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Continuing medical education: Methods of rapid diagnosis

Rapid methods for detection of bacterial resistance to antibiotics☆

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ABSTRACT

The most widely used antibiotic susceptibility testing methods in Clinical Microbiology are based on the phenotypic detection of antibiotic resistance by measuring bacterial growth in the presence of the antibiotic being tested. These conventional methods take typically 24 h to obtain results. Here we review the main techniques for rapid determination of antibiotic susceptibility. Data obtained with different methods such as molecular techniques, microarrays, commercial methods used in work routine, immunochromatographic methods, colorimetric methods, image methods, nephelometry, MALDI-TOF mass spectrometry, flow cytometry, chemiluminescence and bioluminescence, microfluids and methods based on cell disruption are analysed in detail.

Métodos rápidos para la detección de la resistencia bacteriana a antibióticos

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RESUMEN

Los métodos más frecuentemente utilizados en Microbiología Clínica para la determinación de la sensibilidad de las bacterias a los antibióticos se basan en un estudio fenotípico, observando el crecimiento bacteriano de una cepa incubada en presencia del antibiótico a estudiar. Estos métodos requieren normalmente un tiempo de unas 24 h para la obtención de resultados. En esta revisión se exponen el fundamento y los resultados de las principales técnicas instrumentales que proporcionan un antibiograma rápido. De manera pormenorizada se exponen datos relativos a técnicas moleculares, *microarrays*, métodos comerciales utilizados en el trabajo de rutina, técnicas inmunocromatográficas, métodos colorimétricos, métodos de imagen, nefelometría, espectrometría de masas MALDI-TOF, citometría de flujo, quimioluminiscencia y bioluminiscencia, microfluidos y métodos de lisis bacteriana.

Introduction

To achieve a favourable clinical course in patients who suffer from infections, the clinical microbiology laboratory should report the agent causing the infection as quickly as possible. If it is a bacterium that does not have uniform sensitivity to antibiotics, the antibiogram should also be reported, since the significant increase in antimicrobial resistance represents an obstacle to empirical treatment in some cases.

Routine antibiogram techniques are based on a phenotypic study in which microbial growth is observed in the presence of different antibiotics. These techniques include agar dilution (the gold standard for the antibiogram), broth macrodilution and microdilution, and strips with an antibiotic gradient. They yield results in around 17 h. To shorten this time, it would be desirable to have fast and reliable antibiogram results. To evaluate reliability, according to the US Food and Drug Administration (FDA),[1] the results of a rapid antibiogram are classified, compared to the antibiogram obtained through the gold standard, as agreements (concordance), minor errors (erroneous intermediate sensitivity result), major errors (false resistance) and very major errors (false sensitivity).

There are many instrumental techniques that allow an antibiogram to be made quickly. Notable among these are molecular techniques, microarrays, commercial methods used in

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routine work, immunochromatographic techniques, colourimetric methods, imaging methods, nephelometry, MALDI-TOF mass spectrometry, flow cytometry, chemiluminescence and bioluminescence, microfluids and bacterial lysis methods. The basis of each of these techniques and the results obtained are presented below.

Molecular techniques

Molecular techniques enable the detection of genetic material, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Polymerase chain reaction (PCR) is the molecular technique that has acquired the greatest diagnostic value, since it not only allows the infectious agent to be accurately identified, but is also the leading method to characterise its resistance and virulence genotypes. Conventional PCR requires approximately 12 h to perform and consists of 3 steps. The first step consists of extraction of genetic material. The second step, performed in a thermocycler, consists of DNA amplification. The thermocycler reaches the optimal temperatures required for each of the 3 steps comprising an amplification cycle (denaturation of the DNA to be used as a mould, ringing of synthetic primers and extension catalysed by the polymerase DNA of the primers) to take place. Amplification is repeated a certain number of times, generally 25–35. Each time, the number of product molecules (amplicons) is duplicated. Thus a high number of amplicons is synthesised, which allows very small initial amounts of DNA to be detected.[2] The third and final step of PCR consists of detection of amplicons through agarose gel electrophoresis. Real-time PCR was designed to shorten the time to diagnosis of conventional PCR. In real-time PCR, amplification and detection of the amplicons synthesised take place at the same time through different methods. Thus, real-time PCR yields results in a few hours.[2]

