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Review

Roadmap for emerging antimicrobial susceptibility testing and its clinical adaptation, from bench to bedside

Pradhapsingh Bharathiraja , Elavarasan Tamilmani *

Matrix Labs Private Limited, Chennai 600077, Tamil Nadu, India

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ABSTRACT

The rapid emergence of antimicrobial resistance (AMR) underscores the urgent need for accurate and timely antimicrobial susceptibility testing (AST) to guide effective antibiotic treatment. Currently, various AST techniques have evolved, however, no single, globally adopted rapid diagnostic technology available for AST diagnosis. Disk diffusion remains a widely accepted technique around the world for AST detection, despite its limitations and ineffective antibiotic efficacy. Regardless of antimicrobial stewardship program, these delayed diagnosis lead to the overuse of broad-spectrum antibiotics to primarily treat the infections and contribute to AMR development. Although automated AST systems offer improved sensitivity and effective in rapid diagnosis, their limited accessibility in low-resource settings and expensive test cost hinders its widespread adaptation. Emerging innovative AST technologies show promise in point-of-care testing but require validation and integration into clinical workflows. Clinical AST market demands differentiated solutions like high-throughput automation for well-resourced settings and affordable semi-automated solutions for low-resource healthcare areas. Therefore, this review extensively addresses the integration of existing AST technologies with emerging techniques, the key challenges in development, regulatory compliance, and clinical implementation. Additionally, highlight the urgent need for next-generation semi-automated AST technology to balance efficacy and affordability for improving individual patient outcomes in the global fight against AMR.

1. Introduction

Antimicrobial resistance (AMR) poses a significant global hazard for public health and has comprehensive economic implications. Microbes such as bacteria, fungi, viruses and parasites impose defensive mechanisms during infection to evade the effects of different classes of antimicrobial drugs, which emerge as AMR (Lim et al., 2024). Globally, every year an estimated 4.95 million deaths are associated with drug resistant bacterial infections and.

1.27 million deaths are attributed to pathogens which are resistant to already available antibiotic drugs (Okeke et al., 2024). It is estimated that the AMR will significantly cause the death of.

10 million people by 2050 (Tang et al., 2023). Approximately 50 % of antibiotic treatments are wrongly prescribed without proper diagnosis of the pathogen and its susceptible drugs (Vasala et al., 2020). The World Health Organization (WHO) launched the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to foster the surveillance of monitoring AMR in patients consuming antimicrobial drugs. The GLASS report 2022 stated that blood culture isolates of *Escherichia coli, Streptococcus pneumoniae,* and *Klebsiella pneumoniae* species showed resistance to first-line antibiotics such as ampicillin, co-trimoxazole, and ciprofloxacin (GLASS World Health Organization, 2022). Urinary tract infections (UTI) are predominantly gram-negative pathogens, such as *E. coli, K. pneumonia, Proteus mirabilis*, and *Pseudomonas aeruginosa* can produce β-lactamase, an enzyme that inactivates beta-lactum antibiotics and ultimately leads to AMR (Pariyar et al., 2023). Improper diagnosis of pathogenic bacterial infections and subsequent overdose or misuse of antibiotics lead to severe health concerns in clinical platforms. Thus, the early detection of pathogens and understanding their resistance mechanisms are essential for overcoming AMR and paves the way for precision medicine.

Over the decade, antimicrobial susceptibility testing (AST) emerged as the most prominent diagnostic system for the detection of AMR and guiding the appropriate use of antimicrobial drugs (Chen and Hong, 2021; Karlowsky and Richter, 2015; Ramzan et al., 2024). In 2023, the global AST market value was at USD 4.2 billion and projected to reach $5.6 billion by 2029, driven by the rising occurrence of infectious diseases, development of multidrug resistance, and increased hospital-

* Corresponding author.
E-mail addresses: pradhap742@gmail.com (P. Bharathiraja), elaz.au@gmail.com (E. Tamilmani).

