New Microbiologica, 41, 1, 77-79, 2018, ISN 1121-7138

CASE REPORT

Fast and reliable diagnosis of XDR *Acinetobacter baumannii* meningitis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Grazia Brunetti[1], Giancarlo Ceccarelli[1], Alessandra Giordano[1], Anna Sara Navazio[2], Pietro Vittozzi[1], Mario Venditti[1], Giammarco Raponi[1]

[1]*Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy;*
[2]*Department of Molecular Medicine Sapienza University of Rome, Italy*

SUMMARY

Bacterial meningitis is a medical emergency needing quick and timely diagnosis. Even though meningitis caused by *Acinetobacter baumannii* is relatively rare, it is associated with high mortality rates especially in neurosurgery patients and represents a serious therapeutic problem due to the limited penetration of effective antibiotics into the cerebrospinal fluid. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) has been effectively used as a rapid method for microbial identification. In this case report we identified *A. baumanni* by MALDI-TOF technique directly from the CSF drawn from the external ventricular drainage of a patient with severe confusional state and signs of meningism. Simultaneously the antibiotic susceptibility test was performed by automated method from the pellet of the broth-enriched sample. The MALDI-TOF technique allowed microbial identification in less than 30 minutes, and the susceptibility test result was available in eight hours, thus allowing a fast diagnosis ready for prompt and targeted antimicrobial therapy.

Received July 27, 2017 *Accepted* September 25, 2017

INTRODUCTION

Bacterial meningitis is a medical emergency associated with a 15% death rate that requires prompt diagnosis and treatment (Thigpen *et al.,* 2011). Meningitis caused by *Acinetobacter baumannii* is relatively rare, but high mortality rates (70%) were reported especially in neurosurgery patients (Bayramoglu *et al.,* 2012). The treatment of *A. baumannii* meningitis is a serious therapeutic problem owing to the multiple antibiotic resistance shown by this bacterial species and the limited penetration of antibiotics into the cerebrospinal fluid (CSF) (Kim *et al.,* 2009; Rodrìguez Guardado et al., 2008). Nowadays the Gram staining of CSF and the immunological agglutination method for a selected number of species still represent cornerstones in the microbiological diagnosis of meningitis and bacterial identification gained from culture methods delays the diagnosis. Recently, molecular methods based on the identification of bacterial genome sequences have been introduced and might represent powerful tools in shortening the time to diagnosis. However, these methods suffer from high costs and expertise and, therefore, are not always available in clinical laboratories (Woo *et al.,* 2008). Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) has been effectively used as a rapid method for identifying microbial species (Croxatto et al., 2012; Patel, 2015), with remarkable differences in time and cost per isolate compared to biochemical and molecular identification (Croxatto *et al.,* 2012; Patel, 2015; Cherkaoui *et al.,* 2010; Cloud *et al.,* 2010). MALDI-TOF directly applied to clinical samples such as blood, urine and CSF, could bypass the need for culture, directly detecting pathogens in these clinical specimens.

This case report describes the rapid identification of *A. baumanni* by MALDI-TOF technique applied directly to the clinical sample and the execution of the antibiotic susceptibility test directly from the CSF drawn from the external ventricular drainage (EVD) of a patient with signs of meningism.

CASE REPORT

A 57-year-old immune competent female was admitted to the Neurosurgical Intensive Care Unit for headache, vomiting, balance disorder and loss of consciousness. MR scan revealed a mass with moderate contrast enhancement of the fourth ventricle causing obstructive hydrocephalus. For this reason, the patient underwent surgical removal of the lesion with placement of external ventricular drainage (EVD). The histological diagnosis was ependymoma. On day 16 after neurosurgical treatment the patient presented fever (up to 39,3°C) associated with severe confusional state and meningism. CSF analysis, performed from EVD, revealed glucose concentration of 3.94 mmol/L, protein level of 33 mg/dL, and leukocytes cell count of 36 cells/μL

Key words:
Acinetobacter baumannii, Meningitis, MALDI-TOF, Diagnosis, CSF.

