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Direct application of MALDI-TOF mass spectrometry to cerebrospinal fluid for rapid pathogen identification in a patient with bacterial meningitis

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ABSTRACT

Background: Bacterial meningitis is a neurological emergency. Early diagnosis and rapid initiation of antimicrobial therapy are vital.
Methods: Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is increasingly used as a rapid and accurate microbial diagnostic method for species identification of pathogens. Although this technology requires a growth step to obtain bacterial colonies for the acquisition of substantial spectra in most cases, it can also be used to analyze clinical specimens such as urine and cerebrospinal fluid for direct bacterial identification. There are very few reports describing the use of MALDI-TOF MS for the direct detection of microorganisms causing bacterial meningitis.
Results: We describe a case of bacterial meningitis caused by *Klebsiella pneumoniae* in which MALDI-TOF MS provided a rapid bacteriological diagnosis, thus enabling early and appropriate treatment.
Conclusions: Identification of microbes based on MALDI-TOF MS is now an important technology in clinical microbiology laboratories that are required to provide a rapid diagnosis of bacterial meningitis.
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1. Introduction

Bacterial meningitis is one of the most serious and life-threatening manifestations of bacterial infection. Early diagnosis and rapid initiation of antimicrobial therapy are vital [1]. Although the incidence of bacterial meningitis in children and young adults has been decreasing over the last few decades, it is not uncommon in older patients [2].

Conventionally, pathogens responsible for bacterial meningitis have been detected by Gram staining of cerebrospinal fluid (CSF) and by immunological methods for a limited number of species, followed by biochemical tests that require long incubation periods. Molecular methods such as ribosomal RNA sequencing are powerful but are still expensive and require sophisticated expertise [3]. Recently, the development of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has revolutionized the identification of bacteria, yeast, and molds in clinical microbiology by introducing simple, reliable, rapid, and low-cost techniques [4–6].

Although MALDI-TOF MS identification requires a growth step to obtain bacterial colonies for the acquisition of substantial spectra in most cases, this technology can also be used to analyze clinical specimens such as urine for direct bacterial identification [7].

2. Case report

A 43-y-old female experienced dysarthria, dysphagia and gait disturbance and MRI revealed large tumor behind the medulla oblongata in the posterior fossa. Complete resection could be achieved, and pathological diagnosis is ependymoma. Fourteen days after the operation, she had fever and a severe headache and was suspected to have developed meningitis. Examination of CSF revealed an elevated cell count

Abbreviations: MALDI-TOF MS, Matrix-assisted laser desorption/ionization time of flight mass spectrometry; α-CHCA, alpha-cyano-4-hydroxycinnamic acid.

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(2853/μl, neutrophils 98.8%), an elevated protein concentration (1169 mg/dl), and a markedly decreased glucose level (<1 mg/dl). Bacterial meningitis was diagnosed, and the patient was immediately treated with initial antibiotics (ceftriaxone and vancomycin). After the causative bacterium was identified as *Klebsiella pneumoniae*, vancomycin was discontinued.

3. Methods

3.1. Sample preparation for MALDI-TOF MS

CSF (1000 μl) was centrifuged for 5 min at 13,500 ×*g*. Blood cell components, mostly leukocytes, were removed by aspiration, and the resulting pellets were used for MALDI-TOF MS analysis.

The pellets were washed with 300 μl of HPLC deionized water and 900 μl of absolute ethanol and were then centrifuged again for 5 min at 13,500 ×*g*. The dried pellets were treated with 50 μl of 70% formic acid and 50 μl of 100% acetonitrile for 1 min to extract bacterial cell contents. The extract was centrifuged for 5 min at 13,500 ×*g*, and aliquots of the supernatant were subjected to bacterial identification by MALDI-TOF MS.

3.2. MALDI-TOF MS analysis

One microliter of the supernatant was deposited directly on an MTP BigAnchorChip 384 TF target plate (Bruker Daltonics). The preparation was overlaid with 1 ml of α-CHCA matrix solution, which was a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile—2.5% trifluoroacetic acid. It was then air dried at room temperature to enable co-crystallization with the experimental samples.

To identify the isolates, MALDI-TOF MS spectra were generated using a Bruker Microflex LT instrument according to the manufacturer's instructions and using MALDI Biotyper 3.1 software and the reference database 3.3.1.0. The peak lists generated were used to find matches against the reference library by direct use of the integrated pattern-matching algorithm of the software. The result of the pattern-matching process was expressed as described by the manufacturer, with scores ranging from 0 to 3; scores below 1.7 were regarded as unreliable identification; a score of 1.7–2.0 was regarded as genus level identification; and a score of >2.0 was regarded as species level identification.

3.3. Conventional identification procedures

The dried bacterial pellets were subjected to Gram staining. An aliquot of CSF was placed on an appropriate agar plate (BY Chocolate Agar and Trypticase Soy Agar II with 5% sheep blood; both from Nippon Becton Dickinson). After Gram staining and determination of catalase and oxidase activities, the isolates were identified by phenotypic tests using the MicroScan WalkAway system (Siemens Healthcare Diagnostics).

4. Results

Gram staining of CSF showed numerous gram-negative rods (Fig. 1), but they could not be identified definitely from their appearance. Spectral fingerprints obtained from the CSF pellets after removing white blood cells are shown in Fig. 2. The MALDI-TOF MS spectra were equivalent to those of *K. pneumoniae* with a score of 2.09, indicating that the identification was at the species level. After a growth step to obtain bacterial colonies, the pathogen was identified as *K. pneumoniae* by MicroScan WalkAway system using MicroScan NegCombo 3.11C (Siemens) with a 99.77% probability. Conventional identification confirmed the results of MALDI-TOF MS analysis.

