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Popular Article

Rapid and Emerging Technology for Antibiotic Susceptibility Testing

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Abstract

Antimicrobial resistance (AMR) is a global problem resulting in a year-on-year increase in the incidence of drug resistant infections. AMR is expected to be responsible for 10 million deaths annually by 2050. The emergence and spread of antibiotic-resistant bacteria are aggravated by incorrect prescription and use of antibiotics. A core problem is that there is no sufficiently fast diagnostic test to guide correct antibiotic prescription at the point of care.

Introduction

Nowadays increasing emergence and spread of antibiotic-resistant bacteria, a key factor in correct treatment of infections is the ability to rapidly identify the antibiotic susceptibility profile of the infecting species to assure the use of an efficacious antibiotic and reduce the need for broad spectrum drugs. Phenotypic antibiotic susceptibility tests are typically based on the detection of differential bacterial growth with and without antibiotics in liquid cultures or on solid agar plates. In broth tests, detection is based on the change in OD, whereas the disk diffusion method is used on solid agar plates to identify inhibition zones. These methods are generally reliable for detecting resistance and determining the antibiotic concentration that prevents bacterial growth, making them predictive of the therapeutic utility of different antibiotics. However, because it typically takes 1–2 d to get a reliable readout, these methods fail to guide treatment in the early, often critical, stages of infection.

Genotypic ASTs are based on detection of a specific genetic marker (plasmids, genes, or mutations) associated with resistance phenotypes by using the common genetic tools (e.g., sequence-specific amplification by PCR, probe-mediated rolling circle amplification, or whole-genome sequencing). These tests are highly sensitive and can limit the detection time to what is needed to amplify selected DNA sequences to detectable levels, but they require detailed advance knowledge of which resistance markers to test for.

Limitations

If new resistance mechanisms arise, these would go undetected and result in false negatives. The presence of certain resistance genes/mutations does not necessarily translate into phenotypic resistance.

Microfluidic chip-based susceptibility testing

Antibiotic susceptibility testing is done in less than 30 minutes using direct single-cell imaging in the microfluidic chip. In this method, capture bacterial cells directly from samples with low bacterial counts (104 cfu/mL) using a custom-designed microfluidic chip and monitor their individual growth rates using microscopy. By averaging the growth rate response to an antibiotic over many individual cells, push the detection time to the biological response time of the bacteria.

Raman Micro spectrometry-based susceptibility testing

Raman micro spectroscopy has been proposed as a means to achieve antibiotic susceptibility testing. Raman micro spectrometry is a particularly attractive option. The microbial Raman information is known for long to provide identification information down to the strain level. More recently, it was used to probe the differences between resistant and susceptible phenotypes of microbial cell clusters in the presence of antimicrobials.

MALDI-TOF MS-based susceptibility testing

MALDI-TOF MS (Matrix- Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) is an innovative tool that's has been recently integrated into the microbiology laboratory workflow as an easy to use, rapid, accurate, and cost-effective technique-with more specificity which has revolutionized bacterial identification in clinical microbiology laboratories. MALDI-TOF MS, introduced in 2000, is another sensitive method for bacterial identification. The newly developed MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA) is a more-straightforward and cost-effective modulation of MALDI-TOF MS used for both AST and MIC determination.

Table 1: Technologies for rapid phenotypic growth-based antimicrobial susceptibility testing

S. No.	Technology	Short description
1.	Disk-tube method	Growth-based method in liquid medium with visual evaluation of turbidity
2.	Colorimetric method utilizing a pH indicator	Bacterial growth is measured by using a medium containing a pH indicator (phenol red)
3.	Colorimetric method utilizing a redox indicator	Bacterial growth is detected by using a medium containing a redox indicator (resazurin)
4.	Microfluidic agarose channel system with microscopic single cell growth tracking	Bacteria immobilized in the agarose matrix in a microfluidic channel; the growth of single cells is monitored using microscopy
5.	Forward laser light scattering	Optical growth detection in a liquid sample by the laser scattering method
6.	Digital time lapse microscopy	Optical growth detection by serial imaging in a liquid sample
7.	Microbial cell mass measurement	High resolution mass measurement using microchannel cantilevers
8.	Real-time PCR	After an incubation in liquid medium, real-time PCR is used for quantification of DNA copies of either the 16SRNA genes or rpoB
9.	ATP-bioluminescence	Luciferin-luciferase assay produces light in the presence of ATP. The produced light is proportional to the bacterial ATP and, thus, to the microbial concentration
10.	Morphokinetic cellular analysis	Bacterial cells are immobilized on a surface, digital microscopy records microbial response to a single concentration of an antibiotic and software derives MIC values
11.	Flow cytometry	Assessment of drug-induced microbial lesions that lead to changes in morpho-functional parameters (e.g. membrane potential, cell size, amount of DNA)

Conclusions

Overall, the rapid antibiotic susceptibility testing approach shows utility for the rapid detection of antibiotic susceptibility across a range of clinically important pathogen–antibiotic combinations. The simplicity of the technique suggests that the method is suitable for a new generation of rapid tests for the clinical laboratory. Only affordable and easy-to-use rapid antibiotic susceptibility testing methods will have the chance to become widely accepted.

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