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Trends in infectious diseases

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What are the most relevant publications in Clinical Microbiology in the last two years?

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ABSTRACT

This minireview describes some of the articles published in the last two years related to innovative technologies including CRISPR-Cas, surface-enhanced Raman spectroscopy, microfluidics, flow cytometry, Fourier transform infrared spectroscopy, and artificial intelligence and their application to microbiological diagnosis, molecular typing and antimicrobial susceptibility testing. In addition, some articles related to resistance to new antimicrobials (ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, and cefiderocol) are also described.

Key words: innovative diagnostic technologies, microbiological diagnosis, antimicrobial susceptibility testing, resistance to new antimicrobials

INTRODUCTION

Over the last years, the development of new technologies has also reached clinical microbiology laboratories and transformed the care of infectious diseases. The use of multiplex syndromic panels and point of care testing for infectious diseases, molecular diagnostics for fungal infections, rapid genotypic and phenotypic antimicrobial susceptibility testing, introduction of next-generation sequencing into clinical diagnostics and laboratory diagnostic stewardship have improved patient care. In addition, the diagnostic potential of innovative molecular technologies and harnessing big data through artificial intelligence in clinical microbiology offer a great opportunity to move forward.

On the other hand, antimicrobial resistance has even reached the most recently introduced new antimicrobials and is a cause for concern that needs to be rapidly detected to prevent its spread.

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This minireview describes some of the articles published in the last two years related to innovative technologies and resistance to new antimicrobials.

NEW TECHNOLOGIES APPLIED TO MICROBIOLOGICAL DIAGNOSTICS

The manuscript of Zhao L, et al [1] is an excellent review describing the usefulness of the CRISPR-Cas13a system as a tool for molecular diagnostics, gene therapy, gene editing, and RNA imaging. The clustered regularly interspaced short palindromic repeats (CRISPR) system is a natural adaptive immune system of prokaryotes. Additional elements of the CRISPR system include the leader sequence and CRISPR-associated (Cas) genes. Cas13a possesses two RNase activities, one for pre-crRNA processing and the other for cis-cleavage of the target RNA and trans-cleavage of non-specific RNAs. Cas13a processes pre-crRNA into mature crRNA independently and combines with its crRNA to form the surveillance complex that recognizes the foreign target RNA and cleaves the target and surrounding ARNs. Subsequently, products trans-cleaved by Cas13a system are recognized with fluorescence or lateral flow readouts. Because of this trans-cleavage activity and the high specificity of its CRISPR RNA, the CRISPR/Cas13a system has been the most widely characterized for its application in molecular diagnostics including the detection of pathogens like viruses, bacteria, parasites, and fungi. The CRISPR-Cas13a system can improve sensitivity, specificity, operability, portability and cost of other diagnostic methods such as antigen detection, PCR or ADN sequencing. One example of application of the CRISPR-Cas13a system is the study of Fozouni P, et al [2] for the detection and quantification of SARS-CoV-2 directly from nasopharyngeal samples without pre-amplification. The viral load was directly quantified using enzyme kinetics and the assay is integrated with a reader

device based on a mobile phone that allows for portable and sensitive readout. The sensitivity was ~100 copies/µL in under 30 min of measurement and accurately detected pre-extracted RNA from a set of positive clinical samples in under 5 min. This low-cost assay has the potential to be used as point-of-care screening for SARS-CoV-2.

Another innovative technology is the surface-enhanced Raman scattering (SERS). This is a spectroscopic technique based on vibrational analysis and fluorescence detection. Its advantages include fluorescence quenching, short time of measurement, high quality spectra, and low costs of analysis, being an appropriate method for biomedical applications and has been applied for the diagnosis of various infectious diseases [3]. By using this technique, the study of Berus SM, et al. [4] detected the presence of *Neisseria gonorrhoeae*, *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum*, and *Haemophilus ducreyi* directly in men's urethra swabs in less than 15 min. The SERS spectra were analyzed by applying PCA (principal component analysis) partial least square discriminant analysis (PLS-DA), and soft independent modelling of class analogies (SIMCA) and identified the individual species of the *Neisseria* genus with high accuracy. The prediction accuracy reached 89% for SIMCA and 100% for PLS-DA. The authors indicate that the simplicity, high sensitivity, reproducibility, and specificity, open a new path in the improvement of the point-of-care applications.

