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Rapid Identification of Microbes in Positive Blood Cultures by Use of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System

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Sepsis is a major cause of death worldwide among nonhospitalized people and hospitalized patients. A wide range of pathogens are involved, and the correct identification and correct antimicrobial therapy are critical to ensure optimal clinical outcomes. With the recent introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), rapid identification of bacteria and fungi is now possible. The purpose of this study was to develop a rapid technique for identifying organisms in positive blood cultures using the Vitek MS system (bioMérieux). This technique is a lysis centrifugation method which involves a four-step washing and centrifugation procedure. A total of 253 positive monomicrobial blood cultures (Bactec Plus aerobic, anaerobic, and pediatric bottles) were tested using the Vitek MS system (KnowledgeBase version 2.0), with 92.1% and 88.1% of organisms overall being identified to the genus level and the species level, respectively. Of 161 Gram-positive bacterial isolates, 95.7% and 90.1% were identified to the genus level and the species level, respectively; of 92 Gram-negative bacterial isolates, 84.7% and 83.7% were identified to the genus level and the species level, respectively. The results obtained using this method demonstrate that the Vitek MS system can be used for rapid and effective identification of bacteria from positive blood cultures within 30 to 45 min after the positive signal has been provided by the Bactec FX blood culture system (Becton, Dickinson). This will lead to faster administration of the appropriate antimicrobial therapy and increase the chances for optimal clinical outcomes for patients.

Sepsis is one of the most common causes of death in seriously ill patients. About 18 million cases occur worldwide each year, with a mortality rate of around 30% (1). The first 24 h of patient care is critical for determining and administering the appropriate antimicrobial therapy, and mortality rates increase by approximately 7% for every hour a septic patient remains untreated or receives inappropriate antimicrobial therapy (2). The present turnaround time for the identification of causative organisms, from the time of positive results from the blood culture system, is about 18 to 24 h.

Faster identification technology, such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), provides faster and more accurate identification of bacteria and fungi. The objective of this work was to use the Vitek MS system (bioMérieux) to attempt to identify bacteria directly from positive blood culture bottles within 30 to 45 min after the time of the positive signal from the Bactec FX (Becton, Dickinson) blood culture system. Various methods and techniques (both commercial and in-house) have been used for direct identification of microorganisms from positive blood culture broths, for example, the Sepsityper kit (Bruker), the saponin method for bacterial extraction (3, 4), lysis centrifugation methods (5), and the serum separator method (6).

All of these techniques show better results for the identification of Gram-negative bacilli than Gram-positive organisms, including the differentiation of staphylococcal species. This new technique not only maintains the high level of identification of Gram-negative bacilli seen with previous methods but also achieves a high level of identification of Gram-positive organisms, including staphylococcal species, with 126/127 staphylococcal isolates (99.2%) being identified to the species level.

MATERIALS AND METHODS

A mixture of 253 positive aerobic Bactec Plus Aerobic/F, anaerobic Bactec Lytic/10 Anaerobic/F, and pediatric Bactec Peds Plus/F blood culture bottles were incubated in an automated Bactec FX blood culture system (Becton, Dickinson, Cockeysville, MD). When new positive bottles were detected, broth samples from the bottles were assessed using a Vitek MS system (bioMérieux, Australia).

For each positive blood culture bottle, 7 ml of broth was removed, added to 3 ml of wash buffer 1 (2.5 ml of sterile distilled water and 0.5 ml of Spree Multiaction lemon dishwashing detergent [Colgate-Palmolive, Sydney, Australia], with the following composition: sodium laureth sulfate, 0 to <5%; lauramidopropyl dimethylamine oxide, 0 to <5%; myristamidopropylamine oxide, 0 to <1%; tetrasodium EDTA, 0 to <1%; formaldehyde, <0.01%; isothiazolinones, <0.001%) (http://www.colgate.com.au/app/Colgate/AU/HC/Product-Ingredients/SpreeDish.cvsp), and then centrifuged for 2 min at 3,000 × *g*. The supernatant was removed, the pellet was resuspended in 10 ml of wash buffer 2 (9.5 ml of sterile distilled water and 0.5 ml of Spree dishwashing detergent concentrate), and the suspension was centrifuged for 2 min at 3,000 × *g*. The same procedure was then followed with wash buffer 3 (10 ml of sterile distilled water containing 0.1 g *N*-acetyl-L-cysteine) and 10 ml of sterile distilled water. The supernatant was removed, 1 μl of pellet was placed on a MALDI-TOF MS slide, 0.5 μl formic acid (Vitek MS-FA) was added to each well, and the slide was air dried. One microliter of matrix (Vitek MS-CHCA) was added, and the mixture was air dried. The slide

Received 27 June 2013 Returned for modification 23 July 2013
Accepted 23 August 2013
Published ahead of print 28 August 2013
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doi:10.1128/JCM.01679-13

was analyzed in the MALDI-TOF MS system (bioMérieux Vitek MS) to obtain the identification. The procedure required approximately 30 min. Each isolate was then identified from culture plates the following day, using MALDI-TOF MS (bioMérieux Vitek MS), Vitek 2 testing, and other biochemical laboratory tests to confirm the identification of the isolate.