Several real-time PCRs which identify several pathogenic agents and genes that directly confer antibiotic resistance based on different samples have been marketed. These PCRs are fully automated: samples are processed in just a few minutes. The Verigene® system (Nanosphere) is based on grown blood culture bottles. Detection of amplicons takes place through hybridisation with synthetic specific oligonucleotides marked with gold nanoparticles. In Gram-positive bacteria, it detects 9 species and 4 genera of bacteria, as well as the resistance genes *mecA* and *vanA/B*, in less than 3 h with a sensitivity and a specificity very close to 100%.[3] In Gram-negative bacteria, it detects 5 species and 4 genera of bacteria, as well as the resistance genes that encode CTX-M extended-spectrum beta-lactamases (ESBLs) and KPC, NDM, VIM, IMP and OXA carbapenemases, with the same response time and with a sensitivity and a specificity in excess of 93%.[4] The FilmArray Blood Culture Identification Panel system (BioFire Diagnostics) is also applied to the grown blood culture bottle. In this case a nested PCR is performed. First, a region of DNA that contains the target segment is amplified. Next, this amplification product is used as a mould for a second PCR which takes place in a matrix with wells that contain the primers for the different assays. Finally, the instrument uses the fluorochrome LCGreen® Plus (BioFire Diagnostics) to evaluate the fusion curve of the DNA in each well of the matrix to determine whether a PCR product appears in said well. In an hour, this system detects 11 species and 15 genera of Gram-positive and Gram-negative bacteria and 5 species of yeast. It also detects the resistance genes *mecA*, *vanA/B* and *KPC*. Sensitivity ranges from 83% to 100%, and specificity is more than 99%, depending on the pathogen studied.[3] The GeneXpert® system performs real-time PCR in single-use disposable cartridges. There are many cartridges for performing different analyses. To detect *Staphylococcus aureus* and its methicillin resistance in clinical samples, 2 cartridges are available: the Xpert® MRSA/SA BC cartridge, which uses grown blood culture bottles, and the Xpert® MRSA/SA SSTI cartridge, which uses swabs to diagnose skin and soft-tissue infections. Both tests yield results in an hour and have a sensitivity and a specificity very close to 100%.[3,5] The Xpert MTB/RIF® cartridge detects *Mycobacterium tuberculosis* and its rifampicin resistance in sputum and biological fluids in 2 h. In this case a semiquantitative nested PCR takes place. It has been observed that in sputum and bronchoalveolar lavage, the test has a sensitivity and a specificity of 86.8% and 93.1%, respectively, thereby improving sputum smear microscopy results.[6]

Many PCRs – "in-house", commercial and automated – and PCR kits that accurately detect, with a sensitivity and a specificity of practically 100%, a large number of genes that confer antibiotic resistance have been described in the literature.[7] It should be noted that these methodologies do not provide microbial identification and that they are applied, in the majority of cases, to colonies grown on isolation plates.

The main commercial automated real-time PCRs are detailed below. The NucliSENS EasyQ® KPC platform (bioMérieux) detects genes that encode KPC carbapenemases in 2 h.[8] The Xpert® Carba-R cartridge from GeneXpert® detects genes that encode KPC, NDM, VIM, IMP and OXA-48 carbapenemases in an hour.[9] The eazyplex® system (Amplex Biosystems GmbH) consists of a platform that performs nucleic acid amplification through the loop-mediated isothermal amplification (LAMP) technique. This technique is based on DNA amplification through chain displacement at a constant temperature; thus, the system does not require a thermocycler. To carry out amplification, the DNA extracted is inserted in a tube containing a lyophilisate with the reagents required to perform nucleic acid amplification; next, this tube is inserted in the Genie® II apparatus (OptiGene), which keeps the tube at a constant temperature and detects the amplicons formed in real time. For this platform, several kits have been marketed that detect genes that confer antibiotic resistance in less than 30 min. Notable among them is the eazyplex® SuperBug CRE which detects genes that encode CTX-M ESBL and VIM, NDM, KPC and OXA-48 carbapenemases, both in colonies grown on isolation plates and directly from urine samples and grown blood culture bottles.[10] AID Autoimmun Diagnostika GmbH has marketed a PCR based on line probe assays. In this case, once the PCR has been performed, reverse hybridisation of the amplicons takes place with complementary probes anchored to a nitrocellulose strip. Hybridisation is detected using a biotin marker present in the primers used in the PCR. Thus a band pattern is obtained which is interpreted visually or with a scanner. Notable among antibiotic resistance kits is the AID ESBL, which detects genes that encode TEM, SHV and CTX-M ESBLs and KPC carbapenemases in 5 h.[11]

Notable among the kits marketed to detect genes that confer bacterial resistance to antibiotics are the following: LightMix (Roche Diagnostics) and Check-Direct CPE (Check-Points Health B.V.). The LightMix kit, using the LightCycler® 480 Instrument II platform (Roche Diagnostics), detects KPC, NDM, VIM, IMP and OXA-48 carbapenemases in an hour and a half. It should be noted that this kit may also be applied to grown blood culture bottles.[12] The Check-Direct CPE kit may be used on several real-time PCR platforms, such as the ABI 7500 (Applied), CFX96™ (Bio-Rad), LightCycler® 480 system I & II (Roche), Rotor-Gene Q (Qiagen) and BD MAX™ (Becton Dickinson) platforms. This kit includes the reagents required to detect genes that encode KPC, NDM, VIM and OXA-48 carbapenemases in 2 h.[9] In addition, some kits to perform a ligation-mediated PCR have been marketed. Briefly, this PCR uses 2 DNA probes that recognise one of the 2 strands of the gene to be detected. If hybridisation occurs, the probes remain adjacent and a DNA ligase binds them, thereby generating a double-chain DNA. This ligation step is performed in the MyCycler apparatus (Bio-Rad). Finally, real-time PCR takes place on the ABI 7500 (Applied) platform using some universal primers. Notable among the kits that detect genes that encode bacterial resistance to antibiotics