Antimicrobial Susceptibility Testing: A Comprehensive Review of Current Methods and Challenges

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ABSTRACT

In the face of a growing global public health threat posed by antimicrobial resistance (AMR), accurate antimicrobial susceptibility testing (AST) has become increasingly crucial for effective clinical diagnostics. This comprehensive review article delves into the intricacies of current AST methods, shedding light on their limitations and their role in addressing the evolving challenges of AMR in healthcare. The review commences with a historical exploration of AST, tracing its development and establishing its significance in guiding therapeutic decisions. It then embarks on a detailed analysis of the diverse AST methods and technologies employed in clinical practice, providing insights into their respective strengths and weaknesses. The clinical relevance of AST results is emphasized, highlighting the pivotal role these tests play in the selection of effective antimicrobial agents. Furthermore, the review scrutinizes the pressing limitations and challenges faced in contemporary AST. By bridging the gap between laboratory-based AST methods and clinical realities, this review contributes significantly to the ongoing efforts to enhance diagnostic precision, optimize treatment regimens, and combat AMR. It serves as a valuable resource for clinicians, microbiologists, and researchers engaged in the fight against infectious diseases and antimicrobial resistance, offering a roadmap for future advancements in clinical diagnostics and patient care.

KEYWORDS: Antimicrobial Susceptibility Testing (AST), Antibiotic Resistance, AST Methods, Minimum inhibitory concentration (MIC), Diagnostic methods

1.0 INTRODUCTION

Antimicrobial resistance (AMR), an ever-growing global health threat, demands the development and implementation of accurate and reliable antimicrobial susceptibility testing (AST) methods for clinical diagnosis. The roots of AST can be traced back to the early 20th century, with the pioneering work of Paul Ehrlich, who recognized the importance of matching antimicrobial agents to specific pathogens. Ehrlich’s groundbreaking work laid the foundation for the development of in vitro AST methods, which have since become indispensable tools in the fight against infectious diseases¹. AST provides clinicians with crucial information about the susceptibility of bacterial pathogens to various antimicrobial agents. This information is essential for selecting appropriate antimicrobial therapy, ensuring effective treatment of infections, and minimizing the risk of antimicrobial resistance development. This comprehensive review delves into the intricacies of current AST methodologies, placing a particular focus on the Kirby Bauer disc diffusion assay, E-test, Broth Dilution, Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), VITEK 2 systems, Fluorescence-Activated Cell Sorting, and PCR-based methods. These diverse AST techniques represent a critical arsenal in the fight against AMR, each offering unique advantages and facing
distinct limitations. AST has evolved into an indispensable tool for clinicians, enabling them to effectively diagnose and treat infections, particularly in the face of escalating antimicrobial resistance. This review traces the historical development of AST techniques, contextualizing their significance in informing clinical decisions. We then embark on a thorough examination of the AST techniques, highlighting their respective strengths, limitations, and clinical relevance. Contemporary AST practices face several challenges, particularly in the context of escalating multidrug-resistant pathogens. This review scrutinizes these challenges, discussing patient-specific factors that influence treatment outcomes and the growing urgency for rapid AST methods to address emerging microbial threats promptly. In bridging the gap between laboratory-based AST methodologies and clinical realities, we aim to provide valuable insights for healthcare professionals, microbiologists, and researchers engaged in combating infectious diseases and AMR. Our collective goal is to improve the diagnosis and treatment of infectious diseases, including those caused by antimicrobial-resistant pathogens, by enhancing our understanding of the strengths and limitations of AST methods.