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acquired infections (MarketsandMarkets™, 2024). AST is typically performed based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). ASTs generally use the lowest concentration of an antimicrobial drug, which inhibits visible microbial growth, referred to as minimum inhibitory concentrations (MIC) breakpoints calculated as per CLSI and EUCAST guidelines (Gaur et al., 2023). However, the identification of microbial pathogens and their susceptible drugs often takes more than one or two days for accurate diagnosis. Thus, the clinicians are compelled to prescribe typical broad-spectrum antibiotic therapies before proper diagnosis, which often leads to the development of AMR and shows detrimental effects to the patients (Kaprou et al., 2021). Microorganisms tend to continuously adapt and alter their susceptibility pattern to newly developed structurally unsimilar antibiotics. Therefore, obtaining the antimicrobial susceptibility profile rapidly after its confirmation and isolation is crucial (Salam et al., 2023).

Several AST techniques, including manual AST methods and automated systems, have been developed and are currently utilized in clinical settings based on phenotypic and genotypic principles. The automated AST systems are primarily developed by in vitro diagnostic (IVD) companies such as BioMérieux (France), Becton Dickinson (BD) Diagnostic Systems (USA), Beckman Coulter (USA), Thermo Fisher (USA), and Bio-Rad Laboratories (USA). Automated systems are more consistent, offer greater sensitivity, reduce the hands-on time, and minimize human handling errors prevalent in manual methods like disk diffusion or micro dilution. Additionally, the innovation in artificial intelligence, machine learning, biosensor techniques, microfluidics, optics, electrochemical, DNA amplification, and hybridization techniques has conceded novel approaches in AST. However, these technologies do not directly relate to the practical needs of point-of-care testing (POCT), and only limited techniques have been translated to the clinical market (Vasala et al., 2020). Therefore, this review discusses the commercially available systems, recent advances in the AST methods and their diagnostic tools, highlighting their role in improving precision medicine in clinical microbiology.

2. Evolution and market growth of AST systems

The conventional phenotypic AST methods were developed in the 1920s, including agar and broth dilution methods, and their final version was revised in the 1940s (Wheat, 2001). These techniques involve exposing microbes to certain antibiotics present in the agar plate or broth medium to determine their inhibitory concentration. While these techniques are labor intensive, Kirby and A.W. Bauer in 1959 introduced a well-defined AST determination technique called the disk diffusion method. Further, they redefined this method and later got it approved by the WHO in 1961 and published the study in 1966 (Bauer et al., 1966). Moreover, this disk diffusion method is still used in several clinical microbiology laboratories for the determination of AST. In the following years, the automated AST systems started entering the IVD diagnostic markets, and they greatly reduced the manual workload. The automated AST market has undergone a decade-by-decade breakdown of market trends, each reducing diagnostic turnover time, accuracy, and global adaptation. In 1974, the first automated AST system, known as the "Autobac disc elution system", was introduced by Pfizer Diagnostics. Then, the McDonnell Douglas corporation, the predecessor of Biomérieux's Vitek system, developed an automated AutoMicrobic system (Felmingham and Brown, 2001). Biomérieux, in 1988, launched a fully automated AST system called Vitek®, which revolutionized the entire automated AST system. Further, in 2000, Becton Dickinson introduced the "BD Phoenix™" automated ID/AST systems. During 1960–2000, lab automation began to replace the manual phenotypic AST methods and consequently entered the global IVD market (Wheat, 2001).

In the early 2000s, EUCAST and CLSI started standardizing the guidelines and MICs for AST determination. This standardization enabled automated instruments to deliver accurate diagnostic results aligned with EUCAST and CLSI MIC values. Again, Biomérieux introduced the Vitek 2 Compact fully automated ID/AST system in the US and French markets. Followed by they have cleared FDA approval in 2005 and subsequent commercialization in all other countries. In 2015, the Alfred 60 AST system was launched in Italy by Alifax.s.r.l. for automated bacterial culture and susceptibility testing. Followed by them, Accelerate Diagnostics in 2017 introduced the Accelerate Pheno® system for optimal MIC-based AST testing. Then, Thermo Fisher Scientific introduced the Sensititre ARIS HiQ AST system for automated AST detection in 2019. As in the past decade, MALDI-TOF mass spectroscopy-based analytical instruments started revolutionizing bacterial ID/AST determination, in 2019, BD launched BD Kiestra™ TLA (total automation system) system, coupled with BD Kiestra™ IdentifA/SusceptA and BD Phoenix™ M50, which automates all comprehensive processes from specimen processing to ID/AST determination. This BD Kiestra ID/AST system is a total laboratory automation system that provides automated specimen inoculation, incubation, and culture visualization. Further, it prepares samples for MALDI-TOF-based ID and AST determination (Jacot et al., 2021).