All organisms were placed in four wells of a Vitek MS slide. If the identifications of two or more wells were given with high confidence levels, then this was considered an acceptable identification. A misidentification result was given when two or more identifications of the four wells had high confidence levels but did not match the identification given by the Vitek MS system for the subculture the following day. No identification result was given when two or more identifications had poor confidence levels or no identification was given by the Vitek MS system for all 4 wells.

RESULTS

Of the 253 positive monomicrobial blood cultures tested, 92.1% were successfully identified to the genus level and 88.1% were identified to the species level. Of the 161 Gram-positive organisms, 154 (95.7%) were identified to the genus level and 145 (90.1%) were identified to the species level. Of the 127 *Staphylococcus* isolates, 126 (99.2%) were identified to the species level. All 45 *Staphylococcus aureus* isolates (100%) were identified correctly and differentiated from coagulase-negative *Staphylococcus*. Of the 21 *Streptococcus* isolates, 18 (80.1%) were identified to the genus level and 13 (61.9%) were identified to the species level.

Of the 92 Gram-negative organisms identified, 78 (84.7%) were correctly identified to the genus level and 77 (83.7%) were identified to the species level (Table 1). Sixteen positive blood cultures were not included in the study due to mixed growth on subculture. An additional eight positive cultures were not identified due to an absence of the organisms from Vitek MS KnowledgeBase version 2.0. Only 2/253 microbes (0.8%) were misidentified; one was *Streptococcus pneumoniae* misidentified as *Streptococcus mitis/oralis* and one was *Klebsiella pneumoniae* misidentified as *Enterobacter aerogenes*. For 20/253 broths (7.9%), no identification was provided by the Vitek MS system (KnowledgeBase version 2.0). Table 2 presents a list of unidentified and misidentified organisms. Only monomicrobial cultures were used in this study; therefore, 16 broths were excluded due to mixed subcultures.

TABLE 1 Results obtained with the Vitek MS system for 253 positive blood cultures

Organism	No. of isolates tested	No. identified to species level/no. identified to genus level (%)
Gram-positive bacteria		
Staphylococci		
Staphylococcus aureus	45	45/45
Staphylococcus capitis	13	13/13
Staphylococcus warneri	4	4/4
Staphylococcus epidermidis	37	37/37
Staphylococcus hominis	17	17/17
Staphylococcus haemolyticus	6	5/5
Staphylococcus simulans	1	1/1
Staphylococcus lugdunensis	1	1/1
Staphylococcus auricularis	1	1/1
Staphylococcus caprae	2	2/2
Total		126/127 (99.2)[a]
Streptococci		
Streptococcus pyogenes	2	2/2
Streptococcus mitis/oralis	3	0/3
Streptococcus dysgalactiae	2	1/1
Streptococcus pneumoniae	2	0/0
Streptococcus agalactiae	1	1/1
Streptococcus parasanguinis	1	1/1
Streptococcus salivarius	1	0/0
Streptococcus anginosus	1	0/1
Enterococci		
Enterococcus faecalis	5	5/5
Enterococcus faecium	3	3/3
Other		
Micrococcus luteus	1	1/1
Propionibacterium acnes	2	1/2
Bacillus licheniformis	1	1/1
Corynebacterium pseudodiphtheriticum	1	1/1
Bacillus cereus group	5	0/3
Bacillus simplex	1	1/1
Rothia spp.	1	0/1
Finegoldia magna	1	1/1
Gram-negative bacteria		
Enterobacteriaceae		
Escherichia coli	45	43/43
Proteus mirabilis	2	2/2
Proteus penneri/vulgaris	1	0/1
Klebsiella pneumoniae	11	10/10
Klebsiella oxytoca	3	3/3
Enterobacter aerogenes	1	1/1
Citrobacter freundii	1	0/0
Providencia rettgeri	2	2/2
Enterobacter cancerogenus	1	1/1
Enterobacter cloacae/asburiae	2	2/2
Serratia marcescens	2	2/2
Other		
Pseudomonas aeruginosa	3	3/3
Acinetobacter lwoffii	1	1/1
Acinetobacter radioresistens	1	1/1
Stenotrophomonas maltophilia	2	0/0
Acinetobacter baumannii	1	1/1
Neisseria meningitidis	1	1/1
Leptotrichia spp.	1	0/0
Bacteroides vulgatus	1	1/1
Bacteroides thetaiotaomicron	2	2/2
Bacteroides fragilis	1	1/1
Helicobacter spp.	1	0/0
Environmental Gram-negative bacilli	6	0/0
Total	253	223/253 (88.1)[a]; 233/253 (92.1)[b]

[a] Total is for isolates identified to the species level.
[b] Total is for isolates identified to the genus level.