Microfluidic chip technology is a system in which very small amounts of fluid are used. The fluid behaviour at the microscale (microchannels: 10-500 nm) differs from the macroscale since surface tension and energy dissipation are different, the flow is laminar, without turbulence, only diffusion is involved in fluid mixing, and there is a very high surface-to-volume ratio which accelerates chemical reactions. This technology can also be used to integrate the process units required for nucleic acid detection such as sample pretreatment, nucleic acid extraction, target sequence amplification, and signal detection, into one single micron-scale chip (lab-on-a-chip). The manuscript of Gao D, et al [5] reviews the research progress on microfluidic chip-based recombinase polymerase isothermal amplification technology. The advantages of this technology include high integration, compactness, portability and less sample consumption. In the same line, the research study of Ma L, et al. [6] presents a novel example of developing a polymer-based microfluidic device for the identification and antimicrobial susceptibility testing (AST) of *Campylobacter* spp. The assay uses chromogenic agar as selective cultivation medium and employes advanced design of air vents and zigzag channels to prevent the cross-contamination of antibiotics in different testing chambers, ensuring accurate AST results. The chromogenic medium and antibiotics were loaded in the device, and the growth of *Campylobacter* spp. was visualized by color change due to chromogenic reactions. The platform achieved 100% specificity for *Campylobacter* spp. identification. *C. jejuni, C. coli*, and *C. lari* were detected in artificially contaminated milk and poultry meat, with detection limits down to 1×10^2 CFU/ml and 1×10^4 CFU/25 g, respectively. The MICs and susceptibility profiles of *Campylobacter* isolates were also tested by on-chip AST, showing high coincidences in the MIC (91% to 100%) with the conventional agar dilution method against ampicillin, tetracycline and ciprofloxacin. For a presumptive colony, on-chip identification and AST were completed in parallel within 24 h., representing a rapid, portable, and cost-effective approach to detect antibiotic-resistant *Campylobacte*r spp.

Flow cytometry is an analytical method that allows the rapid measurement of light scattered and fluorescence emission produced by suitably illuminated cells. The cells, or particles, are suspended in liquid and produce signals when they pass individually through a beam of light. This technology has been widely used in basic microbiology for the simple and rapid assessment of the viability of a microorganism. In the study of Silva-Dias A, et al [7] the authors evaluate the FASTinov® flow cytometry kit on positive blood cultures for the rapid determination of AST with a time to result of less than 2 h. Two kits were evaluated, one for Gram-negative and other for Gram-positive microorganisms in spiked blood cultures with well-characterized bacteria, and also using positive blood cultures from patients. Results were compared with the standard disk diffusion method, that was used as the reference. After 1 h of incubation at 37°C, bacteria were analyzed using a cytometer. Categorical agreement values for the Gram-negative panel were 96.8% based on EUCAST criteria and 96.4% based on CLSI criteria. The percentages of very major errors (VME) were 0.6% and 0.4% when using EUCAST and CLSI criteria, respectively, and were mainly observed with amoxicillin-clavulanate, cefotaxime, ceftazidime, and gentamicin. The kit also provided information regarding the presence of extended-spectrum beta-lactamases and carbapenemases. For the Gram-positive panel, categorical agreement was 98.6% when using both criteria, and showed 0.4% of VME (gentamicin and *Staphylococcus*) when using EUCAST and no VME when CLSI criteria was used. This study represents the utility of flow cytometry as a rapid alternative for direct antimicrobial susceptibility testing from positive blood cultures.

Fourier transform infrared spectroscopy (FTIR) is a mass spectrometry technology that analyses the absorption in the infrared spectrum of specific bonds present in different groups of molecules (lipids, proteins, carbohydrates and nucleic acids). This automated system has been used for real-time molecular typing for outbreak monitoring. The study of Passaris I, et al [8] presents the validation of FTIR for the capsular typing of *Streptococcus pneumoniae*, and conclude that this is a rapid and cost-effective technique and medium-throughput alternative to the classical phenotypic techniques. The IR Biotyper was first trained with a set of 233 strains comprising 34 different serotypes and the acquired spectra were used to create a dendrogram where strains clustered together according to their serotypes and to train an artificial neural network (ANN) model to predict unknown pneumococcal serotypes. Using 153 additional strains, the accuracy for determining serotypes represented in the training set was

98.0%, and using 124 strains representing 59 non-training set serotypes, the accuracy was 71.1% for the categorization as being non-training set serotypes. Although the method needs to be improved, it is a potential future alternative to conventional methods.

