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Rapid microbial identification and antimicrobial susceptibility testing to drive better patient care: an evolving scenario

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Antimicrobial chemotherapy for septic patients begins with empirical therapy and can be subsequently revised when the results of microbiological testing become available. In recent years, a number of novel technologies for the microbiological diagnosis of sepsis have been developed that return results in a shorter timeframe compared with conventional diagnostic approaches. These novel technologies aid antimicrobial stewardship when treating septic patients by reducing the time to appropriate antimicrobial chemotherapy. Advantages and limitations of these technologies should be well understood upon their introduction in the diagnostic workflow. Increasingly popular DNA-based technologies primarily focus on the rapid identification of pathogens, but information on antimicrobial susceptibility is lacking or limited to a few clinically relevant resistance markers. Thus, DNA-based molecular techniques can complement conventional technologies but cannot replace them. On the other hand, a novel technology that provides both rapid identification of bacterial pathogens and a rapid phenotypic antibiogram with MIC values, and which starts from positive blood cultures, is a very promising approach for fast diagnosis of sepsis. To fully leverage the advantages offered by novel diagnostic technologies for sepsis requires a careful introduction into the laboratory workflow, following an evaluation by a health technology assessment approach. It may also require some reshaping of the workflow (e.g. to process the positive blood cultures on a 24/7 schedule) and of the laboratory organization (e.g. by creating a laboratory subsection for fast diagnosis of sepsis).

Sepsis: balancing antimicrobial chemotherapy with antimicrobial stewardship and the antibiotic resistance crisis

Sepsis is an emergency that requires the prompt administration of antimicrobial chemotherapy active against the causative pathogen(s).[1,2] While a sepsis diagnosis is initially based on clinical evaluation and some clinical chemistry parameters, it takes substantially longer (up to 3–4 days) for identification (ID) and antimicrobial susceptibility testing (AST) of the infecting pathogen(s). Under these circumstances, antimicrobial chemotherapy must start on an empirical basis and can subsequently be revised as soon as the results of microbiological testing become available. Microbiological diagnosis, therefore, remains the rate-limiting step for the selection of definitive antimicrobial chemotherapy, and the development of diagnostic technologies that provide faster results [often referred to as 'fast clinical microbiology' (FCM)] has been strongly advocated to support antimicrobial stewardship programmes (ASPs).[1,3,4]

The recent pandemic of MDR pathogens has greatly increased the need for ASPs, to both improve clinical outcomes and reduce the selective pressures generated by the use of broad-spectrum antibiotics. In fact, the dissemination of MDR pathogens consistently reduces the chances of selecting an appropriate empirical therapy while promoting the empirical use of very broad-spectrum agents.

Emerging FCM technologies in the diagnosis of sepsis

Diagnostic microbiology has remained relatively unchanged for decades, but recent years have witnessed a remarkable effort to develop novel technologies for faster microbiological diagnosis of sepsis. Some of these technologies work with positive blood cultures, while a few work directly from blood specimens. All of them return information on pathogen ID and, in some cases, antimicrobial resistance profiles in a shorter timeframe compared with the conventional diagnostic workflow, which involves subculture followed by ID and AST carried out from isolated colonies. A synopsis of FCM technologies for the diagnosis of sepsis from positive blood cultures is shown in Table 1. The various technologies have

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Table 1. The principal FCM technologies for processing positive blood cultures for the diagnosis of sepsis

Method	No. of ID targets	Panel coverage (%)[a]	ID accuracy, monomicrobial (%)	Resistance markers	No. of AST antimicrobials	References
BioFire FilmArray® BCID panel[b]	8 Gram-positive/11 Gram-negative bacteria + 5 fungi	80–93	82–92	*mecA*, *vanA/B*, bla_{KPC}	–	5
PNA FISH/QuickFISH®[c]	4 Gram-positive/4 Gram-negative bacteria + 5 fungi	–	90–100	–	–	6,7
Prove-it™ Sepsis	60 bacteria + 13 fungi	86	95–98	*mecA*	–	8
Unyvero BCU Blood Culture Application[b]	12 Gram-positive/ 14 Gram-negative bacteria + 8 fungi	90	96	*vanA*, *vanB*, *mecA*, *mecC*, *ermA*, *aac(6′)aph(2″)*, *aacA4*, bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{CTX-M}, bla_{IMP}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-48}, bla_{OXA-58}	–	9
Verigene® (GP/GN)[b]	12 Gram-positive/8 Gram-negative bacteria	90–97	84–99	*mecA*, *vanA/B*, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA}	–	10,11
MALDI-TOF MS (Bruker/Vitek® MS)[b]	NA	100	61–98	–	–	12,13
Short-term culture + MALDI-TOF MS	NA	100	78–92	–	–	14
Accelerate Pheno™ system[b]	7 Gram-positive/8 Gram-negative bacteria + 2 fungi	81–83	86–100	methicillin resistance and MLSb phenotypic screens	8 Gram-positive/ 15 Gram-negative	15

NA, not applicable.
[a]Species coverage rate.
[b]European Conformity (CE) marked and FDA cleared.
[c]Bacterial *Quick*FISH is CE marked and FDA cleared. *Candida Quick*FISH is not FDA cleared.

advantages and limitations that should be considered upon their introduction into the diagnostic laboratory workflow.