2.0 HISTORY AND PRINCIPLES OF CURRENT ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS

1. Kirby Bauer disc diffusion assay

The Kirby-Bauer disk diffusion assay (KB disk diffusion test) is a standard microbiological method for determining the in vitro susceptibility of bacteria to antimicrobial agents. It was first developed in the early 1950s by William Kirby and A. Wade Bauer, and was standardized by the World Health Organization in 1961. The KB disk diffusion test is still widely used today, both in clinical and research laboratories. This widely used method in clinical microbiology laboratories has stood the test of time and is considered one of the most reliable techniques for antimicrobial susceptibility testing. Even today, it remains the preferred manual method for AST, especially in laboratories with low to medium testing volumes, as affirmed by the Clinical and Laboratory Standards Institute (CLSI). In a disk diffusion test, bacteria are examined to determine their sensitivity to various antibiotics. The presence of a clear and distinct circular area around the antibiotic disk, called the zone of inhibition (ZOI), indicates that the antibiotic effectively inhibits bacterial growth, classifying it as "susceptible." Conversely, if bacterial growth is unaffected by an antibiotic, it is considered "resistant" or ineffective. The CLSI-modified Kirby-Bauer disk diffusion technique is now recommended by the WHO because it promotes reproducibility and comparability between laboratories. In this procedure, a standardized amount of the test organism (0.5 McFarland turbidity, approximately 1.5 x 10^8 colony-forming units/mL) is spread onto a 150 mm diameter plate containing Mueller-Hinton agar (MHA). Then, commercially available or in-house prepared antibiotic paper disks containing a specific concentration of antibiotics (approximately 6 mm in diameter) are placed on the bacterial lawn (12 disks on a 150 mm MHA Petri plate). The agar plate is then incubated at 35-37 °C, usually overnight, to allow the antimicrobial agents to diffuse into the agar and inhibit bacterial growth. The zone of inhibition (ZOI) is manually measured in millimeters using a sliding calliper or ruler. The ZOI diameter provides qualitative results indicating susceptibility, intermediate resistance, or full resistance of the bacteria. The CLSI-modified Kirby-Bauer disk diffusion method using Mueller-Hinton agar (MHA) has been effectively used to determine antimicrobial susceptibility of fastidious bacteria such as Streptococci, Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, and Haemophilus parainfluenzae. Other media such as nutrient agar (NA), Tryptone soy agar (TSA), Columbia blood agar base (CBA), and peptone water and agar (PWA) have been shown to produce comparable results to MHA when following CLSI standards for AST, but MHA remains the most widely used medium, likely due to its low cost and ready availability.

2. E-Test

The Etest was developed in the late 1980s by AB BIODISK (now bioMérieux) in Sweden. It was first introduced commercially in 1991 and has since become a widely used method for antimicrobial susceptibility testing in clinical microbiology laboratories. The Etest revolutionized the way microbiologists and healthcare professionals assess the susceptibility of bacteria and other microorganisms to antibiotics. At its core, the Etest operates on a straightforward yet ingenious principle, combining the principles of both diffusion and dilution techniques to provide a quantitative measure of the microorganism’s susceptibility
to a particular antimicrobial agent. The Etest is valuable in clinical microbiology and research settings, and its principle is based on the minimum inhibitory concentration (MIC), the lowest concentration of an antimicrobial agent that effectively inhibits the growth of a microorganism. The Etest employs a gradient strip containing a predefined concentration gradient of the antibiotic to be tested. A culture of the target microorganism is inoculated onto the surface of a solid growth medium, and the Etest strip is placed on the agar. The antibiotic then diffuses from the strip into the agar, forming a concentration gradient. As the antibiotic diffuses, it inhibits microbial growth, creating an elliptical zone of inhibition. The MIC can be determined by identifying the point at which the ellipse intersects the Etest strip. The lower the MIC, the more susceptible the microorganism is to the antibiotic.

3. Broth Dilution

The broth microdilution method for antimicrobial susceptibility testing was first developed in the 1960s and has since been adapted to test a wide range of microorganisms, including bacteria, fungi, and yeasts. It encompasses two techniques: microdilution and macrodilution. Both are widely used, but they differ in scale, volume, and precision. In broth macrodilution, a serial dilution of antimicrobial agents is prepared in a liquid medium and dispensed into test tubes (also known as the tube dilution method). It is one of the most established and widely practiced AST methods. Both broth microdilution and macrodilution methods are used to measure the minimum inhibitory concentration (MIC) of antibiotics, which provides valuable information on antimicrobial susceptibility. Broth macrodilution follows a standardized procedure to ensure accurate and reproducible results. A stock solution of the antimicrobial agent is diluted in liquid broth to produce a series of decreasing concentrations, typically two-fold dilutions, allowing for a precise determination of the MIC. The isolate under investigation is then added to each of the antibiotic dilutions. A standardized inoculum size is crucial to achieve consistent outcomes. The tubes are then incubated at an appropriate temperature for a defined period, which may vary depending on the bacterial species being tested. After incubation, the tubes are observed for visible antimicrobial growth. The absence of growth in a particular concentration indicates bacterial inhibition by the antibiotic. The MIC is recorded as the lowest antibiotic concentration at which no visible growth is observed. In broth microdilution, the antimicrobial agent is diluted in a liquid broth and dispensed into a microdilution plate, where the microbial growth is assessed at different concentrations according to CLSI standards. Each dilution represents a decreasing concentration of the antibacterial agent. The bacterial isolate under investigation is then added to each well of the microdilution plate. A standardized bacterial inoculum is crucial to maintain consistency. The microdilution plate is then incubated at the appropriate temperature and for a defined period, allowing bacterial growth under specific conditions. After incubation, the wells are examined for visible bacterial growth. The MIC is determined as the lowest concentration at which no visible growth is observed.

4. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF-MS is an analytical technique invented in the 1980s for protein identification and characterization. It has since evolved to be an efficient method for identification of bacterial isolates. The use of MALDI-TOF MS for antimicrobial susceptibility testing (AST) is a relatively new development. The first studies evaluating the use of MALDI-TOF MS for AST were published in the early 2000s. These studies showed that MALDI-TOF MS could be used to identify microorganisms and determine their susceptibility to a variety of antimicrobial agents. Since then, there has been a growing body of research on the use of MALDI-TOF MS for AST. A number of different MALDI-TOF MS-based AST methods have been developed, and some of these methods are now being used in clinical laboratories. One of the most promising MALDI-TOF MS-based AST methods is called direct-on-target microdroplet growth assay (DOT-MGA). In DOT-MGA, microorganisms are grown in microdroplets on a MALDI target plate in the presence of antimicrobial agents. The growth of the microorganisms is then assessed using MALDI-TOF MS. Another promising MALDI-TOF MS-based AST method is called minimal profile change concentration (MPCC). In MPCC, the protein profiles of microorganisms are compared before and after exposure to antimicrobial agents. The MPCC is the lowest concentration of an antimicrobial agent that causes a significant change.
...in the protein profile of the microorganism. This revolutionary technique operates on the principle of ionization and detection of microbial biomolecules, primarily proteins, to create mass spectra specific to each microorganism. MALDI-TOF MS can be used to identify microorganisms at the genus, species, and even strain levels by comparing their peptide mass fingerprint (PMF) patterns to a vast open database of PMFs associated with ribosomal proteins. Samples for MALDI-TOF MS are obtained from pure microbial colonies and deposited onto a MALDI target plate. The colony is then overlaid with a matrix solution, such as α-cyano-4-hydroxycinnamic acid. The sample plate is allowed to air-dry, forming crystallized spots of the microbial-matrix mixture. The prepared target plate is loaded into the MALDI-TOF MS instrument, and the instrument’s laser is used to ionize the microbial molecules in each spot. The resulting ions are detected and analyzed, and the generated mass spectra are compared to an extensive database containing reference spectra of known microorganisms. The instrument software identifies the microorganism based on the closest match between the sample’s mass spectrum and the reference spectra.

5. VITEK 2 systems

The VITEK 2 system is an automated system for the identification and antimicrobial susceptibility testing (AST) of bacteria. It was developed by bioMérieux and first released in 2005. The VITEK 2 system is widely used in clinical microbiology laboratories around the world. The VITEK 2 system is based on the VITEK system, which was first introduced in the 1970s. The VITEK system was one of the first automated systems for the identification and AST of bacteria. It was originally developed to test for urinary tract infections, but it was later adapted to test for a wider range of infections. The VITEK 2 system is an improved version of the VITEK system. It is faster, more accurate, and more versatile than the VITEK system. The VITEK 2 system can also be used to test for a wider range of antimicrobial agents. The VITEK 2 system uses the broth microdilution method to test for antimicrobial susceptibility. In broth microdilution, a series of two-fold dilutions of the antimicrobial agent are prepared in broth. The bacteria to be tested are then added to each dilution. The tubes are incubated overnight and then examined for turbidity. The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevented visible growth of the bacteria. The VITEK 2 system automates the broth microdilution method. The system uses a special card that contains 64 wells. Each well contains a different concentration of the antimicrobial agent to be tested. The bacteria to be tested are added to a single well on the card. The card is then placed in the VITEK 2 system. The VITEK 2 system incubates the card and then measures the turbidity of each well. The system then calculates the MIC for each antimicrobial agent. The results are displayed on the VITEK 2 system’s monitor. The VITEK 2 system is a valuable tool for clinical microbiology laboratories. It provides rapid and accurate results for AST, which can help clinicians to select the most appropriate antibiotic therapy for their patients.

