Speeding up antimicrobial susceptibility testing

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Infectious diseases are initially treated empirically until the identity and antimicrobial susceptibility of the responsible microorganism is established and the treatment can be directed to the specific strain of the patient. Traditional microbiological diagnosis, based on the isolation of a microorganism for subsequent identification and susceptibility testing, is a process that usually takes up to 48-72 h.

Numerous studies have reported that the rapid initiation of directed therapy has a positive effect on the clinical evolution of the patient, especially in serious infections.[1–6] In patients with bacteremia, inappropriate treatment can double mortality, which increases by 7,6% each hour that therapy adjustment is delayed.[3] When evaluating these studies, it is necessary to take into account the criteria for case inclusion, i.e. the infection type, as well as the methodology used to shorten the time for microbiological response (techniques performed directly on the clinical sample or using positive culture). The clinical circuit should also be born in mind: the laboratory schedule, response pathways to the clinician, possibility of consultation with an infectious disease specialist, etc. All these variables can justify the discrepancies between the different studies. Despite these discrepancies, however, there is a consensus that reducing the time for provision of antimicrobial susceptibility test results contributes to decreasing the morbidity and mortality rates of patients as well as the cost of patient care. Savings in laboratory and pharmacy costs have been reported as between 2 and 5 million dollars a year in the hospitals where the studies were conducted.[1,2,5] Finally, the potential impact of inappropriate use of antimicrobials should also be mentioned. Even with effective treatments for an infectious process, the use of broad-spectrum antimicrobials facilitates the selection of multi-resistant microorganisms in the flora of both patient and the hospital environment.[7]

As mentioned, microbiological diagnosis based on isolation by culture and subsequent study of the microorganism tends to be quite slow. This delay in generating information, as well as a growing awareness of microorganisms that cannot be cultured in the laboratory, has prompted the development of different molecular techniques for rapid diagnosis.[8–11]

If rapid techniques are to have an impact on empirical treatment, it is necessary to know not only the identity of the microorganism (and therefore its natural antimicrobial resistance), but also its acquired resistance patterns. This is due to the rapid evolution of resistance observed in practically all the microbial groups.[12,13] It is within this context that the *Theranostics* concept has arisen, which emphasizes the importance of rapid diagnostic techniques that can identify both the infectious agent and its pattern of antimicrobial susceptibility for successful initial treatment.[9] Many companies have been working actively in this field.[8,10,11]

Ideally, the microorganism and its sensitivity profile should be determined directly in the clinical sample. Some commercial kits authorized by the American Food and Drug Administration (FDA) or the *Conformité Européenne* (CE) are already available on the market for this purpose. The majority of these kits are based on the detection of nucleic acids by the polymerase chain reaction applied to the etiological diagnosis of specific infections (e.g., sepsis, meningitis, respiratory infection).[8,14] These rapid diagnostic techniques have been reviewed in different workshops of the Transatlantic Task force on Antimicrobial Resistance (TATFAR) with members from the European Union and United States of America.[15]

Although a major step forward, these systems have certain limitations, including the volume of sample needed, the level of clinical sensitivity, and a restricted number of detection targets.[8,10,16] In addition, the few commercial kits available for detecting resistance genes tend to analyze only a small number of genes.[8,10,16] While these targeted genes are of great clinical importance (for example, those encoding carbapenemases and extended-spectrum beta-lactamases or the ones conferring resistance to vancomycin or methicillin), accurately directed treatment requires a high number of genes or resistance mechanisms to be determined.

Furthermore, the only resistance mechanisms that can be detected directly in the sample are those previously determined and targeted by a commercial kit. The molecular basis for resistance to some antibiotics may involve only one or a few easily detectable genes (for example, rifampicin, methicillin, vancomycin, quinolones), but in other cases (for example, beta-lactams and aminoglycosides) a wide variety of mechanisms with multiple genes are implied, therefore requiring multiple target detection. Sometimes it is not enough to determine the presence or absence of a gene but also its expression level and if it has undergone mutations (for example, expressing TEM, SHV and OXA type beta-lactamases that confer resistance to beta-lactams). In Gram-negative bacilli, for example, hundreds of genes confer resistance to beta-lactams and aminoglycosides, and numerous mutations affect fluoroquinolones, aminoglycosides and macrolides, making their detection by molecular techniques highly challenging.[17] A possible solution to these limitations is the implementation of whole genome sequencing techniques, which together with data interpretation software can provide information on the identity of the organism and resistance genes. However, their high cost and complexity currently preclude their routine use in health care laboratories. Also, they do not resolve issues such as the detection of resistance by unknown mechanisms or gene expression levels.[18]

