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Rapid and cost-effective identification and antimicrobial susceptibility testing in patients with Gram-negative bacteremia directly from blood-culture fluid

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ABSTRACT

Rapid pathogen identification (ID) and antimicrobial susceptibility testing (AST) in bacteremia cases or sepsis could improve patient prognosis. Thus, it is important to provide timely reports, which make it possible for clinicians to set up appropriate antibiotic therapy during the early stages of bloodstream infection (BSI). This study evaluates an in-house microbiological protocol for early ID as well as AST on Gram negative bacteria directly from positive monomicrobial and polymicrobial blood cultures (BCs).

A total of 102 non-duplicated positive BCs from patients with Gram-negative bacteremia were tested. Both IDs and ASTs were performed from bacterial pellets extracted directly from BCs using our protocol, which was applied through the combined use of a MALDI-TOF MS and Vitek2 automated system.

The results of our study showed a 100% agreement in bacterial ID and 98.25% categorical agreement in AST when compared to those obtained by routine conventional methods. We recorded only a 0.76% minor error (mE), 0.76% major error (ME) and a 0.20% very major error (VME). Moreover, the turnaround time (TAT) regarding the final AST report was significantly shortened (ΔTAT = 8–20 h, $p < 0.00001$).

This in-house protocol is rapid, easy to perform and cost effective and could be successfully introduced into any clinical microbiology laboratory. A final same-day report of ID and AST improves patient management, by early and appropriate antimicrobial treatment and could potentially optimize antimicrobial stewardship programs.

1. Introduction

Timely identification of infectious agents and the early introduction of an appropriate antimicrobial therapy are crucial in clinical practice, particularly in severe infections, such as bloodstream infection (BSI) caused by Gram-negative rods that could rapidly progress to sepsis/septic shock. BSIs are characterized by a high morbidity and mortality, mostly when they occur in patients admitted to intensive care units (ICU) (Vincent et al., 2009). In such infections, the early startup of appropriate therapy is essential to guarantee a favorable patient outcome. Instead, an inappropriate antimicrobial therapy by administering broad-spectrum antibiotics could worsen the outcome, induce antimicrobial resistance and spread, and increase hospital length of stay and costs (Tumbarello et al., 2010; Cain et al., 2015). MALDI-TOF MS technology has been shown to be a suitable tool for rapid and accurate pathogen identification (ID) (Bizzini and Greub, 2010). To this aim, this technology has been adopted in various protocols which make it possible to identify microorganisms directly from positive blood-culture (BC) broth. Some of these protocols are commercially available, such as the Sepsityper kit (Bruker Daltonics, Bremen, Germany) and serum separator tubes while others are in-house methods (Martiny et al., 2012; Morgenthaler and Kostrzewa, 2015; Tian et al., 2016; Machen et al., 2014; Schubert et al., 2011).

However, the Sepsityper kit is expensive, whereas in-house methods

Abbreviations: AST, Antimicrobial Susceptibility Test; BC, Blood Culture; BSI, Bloodstream Infection; ESBL, Extended-Spectrum b-Lactamase; ID, Identification; GNNF, Non Fermentative Gram Negative; ICU, Intensive-Care Unit; MALDI-TOF MS, Matrix-assisted laser desorption ionization-time of flight mass spectrometry; MCA, Morphokinetic Cellular Analysis; ME, Major Error; mE, Minor Error; MIC, Minimum Inhibitory Concentration; TAT, Turn Around Time; ΔTAT, Difference in Turn Around Time; VME, Very Major Error

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are cheap but labor-intensive, involving multi-step washing, centrifugation, filtration and enrichment procedures.

In order to accelerate the final report of antimicrobial susceptibility testing (AST) for clinicians, there are many techniques, such as rapid phenotypic ASTs, proteomic methods and molecular ASTs (Waldeisen et al., 2011; Grundt et al., 2012; Hrabák et al., 2012; Rondinelli et al., 2010; Favaro et al., 2014; Sakarikou et al., 2017). All of these have been reported in several studies and also many of them have been used in practical routines. Nevertheless, they show some limitations. Genotypic assays, based on the detection of target drug resistance genes, have the ability to detect resistance but not susceptibility and in cases where there is a drug resistance gene present, they cannot predict its real expression. Moreover, bacteria rapidly develop new resistance patterns that molecular assays could fail to identify. In fact, the diversity of genetic mechanisms may exceed the capabilities of current molecular technology to detect them. Although results can be obtained rapidly, many molecular methods are labor-intensive, expensive, and lack standardization (Waldeisen et al., 2011; Favaro et al., 2014). Unlike molecular assays, proteomic assays provide timely and cost-effective information on gene expression by monitoring the activity of enzymes responsible for drug resistance, thus predicting susceptibility patterns. However, these assays focus particularly on the enzymatic mechanisms of antibiotic resistance and they are yet to be well standardized (Hrabák et al., 2012; Sakarikou et al., 2017). Phenotypic methods will continue to have an advantage when resistance to the same antimicrobial agent may be caused by several different mechanisms. Some rapid phenotypic methods are relatively ineffective because only a few drugs are tested and some others require a 2–4 h pre-enrichment step, usually in brain heart infusion broth, which lengthens working time (Tian et al., 2016; Waldeisen et al., 2011; Grundt et al., 2012; Hrabák et al., 2012; Pulido et al., 2013). Recently, some rapid phenotypic ASTs based on laser light-scattering technology (Sidecar, Alifax) were introduced. Results are available in 3 to 5 h, but they do not return MIC values (Rondinelli et al., 2010). The Accelerate Pheno™ system (Accelerate Diagnostics), which combines pathogen identification by a fully automated fluorescence in-situ hybridization and AST by Morphokinetic Cellular Analysis (MCA), is the first to track phenotypic features such as the size, shape, and division rate of individual live cells growing in micro-colonies while being challenged by antimicrobials. The first one is labor-intensive while the second is really expensive. Therefore, a rapid, simple and inexpensive AST remains an unmet medical need. In this study, we evaluate the performance of an in-house protocol for timely and cost-effective identification as well as rapid antimicrobial susceptibility testing of Gram-negative bacteria, directly from positive blood-culture broth. A total of 102 non-duplicated positive BCs from hospitalized patients with Gram-negative as well as polymicrobial bacteremia were tested. Both identification and antimicrobial susceptibility testing were performed from bacterial pellets extracted directly from BC broth using our in-house protocol, which combined the use of MALDI-TOF MS and the Vitek2 automated system.