DISCUSSION

Problems in previous studies included the identification of Gram-positive organisms such as *Staphylococcus* spp. and *Streptococcus* spp. Various techniques and methods using commercially available kits, such as the Sepsityper kit (Bruker Daltonics, Billerica, MA) (3, 4, 7), showed poor results in the identification of Gram-positive organisms, including staphylococci; this has been overcome with the present technique. However, misidentification of *S. pneumoniae* caused by a lack of data in the Vitek MS database (KnowledgeBase version 2.0) was also evident in this and other studies (5, 8). Difficulties with the identification of encapsulated organisms (e.g., *Klebsiella* spp.) experienced in previous studies (9) have also been overcome using the present technique. All 45 *Staphylococcus aureus* isolates (100%) and 81/82 coagulase-negative *Staphylococcus* isolates (98.8%) were identified to the species level. This is of major clinical importance, as it enables clinicians to distinguish between *Staphylococcus aureus*-related sepsis and possible skin contamination due to other staphylococci.

The cost of this technique was $2.26 per positive broth tested, which is cheaper than Sepsityper (Bruker) testing ($5.15) (10). In addition, this technique has the same turnaround time as Sepsityper testing, i.e., 30 to 45 min. This new technique is now being used routinely in our laboratory, and we are currently using it as a screening test in a trial study using the Xpert MRSA/SA BC kit (Cepheid, Sunnyvale, CA) for identification of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) directly from positive blood culture broths. The retail price of the Xpert MRSA/SA

TABLE 2 Unidentified and misidentified isolates

Organism	No. of unidentified isolates	No. of misidentified isolates
Staphylococcus haemolyticus	1	
Streptococcus pneumoniae	1	1[a]
Bacillus cereus group	2	
Streptococcus dysgalactiae	1	
Propionibacterium acnes	1	
Streptococcus salivarius	1	
Escherichia coli	2	
Klebsiella pneumoniae		1[b]
Citrobacter freundii	1	
Stenotrophomonas maltophilia	2	
Leptotrichia spp.	1[c]	
Helicobacter spp.	1[c]	
Environmental Gram-negative bacilli	6[c]	
Total	20	2

[a] Identified as *Streptococcus mitis/oralis*.
[b] Identified as *Enterobacter aerogenes*.
[c] Not in Vitek MS database.

BC kit is $80.72 (11). In a period of 2 months, our laboratory had 150 positive blood cultures with staphylococci isolated. Of those 150 cultures, *Staphylococcus aureus* was isolated from 50 positive blood culture broths and the remaining 100 broths contained coagulase-negative *Staphylococcus*. If all 150 positive blood cultures were tested using the Xpert MRSA/SA BC kit, then the cost would be $12,108. With the use of this new technique as a screening test, only 50 positive broths would be tested, reducing the cost to $4,036 and giving savings of $7,846 when the cost of the 100 coagulase-negative *Staphylococcus* screening tests is included. It has been demonstrated that rapid identification, such as with this technique, can significantly decrease the lengths of hospital stays for patients and decrease hospital costs (12, 13).

These results demonstrated that it was possible to obtain rapid accurate identification of bacteria directly from positive blood cultures using the Vitek MS system (KnowledgeBase version 2.0). Using this lysis centrifugation technique, it was possible to identify the causative organism approximately 30 to 45 min after a new blood culture was detected as positive by the Bactec FX system (Becton, Dickinson). Organism identification therefore would be available for the medical staff at virtually the same time as the Gram staining result. This would enable important decisions to be made regarding the clinical significance of the isolate and the use of appropriate antimicrobial therapy.

This method will allow prompt differentiation between *Staphylococcus aureus* and other staphylococci (the majority of which are contaminants), provide early clinically important information, and reduce the inappropriate use of antibiotics. It has also been demonstrated that this method can provide large savings in the costs of rapid identification of *Staphylococcus* species in positive blood cultures.

The only limitations of this procedure were with mixed cultures, broths from patients with very high white cell counts (as seen in chronic myelogenous leukemia) not being able to yield clean spun pellets, resulting in no identification by the Vitek MS system, and the current database of the Vitek MS system (KnowledgeBase version 2.0). Overall, the data suggest that this method will provide considerable patient benefits and contribute to reducing mortality rates for septicemia.

ACKNOWLEDGMENTS

I thank Stephen Graves, Chris Ashhurst-Smith, Patrick Harris, and Mahesh Menon, who contributed to this study in various ways.

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