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Commentary

Latest Developed Methods for Antimicrobial Susceptibility Testing

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DESCRIPTION

Antimicrobial susceptibility testing is used to determine which specific antibiotics a particular bacterium or fungus is susceptible to mostly. In most cases, this test complements the Gram stain and culture and provides much faster results. Susceptibility is the term used when microorganisms such as bacteria and fungi cannot grow in the presence of one or more antimicrobial agents. A susceptibility test is performed on a bacterium or fungus that causes infection in humans after it has been obtained by culturing a sample. Performing Antimicrobial Susceptibility Testing (AST) of bacterial pathogens is an important task for determining susceptibility to antimicrobial agents and detecting potential drug resistance in clinical microbiology laboratories. The Kirby-Bauer agar diffusion method is a well-documented and standardized method for determining antimicrobial susceptibility. A susceptibility test is an overall profile of antimicrobial susceptibility testing results for a particular organism to various antimicrobials. Rapid identification of pathogens and their antimicrobial resistance followed by appropriate antimicrobial therapy is essential for accurate patient outcomes. Traditional methods for detecting bacterial resistance, such as disc diffusion, culture micro dilution, and automated instrumentation, are still widely used and largely standardized. Some of the AST phenotypic methods that detect phenotypic resistance by measuring bacterial growth in the presence of a tested antimicrobial are among the most modern diagnostic tools in clinical microbiology laboratories, including their advantages over genotypic methods. The first advantage is the ability to predict drug resistance and susceptibility. The second is the ability to enumerate the degree of pathogen susceptibility to antimicrobial agents (quantitative AST).

Classical Antimicrobial Susceptibility Testing (AST) methods

Disk diffusion methods: Developed in 1940, the agar disk diffusion assay is one of the oldest methods of routine AST and one of the most common manual techniques of AST in clinical microbiology laboratories. The main advantages are simplicity, reproducibility, easy modification of antimicrobial discs, potential use as a screening test against a large number of isolates. Disc diffusion refers to the diffusion of a specific concentration of antimicrobial agent from a disc, tablet or strip into solid culture medium inoculated with a selected inoculum isolated in pure cultures. Disc spreading is based on the determination of a zone of inhibition that is proportional to the susceptibility of bacteria to antimicrobial agents present in the disc.

Antimicrobial gradient method: The antimicrobial gradient strip method combines the principles of dilution and diffusion methods. ETEST, MIC Test Strip, MIC Evaluator, and Ezy MIC Strip are commercial versions of this method. The antimicrobial susceptibility dilution method appears to be more reproducible and quantitative than agar disk diffusion. However, antibiotics are typically tested at 2-fold dilutions, which can lead to inaccurate Minimum Inhibitory Concentration (MIC) data.

Dilution methods: The dilution method is the AST reference method and is used to determine the MIC value of antimicrobials tested on agar plates (agar dilution) or broth media (broth micro dilution or macro dilution). Both agar and broth dilution methods can be used to quantitatively measure antimicrobial activity against fastidious or capricious bacteria yeasts and molds. Dilution methods are useful for comparative testing of new antibiotics for resistance after disk test surveillance is inconclusive, for susceptibility testing of strains where disk testing may not be reliable, and for quantitative testing in clinical management. Broth dilution is a technique in which a given optimal or appropriate concentration of bacterial suspension is tested against various concentrations of an antimicrobial agent (usually two-fold serial dilutions) in liquid media of a given, documented formulation. The broth dilution method can be performed in tubes with a minimum volume of 2 macro dilution (mL) or in small volumes using microliter plates (micro dilution).

Broth macro dilution: The broth macro dilution (or tube dilution) method was one of the earliest AST methods, but was replaced by the broth micro-dilution method. Its major drawbacks include the relatively large space and reagents required its labor-intensive nature, and the potential for error in

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the preparation of the antibiotic solution for each test. However, the advantage of this method is that it gives quantitative results.

Broth micro dilution: The miniaturization of the test has made the broth micro dilution method more convenient and popular. This technique is typically performed using small sterile disposable polystyrene microliter plates containing 96 wells. Advantages of broth micro dilution include reproducibility, low sample volumes required, and low cost allowing for large numbers of replicates. This method is more efficient and easier than the macro dilution methods.

Agar dilution: The agar dilution procedure is performed by introducing various concentrations of antimicrobial agents into molten agar, typically using two-fold serial dilutions, followed by inoculation of standardized microbial inoculum onto the surface of the agar plates. This method is suitable for both antibacterial and antifungal susceptibility testing and is used as a standardized AST method for fastidious organisms such as anaerobes and Helicobacter spp.

Other bacterial AST and specific antimicrobial resistance tests: Bacterial antibacterial MICs can also be obtained using commercially available gradient strips that distribute predetermined concentrations of antibiotics. However, the use of gradient strips can be very expensive, and when testing certain combinations of bacteria and antimicrobial agents, researchers have found discrepancies in MIC compared to agar dilution results. Bacterial antibacterial MICs can also be obtained using commercially available gradient strips that distribute predetermined concentrations of antibiotics. However, the use of gradient strips can be very expensive, and MIC may be inconsistent compared to agar dilution results when testing certain combinations of bacteria and antimicrobial agents.

The use of genotypic approaches to detect antimicrobial resistance genes is being promoted as a way to improve the speed and accuracy of susceptibility testing. Methods utilizing comparative genomics, gene probes, microarrays, nucleic acid amplification techniques, and DNA sequencing promise to improve the sensitivity, specificity, and speed of detection of certain known resistance genes. New technological advances will facilitate the ability to rapidly and inexpensively screen bacterial species for a large number of antimicrobial resistance genes, potentially providing additional relevant data for surveillance and surveillance programs.