6. PCR-based Antimicrobial Susceptibility Testing

PCR-based methods for antimicrobial sensitivity testing are designed to detect specific genetic markers associated with antibiotic resistance in bacterial strains. PCR-based AST methods were first developed in the early 1990s. The basic principle of these methods is to use PCR to amplify a DNA sequence that is associated with antimicrobial resistance. The amplified DNA sequence is then detected and analyzed to determine the presence or absence of the resistance gene(s). The presence or absence of resistance genes in the amplified products indicates the susceptibility or resistance of the bacterial isolate to specific antibiotics. The principle of PCR-based AST lies in identifying the genetic determinants that confer resistance to certain antimicrobial agents. The first step in PCR-based AST typically involves extracting bacterial DNA from the microbial isolate. Various DNA extraction methods, such as heat lysis or commercial DNA extraction kits, are used to obtain pure DNA. Specific primers are then designed to target the resistance genes of interest. PCR amplification is then performed, where the extracted DNA is subjected to multiple cycles of denaturation, annealing of primers, and extension using a thermostable DNA polymerase. This process amplifies the target gene if it is present in the bacterial DNA. Different methods are available for detecting PCR-amplified products to verify the existence of resistance genes. These include electrophoresis, Southern blotting, restriction fragment-length polymorphism (RFLP), single-strand conformation polymorph...
A method called loop-mediated isothermal amplification (LAMP) has also been employed for rapid, specific, and efficient antimicrobial susceptibility testing. LAMP amplifies DNA at a constant temperature of 60–65 °C using Bst DNA polymerase, instead of Taq polymerase. Another technique, real-time PCR, enables the quantification of amplified genes for AST purposes, utilizing hydrolysis probes, hybridization probes, or double-stranded DNA-binding fluorescent dyes. One of the most intriguing aspects of PCR-based methods is that the samples do not require sterility and can contain mixtures of bacteria. This makes PCR-based AST a powerful tool for clinical microbiology laboratories, as it can be used to test specimens directly from patients, such as blood, sputum, and urine.

7. Fluorescence-activated cell sorting (FACS)

Fluorescence-activated cell sorting (FACS) was first developed in the late 1960s by Bonner, Sweet, Hulett, Herzenberg, and others. The original goal of FACS was to develop a method for sorting cells based on their size and fluorescence. However, it was soon realized that FACS could also be used to sort cells based on the presence or absence of specific cell surface markers. In the early 1970s, FACS was first used to study the immune system. Researchers used FACS to sort different types of immune cells, such as T cells, B cells, and macrophages. This allowed them to study the function of each type of cell in more detail. In the 1980s, FACS began to be used for antimicrobial susceptibility testing (AST). FACS-based AST methods work by sorting bacteria based on their resistance to specific antibiotics. This allows researchers to quickly and accurately identify the best antibiotics to use to treat an infection. Fluorescence-activated cell sorting (FACS) is a specialized form of flow cytometry that utilizes optical and fluorescence characteristics to count and sort specific cells of interest from a heterogeneous mixture of biological cells into separate containers, one cell at a time. FACS is an invaluable tool used for various bacterial analyses, ranging from identification and counting of bacteria to investigating changes in cellular and metabolic activity, as well as identifying differential gene expressions. FACS can also be used to determine the viability and antibiotic susceptibility of bacteria in a mixture by interpreting the emitted light spectrum resulting from excitation within a short time of 2–3 hours. Permissible cells, when bound with fluorescent stains to their nucleic acids, emit stronger fluorescence than non-permissible cells, and fully lysed cells exhibit even greater fluorescence. However, it is important to note that the distinction of single cells from cell aggregates by flow cytometry may be inadequate, and thus, viability does not always correlate directly with the amount of fluorescence activity. Compared to conventional AST methods that take around 24 hours, FACS provides rapid results in just about 2 hours, as it relies on detecting physiological changes in bacteria caused by antibiotics rather than the inhibition of bacterial growth processes. FACS is an automated technique capable of processing thousands of cells per second, making it accurate, sensitive, and timesaving.

