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Continuing medical education: Methods of rapid diagnosis in clinical microbiology

Methods of rapid diagnosis in clinical microbiology: Clinical needs☆

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ABSTRACT

The diagnostic methods of infectious diseases should be fast, accurate, simple and affordable. The speed of diagnosis can play a crucial role in healing the patient, allowing the administration of appropriate antibiotic treatment. One aspect that increasingly determines the need for rapid diagnostic techniques is the increased rates of serious infections caused by multidrug resistant bacteria, which cause a high probability of error in the empirical treatment. Some of the conventional methods such as Gram staining or antigen detection can generate results in less than 1 h but lack sensitivity.

Today we are witnessing a major change in clinical microbiology laboratories with the technological advances such as molecular diagnostics, digital microbiology and mass spectrometry. There are several studies showing that these changes in the microbiological diagnosis reduce the generation time of the test results, which has an obvious clinical impact.

However, if we look into the future, other new technologies which will cover the needs required for a rapid microbiological diagnosis are on the horizon. This review provides an in depth analysis of the clinical impact that the implementation of rapid diagnostic techniques will have on unmet clinical needs.

Métodos de diagnóstico rápido en microbiología clínica: necesidades clínicas

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RESUMEN

Los métodos para diagnosticar enfermedades infecciosas han de ser rápidos, precisos, sencillos y asequibles. La rapidez en el diagnóstico puede jugar un papel crucial en la curación del paciente, ya que permite la administración de un tratamiento adecuado. Un aspecto que condiciona cada vez más la necesidad de disponer de técnicas de diagnóstico rápido es el aumento de las tasas de infecciones graves causadas por bacterias resistentes a los antibióticos, lo que ocasiona una elevada probabilidad de error en el tratamiento antibiótico empírico. Algunos de los métodos convencionales, como la tinción de Gram o la detección de antígenos pueden generar resultados en menos de una hora pero adolecen de sensibilidad.

En la actualidad estamos asistiendo a un cambio importante en los laboratorios de microbiología clínica, en el que se incluyen avances tecnológicos tales como el diagnóstico molecular, la microbiología digital y las técnicas de espectrometría de masas. Existen diversos estudios que demuestran que dichos cambios en el diagnóstico microbiológico reducen el tiempo de generación de los resultados de las pruebas, lo cual posee un impacto clínico evidente.

Sin embargo, si miramos hacia el futuro, otras nuevas tecnologías están en el horizonte, las cuales permitirán cubrir las necesidades que se requieren en el diagnóstico microbiológico rápido. Esta revisión

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proporciona un análisis en profundidad del impacto clínico que la implementación de técnicas de diagnóstico rápido tendrá en las necesidades clínicas no satisfechas.

Introduction

The "trending topic" in the field of biomedicine right now is personalised medicine, also called precision medicine, stratified medicine, and P4 medicine (predictive, personalised, preventive and participatory). It is understood as using the right drug for the indicated person at the right time. Although this concept has been gaining more importance in the area of cancer, if we consider all the specialties, we could say that clinical microbiology, and specifically diagnostic microbiology, is the paradigm of personalised medicine. Several diagnostic methods can be used ranging from direct methods, by directly detecting the microorganism causing the infection, such as microscopy, cultures, specific gene detection and antigen detection, to indirect methods, such as serology, in which the levels of specific antibodies against certain microorganism antigens are detected. In general, diagnostic methods must be fast, precise, simple and affordable. Evidently, some of the above-mentioned methods, such as Gram-staining, antigen detection or gene detection, present several of these characteristics. However, the primary requirements for a diagnostic method are high specificity and sensitivity. Other interesting collateral properties, although not essential, would be the possibility of being automated and being cost-effective.

For some infections, early diagnosis and treatment may have a crucial role in curing the patient or in reducing their morbidity and mortality, since the right antibiotic treatment is administered at the right time when needed. One aspect that is increasingly conditioning the need for fast diagnostic techniques is the rise in the rate of severe infections caused by antibiotic-resistant bacteria, which causes a high probability of error in empirical treatment.