2. Materials and methods

2.1. Specimen collection

A total of 102 consecutive non-replicated BCs were processed both by a new in-house protocol and traditional culturing methods. The blood specimens were collected in BD BACTEC™ Plus Aerobic/F and BD BACTEC™ Plus Anaerobic/F vials and incubated in a Bactec FX automated system (Becton Dickinson, NJ, USA). They were then collected from consecutive patients admitted to the Polyclinic “Tor Vergata” Foundation of Rome, Italy. Specimens were delivered and processed in the Microbiology Laboratory at the same hospital. When BCs were flagged as positive by the Bactec FX system, they were removed and promptly processed by preparing Gram-smears and by culturing an aliquot of broth on solid plate media. Only positive BCs for which microscopic observation demonstrated the presence of Gram-negative rods, even when in mixed flora, were included in the study. This study was performed in public health practice as in a routine clinical setting, so no ethical approval was required.

2.2. Sample preparation for identification assays

After the BC was flagged as positive, Gram staining was performed. In the presence of Gram negative rods, MALDI-TOF MS identification was carried out directly from 1 mL of broth from positive BC, and processed using a MALDI Sepsityper kit (Bruker Daltonics, Bremen, Germany). An ethanol-formic acid extraction was then performed, according to the manufacturer's instructions, and analyzed in an autoflex speed mass spectrometer (Bruker Daltonics, Bremen, Germany). In parallel, an additional 1 mL of BC fluid was transferred to a sterile Eppendorf tube and centrifuged at 2000 rpm for 2 min. The supernatant was mixed with 1 mL of ammonium chloride home-made hemo-lysis buffer (0.15 M NH_4Cl, 0.02 M $NaHCO_3$, 0.001 M EDTA) and centrifuged at 13,500 rpm for 3 min. The supernatant was discarded, and the pellet was washed with 1.5 mL of deionized water and again centrifuged. The supernatant was again discarded. For the identification assay, an ethanol-formic acid extraction was performed on the bacterial pellets obtained using the home-made hemo-lysis buffer and processed by using MALDI TOF MS as reported above. Each sample was tested in quadruplicate to minimize random effects and ensure the reproducibility of spectra. All measurements were performed on an autoflex speed mass spectrometer (Bruker Daltonics, Bremen, Germany), within a mass range of 2.000 to 20.000 Da, and the spectra were automatically analyzed using the MALDI Biotyper 3.1 software (Bruker Daltonics Bremen, Germany), a proprietary algorithm for spectral pattern matching which produces a logarithmic score from 0 to 3. The software compares acquired sample spectra to reference spectra in the provided database (Bruker Taxonomy database 3.3.1; Bruker Daltonics, Bremen, Germany) and compiles a list of best matching database records. Identification scores were interpreted according to the manufacturer's recommended criteria: a score of 3.0–2.0 indicated species level identification; a score of 1.70–1.999 indicated identification at genus level, and a score of $\leq$1.70, was interpreted as “no reliable identification”. The obtained spectra are not reported in this section because of space limitations.

2.3. Sample preparation for AST assays

5 mL of positive BC broth were placed in a serum gel-separator tube (BD Vacutainer SSTII Advance, USA) and centrifuged at 3000 rpm for 12 min. The supernatant was carefully discarded, while the layered cells that were deposited on the gel, were gently suspended through vortexing with 1.5 mL of sterile distilled deionized water. The sample was transferred into a new sterile Eppendorf tube and centrifuged at 13.500 rpm for 2 min. The supernatant was discarded, and the pellet was suspended in 0.45% sterile saline solution and adjusted to a turbidity of 0.5–0.55 on the McFarland scale. This suspension was used for the inoculation of AST N-202 cards (bioMérieux) which were processed by using a Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions, in order to determine the antimicrobial susceptibility. A conventional AST was also performed from subcultures of BC-isolate growth on chocolate PolyViteX agar plates (bioMérieux) after overnight incubation at 37 °C. The MIC values, obtained from both assays, were clinically categorized according to EUCAST clinical breakpoint version 7.1, 2015. Comparisons between the rapid-direct and conventional AST methods were expressed as categorical agreement, very major error (false susceptibility), major error (false resistance), or minor error (intermediate versus susceptible or resistant). The obtained AST reports are not reported in this section because of space limitation.