In the following years, molecular platforms started to be employed in AST determination, which led to low turnaround time and extensively improved their speed. The discovery of antibiotic resistance genes such as blaTEM for β-lactamse (Datta and Kontomichalou, 1965) and mecA for methicillin resistance (Kreiswirth et al., 1993) paved the way for genotypic AST determinations using molecular platforms such as nucleic acid amplification technology (NAAT) and started evolved into a rapid diagnosis combined with the hybridization techniques (Vasala et al., 2020). These NAAT-based genotypic AST techniques include PCR, DNA microarray, and DNA sequencing, which systematically identify the specific strains or particular resistance-causing genes (Gerace et al., 2022). Early genotypic methods are majorly focused on PCR amplification to probe specific resistance genes, such as mecA for methicillin-resistant *S. aureus* (MRSA) and multiple genes simultaneously using multiplex PCR (Huletsky et al., 2004; Murakami et al., 1991). In 2009, Cepheid launched the Xpert MRSA/SA Blood culture assay system based on PCR for the identification of the mecA gene in *S. aureus*. Followed by GeneXpert MTB/RIF assay based on real-time PCR technology to rapidly diagnose rifampicin resistance in tuberculosis. Further, the high-throughput technologies like DNA microarrays enabled the detection of multiple genes simultaneously and efficiently reduced the AST turnaround time (Perreten et al., 2005). The Verigene Gram +ve and Gram –ve blood culture nucleic acid tests were launched in 2013 as a microarray-based diagnostic kit for resistance markers such as mecA, vanA/B, and blaCTX-M, blaKPC (Kim et al., 2016).

Further, next-generation sequencing (NGS) started revolutionizing microbial diagnosis and allows comprehensive identification of multi-drug resistance mechanisms and genetic mutations in microbes. Köser et al. (2012) reported that whole genome sequencing can rapidly investigate the neonatal MRSA outbreak in intensive care units (Köser et al., 2012). Furthermore, computational databases such as the comprehensive antibiotic resistance database (CARD) (card.mcmaster.ca), ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), and National Database of Antibiotic Resistant Organisms (NDARO) (www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/) standardized the AMR gene annotations and act as a centralized hub for accessing AMR data for surveillance of pathogenic organisms. BioFire launched BioFire Blood Culture Identification 2 (BCID2) and received FDA clearance in 2022. These BCID2 tests identify 43 targets for bloodstream infections and AST markers using a multiplex RT-PCR system within one hour of a positive blood culture sample. Moreover, recent advances in genome editing using CRISPR-Cas9 mediated systems and metagenomic sequencing analysis have been developed for AMR diagnosis and prediction directly from complex clinical samples (Allan-Blitz et al., 2023; Halpin et al., 2025; Moragues-Solanas et al., 2024). These genotypic AST methods greatly reduced the diagnostic turnaround time and can be

performed directly from the clinical specimens. However, they are limited to detecting known resistance-making genes, cannot predict the phenotypic susceptibility of a particular drug due to uncharacterized resistance mechanisms (Hattab et al., 2024).

3. Conventional phenotypic AST

The conventional phenotypic methods, also known as growth-based assays, primarily rely on the observation of microbial growth. Techniques such as broth dilution or Kirby-Bauer disc diffusion methods are used to determine the susceptibility or resistance of bacteria to particular antibiotics.