3.0 LIMITATIONS OF CURRENTLY USED ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS

The Disk Diffusion Method

The Disk Diffusion Method, while providing valuable insights into bacterial susceptibility to antibiotics, has certain limitations that must be considered for accurate interpretation of results and informed treatment decisions. The visual assessment of inhibition zones introduces subjectivity, as different laboratory technicians may have varying interpretations of zone sizes, potentially affecting the accuracy and reproducibility of results. Moreover, despite standardized guidelines, inconsistencies in media composition, inoculum density, and incubation conditions across laboratories can lead to disparate results and hinder the comparability of susceptibility data between studies and institutions. Furthermore, the Disk Diffusion Method primarily detects resistance mechanisms that manifest as visible growth inhibition zones. It may fail to detect certain resistance mechanisms, such as efflux pumps or enzymatic inactivation of antibiotics, which do not produce visible zones. This limitation can lead to false susceptibility results and potentially influence treatment decisions. Additionally, the Disk Diffusion Method’s limited antibiotic panel allows for the simultaneous testing of only a predefined set of antibiotics. The choice of antibiotics on the panel may not always align with the specific resistance patterns of the tested bacteria, resulting in incomplete or inadequate susceptibi-
lity information. Another limitation of the Disk Diffusion Method is its time-consuming nature. Overnight incubation is required for the formation of visible inhibition zones, which can delay the availability of susceptibility results. This delay can be particularly problematic in urgent clinical situations where prompt treatment decisions are crucial. Moreover, unlike other methods such as broth dilution, the Disk Diffusion Method does not provide information about the minimum inhibitory concentration (MIC) of the tested antibiotics. MIC values are essential for determining the appropriate dosing regimen and accurately predicting treatment outcomes. Furthermore, the applicability of the Disk Diffusion Method to fastidious organisms is limited. Some fastidious or slow-growing bacteria may not thrive on standard culture media, making it challenging to perform the Disk Diffusion Method effectively. These organisms may require specialized growth conditions, which can restrict the method’s applicability. Lastly, with emerging breakpoints and evolving resistance patterns, the Disk Diffusion Method may not always reflect the most up-to-date breakpoints for susceptibility interpretation. This can result in misclassification of susceptibility or resistance and potentially impact treatment decisions. The limitations of the Disk Diffusion Method highlight the need for continuous development and evaluation of antimicrobial susceptibility testing methods to ensure accurate and timely information for informed treatment decisions and effective management of antimicrobial resistance.

2. E-test Method

The E-test method, while offering a convenient and quantitative approach to antimicrobial susceptibility testing, has several limitations that must be considered for accurate interpretation and clinical decision-making. The diffusion of antibiotic gradients from an impregnated strip can be influenced by factors such as evaporation, diffusion barriers, and variations in inoculum density, leading to potential inaccuracies in the shape of the resulting elliptical zone of inhibition. These variations can make it challenging to interpret susceptibility accurately. Additionally, the E-test method may not fully reflect the complex in vivo conditions encountered by bacteria. The agar medium used in the test does not replicate the intricate environment found in host tissues or biofilms, where bacterial susceptibility can be affected by variations in growth conditions and interactions with host factors. As a result, discrepancies between E-test results and clinical outcomes may arise. Another limitation of the E-test method is the restricted number of antibiotics that can be tested simultaneously using E-test strips. A comprehensive antibiotic panel is crucial for accurately reflecting local resistance patterns and guiding appropriate treatment choices. However, the limited number of antibiotic options available on E-test strips may lead to incomplete susceptibility profiles, particularly when dealing with multidrug-resistant organisms. Furthermore, the interpretation of E-test results can be subjective, relying on visual assessment of the elliptical zone of inhibition. Variations in interpretation among different laboratory technicians can introduce subjectivity and impact the reproducibility of susceptibility data. Moreover, the E-test method may not provide complete information about specific resistance mechanisms underlying bacterial susceptibility. It primarily determines the MIC, which represents the lowest concentration of an antibiotic that inhibits visible growth. However, certain resistance mechanisms, such as enzymatic inactivation or efflux pumps, may not manifest as growth inhibition at the tested concentrations, potentially leading to false susceptibility results. This highlights the need for complementary methods that can provide more in-depth insights into resistance mechanisms and guide the selection of effective treatment strategies.

