CASE DESCRIPTION

An 82-year-old man with dementia presented to his primary care physician with a chief complaint of left eye swelling for 2 days. He was prescribed ciprofloxacin ophthalmic drops. A few days later, his symptoms had not improved, and he presented to the emergency department at an outside hospital with ongoing eye pain. He had loss of vision in his left eye. He noted that he may have fallen approximately 2 weeks earlier but did not seek medical care. Antimicrobial therapy with intravenous ceftriaxone and vancomycin was initiated. MRI demonstrated detachment of the left retina, significant inflammation of the circumference of the eye, and an irregular globe contour that was of concern for rupture. On the basis of these findings, he was transferred to our hospital for further management; an emergent ophthalmology consultation revealed fulminant endophthalmitis. The patient’s antimicrobial therapy was changed to intravenous cefazolin.

An evisceration of his left eye was performed the following day without complication. An ocular swab was sent to the microbiology laboratory for aerobic culture and Gram stain, and the intraocular contents were sent to pathology. Pathology confirmed acute panophthalmitis, and bacilli were documented in the sample with Gomori methenamine silver stain. Gram stain of material from the swab showed abundant polymorphonuclear leukocytes but no organisms. The next day, rare \( \text{β}- \) hemolytic colonies were noted on 5% sheep’s blood agar (Remel) and rare gray colonies were growing on chocolate agar plate (Remel). The organism was determined to be a catalase-positive, spore-forming, gram-positive \( \text{Bacillus} \).

The organism was identified as \( \text{Bacillus anthracis} \) by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics) with a confidence score value that would be acceptable for a species-level identification. In accordance with laboratory protocols for identification of select agents (potential biothreat/bioterrorism agents), the case was brought to the attention of the medical director. The isolate was confirmed to be motile. Because the organism was \( \text{β} \)-hemolytic and motile, \( \text{B. anthracis} \) was rapidly ruled out, and the organism was presumed to be a member of the \( \text{B. cereus} \) group on the basis of morphologic and biochemical findings. Susceptibility testing was performed with a gradient diffusion method (Etest, bioMérieux), and the isolate was found to be susceptible to ciprofloxacin, clindamycin, and vancomycin and resistant to trimethoprim-sulfamethoxazole. The patient’s antimicrobial therapy was streamlined to oral moxifloxacin and topical erythromycin ointment; he was discharged 6 days after his surgery to a skilled nursing facility and has continued to do well.

DISCUSSION

Posttraumatic endophthalmitis makes up 25%–30% of all infectious endophthalmitis cases (1). Infection is derived either from inoculation of the endogenous flora from the periorbital region into the open wound or direct inoculation of environmental organisms into the eye during trauma (2). Most cases are caused by gram-positive bacteria, with approximately 20% of cases attributed to \( \text{Bacillus} \) spp. (1). These \( \text{Bacillus} \) infections can be very severe (a total loss of vision occurs in \( \approx70\% \) of cases (3)), and enucleation is the outcome in >50% of \( \text{Bacillus} \)-associated cases (1, 3). The most common species of \( \text{Bacillus} \) associated with posttraumatic endophthalmitis

QUESTIONS TO CONSIDER

1. What is the principle of MALDI-TOF MS for microorganism identification?
2. What are the analytical performance characteristics of MALDI-TOF MS for microorganism identification? Which organisms can be challenging to accurately identify with MALDI-TOF MS, and why? How might rapid identification of microorganisms in culture contribute to optimization of antimicrobial therapy?
3. What is the phylogenetic relationship between \( \text{Bacillus anthracis} \) and \( \text{B. cereus} \)?
4. What is the role of a “sentinel laboratory” within the Laboratory Response Network?
5. What is the clinical significance of recovering \( \text{Bacillus} \) spp. from an ocular sample?
are *B. cereus*, *B. subtilis*, and *B. licheniformis*. The symptoms of a *Bacillus* infection can begin within 18–24 h and may include severe pain, periorbital edema, proptosis, and corneal opacification (1).

*B. cereus* is frequently associated with severe and rapid disease onset owing to its ability to rapidly grow in the vitreous humor and produce specific virulence factors, including proteases and hemolysins. Additionally, *Bacillus* spp. have been shown to rapidly migrate to all tissues within the eye, allowing the organism to move to protected regions of the eye, thus evading antimicrobial treatment (3). For the best outcome, posttraumatic infectious endophthalmitis treatment should be initiated within 24 h, and both wound closure and systemic antimicrobial treatment are recommended (4).