3.1. Phenotypic manual methods

Several phenotypic manual methods are widely used in clinical laboratories for susceptibility testing. A gold standard commonly used method is Kirby-Bauer's disk diffusion method, which classifies bacteria as susceptible, intermediate, or resistant to antibiotics based on universal MIC values and guidelines defined by CLSI (CLSI-M100, 2025). Similarly, the agar dilution method, a pioneering quantitative AST method widely used for determining the MIC of a particular new antibiotic drug (Wiegand et al., 2008). In this method, the MIC is identified as the lowest antibiotic concentration at which no bacterial colony growth or colony-forming units (CFU) are observed. Also, several chromogenic antibiogram methods were developed to identify the drug resistant bacterium based on the colony color changes and also obtains antibiogram. Chromogenic media contain substrates which are enzymatically cleaved by specific bacteria to form a colored compound. When chromogenic agar media incorporated with specific antibiotics, the growth of the colored colonies indicates the susceptibility profile (Cugmas et al., 2022). Biomérieux offers CHROMID® culture media like CHROMID MRSA REF, VRE REF, Carba REF chromogenic ready to use agar plates to identify methicillin resistant *S. aureus*, vancomycin resistant *Enterococci* (VRE), Carbapenemase producing *Enterobacteriaceae* and also other drug resistant UTI and healthcare-associated infection (HAI) pathogens. CHROMagar™ ESBL manufactured by CHROMagar, France can identify the Extended Spectrum β-Lactamases (ESBL) producing gram-negative *Enterobacterales.* Similarly, the broth dilution method follows the same dilution principle but in liquid media. Antibiotic dilutions are prepared in broth and bacterial growth is assessed visually or through turbidity (Wiegand et al., 2008). Notably, the resazurin-based broth microdilution method offers AST determination using colorimetric indication (Jia et al., 2020). When a sample contains metabolically active bacterial cells, the blue color non-fluorescent resazurin is reduced to the pink color of fluorescent resorufin, indicating bacterial growth. Based on the color differences, the blue, pink, and violet color indicates susceptible, resistant, and intermediate to particular antibiotic drug treatments which can be detected either visually or colorimetrically. Antimicrobial gradient is another phenotypic method for AST and MIC determination in agar plate using a concentration gradient strip (Gajic et al., 2022). Here, the MIC values are manually compared with standard CLSI or EUCAST reference breakpoints. These strips are easy to use, provide accurate MIC values, and are particularly suitable for testing slow-growing or fastidious organisms.

Besides these advantages, these manual AST methods show inconsistent test patterns for certain antibiotics and organisms. The disk diffusion method, in particular, is qualitative, time-consuming, labor-intensive, and limited by variations in antibiotic efficacy. Overall, the factors that influenced the disk diffusion are the physicochemical properties of antibiotics, such as solubility, pH, zone of clearance, and molecular weight, unlike the broth microdilution, which completely dissolves antibiotics and provides true MIC without any interferences. These abnormalities were counteracted by doubling the antibiotic concentration in disks or using two disks to match the efficacy of antibiotics in broth dilutions (Alagumaruthanayagam et al., 2009). In most cases, a laboratory technician visually interprets the disk diffusion data values, which are not accurately measured and entered into electronic medical records. Although the broth microdilution in both calorimetrically and turbidity-based methods provides true MICs, the complex manual handling and labor-intensive task increases the risk of false positive results and inconsistent reproducibility. Chromogenic agar plates can identify resistant pathogens, but do not efficiently generate a full susceptibility profile. Gradient strips, while beneficial for minimal handling and direct MIC determination, can produce subjective results. The elliptical zone of inhibition may be interpreted differently based on the technician's experience and is often inconsistent for organisms that exhibit swarming growth. Due to the error-prone nature of these manual methods, the development and adoption of automated AST systems has gained its attention.

3.2. Phenotypic automated systems

In recent decades, several automated AST instruments have revolutionized clinical microbiology platforms by offering rapid and efficient results compared to the conventional manual AST methods. These automated AST instruments have significantly reduced the turnaround time of manual methods and reduced human handling errors. Notably, the growth of automated AST improves treatment efficacy and subsequently reduces the patient recovery time and hospital stay.