The crucial factors for FCM technologies are cost-effectiveness, timeliness of the results and the value of the returned information. There is no universal consensus on the definition of 'fast', but it is reasonable to define it as obtaining the result within a working day shift (i.e. ∼8 h).[16] Concerning the value of the returned information, it is without doubt that detailed and accurate information on the ID and the AST of the pathogens is desired. The species coverage rate of clinical isolates with these technologies is in general high, reaching 100% in the case of MALDI-TOF MS. On the other hand, with molecular technologies based on DNA analysis, coverage depends on the panel of probes included in the test and may be broad spectrum or focused on specific subsets of sepsis pathogens (e.g. Gram-positives or Gram-negatives) (Table 1). Unfortunately, the majority of the current fast technologies focus on rapid ID of microorganisms. The information on the antimicrobial susceptibilities of the pathogens is not available or limited to a few clinically relevant resistance genes (e.g. *mecA* for methicillin resistance in staphylococci, *vanA* and *vanB* for glycopeptide resistance in enterococci, and carbapenemase and ESBL genes in Gram-negatives). Moreover, technologies based on the detection of resistance genes only include a limited number of resistance markers. The presence of these markers only indicates a possible resistance phenotype to some antibiotics and does not reliably inform on the complete susceptibility profile or return MIC values. These are major limitations of the so-called molecular antibiogram.[17] Therefore, there is an obvious need for fast diagnostic technologies with phenotypic AST, such as the Accelerate PhenoTest® BC Kit, in which fluorescence *in situ* hybridization (FISH) to provide organism ID within 90 min is combined with morphokinetic cellular analysis (MCA) to provide a phenotypic antibiogram with MIC values in a timeframe of 6–7 h starting from a positive blood culture, as a fully automated standalone system.[15]

Given their limitations, most of the fast diagnostic technologies are solely regarded as complementary tools to standard methods in diagnostic clinical microbiology, such that the results obtained from these tests are delivered to clinicians only as preliminary results that should then be confirmed by standard methods. From this perspective, the availability of fast diagnostic technologies, such as the one combining FISH and MCA, that provide the same information and could replace standard methods represents a

significant advance. It is therefore time to re-evaluate the actual need for confirmatory standard methods for these fast diagnostic technologies in the clinical routine.

The available evidence for FCM

The ultimate goal of establishing fast diagnostic technologies in clinical microbiology is to improve patient care. However, it is very challenging to evaluate the clinical impact of these technologies (i.e. mortality and morbidity rates of patients with sepsis) due to the complexity of the pathogenesis of sepsis, the local microbiological epidemiology, including the types and antimicrobial susceptibility profiles of microorganisms recovered from blood cultures, and the local antimicrobial policies that play a decisive role in the eventual outcome of septic patients.

A recent systematic review and meta-analysis showed that implementation of molecular methods for the rapid diagnosis of sepsis has clear advantages in terms of reducing the time to administration of appropriate antimicrobial chemotherapy and reducing the defined daily dose of antimicrobial medications.[16] However, the study design and the populations evaluated by these studies were heterogeneous, and the quality of studies was variable. Therefore, additional evidence is warranted for a better assessment of the impact of fast diagnostic technologies on outcomes of sepsis, including an evaluation of the importance of the accuracy of the test results, the coverage of the test panel, the information obtained from susceptibility testing, and eventually the post-analytical factors, including overall antimicrobial use, which can also be influential.[18,19]

Exploitation of FCM: the need to reshape the laboratory workflow

Full exploitation of the advantages offered by FCM requires a thoughtful introduction of the various technologies in the routine laboratory workflow, as well as some reshaping of the workflow itself.[20] A substantial shortening of the time to result is possible: down to 1–2 h for ID and detection of resistance determinants with molecular technologies. Using two technologies in a single assay has recently made it possible to obtain ID results in 90 min with FISH and complete phenotypic AST with MCA in 7 h. This shortening, however, possibly mandates processing the positive blood cultures on a 24/7 schedule, although a consistent advantage in terms of time-to-result reduction can also be achieved in laboratories processing positive blood cultures on a 12/6 schedule (which is still adopted by many laboratories).

The introduction of novel diagnostic technologies into the established routine laboratory workflow has an impact on human resources requirements and laboratory budget. Since most of the novel diagnostic technologies are additions to the conventional workflow, their introduction usually requires a net increment of budget and personnel, which should be subjected to an evaluation by a health technology assessment approach.[21] From this perspective, fast technologies such as FISH and MCA, that can be substitutive rather than complementary to the standard technologies, can be advantageous, since they eventually have a lower impact on the additional resources needed.

Concluding remarks

The recent development of state-of-the-art fast technologies can potentially improve the impact of clinical microbiology results on clinical outcomes for sepsis. Modern laboratories, equipped with a repertoire of possibilities for the laboratory diagnosis of sepsis, however, face a challenge in establishing these methods in the clinical routine. Positive blood culture followed by subcultures on agar plates and standard phenotypic identification and AST was a 'one protocol for all samples' approach, but is quickly becoming out of date as a stand-alone approach. On the other hand, for large laboratories, it is currently almost impossible to routinely implement fast technologies on all clinical samples. Therefore, there is an increasing need to establish algorithms for the use of fast technologies with selected clinical samples as seen in the triage models implemented in emergency rooms.

In the organization of the clinical microbiology laboratory, a subsection dedicated to sepsis-patient diagnostics incorporating the fast microbiology technologies should possibly be foreseen, in which suitable human and technological resources and specific clinical competences are present. In fact, interpretation of the fast microbiology results can be a challenge for clinical laboratories as well as clinicians, and at the same time these results can be crucial to the implementation of efficient ASP.

The concept of personalized treatment has long been established in cancer and autoimmune diseases, including multiple sclerosis and rheumatoid arthritis. Revising the diagnosis and treatment of autoimmune diseases towards personalized medicine will be a revolutionary step towards having more effective and safer therapeutic options.[22] In the era of the establishment of state-of-the-art diagnostic methods for sepsis, we believe it is time to start discussing the term 'personalized diagnostics' in clinical microbiology.

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