3. The Broth Dilution Method

While the Broth Dilution Method offers a quantitative and widely utilized approach to antimicrobial susceptibility testing, it is crucial to consider its limitations for accurate interpretation and clinical decision-making. The method’s resource-intensive nature, requiring specialized equipment and technical expertise, makes it less accessible compared to other methods. Setting up and performing the Broth Dilution Method can be time-consuming, demanding skilled personnel to ensure the accuracy of antibiotic dilutions and inoculum density. Additionally, the limited number of antibiotics that can be tested simultaneously may restrict the method’s ability to provide a comprehensive overview of the tested bacteria’s susceptibility profile. This can result in incomplete susceptibility information, particularly when dealing with local resistance patterns that may not align with the predetermined antibiotic panel. Furthermore, the Broth Dilution Method may not accurately reflect the in vivo conditions encountered by bacteria in the host. The growth conditions in
broth, lacking host factors and exposing bacteria to high antibiotic concentrations, differ significantly from the complex environment of tissues or biofilms. This discrepancy between in vitro and in vivo conditions may lead to discrepancies in susceptibility results and limit the clinical relevance of the method. Moreover, the subjective interpretation of turbidity or growth inhibition endpoints in the Broth Dilution Method can introduce variability in results. Variations in interpretation among different laboratory technicians can impact the accuracy and reproducibility of susceptibility data. Additionally, the method does not provide information about specific resistance mechanisms underlying bacterial susceptibility. It primarily determines the MIC, representing the lowest concentration of an antibiotic that inhibits visible bacterial growth. However, certain resistance mechanisms, such as efflux pumps or enzymatic inactivation of antibiotics, may not result in growth inhibition at the tested concentrations, leading to false susceptibility results. Furthermore, the Broth Dilution Method requires sufficient incubation time, which can result in delays in obtaining susceptibility results. This delay may not be ideal in urgent clinical situations where prompt treatment decisions are crucial. To overcome these limitations, combining the Broth Dilution Method with other complementary techniques, such as molecular-based methods or alternative susceptibility testing methods, can enhance the accuracy and reliability of antimicrobial susceptibility testing. Molecular methods can detect specific resistance genes or mutations, providing insights into resistance mechanisms not captured by traditional methods. Alternative methods, such as rapid automated systems, offer faster turnaround times and broader antibiotic panels, enabling more efficient and comprehensive susceptibility testing.

4. VITEK 2 Systems

Despite their automated and rapid approach to antimicrobial susceptibility testing (AST), VITEK 2 systems’ dependence on predefined breakpoints and potential difficulties with certain organisms warrant careful interpretation and the consideration of complementary testing methods to ensure accurate clinical decision-making. These fixed breakpoints may not always align with evolving resistance mechanisms, leading to potential discrepancies between reported susceptibility results and actual clinical outcomes. This is particularly relevant as new resistance mechanisms emerge, which may not be adequately captured by existing breakpoints. Additionally, the effectiveness of VITEK 2 systems can be compromised when dealing with challenging organisms. Fastidious microbes or those with complex resistance mechanisms may not be reliably detected, potentially leading to misclassification of susceptibility. For instance, certain bacterial species exhibit heterogeneous resistance patterns within a single isolate, a phenomenon known as heteroresistance. Unfortunately, VITEK 2 systems lack the capability to detect and report heteroresistance, which could significantly impact the selection of appropriate treatment strategies. To address these limitations, a cautious approach to interpreting AST results is crucial, especially considering the potential for discordance between reported susceptibilities and clinical outcomes. Incorporating complementary testing methods, such as molecular-based assays or broth microdilution, can provide additional insights into resistance mechanisms and enhance the accuracy of susceptibility profiles. Additionally, continuous updates to breakpoints are essential to ensure that VITEK 2 systems remain aligned with the evolving landscape of bacterial resistance. By combining these strategies, healthcare professionals can make informed treatment decisions based on VITEK 2-generated AST data, while acknowledging the potential limitations and taking appropriate measures to mitigate them.