Numerous automated systems are available in the market. Remarkably, Vitek® 2 and Vitek® 2 compact systems are the automated ID/AST detection instruments generally based on the principle of the broth microdilution method originally invented by Biomérieux in France. This system works with cost economical, user friendly disposable cards for ID/AST detection. Moreover, it has an advanced expert system uses phenotype based database for more than 3800 phenotypes and 15,000 bug-drug combinations greatly helping clinicians worldwide for ID/AST diagnosis (Galar et al., 2012; Paluch et al., 2023). Although, the Vitek® 2 system has its own predefined database, it may have limitations like not including rare or emerging MDR pathogens. Similarly, the BD Phoenix™ M50 automated ID/AST system also employs the broth microdilution technique for susceptibility testing. It integrates dual technologies—redox potential measurement and turbidity detection to monitor bacterial growth under various antibiotic exposures. This instrument can detect emerging resistance and is the only automated system that can identify extended spectrum β-lactamase in all gram negative panels and first to include MRSA detection for cefoxitin and oxacillin resistance (Swenson et al., 2007). However, having some limitations in the detection of colistin resistance and showing frequent false positive results for carbapenemase-producing organisms (Hong et al., 2019; Jonas et al., 2021). The DxM MicroScan WalkAway® system by Beckman Coulter Diagnostics is another widely used automated platform. It also applies the broth microdilution method and is especially compatible for detecting critical and emerging antimicrobial resistance patterns. It provides MIC values across a broad antibiotic range with high accuracy, making it a gold-standard solution for clinical diagnostics. This Microscan WalkAway system can provide accurate results for emerging resistance pathogens, including MRSA, vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), and carbapenem-resistant *Enterobacteriaceae* (CRE) (Singh et al., 2019). However, this system reported to show false-susceptible results and MIC overestimation of fosfomycin (Bondi et al., 2023) and failed to detect heteroresistance to carbapenems in patients with *E. aerogenes* bacteremia (Gordon and Wareham, 2009). In contrast, the Alfred 60AST system, developed by ALIFAX S.r.l. (Italy), is a fully automated platform capable of performing bacterial culture, residual antimicrobial activity (RAA) assessment, and AST via utilizing an integrated turbidimeter and McFarland monitor to detect the 0.5 McFarland turbidity standard, ensuring reliable sample preparation and analysis. The reagent costs are relatively low and faster turnaround time

compared to other rapid testing system. However, it is less reliable for providing AST results of gram positive bacteria and potentially leads to false positive results. Moreover, the Accelerate Pheno™ System, developed by Accelerate Diagnostics, Inc. (USA), combines both rapid genotypic identification using fluorescence in situ hybridization (FISH) and phenotypic AST via morphokinetic cellular analysis. It uses time-lapse imaging to track bacterial responses in real-time, offering rapid and comprehensive results from positive blood cultures. This complete automation requires only 2 min to load one kit and can perform ID/AST directly from clinical samples and no manual McFarland preparation. Although, it offering faster turnaround times, the cost of the instrument is expensive and struggle to accurately identify polymicrobial cultures (Marschal et al., 2017). FASTinov, Portugal has developed a flow cytometry based AST system for rapid diagnosis directly from positive blood culture samples. This FASTinov system assess the bacterial cell damage directly after exposure to antibiotics rather rely on bacterial growth, so that it can provide susceptibility profile rapidly in 2–3 h (Cintora-Mairal et al., 2025). Thermo Fisher's Attune NxT flow cytometer, using Flow Cytometry–Assisted AST (FAST) technology, distinguishes live and dead bacterial cells after exposure to antibiotic gradients and enables rapid MIC determinations. This approach delivers breakpoint AST results within 30 mins, offering a much faster alternative to conventional broth microdilution methods. Although it has wider application, the expensive reagent cost, significant expertise for data analysis and limited clinical validation data limit its accessibility in most of the laboratories.

