Fighting the Resistance: How Rapid Microbial ID with MALDI MS and Antimicrobial Susceptibility Testing Improves Patient Care

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The global emergence and spread of antimicrobial resistance among human pathogens is a serious public health concern worldwide. Infectious diseases caused by multi-drug resistant (MDR) bacteria are at the forefront of this problem, as the number of potential treatment options is drastically reduced. Fast, reliable antimicrobial susceptibility testing (AST) in microbiology laboratories is therefore critically important, for rapid patient treatment with the most effective drugs possible. Current methods for AST provide accurate phenotypic detection of common antimicrobial resistance mechanisms, but there is a need for novel diagnostic systems that can be quickly updated if new mechanisms emerge, or if breakpoint values of common drugs change or new antibiotics become available.

The integration of matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS), for rapid microbial identification, with innovative test plate design, based on the broth microdilution method (BMD), to determine true minimum inhibitory concentration (MIC) values for accurate AST, is enabling the Department of Microbiology at the Santa Maria Misericordia University Hospital, Udine and the Angelo Hospital, Mestre, Italy, to provide patients with targeted antimicrobial therapy in shorter time frames, therefore improving overall health outcomes. The two sites are specialised centres of laboratory medicine, providing diagnosis, treatment and prevention of infectious diseases.

The emerging threat of antimicrobial resistance

Antimicrobial resistance is a prevalent and growing threat to global public health, and is particularly dangerous to certain patient groups, such as those undergoing organ transplants or chemotherapy. Millions of infections worldwide are caused by MDR bacteria, often leading to prolonged hospital stays for patients and increased costs to the healthcare system. In the US, it is estimated that eight million extra days of hospitalisation, 23,000 deaths and over $20 billion in additional healthcare costs result from bacterial resistance to first-line antibiotics [1].

In a report published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), MDR bacteria has been identified as one of the major threats to public health, due to its ability to greatly narrow effective treatment options [2] (*Figure 1*).

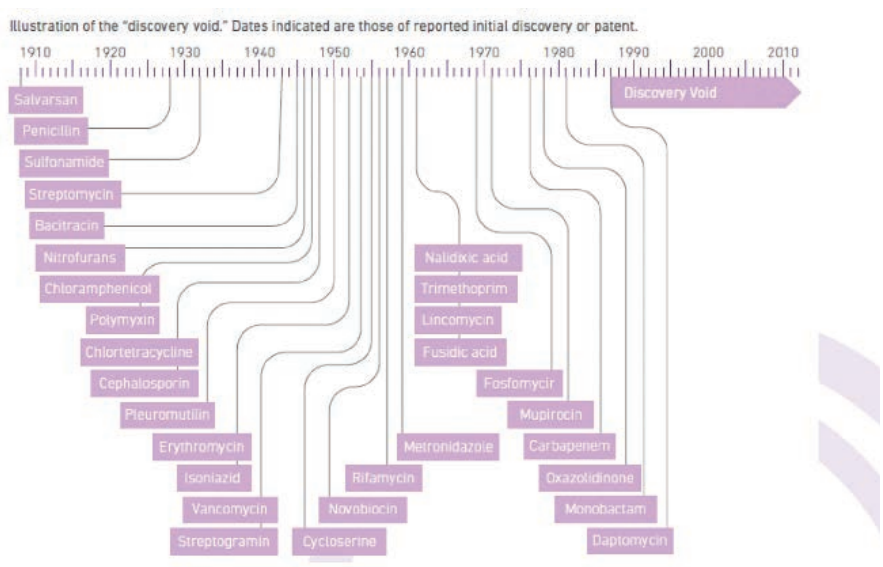

Figure 1: Dates of discovery of distinct classes of antibacterial drugs

Determining true MIC values

The amount of antibiotic required to inhibit growth of microorganisms is defined as minimum inhibitory concentration (MIC). During MIC tests, microorganisms are subjected to a range of antimicrobial concentrations in a solid or liquid medium, for a defined time period under specific temperature conditions. Broth microdilution (BMD) is a standardised and globally-accepted AST reference method for determining the MIC of an antimicrobial agent. Traditional breakpoint AST methods only give information on the interpretive category (whether the microorganism is susceptible, intermediate or resistant to the test drug), whereas multiple concentration AST covers a broad range of drug concentrations and MIC is additionally determined for each agent tested. Multiple drug concentrations also provide more precise information about the resistance of bacteria, and is helpful in antibiotic dosage finding.