Artificial intelligence (AI) is a technology that for some years now has been present in our daily lives, including microbiological diagnostics. AI is a combination of algorithms to create machines that mimic human intelligence to perform tasks and can improve as they gather new information. AI is applicable to the diagnosis of infectious diseases including the detection of patients at risk of sepsis, the early detection of infections, the diagnosis of viral respiratory infections, and also as an aid in the radiological diagnosis of pulmonary tuberculosis [9]. One application of AI in clinical microbiology is the reading and interpretation of antibiograms using the disk diffusion method as described in the study of Pascucci M, et al. [10]. The system consists of an offline smartphone application for antibiogram analysis that captures antibiogram images with the phone's camera, and the user is guided throughout the analysis on the same device by a user-friendly graphical interface. An embedded expert system validates the coherence of the antibiogram data and provides interpreted results. In the study, the authors validate this fully automatic measurement procedure and obtained an overall agreement of 90% on susceptibility categorization against a hospital-standard automatic system and 98% against manual measurement (gold standard), with reduced inter-operator variability. The automatic reading of AST is entirely feasible on a smartphone. The authors conclude that this application is suited for resource-limited settings, and has the potential to significantly increase patients' access to AST worldwide.

RESISTANCE TO NEW ANTIMICROBIALS

Carbapenemase-producing Enterobacterales, mainly *Klebsiella pneumoniae*, have emerged and spread world-wide as a major cause of infections associated with high morbidity and mortality. In recent years, novel beta-lactam-beta-lactamase inhibitor combinations, such as ceftazidime/avibactam (CZA), meropenem/vaborbactam (MVB), and imipenem/relebactam (IMR), have been introduced in clinical practice, and are useful to treat infections caused by KPC-producing *K. pneumoniae* (KPC-Kp). However, acquired resistance to these combinations has been reported in KPC-Kp. Di Pilato V, et al [11] described an outbreak caused by CZA-resistant KPC-Kp, which was also variably resistant to MVB and IMR. Whole-genome sequencing revealed that the outbreak was multi-clonal and resistance to CZA was primarily mediated by overproduction of KPC-3 associated with increased gene dosage, a mechanism accounting for cross-resistance to MVB in most cases, and to IMR in a single KPC-Kp isolate; multiple alterations of the OmpK36 porin were also detected and mutated KPC (KPC-53) was detected in a single case. All cases were associated with previous CZA exposure. Nicola F, et al [12] described a clonal outbreak caused by 6 isolates of KPC-Kp sequence type 11 producing KPC variants resistant to CZA: KPC-31 variant in 5 cases and a novel variant, named KPC-115, in one case. Three patients had previously received CZA. These two studies reflect the need to monitor the evolution of the resistance, possibly coupled with a genomic analysis in order to understand the mechanisms of resistance to these antimicrobial combinations.

Regarding metallo-beta-lactamases (MBLs), these belonging to the NDM group are the most frequently identified acquired carbapenemases worldwide. MBLs hydrolyze all beta-lactams except monobactams, and they are not inactivated by currently commercialized beta-lactamase inhibitors. Nevertheless, very recent therapeutic options are promising, such as cefiderocol (FDC) and aztreonam/avibactam (ATM-AVI). The study by Poirel L, et al [13] described a ST167 *Escherichia coli* clinical isolate that produced NDM-35 and was resistant to carbapenems, ATM-AVI, and FDC, showing a 10-fold increased hydrolytic activity against cefiderocol compared to NDM-1. The isolate also produced a CMY-type beta-lactamase, exhibited a four amino-acid insertion in PBP3, and possessed a truncated iron transporter CirA protein (resistance to FDC), leading to resistance to virtually all beta-lactams. Finally, the study of Lan P, et al [14] from China, presents 2 strains of *K. pneumoniae* isolated from bloodstream infections that were resistant to FDC. One isolate carried the beta-lactamases SHV-12, DHA-1 and NDM-1, and the other carried the MBL NDM-5 and also presented a deficiency in the CirA protein, leading to high-level cefiderocol resistance (MIC >256 mg/L). It is noteworthy that these strains were isolated prior to the approval of cefiderocol clinical use in China.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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