hydroxycinnamic acid matrix solution placed onto the dried sample spot in duplicate.[12,16]

Mass spectra profiles were acquired using a microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) following the manufacturer's settings. Spectra are recorded in the linear positive mode at a laser frequency of 60 Hz within a mass range from 2000 Da to 20,000 Da. All bacteria identifications were performed by MALDI-TOF Biotyper RTC and the Bruker MALDI Biotyper 3.1 software and library (4613 isolates; Bruker Daltonics). Criteria used for microorganism analysis and identification were as recommended by the manufacturer. A score of <1.700 was interpreted as no identification, a score of 1.700—1.999 indicates identification to genus level, and a score of ≥ 2.000 indicates identification to species level.[18,19]

Time saved by earlier identification of BSI microorganisms using BACTEC FX and MALDI-TOF MS

Bacterial colonies of *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. haemolyticus*, and *S. aureus* were harvested from the second subculture to prepare a suspension in sterile blood coming from healthy adult volunteers. A volume of 5 mL of blood specimen was inoculated into BACTEC aerobic bottles. After inoculation, the culture bottles were loaded into a BACTEC FX instrument as per the manufacturer's instructions. Measurements of the real concentrations of the inocula were performed by subculturing 0.1 mL of the bacterial suspension on blood agar plates prior to introducing the bottles into the instrument. Culture bottles flagged as positive were removed from the data units and processed, and the TTD was recorded.

We then performed a sequential reduction in incubation time (per 1 hour) to obtain a minimal incubation time required for positive identification of the microorganism by MALDI-TOF MS. The time saved by earlier identification of common BSI pathogens was calculated by TTD minus the minimal incubation time. The corresponding bacterial concentrations applied to MALDI-TOF MS and in blood culture bottles at minimal incubation times were also determined.

Results

Of the 108 consecutive clinical BSI isolates detected in BACTEC FX blood culture bottles, 45 (41.6%) were Gram-positive bacteria, 55 (50.9%) were Gram-negative bacteria, six (5.6%) were fungi, and two (1.9%) were mixed infection. The 10 leading pathogens were coagulase-negative *Staphylococcus* (22.2%), *E. coli* (16.7%), *S. aureus* (12.0%), *Acinetobacter baumannii* (7.4%), *K. pneumoniae* (6.5%), *Streptococcus agalactiae* (4.6%), *Candida albicans* (4.6%), *Aeromonas hydrophila* (3.7%), *P. aeruginosa* (2.8%), and *Enterobacter cloacae* (2.8%). The minimal microorganism concentrations required for blood culture bottles flagged positive using BACTEC FX blood culture system were more than 10^7—10^8 CFU/mL for most of the BSI pathogens (Table 1). The average minimal bacterial concentrations of Gram-positive and Gram-negative bacteria required for positive blood culture were 2.6×10^8 CFU/mL and 3.2×10^8 CFU/mL, respectively. The average minimal microorganism concentration for fungi (2.9×10^7 CFU/mL) was 1 log lower than those of Gram-positive and Gram-negative bacteria.

For the 18 common BSI pathogens identified at National Cheng Kung University Hospital in 2012, their minimal microorganism concentrations required for identification using MALDI-TOF MS were $> 10^7$ CFU/mL (Table 2). Several microorganism concentrations required for identification of BSI pathogens using MALDI-TOF MS were less than those by positive BACTEC FX. All the microorganisms identified by the MALDI-TOF MS in this study had been confirmed by the conventional bacterial identification method, and the correlation rate was 100%.

The bacterial loads, TTD, and bacterial counts for positive BACTEC FX, and TTD and bacterial counts for positive MALDI-TOF MS identification of simulated BSI of five

Table 1 Determination of the minimal microorganism concentrations of common blood stream infection pathogens required for blood culture bottles flagged as positive using BACTEC FX blood culture system

Microorganism	No. of strains	Concentration of microorganism (CFU/mL)
Coagulase-negative *Staphylococcus*	24	1.9×10^8
Escherichia coli	18	3.7×10^8
Staphylococcus aureus	13	3.7×10^8
Acinetobacter baumannii	8	9.3×10^7
Klebsiella pneumoniae	7	5.4×10^8
Streptococcus agalactiae	5	4.8×10^8
Candida albicans	5	3.4×10^7
Aeromonas hydrophila	4	1.4×10^8
Pseudomonas aeruginosa	3	2.0×10^8
Enterobacter cloacae	3	1.0×10^9
Klebsiella oxytoca	2	1.7×10^7
Enterobacter gergevice	2	2.0×10^8
Achromobacter xylosoxidans	2	3.4×10^7
Pseudomonas putida	2	4.5×10^7
Burkholderia cepacia	2	7.3×10^7
Gram-positive bacilli	2	1.6×10^7
Serratia marcescens	1	2.5×10^8
Cryptococcus curvatus	1	1.9×10^8
Chryseobacterium meningoseptium	1	5.0×10^8
Peptostreptococcus magnus	1	4.0×10^6
Mixed infection[a]	2	6.6×10^7
Gram-positive bacteria	45	2.6×10^8
Gram-negative bacteria	55	3.2×10^8
Fungi	6	2.9×10^7

[a] Pathogens of mixed infections were *Stenotrophomonas maltophilia* plus *Enterococcus faecium* and *Stenotrophomonas maltophilia* plus *Acinetobacter baumannii*.
CFU = colony-forming unit.