For AST by BMD, a suspension with a defined cell count of the microbial test strain is prepared in the test medium, before being inoculated onto the microtitration plates. After plate incubation under the defined conditions, the MIC that represents the lowest concentration of an antimicrobial agent that prevents visible growth is read either manually, or by semi- or fully-automated systems. MIC results are interpreted according to European (i.e. European Committee on Antimicrobial Susceptibility Testing [EUCAST]) or international standards (i.e. Clinical and Laboratory Standards Institute [CLSI]), by comparing the evaluated MIC value with breakpoints listed in the respected documents [3, 4].

However, many microbiology laboratories still conduct AST using the agar diffusion method. Here, a defined cell count of the test bacterium is spread on an agar plate, and paper disks loaded with specific concentrations of an antibiotic agent are placed onto the agar. After a defined incubation period, the diameter of the growth inhibition zone is visually inspected and manually measured. Despite its widespread use, the agar diffusion method is only viable for categorising bacteria as susceptible, intermediate or resistant, but it is not appropriate for determining the MIC.

BDM provides true MIC values and is also recognised by EUCAST as the recommended method for highly effective backup drugs, such as tigecycline and colistin. Colistin is of great importance for the treatment of MDR, carbapenemase-producing Gram-negative bacteria. BMD also provides a good correlation between the in vitro data and the clinical outcome. In this age of MDR bacteria, which have developed resistance to some of the previously most effective antimicrobial treatments, AST based on true MIC values is critical to provide clinicians with detailed information for an optimal therapeutic strategy and, ultimately, a better patient outcome.

Innovative technologies

Dr Claudio Scarparo, Microbiology Manager at the Santa Maria Misericordia University Hospital and Angelo Hospital coordinates the diagnostic activity in the laboratories at the two sites. He has recently introduced a new system, based on the broth microdilution method, for phenotypic MIC determination. The system includes microtitration plates that are customisable (individual defined antibiotic configuration) to suit the requirements of the laboratories in Udine and Mestre. Different microtitration plates can be prepared for different groups of microorganisms (Gram-negative bacteria, Gram-positive bacteria,

fastidious bacteria, streptococci, and urinary pathogens). The laboratory has also observed an increase in standardisation of the inoculation since adopting this new method, as well as process traceability, and automatic reading and interpretation of the antibiogram.

The customised plates used by the laboratory for determining and interpreting true MIC values are integrated with rapid bacterial identification by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics), which enables the identification of over 2,600 microbial species. This leads to more detailed information about the infecting organism and allows clinicians to de-escalate from an empiric therapy with broad-spectrum antibiotics, to a narrower, more targeted therapy. The automation of the entire system increases standardisation and accuracy of results, and decreases the risk of human error (*Figure 2*).