The utilization of Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for antimicrobial susceptibility testing (AST) is advantageous due to its rapid and accurate bacterial identification capabilities; however, certain challenges associated with this technique demand careful consideration to ensure its optimal implementation. The intricate and time-consuming nature of sample preparation procedures associated with MALDI-TOF MS can introduce variability in results, potentially affecting the reproducibility of AST outcomes. Additionally, MALDI-TOF MS, while excelling at species-level
identification, may not provide sufficient discriminatory power at the strain level to detect emerging resistance mechanisms that require high-resolution typing. Furthermore, the lack of standardized breakpoints for numerous bacterial species complicates the interpretation of MALDI-TOF MS AST results. Without universally accepted criteria for susceptibility categorization, the comparability and reliability of AST outcomes may be compromised, limiting the effectiveness of treatment decisions. Moreover, the effectiveness of MALDI-TOF MS in detecting slow-growing or non-viable bacteria poses an additional limitation, which could impact the accuracy of susceptibility results, particularly for organisms with atypical growth patterns or those harboring subpopulations with differing susceptibility profiles. To effectively integrate MALDI-TOF MS into routine AST protocols, a thorough understanding of its capabilities and limitations is crucial. Complementary methodologies, such as broth microdilution or molecular-based assays, can provide additional insights into resistance mechanisms and enhance the accuracy of susceptibility profiles. Rigorous standardization efforts and the establishment of species-specific breakpoints are essential steps toward maximizing the utility of MALDI-TOF MS in informing antimicrobial treatment decisions and ensuring patient-centered care.

6. Fluorescence-Activated Cell Sorting (FACS)

Fluorescence-Activated Cell Sorting (FACS) is a powerful tool for antimicrobial susceptibility testing (AST), but it is not without its limitations. One major challenge is the requirement for fluorescent labeling of microbial cells, which can potentially perturb their natural physiological state and alter their responses to antimicrobial agents. Additionally, FACS is designed to analyze bulk populations, masking the presence of minor subpopulations with distinct susceptibility profiles. This becomes particularly problematic when dealing with heteroresistance, where subsets of cells within an otherwise susceptible population exhibit varying degrees of resistance. Furthermore, FACS encounters difficulties in accurately distinguishing and quantifying different species within complex polymicrobial infections. Effective utilization of FACS for AST demands specialized expertise and meticulous data analysis. Careful calibration of gating strategies is essential to avoid misinterpretation and ensure reliable results. Despite these limitations, ongoing advancements in fluorescence labeling techniques and data analysis methodologies hold the potential to address some of the challenges associated with FACS-based AST. By recognizing and actively mitigating these limitations, FACS can contribute significantly to the refinement of targeted antimicrobial therapies. However, it is crucial to diligently consider and manage the constraints of FACS to ensure its accurate and effective application in AST.

7. PCR-Based Methods

PCR-based AST methods offer a powerful approach for detecting resistance genes, but their effectiveness is influenced by the choice of downstream analysis techniques. Gel electrophoresis, while widely used for visualizing PCR products, lacks the sensitivity to detect minor variations in amplicon sizes, potentially leading to missed mutations or polymorphisms within resistance genes. Southern blotting, while specific, suffers from low throughput and the requirement for specialized reagents, which can limit its practicality for routine clinical testing. Restriction fragment-length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) analyses, while providing valuable information about DNA sequence variations, can be labor-intensive and require specialized equipment, limiting their widespread adoption. DNA fingerprinting, while useful for epidemiological studies, may have limited relevance for direct AST due to its focus on strain typing rather than resistance gene detection. Molecular beacons offer real-time detection of PCR amplicons, but their design complexity and potential for non-specific binding can compromise assay specificity, leading to inaccurate discrimination of resistance gene variants. DNA sequencing analysis techniques, including Sanger sequencing and next-generation sequencing (NGS), provide high-resolution genetic information, but their broad applicability to routine AST might be hindered by the need for specialized bioinformatics expertise, data processing, and the potential for high costs. Loop-mediated isothermal amplification (LAMP), while rapid and amplification-efficient, primarily detects presence/absence of target genes, making it difficult to quantify resistance gene levels and correlate gene presence with phenotypic resistance. To effectively incorporate PCR-based methods into clinical practice, care-
ful consideration of these limitations is crucial. Combining multiple PCR-based approaches, complementing them with phenotypic assays, and leveraging bioinformatics tools can enhance their accuracy and utility in guiding targeted antimicrobial therapies.