The *B. cereus* group includes several closely related members including *B. anthracis* and *B. thuringiensis*; these genetically similar gram-positive, spore-forming bacilli possess 16S rRNA gene sequence homology of ≥99%. The species are differentiated on the basis of their plasmid DNA, which accounts for the species-specific disease phenotypes. For example, *B. anthracis* contains 2 plasmids, pX01 and pX02, that encode specific virulence genes, whereas *B. thuringiensis* contains the pBT plasmid, which encodes genes that give the bacteria its insecticidal properties (5). *B. anthracis*, the causative agent of anthrax, is classified as a select agent because of its ability to be aerosolized and its potential to be used in a bioterrorism event. Special handling is required to avoid infection of laboratory workers if the presence of this organism is suspected.

MALDI-TOF MS is changing the way clinical microbiology laboratories identify microorganisms. Traditional identification methods rely on biochemical activity or metabolic profiling of microorganisms; these techniques can have prolonged turnaround time, and their accuracy is variable. In contrast, MALDI-TOF MS is based on creating a protein fingerprint from a single colony of a microbe and then comparing this protein fingerprint to a reference library to generate an identification. MALDI-TOF MS can produce rapid and accurate identifications of gram-positive and -negative bacteria, anaerobes, mycobacteria, yeast, and filamentous fungi. For most bacteria and yeast, only a small amount of growth is required, and the colony is directly spotted onto a MALDI-TOF MS target plate and overlaid with a matrix. For other organisms, such as mycobacteria and filamentous fungi, additional sample preparation steps may be required before analysis (for both biosafety reasons and analytical purposes), such as chemical treatment or mechanical disruption. In general, the spectrum that is generated will include peptides of 2–20 kDa, with predominantly ribosomal proteins being detected. These proteins are excellent targets for microbial identification, since ribosomal proteins are usually highly conserved within a species but vary between species (6, 7).

**POINTS TO REMEMBER**

- Microorganism identification on the basis of proteomic profiling with MALDI-TOF MS is rapid and accurate; this identification method is revolutionizing clinical microbiology laboratories.
- MALDI-TOF MS may not be able to reliably differentiate microbial species that have a high degree of similarity at the 16S rRNA gene sequence level.
- It is critical that clinical microbiology laboratories retain competence in classic microbiologic methods, including understanding Gram stain and colony morphology, for both safety of laboratory workers and ongoing quality assurance.
- The Laboratory Response Network for Biological Threat Preparedness and Response establishes rules for sentinel laboratories, which includes many clinical microbiology laboratories, for the handling and identification of bioterrorism/select agents. The sentinel laboratories’ role is to rule out and refer these agents, which can include the examination of growth and conventional biochemical tests. It is not acceptable for sentinel laboratories to use automated identification methods, such as MALDI-TOF MS, to identify select agents.
- Recovery of *Bacillus* spp. from an ocular sample should be regarded as a medical emergency, and the results should be communicated to the provider as soon as they are available.

At this time, there are 2 commercially available MALDI-TOF MS platforms for microbial identification, the Bruker MALDI Biotyper and the bioMérieux VITEK MS. Each platform has its own reference database and computational algorithms for determining the quality of the identification. The accuracy of the identification is based largely on accuracy and breadth of the database being used, as well as the software algorithms. Not all organisms are represented in the database, and some organisms have a larger number of spectra present (6, 7). Depending on the library acquired by a laboratory, some groups of organisms may be missing from the reference collection. For example, for the Bruker Biotyper, select agents are not part of the conventional library but may be acquired as a separate “security-relevant library” for bioterrorism/select agents.

Despite the availability of the security-relevant library and rapid identification provided through the use of MALDI-TOF MS, it is necessary that clinical microbiology laboratories continue to follow the regulations established by the Laboratory Response Network for Biological Threat Preparedness and Response. Most hospi-
tal microbiology laboratories are classified as sentinel laboratories; the role of these facilities is to “rule out and refer” any potential bioterrorism/select agents to designated reference laboratories (8).

