



Current Trends in Clinical Microbiology and Antimicrobials

Christina Sakarikou*

Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy

*Corresponding author: Christina Sakarikou, Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy, Tel: + 39 3937730866; E-mail: cristina_sakarikou@yahoo.it

Received: December 04, 2017, Accepted: December 06, 2017, Published: December 08, 2017

Copyright: © 2017 Sakarikou C. This is an open-access article distributed under the terms of the creative commons attribution license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Editorial

Timely identification of infectious agents and the early introduction of an appropriate antimicrobial therapy is crucial in clinical practice, particularly in severe infections, such as bloodstream infection. However, rapid species identification is only the first step in the clinical microbiology diagnostic workflow. Surveillance and early detection of antimicrobial resistant strains should be the priority of the diagnostic microbiology as resistant bacteria are involved into hospital outbreaks as well as in sepsis. Antimicrobial stewardship and antimicrobial resistance issues have recently hold considerable political attention at national and international level [1-6]. Thus, rapid, accurate and cost effective diagnostic methods are necessary to provide timely information for antimicrobial treatment and infection control measures in clinical microbiology laboratories.

In this editorial we aim to discuss the current technological trends in clinical bacteriology related to laboratory automation, high level information availability and reducing the overall time to identification, antimicrobial susceptibility testing (AST) and epidemiological typing final reports with cost effectiveness and high- accuracy.

Among the so-called "omics" technologies proteomics and genomics are being successfully introduced allowing the laboratory automation. Several of the time-consuming culture-based technologies in routine diagnostics were replaced by culture-free tests based on nucleic acid and protein profile detection.

During the past few years specific real-time PCR and multiplex-PCR techniques for all clinically relevant micro-organisms detection and for a variety of antimicrobial resistance genes monitoring were developed, some of which are now fully automated [7]. Genomic techniques like amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE), and multi locus sequence typing (MLST) are used for subtyping of bacteria for epidemiological purposes in many laboratories [8]. Furthermore, several systems for molecular AST have been proposed but a real-time PCR antibiogram is still only a research tool [9]. Recently several studies have been reported that PCR could be successfully used for determining minimum inhibitory concentrations (MICs) of antibiotics [10].

Mass spectrometry was successfully introduced as a new diagnostic gold standard method in clinical microbiology for rapid, accurate and cost-effective microbial species identification [11].

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) technology has been shown to be a suitable tool for high throughput and complete automation in clinical microbiology laboratory. Furthermore, the microbial species identification directly from the blood culture fluid by MALDI-TOF MS technology has been widely proved. This technology is also well

positioned to be applied for rapid antimicrobial susceptibility testing implementing the stewardship programs.

Recently, a semi-quantitative MALDI-TOF MS-based method for the detection of antibiotic resistance was described in several studies [12,13]. This assay namely MBT-ASTRA allows rapid identification and susceptibility testing of both Gram negative and Gram-positive pathogens in a single assay within approximately 3-4 h. MBT-ASTRA assay measures the relative growth rates of bacterial isolates exposed to antibiotic compared to untreated controls during a short incubation step. This assay is also applicable for AST directly from blood culture fluid with high sensitivity, specificity, and an overall accuracy rate of 95%. However, this alternative AST assay cannot be used to classify intermediate resistance in its current configuration.

MALDI-TOF MS could be used also as a screening tool for detection of resistance to β -lactam antibiotics and to discriminate vancomycin-resistant from vancomycin-susceptible *Enterococcus faecium* strains as well as carbapenemase production in anaerobic bacteria as early as 3 h. Furthermore, identification and differentiation of carbapenemase producing clinical strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* from metallo- β -lactamase producing strains has been reported in several studies. [14,15]. However, as a species-specific resistance to antimycotic agents is observed in many molds and zygomycetes, drug resistance in fungal isolates may be detected by identification of the fungal species by MALDI-TOF MS [16].

Proteomics represents the functional aspect of genomics and can be used as a taxonomic tool. Recently has been shown the applicability of MS for bacterial taxonomy and strain typing. However, the use of MALDI-TOF MS for strain typing of fungal isolates does not successful as in bacteria. In contrast to yeasts, it has been difficult to type molds since they have complicated phylogenetic relationships and more complicated morphology. Strain typing by MALDI-TOF MS was, however, reported to be feasible with *C. albicans* and *C. parapsilosis* [17].

MALDI typing is a simple, cost effective and time-saving method in contrast to gold standard method, PFGE. It could allow broad and prospective typing of all clinical isolates detected in clinical settings. However, application of MALDI-TOF MS for rapid susceptibility testing or epidemiological studies is currently hampered by the lack of standardized protocols and universal guidelines for data interpretation. Furthermore, laboratory equipment is expensive and in need of costly maintenance although the operating costs are low.

