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CASE REPORT

Utilizing MALDI-TOF MS from Positive Blood Culture for Diagnosing Anaerobic Bacteremia

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ABSTRACT

Anaerobic bacteraemia is a significant cause of morbidity and mortality among hospitalized patients worldwide. Early identification of these microorganisms from blood culture is pivotal in diagnosing and can improve treatment and outcomes. We present a case of anaerobic bacteraemia in a patient with diabetic foot ulcer, which was detected rapidly by the Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) from a positive blood culture bottle in a laboratory setting with minimal facilities for anaerobic culture methods.

KEYWORDS: Anaerobe; Antimicrobial stewardship; Bloodstream infection; Matrix Assisted Laser Desorption Ionization Time of Flight; Mass spectrometry

INTRODUCTION

Bloodstream infection is a severe condition and a leading cause of death worldwide. These patients require utmost care from the moment they seek medical attention. It includes early recognition, emergency care, targeted antimicrobial therapy, infection source control, intensive monitoring, detection of clinical deterioration, and prevention of organ failure and complications. Effective and efficient diagnosis and care can further reduce mortality and morbidity. Blood culture is the gold standard in diagnosing bloodstream infections, but it takes time. In the routine practice of blood culture, positive blood culture bottles are subcultured on solid media. Following overnight incubation, the colonies are used for identification by the Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) mass spectrometry (MS) system. Rapidly identifying a pathogen in blood culture is critical in supporting an effective antimicrobial stewardship program and patient management. By shortening the incubation time of subculture on agar plates for routine identification, bacteria can be identified earlier with direct detection of pathogens by using MALDI-TOF MS.[1-3]

We report a case of diabetic foot ulcer and septic shock due to anaerobic infection, identified by MALDI-TOF MS from a positive blood culture sample.

CASE PRESENTATION

A 52-year-old male patient presented to the general surgery department with complaints of an ulcer over the left foot for the past 45 days, which was insidious in onset and associated with pain and pus discharge from the ulcer site. He had a fever, which was associated with chills. The patient had similar complaints in the

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past, for which amputation has been done for the right great and second toes. He was a known case of type II diabetes mellitus. He was diagnosed with a diabetic foot ulcer, uncontrolled diabetes, and septic shock. His vitals included a pulse of 108/min, blood pressure-130/70mmHg. On examination, an ulcer was found in the plantar aspect of the left foot, measuring 13X7 cm^2 with slough. No erythema or tenderness was present, and there was no local rise in temperature.

Debridement was done, and a blood culture was sent due to fever spikes. Debrided tissue sent for culture yielded no growth. He was started on injection piperacillin-tazobactam 2.25 gm IV Q8H over 3 hours infusion (a time-dependent antibiotic) and injection clindamycin 600 mg Q12H after sending blood for culture (one aerobic and one anaerobic). The anaerobic blood culture flagged positive after two days of incubation, and sub-cultures were performed. Direct Gram stain showed Gram-negative bacilli, and the sample was processed as per standard protocols by subculturing on MacConkey and sheep blood agar and for direct antimicrobial susceptibility test for Gram-negative bacilli as per CLSI protocol.[4] There was no growth by the second day of aerobic incubation. Suspecting that the microorganism might be an anaerobe, we attempted anaerobic culture methods to detect the pathogen with the available resources, which was unsuccessful due to quality control failure. We decided to detect the microorganism by MALDI-TOF (Vitek MS Prime®, BioMerieux, France) directly from the positive blood culture bottle. A three ml blood sample (BactAlert Bottle sample) was taken in a sterile test tube and centrifuged at 3500 rpm for eight mins. The supernatant was discarded, and the pellet was suspended in one ml of sterile normal saline and centrifuged at 8000 rpm for ten minutes. The supernatant was discarded, and the pellet was resuspended in normal saline; the resulting sediment was smeared on the MALDI slide, and one µl of matrix (α-Cyano-4-hydroxycinnamic acid) was added and subjected to MALDI-TOF. The organism was detected to be *Bacteroides fragilis*. Since the patient had already started on piperacillin-tazobactam empirically, he responded well to the treatment.

To replicate the protocol, two ml of 0.5 McFarland turbidity standard of an ATCC 25922 *Escherichia coli* strain was inoculated in a BactAlert bottle, and 5 ml of blood was added from a healthy volunteer. The bottle was incubated, and when it flashed positive, the same protocol was used as described above, and MALDI-TOF could identify the strain as *E. coli*.

DISCUSSION

MALDI-TOF MS has been routinely used to identify bacteria and fungi from culture. MALDI-TOF instruments use an ionizing laser to vaporize bacteria and yeasts' structural elements (primarily ribosomal proteins) to analyze each particle's weight and relative abundance to generate a spectrum. The spectra generated are compared to a computer database of reference, and identification is obtained by matching the most similar spectrum in the database to the unknown organism. More recently, protocols for directly identifying pathogens from positive blood culture broths have been developed.[5-9] Although blood culture broths are usually mono-bacterial (or mono-fungal) cultures, the presence of proteins from red cells, white blood cells, and serum interferes with the analysis by adding spectral peaks not found in the organism database. Furthermore, interfering substances such as charcoal (when present) and low organism numbers (as might be encountered with slow-growing or contaminating bacteria) present additional challenges in using and interpreting MALDI-TOF spectra to identify pathogens directly from positive blood cultures.[10] Not surprisingly, turnaround time was greatly improved by using MALDI-TOF to identify organisms directly from positive blood cultures. Vlek et al. recently demonstrated that the direct performance of MALDI-TOF MS on positive blood culture broths significantly reduced time to organism identification by 28.8 hours compared with identification by conventional methods.[11] Rapid identification at the genus level would be instrumental in guiding clinical management, such as the rapid differentiation of Gram-negative bacteria to the genus level (for example, *Acinetobacter* and *Klebsiella*). Significantly, fast and precise results were associated with an 11.3% increase in the proportion of patients receiving appropriate antibiotic treatment within one day of culture positivity.[11]

CONCLUSION

Faster identification using MALDI-TOF helps the clinician assess the significance of a blood culture isolate earlier. It may allow for the appropriate choice of an antimicrobial agent, even without susceptibility testing, and help narrow down the potential source of infection, providing a focus for further investigation in a timely manner than conventional techniques alone. Furthermore, it helps identify anaerobic organisms from the positive blood culture bottle in a laboratory with minimal facilities to perform anaerobic culture

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methods. By this, we conclude that the routine incorporation of the technique of direct MALDI-TOF from blood culture helps in faster identification, appropriate treatment, reduced turnaround time, and antimicrobial stewardship.

INFORMED CONSENT

Written informed consent was obtained from the patient. Confidentiality of the patient was maintained in the article.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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None

AUTHOR'S CONTRIBUTION

HP: Conceptualization; Data curation; Analysis; Writing the draft

DD: Investigation; Methodology; Resources

SR: Conceptualization; Supervision; Validation; Review & Editing

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