Corresponding author:
Giammarco Raponi MD, PhD
E-mail: giammarco.raponi@uniroma1.it

(30% polymorphonucleocytes); *Gram staining* of the *CSF* demonstrated *Gram-negative* coccobacilli. MALDI-TOF (Bruker Daltonik GmbH, Bremen, Germany) identified *A. baumannii with* scores values ≥2.2, and susceptibility profiles obtained from the pellet displayed an extensively-drug-resistant (XDR) strain with colistin susceptibility only. Intravenous colistin (COL loading dose 9 MUfor 1 dose, then 4,5 MU/q12 h) plus meropenem (MER 2 g/q8h) was administered and EVD was changed. EVD-associated bacterial meningitis was confirmed on the basis of XDR *A. baumannii* isolation from the CSF culture, from the removed EVD and from one concomitant blood culture. Concurrently *A. baumannii* was also isolated from the broncoalveolar lavage fluid and from the rectal swab. According to the antimicrobial susceptibility profile of the bacterial isolate, intrathecal COL (125,000 IU/day) was added to intravenous therapy. The CSF Gram stain and culture became negative on day 4 of antibiotic treatment, whereas the CSF chemistry and CSF leukocytes cell count normalized on day 8. The clinical course and the main laboratory findings, expressed in function of time, are shown in *Figure 1*. With the eradication of *A. baumannii* from CSF, a clinical improvement of patient's condition in terms of Glasgow Coma Score was observed.

For the microbiological diagnosis, the sample of CSF (500 µl) was added to 100 µl of 13% sodium dodecyl sulphate (Sigma-Aldrich Saint Louis, Missouri, USA), vortexed for 10 seconds and centrifuged at 13,000×g for 2 min. The resulting pellet was resuspended with 1 mL of deionized water and centrifuged at 13,000 ×g. for 2 min. After removal of the supernatant the pellet was supplemented with 300 µl deionized water plus 900 µl absolute ethanol and centrifuged again at 13,000 ×g for 4 min. The dried pellets were treated with 50 µl 70% formic acid and 50 µl 100% acetonitrile to extract the bacterial cell content. The extract was then centrifuged at 13,000×g for 4 min. One microliter of the supernatant was directly deposited on an MTP BigAnchorChip 384 TF target plate (Bruker Daltonik). The preparation was overlaid with 1 ml of α-CHCA matrix solution (Bruker Daltonik), which was a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid and then air dried at room temperature. To identify the isolates, MALDI-TOF spectra were generated using a Microflex LT (Bruker Daltonik) instrument and interpreted using the MALDI Biotyper 3.1 software. The results of the pattern matching process were expressed as described by the manufacturer, accepting scores values ≥2.0. In parallel, 500 µl of CSF was incubated with 1 ml of brain-heart-infusion broth at 37° for 30 minute. After incubation the supernatant was removed and the pellet was brought to 0.5 Mc Farland in 0.45% NaCl. For the antimicrobial susceptibility test the AST-N202 card by Vitek2® (Biomerieux, Marcy-l'Étoile, France) was used. Susceptibility to colistin was further analysed by microdilution method (Sensititre™ Gram Negative MIC Plate, Thermo Fisher Scientific, USA). To confirm the results obtained with the above procedure, 10 µl of CSF were placed on agar plates to perform conventional culture identification. After incubation for 24 h at 37° the bacterial load was $>10^5$ CFU/mL. The identification was performed on the isolated colonies both by MALDI-TOF technique and biochemically using GN ID card together with the assessment of the antibiotic susceptibility test by AST-N202 card of the Vitek2® system (Biomerieux, Marcy-l'Étoile, France).

DISCUSSION

The frequency of CNS infections and meningitis caused by Gram-negative bacteria has increased from 12 to 27% of cases (Schiaroli *et al.,* 2015). Multidrug-resistant *A. baumannii* has emerged as a cause of CNS infections after neurosurgery, often associated with the use of external CSF catheters (Wang *et al.,* 2005; Kim *et al.,* 2009), and with high mortality rates (Ceylan *et al.,* 2017). It is well known that the rapid identification of microorganisms in any sample contributes to the early treatment and influences patient outcomes leading to decreased mortality (Deresinski *et al.,* 2007). In the case of suspected meningitis, Gram staining of CSF still represents the first step to address the diagnosis, and usually patients with positive smears are treated with broad-spectrum antibiotics. Nowadays microbial culture is still considered the gold