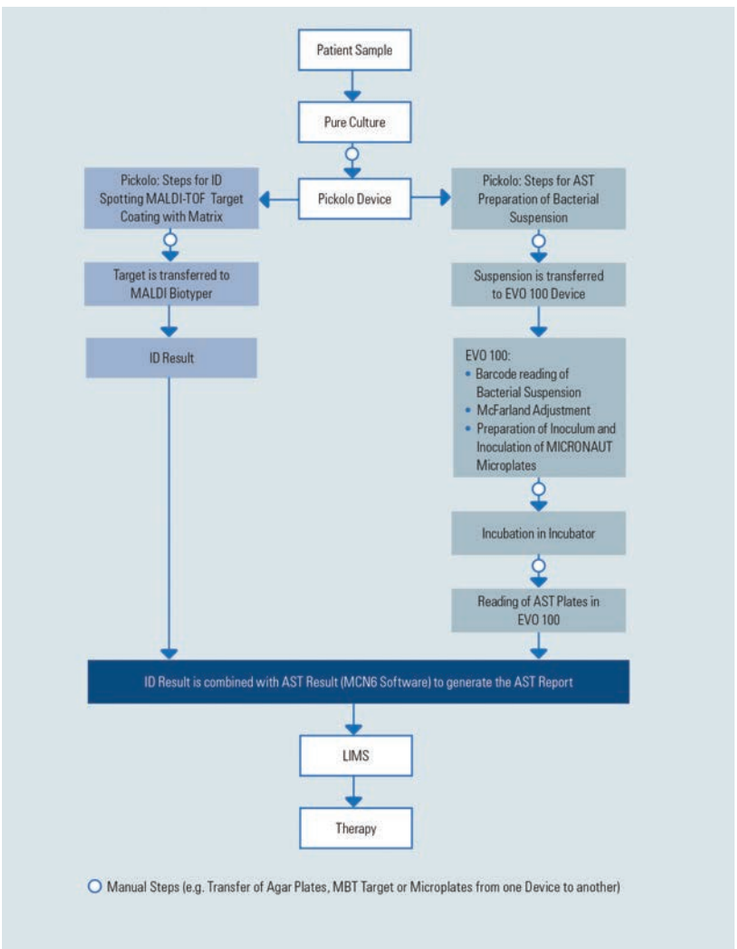

Figure 2: ID/ AST semi-automated sample workflow.
Combining Bruker's MALDI Biotyper & MICRONAUT Technology enables confident ID/ AST/ True MIC results

MALDI-TOF MS technology identifies microorganisms by determining its unique proteomic fingerprint, by measuring highly abundant proteins and matching the pattern with an extensive reference library, which is constantly being updated.

Benefits to the patient and hospital

Given the urgent situation in Europe (*Figure 3*), and specifically Italy, regarding rising antimicrobial resistance, the ability to conduct a reliable AST test with true MIC values not only enables the hospitals to comply with regulatory guidelines, but also protect the health of the local population. Rapid identification using MALDI-TOF MS allows Dr Scarparo and his laboratory to quickly identify the infecting bacterial species, thereby speeding up decision-making on therapeutic strategy and preventing patients from remaining on broad-spectrum antibiotics for too long. If this is allowed to occur, the chance of MDR bacterial strains emerging increases, and costs to the hospital rise dramatically. AST in parallel further strengthens the clinicians' ability to prescribe antimicrobials that are known to be the most effective against that specific pathogen.

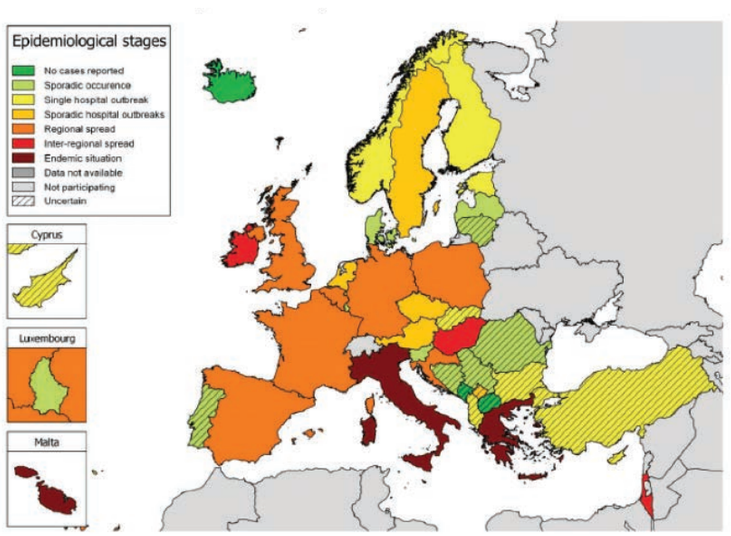

Figure 3: Occurance of carbapenemase-producing Enterobacteriaceae in 38 European countries based on self-assessment by the national experts, March 2013.

The future of AST

There is now a strong clinical trend towards a personalised medicine approach to patient care. Dr Scarparo's approach to combine MALDI-TOF MS analysis for fast identification of microorganisms with the BMD method for AST, to obtain true MIC values using customised layouts, enables the laboratory to adopt a precision medicine approach, by getting relevant therapies to patients sooner. This reduces costs by shortening hospital stays and reducing the initial use of ineffective therapies. Targeted therapy improves clinical outcomes for patients, and contributes to the worldwide effort to narrow antimicrobial treatments to combat the global rise of antimicrobial resistance.

References

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