To date, most clinical microbiology laboratories have implemented different molecular techniques for etiological diagnosis of infectious diseases according to the particularities of each center and the needs of their patients. However, antimicrobial susceptibility tests remain fundamentally based on the more traditional commercially available techniques such as microdilution broth, disk diffusion or the epsilon test.[19] All these systems require bacterial cultures, which usually become available for testing after 18-24 hours, although some commercial kits have tried to reduce this to 4 or 6 hours.[10,11]

Sensitivity studies have long been carried out using methodologies based on the viability of the microorganism in contact with the antimicrobial agents. In recent years, a variety of new approaches have been developed to accelerate the susceptibility tests, applying technologies of microfluidics, microcalorimetrics, cantilever technology, mass spectrometry, nuclear magnetic resonance, microsound detection, Raman and infrared spectroscopy, intrinsic fluorescence detection, flow cytometry, bacteriophages susceptibility, impedance-mediated methods, apoptosis markers, electric noses and luciferase expression vectors, among others.[10,11,18,20] However, much of this methodology has yet to be validated before it can replace the traditional systems. Before a procedure can be incorporated into the routine of microbiological diagnosis, it is necessary to optimize aspects such as automation, suitability for multiple studies and cost.

Presented in this current issue of the journal is a study of a rapid antimicrobial susceptibility test carried out by March Roselló GA *et al*[21] on isolated microorganisms and based on the detection of ATP bioluminescence. The methodology proposed by these authors generates reliable results in only 2 hours. It consists of a microtiter plate of 96 wells containing a series of progressive dilutions of antimicrobial agents and 1.3 μL of bacterial suspension. After incubating the plate for two hours at 35°C, the ATP extraction is performed and the amount of ATP produced and released is quantified in the luminometer. The relative light unit (RLU) from a control drug-free well is compared with those obtained at each antimicrobial agent concentration. Finally, using ROC curves, the authors define the cutoff point of maximum sensitivity and specificity to determine the susceptibility or resistance of the microorganism tested. There is no doubt that this is a very attractive proposal, although in its current form it has two important limitations: the instability of ATP, which requires the RLU to be determined well by well, and the cost of reagents, which the authors themselves have estimated at around 125-130€ per 96-well plate. Despite these drawbacks, this ATP-based system could be optimized and automated relatively easily, providing a useful tool for the study of antimicrobial susceptibility. The system requires little handling by the professional, and provides results rapidly, which can be crucial in serious infections. The use of MALDI-TOF MS in clinical laboratories for the identification of the vast majority of microorganisms, together with techniques such as the aforementioned, allows both microorganism identification (necessary to ascertain susceptibility) and antimicrobial susceptibility to be performed within a few hours.

In conclusion, while many methodological alternatives have been published to determine microorganism susceptibility to antimicrobial agents, either directly from the clinical sample or from culture, several issues still need to be resolved.

Molecular techniques for the detection of resistance genes are difficult to develop given the wide variety of targets, the numerous families of antimicrobials, lack of knowledge of molecular resistance mechanisms, and the involvement of regulatory genes and/or alterations in regulatory regions of expression, which can also alter the antimicrobial susceptibility of a microorganism.

On the other hand, growth-based techniques able to provide susceptibility results within a matter of hours still require greater automation and simplification before they can form part of the laboratory routine. Moreover, their analytical and clinical sensitivity/specificity as well as costs need to be validated. Other important factors to consider are the particularities of each clinical laboratory, namely their working hours (24 hours a day, 7 days a week, or not), availability of qualified personnel, the degree of automation of the commercial kit, and the possibility of immediately contacting the physician responsible for the patient to establish the proper treatment.

There is no doubt that the progress achieved in this field so far will bring about an improvement in patient care, reduced hospital costs, and possibly a lower selection of resistant organisms, and for all these reasons, the research should continue.

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