4.0 CONCLUSION

The ever-evolving landscape of diagnostic medicine necessitates a continuous evaluation of Antimicrobial Susceptibility Testing (AST) methods. This study has meticulously examined these methods, illuminating their distinctive strengths and limitations, with a specific focus on their significance for clinical diagnostics (Table 1). Our in-depth exploration of AST methods, including the Kirby Bauer disc diffusion assay, E-test, Broth Dilution, Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), VITEK 2 systems, Fluorescence-Activated Cell Sorting, and PCR-Based Methods, has yielded several key insights. Firstly, the technical simplicity and cost-effectiveness of certain methods, such as the Kirby Bauer assay, make them invaluable tools for routine clinical utilization. These methods provide rapid results and have made significant contributions to monitoring antibiotic resistance patterns. Secondly, cutting-edge technologies like MALDI-TOF MS and automated systems like VITEK 2 have revolutionized AST, providing rapid bacterial identification and susceptibility data. While these methods excel in many aspects, they require substantial investments in equipment and expertise. However, our analysis has also highlighted persistent challenges. Subjectivity in visual interpretation, variations in test conditions, limited antibiotic panels, and the inability to capture localized resistance nuances are recurring limitations across a spectrum of AST methods. In summary, this research underscores the importance of recognizing AST methods as indispensable instruments for clinical decision-making. These findings enhance the knowledge base of clinicians and laboratories, facilitating informed choices in the diagnosis and treatment of bacterial infections. Moreover, by confronting the strengths and limitations head-on, this study advances the academic discourse surrounding AST methodologies, striving for heightened precision in clinical outcomes and fortifying the global response to antimicrobial resistance. As the diagnostic medicine landscape continues to evolve, this investigation serves as a foundational resource, deepening our understanding of AST methods and their pivotal role in safeguarding public health.

REFERENCE


Table 1. Comparative Assessment of the Strengths and Weaknesses of Common Clinical AST Methods.

<table>
<thead>
<tr>
<th>AST Method</th>
<th>Principle</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirby Bauer Disc Diffusion Assay</td>
<td>Observes zones of inhibition around antibiotic discs on agar plates to determine susceptibility.</td>
<td>- Simplicity and cost-effectiveness. - Established method. - Qualitative results.</td>
<td>- Limited quantitative data. - Subjective zone interpretation. - Variability due to disk potency.</td>
</tr>
<tr>
<td>AST Method</td>
<td>Principle</td>
<td>Pros</td>
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<tr>
<td>E-test</td>
<td>Utilizes predefined gradient strips impregnated with antibiotics to determine MIC values.</td>
<td>- Provides quantitative MIC values. - Requires minimal equipment. - Useful for various bacterial species.</td>
<td>- Costlier than disk diffusion. - Interpretation may be subjective. - Not suitable for all antibiotics.</td>
</tr>
<tr>
<td>Broth Dilution</td>
<td>Determines minimum inhibitory concentration (MIC) by testing antibiotic dilutions in a broth medium.</td>
<td>- Provides quantitative MIC values. - Accurate and reproducible results. - Versatile for different bacteria.</td>
<td>- Requires specialized equipment. - Time-consuming. - Suitable for pure cultures only.</td>
</tr>
<tr>
<td>VITEK 2 Systems</td>
<td>Automated system measuring bacterial growth in the presence of antibiotics.</td>
<td>- Efficient and fast results. - Provides MIC values and susceptibility categorization. - Reduced manual work.</td>
<td>- Expensive equipment and consumables. - Limited availability in certain settings. - May require additional confirmation tests.</td>
</tr>
<tr>
<td>Fluorescence-Activated Cell Sorting</td>
<td>Sorts bacteria based on fluorescence to assess antibiotic susceptibility.</td>
<td>- High specificity and sensitivity. - Potential for single-cell analysis. - Applicable to mixed cultures.</td>
<td>- Specialized equipment and expertise needed. - Limited to fluorescence-labeled strains. - May not capture resistance genes.</td>
</tr>
<tr>
<td>PCR-Based Method</td>
<td>Detects resistance genes or mutations using polymerase chain reaction (PCR).</td>
<td>- Rapid detection of known resistance mechanisms. - High specificity and sensitivity. - Suitable for mixed cultures.</td>
<td>- Limited to known resistance genes. - May miss novel resistance mechanisms. - Requires specialized expertise and equipment.</td>
</tr>
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</table>

**Table 1:** This table provides an overview of various AST methods commonly used in clinical settings. It outlines the principles, advantages and limitations associated with each method, helping clinicians and researchers make informed decisions regarding the diagnosis and treatment of bacterial infections.