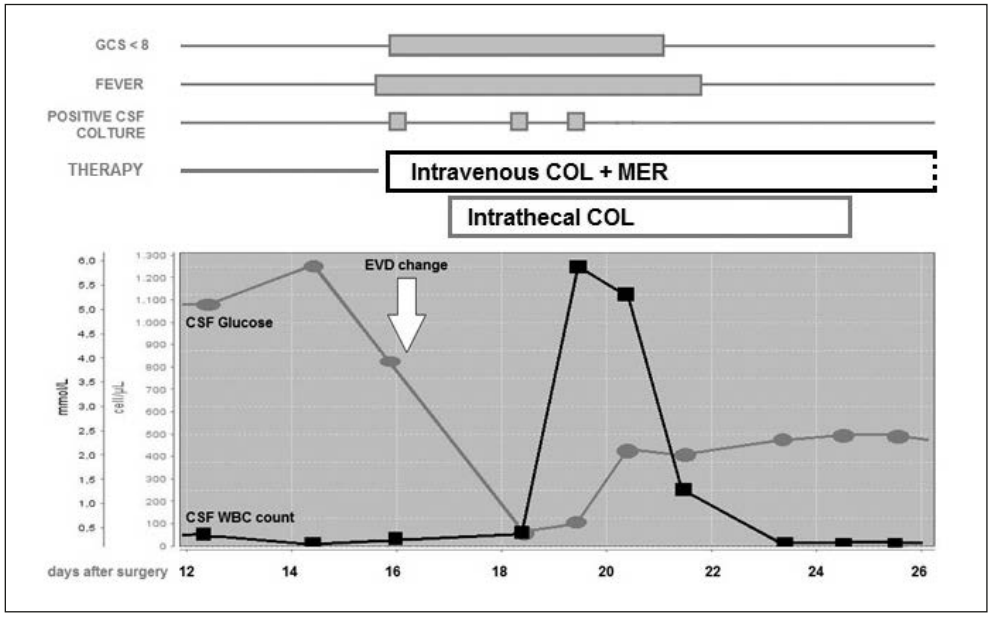

Figure 1 - *Clinical course, microbiological and laboratory monitoring of meningitis caused by A. baumannii. GCS=Glasgow Coma Score, CSF WBC=cerebrospinal fluid white blood cells, CSF=cerebrospinal fluid, EVD =external ventricular drainage.*

standard method for the diagnosis of CSF infection, but it requires an additional day to obtain a definitive identification and obtain the susceptibility profile. The advent of mass spectrometry has revolutionized the world of microbiology, dramatically shortening the turnaround time (TAT). Several studies reported successful microorganism identification directly from blood culture bottles and urine samples (Bhatti *et al.,* 2014; Ferreira *et al.,* 2010), but to our knowledge, few reports have described the use of MALDI-TOF applied directly to CSF (Nyvang *et al.,* 2010; Segawa *et al.,* 2014). In a very recent study (Bishop *et al.* 2017) 44 CSF were directly analysed by MALDI-TOF and a reliable identification was achieved when the infecting microorganisms were Gram negative. In our study the identification of *A. baumannii* was gained in less than 30 minutes by the MALDI-TOF technique, and our procedure allowed the execution of the susceptibility test directly from the CSF pellet, with a TAT for a conclusive diagnosis in less than eight hours. On the other hand, the classical culture method lasted 32 hours for an identical diagnosis. Such a fast diagnosis allowed the CSF catheter to be removed from the patient and to add intrathecal COL to the previous i.v. MER-COL therapy, thus achieving microbial eradication at the fourth day of therapy. The treatment of *A. baumannii* meningitis is a well-known therapeutic problem due to the limited penetration of intravenous COL into the CSF (Markantonis *et al.,* 2009). However, intrathecal or intraventricular COL administration has been reported much more effective than i.v. therapy alone (De Bonis *et al.,* 2016), with no side-effects and with good pharmacokinetics and tolerability (Schiaroli *et al.,* 2015). In our opinion larger studies are needed to assess the clinical implications of MALDI-TOF identification on CSF. As reported in other studies, a possible limitation for successful microbe identification with MALDI-TOF may be the low bacterial load in the single clinical sample and/or the limited volume of sample available.

Note. Informed consent was obtained from the patient for the publication of this case study. Given that interventions and all investigations described in this case report were performed as part of standard health care, no ethical approval was either sought or required.

Funding information. The authors declare that this work received no specific grant from any funding agency.

Conflicts of interest. The authors declare that there is no conflict of interest.

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