Table 2 Determination of the minimal microorganism concentrations required for identification of common blood stream infection pathogens using MALDI-TOF MS

Microorganism	Simulated blood culture broth, CFU/mL ($\times 10^8$)
Acinetobacter baumannii	1.02
Aeromonas hydrophilia	0.57
Bacteroides fragilis	2.10
Candida albicans	0.48
Candida parapsilosis	0.78
Enterobacter cloacae	1.05
Enterococcus faecalis	1.17
Escherichia coli	0.93
Haemophilus influenza	0.81
Klebsiella pneumoniae	0.60
Proteus mirabilis	1.50
Pseudomonas aeruginosa	0.69
Salmonella enteritidis group D	0.93
Staphylococcus aureus	1.20
Staphylococcus haemolyticus	1.20
Streptococcus agalactiae	1.35
Viridian streptococcus	0.45
Vibrio vulnificus	1.11

CFU = colony-forming unit; MALDI-TOF MS = matrix-assisted laser desorption/ionization—time of flight mass spectrometry.

common pathogens are shown in Table 3. The average TTD with positive BACTEC FX was 4.9 hours, 4.7 hours, 6.3 hours, 6.0 hours, and 6.0 hours for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. haemolyticus*, and *S. aureus*, respectively. The average TTD with positive MALDI-TOF MS identification were 3.0 hours, 3.0 hours, 4.0 hours, 4.0 hours, and 3.0 hours for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. haemolyticus*, and *S. aureus*, respectively. Our simulated BSI models showed that earlier and successful identification of BSI pathogens can be achieved using MALDI-TOF MS with earlier sampling from blood culture bottles in BACTEC FX. The time saved by earlier identification of bacteria was 1.7—2.3 hours compared with the current methods using positive BACTEC followed by MALDI-TOF MS.

Discussion

This study demonstrates a method of earlier identification of BSI pathogens based on the understanding of minimal microorganism concentrations required for positive blood culture in BACTEC FX and for positive identification by MALDI-TOF MS. The time saved by earlier identification of five common BSI pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. haemolyticus*, and *S. aureus*) was 1.7—2.3 hours.

MALDI-TOF MS is a valuable and efficient method for direct and rapid identification of microorganisms, and is also an important step beside Gram stain in guiding the empirical treatment of patients with BSI.[20] Integration of MALDI-TOF MS and blood culture systems can speed the identification of causative microorganisms in BSI. The median time to identification by MALDI-TOF MS from positive blood cultures (7.1 hours) was significantly shorter than that of reference standard methods (48.1 hours), working 6 days a week, 12-hour shifts (8 AM/8 PM).[12,21,22] There was a clear association between bacterial concentration and identification scores of MALDI-TOF MS. Bacterial concentrations in blood and urine samples required to provide reliable scores for MALDI-TOF MS identification have been reported in several studies. They needed $>10^8$ CFU/mL in blood culture broth and $>10^5$ CFU/mL in urine specimens, respectively.[16,23,24] Our study revealed that the minimal microorganism concentrations of BSI pathogens required for species identification by MALDI-TOF MS were $>10^7$—10^8 in simulated blood culture bottles and $>10^6$ on the plate for MALDI-TOF MS. There was no significant difference between Gram-positive and Gram-negative bacteria.

For conventional identification of microorganisms, Gram staining and subsequent culture are performed following a blood culture flagged positive by the blood culture system. Several studies have evaluated the TTD in different blood culture systems using clinical or simulated blood cultures.[25—27] The minimal bacterial concentrations of different BSI microorganisms required for blood culture

Table 3 Earlier identification of five common microorganisms from simulated blood culture bottles incubated in BACTEC FX blood culture system using MALDI-TOF MS

Microorganism	Bacterial load (CFU/mL)	TTD with positive BACTEC (h′min)	Bacterial count with positive BACTEC (CFU/mL)	TTD with positive MALDI-TOF MS result (h′min)	Bacterial count in BCB with positive MALDI-TOF MS result (CFU/mL)
Escherichia coli	1.4×10^6	4′51	3.4×10^8	3′00	3.3×10^7
Klebsiella pneumoniae	1.4×10^6	4′40	3.9×10^8	3′00	1.3×10^7
Pseudomonas aeruginosa	9.3×10^5	6′21	1.6×10^8	4′00	3.6×10^7
Staphylococcus haemolyticus	1.1×10^6	5′57	1.3×10^8	4′00	7.8×10^7
Staphylococcus aureus	3.3×10^6	5′57	1.3×10^8	3′00	1.2×10^7

Time saved by earlier identification of the five microorganisms was *E. coli*, 1′51; *K. pneumonia*, 1′40; *P. aeruginosa*, 2′21; *S. haemolyticus*, 1′57; and *S. aureus*, 2′57; respectively.
BCB = blood culture bottle; CFU = colony-forming unit; MALDI-TOF MS = matrix-assisted laser desorption/ionization—time of flight mass spectrometry; TTD = time to detection.

bottles flagged positive by automatic incubation system have not been well investigated.[15,16] This study demonstrated that the average minimal microorganism concentrations required for positive blood culture using BACTEC FX were $>10^8$ CFU/mL for BSI bacteria and $>10^7$ CFU/mL for BSI fungi. We further demonstrated that enough bacterial concentration from blood culture bottles for successful identification of five common Gram-positive and Gram-negative bacteria using MALDI-TOF MS in simulated BSI models can be obtained 1.7—2.3 hours earlier than the usual TTD in blood culture systems. The earlier identification of BSI pathogens may have benefits of earlier therapy choice and on patients' outcomes. Speeding the detection of blood cultures by the blood culture system with 1 log lower minimal microorganism concentrations required for blood culture bottles flagged as positive will be helpful.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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