Next generation sequencing (NGS) is already applied in several medical microbiology laboratories. NGS allows sequencing of the whole genome (WGS) of numerous pathogens in one sequence run. It can be performed directly to clinical specimens for rapid identification of bacteria, outbreak management, molecular case finding, characterization and surveillance of pathogens, taxonomy,

metagenomics approaches on clinical samples, and for the determination of the transmission of zoonotic microorganisms from animals to humans by using a single protocol for all pathogens for both identification and typing applications. It is particularly important in outbreak detection and monitoring the evolution and dynamics of multi-drug resistant pathogens [18]. NGS can differentiate between clones with specific properties and allows the implementation of control measures to avoid the dissemination of resistant bacterial clones providing patient management, infection prevention and evolutionary studies. NGS also allows the detection of novel resistance genes (ARG) in bacteria, both in current as well as in historical strain collections [19]. A rapid and accurate molecular epidemiological surveillance of pathogens at regional and national level also can be performed by NGS. NGS will also reveal differences in previously indistinguishable animal and human bacterial strains regarding the zoonotic transmission of microorganisms. In addition, NGS allows an extensive analysis of how antibiotic use manipulates specific microbiota and the consequences for interspecies transmission increasing the knowledge on microbial evolution through the analysis of bacterial genomes, namely the variable regions, which usually determine host-adaptation and the potential of spread to different hosts [20]. Genome sequencing will be applied for novel generations of AST assays and the first examples regarding genome sequencing and determination of appropriate real-time antimicrobial therapy has already been reported [21]. Consequently, NGS should become routine in the clinical microbiology laboratory. However, further studies are required to improve the workflow for NGS, by shortening the turnaround time for the library preparation and the runs on the NGS platforms, and further reducing costs.

In contrast to proteomics approaches, all genomics techniques described above request highly expertise personnel for performing the assays and for data interpretation. Obviously, all these technological innovations that allow the automation of clinical laboratory procedures can be easily introduced in developed countries laboratories rather than in developing countries laboratories.

References

1. <http://www.who.int/antimicrobial-resistance/global-action-plan/database/en/>
2. <http://www.un.org/pga/71/2016/09/21/press-release-hl-meeting-onantimicrobial-resistance/>
3. <http://www.oecd.org/about/secretary-general/g20-health-ministersmeeting-fighting-antimicrobial-resistance.htm>
4. https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf
5. https://cddep.org/blog/posts/cara_conscience_antimicrobial_resistance_accountability_and_next_big_thing_cddep
6. <http://www.who.int/antimicrobial-resistance/en/>
7. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S (2012) Rapid detection of carbapenemase genes by multiplex real-time PCR. J Antimicrob Chemother 67: 906-909.
8. Murchan S, Kaufmann ME, Deplano A, De Ryck R, Struelens M, et al. (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: A single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41: 1574-1585.
9. Waldeisen JR, Wang T, Mitra D, Lee LP (2011) A real-time PCR antibiogram for drug-resistant sepsis. PLoS One 6: e28528.
10. Beuving J, Verbon A, Gronthoud FA, Stobberingh EE, Wolffs PF (2011) Antibiotic susceptibility testing of grown blood cultures by combining culture and real-time polymerase chain reaction is rapid and effective. PLoS One 6: e27689.
11. Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G (2010) Performance of matrix assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J Clin Microbiol 48: 1549-1554.
12. Jung JS, Hamacher C, Gross B, Sparbier K, Lange C, et al. (2016) Evaluation of a semi-quantitative matrix-assisted laser desorption ionization-time of flight mass spectrometry method for rapid antimicrobial susceptibility testing of positive blood cultures. J Clin Microbiol 54: 2820-2824.
13. Maxson T, Taylor-Howell CL, Minogue TD (2017) Semi-quantitative MALDI-TOF for antimicrobial susceptibility testing in *Staphylococcus aureus*. PLoS ONE 12: e0183899.
14. Hrabak J, Chudackova E, Walkova R (2013) Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. Clin Microbiol Rev 26: 103-114.
15. Sakarikou C, Ciotti M, Dolfa C, Angeletti S, Favalli C (2017) Rapid detection of carbapenemase-producing *Klebsiella pneumoniae* strains derived from blood cultures by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). BMC Microbiol 17: 54.
16. Bader O (2013) MALDI-TOF-MS-based species identification and typing approaches in medical mycology. Proteomics 13: 788-799.
17. Pulcrano G, Roscetto E, Iula VD, Panellis D, Rossano F, et al. (2012) MALDI-TOF mass spectrometry and microsatellite markers to evaluate *Candida parapsilosis* transmission in neonatal intensive care units. Eur J Clin Microbiol Infect Dis 31: 2919-2928.
18. <https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/whole-genome-sequencing-for-public-health-surveillance.pdf>
19. Nijhuis RH, Oueslati S, Zhou K, Bosboom RW, Rossen JW, et al. (2015) OXY-2-15, a novel variant showing increased ceftazidime hydrolytic activity. J Antimicrob Chemother 70: 1429-1433.
20. Harrison EM, Weinert LA, Holden MT, Welch JJ, Wilson K, et al. (2015) A shared population of epidemic methicillin-resistant *Staphylococcus aureus* 15 circulates in humans and companion animals. M Bio 5: e00985-13.
21. Scholl D, Gebhart D, Williams SR, Bates A, Mandrell R (2012) Genome sequence of *E. coli* O104:H4 leads to rapid development of a targeted antimicrobial agent against this emerging pathogen. PLoS One 7: e33637