At present, we are witnessing a significant change in the clinical microbiology laboratories led by automation. This trend is supported by technological advances such as molecular diagnostics, digital microbiology and mass spectrometry techniques (MALDI-ToF and ESI-ToF). These advances open the door to greater standardisation in the processes and results, a new level of operational excellence and performance, as well as better laboratory efficiency. There are several studies demonstrating that such changes in diagnostic microbiology reduce the time for generating test results, which has a clear clinical impact.

Despite the fact that clinical microbiology laboratories are implementing the many advances taking place in our field, if we look towards the future, other new techniques are on the horizon, including next-generation sequencing. Although it is only used in a few laboratories at present, it is undoubtedly one method to keep an eye on, since the bioinformatics analysis time and cost are being optimised.

In this review, we intend to analyse in detail the clinical impact that implementing these rapid diagnostic techniques will have, as well as the unmet clinical needs.

Clinical impact and need for rapid diagnosis

Sepsis

The fact is clear that a delay in starting a suitable antibiotic treatment for sepsis increases the risk of mortality.[1] Until the advent of molecular diagnostic tests, blood cultures were and continue to be the standard method for routinely detecting pathogenic bacteria and fungi in blood. However, blood cultures have limitations inherent to the methodology, including the time delay in obtaining results. At present, implementing direct MALDI-ToF from the positive blood culture along with detecting certain resistance genes (essentially the *mecA* gene and genes coding for ESBLs and carbapenemases), as well as multiplex PCR-based techniques for detecting the pathogens that most often cause bacteraemia and their resistance determinants, have had a significant clinical and economic impact by reducing the time to establish the right treatment to 46 h.[2–5]

Sepsis is generally treated empirically, using broad-spectrum antibiotics. However, broad-spectrum antibiotics are not always sufficient for treatment since resistance to antimicrobials is increasing. Studies have demonstrated that every hour of delay in implementing an effective treatment in sepsis patients leads to a 7.6% increase in mortality.[1] Molecular diagnostic techniques that detect specific genes directly in blood produce results faster than blood cultures since they avoid the antimicrobial growth time. Nevertheless, these new diagnostic techniques also have their limitations. Interpreting the results is sometimes complicated given that these molecular tests detect the DNA of microorganisms rather than live pathogens, in addition to the risk of interference from contamination, the presence of "background" DNA in blood and the lack of an ideal "gold standard".[6] Another limitation is that the antimicrobial sensitivity results cannot always be provided simultaneously. For this reason, such techniques are usually seen more as a potentially useful tool that complement conventional blood cultures and not as a definitive method that would exempt the use of blood cultures altogether.[7] As a result, blood cultures continue to be the cornerstone for diagnosing sepsis, since it is a prerequisite for the antimicrobial sensitivity tests. The main future need for diagnosing sepsis is to identify the causative microorganism and, in addition, to ascertain the antibiotic sensitivity directly from blood. An ideal test would be capable of processing a small volume of blood and be fast, technically simple or automated, low cost, and not require batch processing. An additional advantage would be the possibility of being able to determine the bacterial load directly from the blood. The published data indicate that determining the bacterial load in clinical samples using quantitative PCR (qPCR) potentially represents a useful marker for assessing the efficacy of a treatment and the prognosis in patients with acute bacterial infections.[8]

qPCR-based diagnostic tests will continue to grow in the coming years, however new techniques will emerge, especially based on microfluidics and nanotechnology, which will enable antibiotic sensitivity to be determined directly from the microorganism present in the blood without having to pass through blood cultures.

Respiratory infections

Until recently, when the topic of rapid diagnostics in respiratory infections arose, many continued thinking about the various direct stains for respiratory tract samples. These classic techniques enabled us, and still enable us, to assess the sample cellularity, and thus approximate the clinical value of the isolate on the one hand, and to distinguish the presence of microorganisms typically considered respiratory pathogens on the other. Afterwards, direct immunofluorescence stains were added for diagnosing *Legionella pneumophila* and viral infections. The arrival of