The B. cereus group of organisms illustrates a challenge with MALDI-TOF MS for microorganism identification. Because the 16S rRNA gene sequence is nearly identical for this group of species, these organisms can be difficult to resolve with MALDI-TOF MS. It is necessary to have procedures in place to account for organisms that may be a challenge for MALDI-TOF MS analysis, especially if the organism is of specific clinical or epidemiological significance (such as a select agent). For the B. cereus group, this can include examining the hemolytic pattern on sheep’s blood agar and the motility, with B. anthracis being nonhemolytic and nonmotile. Some other examples of challenging organisms include Escherichia coli, Shigella spp., Streptococcus pneumoniae, the Streptococcus mitis group, Neisseria meningitidis, and other saprophytic Neisseria spp. (6, 9).

This case highlights both the clinical benefit (rapid identification) and potential limitations of the use of MALDI-TOF MS in the clinical microbiology laboratory. The time interval from plating of the sample and reporting the sample Gram stain to the time of organism identification was <17 h. This rapid identification facilitates optimization of antimicrobial therapy and may reduce the need for additional diagnostic procedures. Unfortunately, MALDI-TOF MS cannot identify all microorganisms with 100% accuracy. An understanding of the advantages and disadvantages of MALDI-TOF MS and the establishment of adjunct testing protocols is key to ensure both rapid and accurate microbial identification from clinical samples.

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Commentary
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This case is a wonderful example of the benefits and limitations of MALDI-TOF MS. MALDI-TOF MS has been cleared by the US Food and Drug Administration for the identification of bacteria and some yeast. The methodology is based on creating a protein spectrum, which is then compared to a library of known spectra. MALDI-TOF MS has been implemented in many clinical microbiology laboratories because of its accuracy, speed, and relatively low cost per identification, but well-trained clinical microbiologists are still needed to review the results to ensure they fit with the entire clinical picture. In this case, a classic presentation of Bacillus cereus could have been misidentified as Bacillus anthracis if the MALDI-TOF MS result had been accepted at face value by a less informed medical technologist. Because B. anthracis has the potential to be used as an agent of bioterrorism and is therefore labeled as a select agent, the laboratory would have been required to contact the local or state department of health to report and refer the isolate for confirmatory testing.

MALDI-TOF MS produces accurate results for most organisms. Some known organisms cannot reliably be distinguished, however, because of the similarity of the 16s rRNA proteins they share. In addition to misidentifying B. cereus as B. anthracis, MALDI-TOF MS can misidentify Shigella spp. as Escherichia coli and Streptococ-
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cus mitis as Streptococcus pneumoniae. The limitations of any system need to be fully understood before implementing testing. In addition, a strong microbiology knowledge base is still required to correlate the result from the instrument with what is growing on the plates. In this case, the technologist realized the B. anthracis result could not be correct because the organism that was growing was β-hemolytic and motile (B. anthracis is non-hemolytic and nonmotile).

**Commentary**

Nancy S. Miller*

MALDI-TOF-MS has been embraced as a technological game-changer in clinical microbiology. But, while chanting “MALDI is the new micro,” we must heed the caveats of proteomic biotyping. Equivocal, suspect, or incorrect microbial identifications may occur due to technical, database, or method-based limitations. The current case rightfully highlights Bacillus cereus as an ophthalmic pathogen. Alas, only a swab could be sent for gram stain; tissue or aspirate might have provided a better chance to visualize bacilli before histologic sectioning.

This report also includes lessons for MALDI users when a select agent is presumed to be identified: a Bacillus anthracis false alarm and its implications. Other scenarios then come to mind. The colonial morphology of Bacillus mycoides can mimic B. anthracis; no one should be reassured by a non-anthracis MALDI result. Without a security-relevant database, MALDI could misidentify B. anthracis by defaulting to a non-anthracis Bacillus. What is the risk then for clinical mismanagement, unprotected exposures, or instrument contamination? Basic competencies and select agent protocol compliance trump blind obedience to any one result. How ironic it is that a 2014 Centers for Disease Control and Prevention laboratory gaffe (unintentional release of potentially viable anthrax extract) occurred in association with an organism prepared for MALDI. Evaluation of culture growth provided the alarm!

Some individuals do believe MALDI is feasible for accurate, timely identification of select agents. A small study by Cunningham and Patel in 2013 (1) supported inclusion of Brucella species, Francisella tularensis, and Burkholderia pseudomallei in standard clinical laboratory MALDI libraries. In 2015, Lasch et al. (2) showed that robust databases are critical; Bacillus species remain problematic. Eventually, routine MALDI use may include direct specimen testing. Careful oversight and clinical correlation will be critical to believing a select agent result by direct MALDI before seeing the corresponding culture.

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