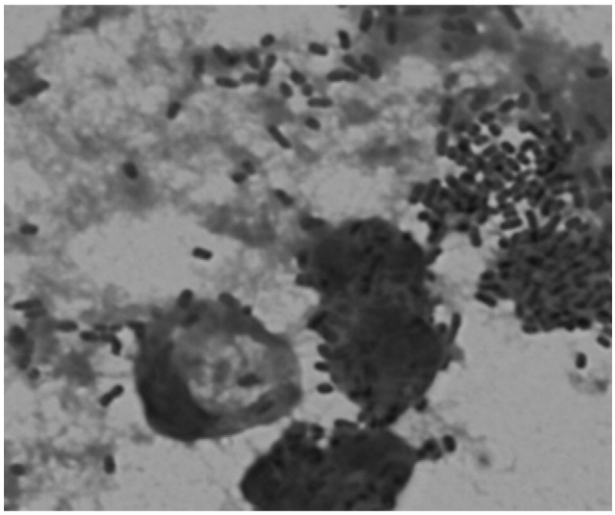

Fig. 1. Gram staining of a cerebrospinal fluid sample identified numerous gram-negative rods.

5. Discussion

The earliest attempt to use mass spectrometry to identify bacteria was made by Anhalt et al. [8], before MALDI was described. They noticed that unique mass spectra of pyrolysis products of phospholipids and ubiquinones rather than bacterial proteins. The discovery of soft ionization techniques such as MALDI and electrospray ionization made it possible to analyze large biomolecules by MS. In 1996, it was reported that MALDI-TOF spectral fingerprints could be obtained from whole bacterial cells without pretreatment prior to MS analysis [4]. This approach was then used by increasing numbers of groups to identify bacteria at the genus and species levels, as reviewed elsewhere [5,6].

The most common use of MALDI-TOF MS in clinical bacteriology laboratories is for the identification of bacteria grown on solid media. Investigators from around the world have reported that percentages of genera correctly identified were 97%–99% and those of species 85%–97% when testing routinely isolated bacteria and yeast using the Bruker Biotyper MALDI-TOF MS system [9].

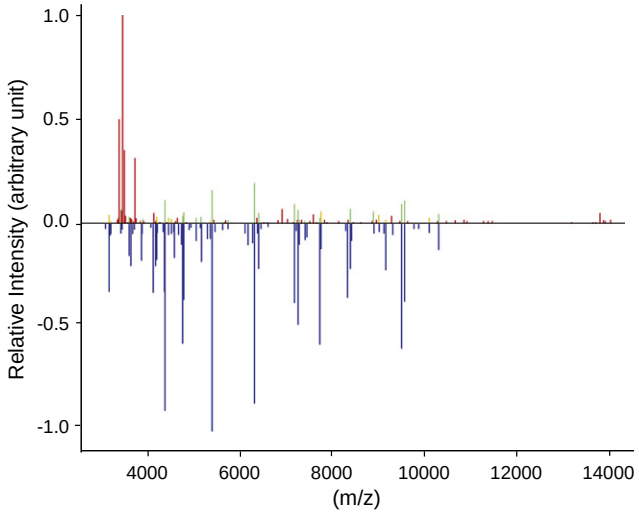

Fig. 2. MALDI-TOF MS spectrum of a cerebrospinal fluid sample from a patient with bacterial meningitis. The sample spectrum presented in the upper column, and the matched database spectrum (*Klebsiella pneumoniae*) in the lower. Green bars in the upper column indicated the matched peaks with the database, red ones mismatched peaks, and yellow ones intermediate peaks.

This technology might be directly applied to clinical samples such as urine and CSF, bypassing the need for culture by detecting the presence of pathogens in clinical specimens. For successful microbe identification with MALDI-TOF MS, approximately 10^4–10^6 CFU are needed on the target plate. These levels can be reached in patients with urinary tract infection, and direct application of MALDI-TOF MS for the identification of urinary pathogens has been reported [7].

Similar to urine, CSF is a promising target for microbe identification with MALDI-TOF MS. At present, the first step in the detection of bacterial pathogens responsible for meningitis is Gram staining of CSF, looking for the presence of bacteria. Patients with positive smears are treated with broad-spectrum antibiotics on the basis of the Gram staining results. It used to take an additional day to obtain a definitive identification on the basis of the culture result, but with the use of MALDI-TOF MS, it may be possible to initiate more specific antibiotic treatment shortly after receiving clinical specimens. There are very few reports describing the use of MALDI-TOF MS for the direct detection of microorganisms causing bacterial meningitis. A case of pneumococcal meningitis in which MALDI-TOF was successfully identified as the pathogen was reported by Hartmeyer et al. [10].

Thus, MALDI-TOF MS is a promising tool for the rapid identification of pathogens in patients with monomicrobial bacterial meningitis, as described in this report. It should be noted, however, that in practice, a low bacterial load and a limited volume of sample available may limit the use of this approach. In the present case, the pathogen was a common bacterium with substantial spectral data available in the commercial database used. This technology, however, is relatively new and databases are still being optimized, which may result in problematic identification of relatively rare bacterial species. Furthermore, this technique is not yet sufficiently powerful for bacterial identification in cases of polymicrobial infection, and its use for the detection of resistance remains to be developed.

6. Conclusions

Direct identification of bacteria in CSF by MALDI-TOF MS can provide a rapid bacteriological diagnosis, which can enable early and appropriate treatment. Identification of microbes based on MALDI-TOF MS is now an important technology in clinical microbiology laboratories that are required to provide a rapid diagnosis of bacterial meningitis.

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