Additionally, isothermal microcalorimetry is a widely recognized method for AST determination by measuring the thermal energy released by metabolically active pathogens, which directly proportionate to its growth under antibiotic treatments (Tellapragada et al., 2020). The CalScreener™ from SYMCEL, Sweden, is a highly sensitive, label-free isothermal microcalorimeter designed for detecting metabolic heat flow of growing microorganisms in real time under isothermal conditions. The instrument's longer turnaround time, combined with its need for specialized training and expert interpretation, often places it beyond the reach of labs with limited budgets or personnel. MALDI Biotyper-Antibiotic Susceptibility Test Rapid Assay (MBT-ASTRA), an alternative automatic system invented by Bruker, utilizes a MALDI-TOF mass spectrometer based rapid analysis of ID and AST (Maxson et al., 2017). Although all these automated systems greatly improve efficiency, are less error prone, and less labor-intensive compared to manual methods. Besides its advantages and automation, the high instrument cost and requirement of expert technicians for optimal performance and maintenance make it less accessible to resource-limited laboratories in developing regions.

4. Genotypic AST

Genotypic AST methods utilize nucleic acid based molecular techniques to detect particular resistance genes or mutations in microbes, which can provide a direct, sensitive, and rapid approach for AMR detection (Hattab et al., 2024). These methods are time consuming and less prone to contamination compared to phenotypic methods. Continuous advancements such as mass spectrometry, PCR, DNA sequencing, and FISH technologies offer an alternative option of genotypic AST in clinical laboratories (Duan et al., 2025). Several commercialized genotypic AST kits and instruments are currently available in the clinical market.

4.1. Manual genotypic methods

Polymerase chain reaction (PCR), a widely used molecular tool for determining the particular gene expression profile of antibiotic resistance genes. Several PCR amplification methods, such as electrophoresis, DNA fingerprinting, and restriction fragment length polymorphism (RFLP), were used for rapid AST determination (Athamanolap et al., 2017; Rohit et al., 2016; Salimizand et al., 2016). The conventional PCR method can identify the presence or absence of target gene expression, while Multiplex PCR can simultaneously detect multiple resistance genes and offers rapid diagnostic efficiency. Reverse transcription PCR (RT-PCR) or quantitative PCR (qPCR) can quantitatively measure the gene copy number by targeting multidrug resistance (MDR) mRNA expression with improved sensitivity and speed. Loop mediated Isothermal Amplification (LAMP), a recent molecular technique for rapid genotypic AST that amplifies DNA using *Bst* polymerase instead of *Taq* polymerase (Rödel et al., 2017). LAMP offers rapid AST determinations in point-of-care testing. Mu et al. (2016) reported that LAMP can sensitively detect the ISAba1-blaOXA-51 resistant gene in *Acinetobacter baumannii* (Mu et al., 2016). These PCR methods don't requires sterile samples and sometimes can analyze a mixture of bacteria. While these methods are celebrated for its accuracy and speed, it has several significant limitations. Most importantly, the PCR based genotypic methods detect the resistance causing target genes, which are designed to find. They provide no information on the susceptibility profile of a particular drug, unlike phenotypic methods. These tests are inherently targeted, may not be suitable for identifying emerging resistance, and pose a major challenge to translate genetic results into a clinical decision.

4.2. Automated genotypic systems

Automated systems utilize molecular techniques to identify resistance markers in microorganisms. These methods are typically nucleic acid-based and enable comprehensive profiling of resistance-associated genes. In addition to rapid diagnosis, they significantly reduce manual labor, making them ideal for efficient diagnosis. Abacus Diagnostics developed the GenomERA® CDX automated system based on a closed tube PCR platform for identifying methicillin resistance in *S. aureus* and *Clostridioides difficile* from stool and swab samples (nasal, throat, and groin/perineum). Further, they provided GenomERA MRSA/SA Multi-swab assay kit for high-sensitivity screening of MRSA from swab samples and GenomERA *C. difficile* direct stool kit detecting tcdB-gene for antibiotic-associated diarrhea (Hirvonen and Kaukoranta, 2013). The major drawbacks of GenomERA are its higher rate of invalid results and potential for detection failures due to target mutations (Paitan et al., 2017). Likewise, the BD MAX™ fully automated system, a widely used diagnostic tool that uses real-time PCR for identification of various pathogens, including enteric viruses, respiratory viruses, and sexually transmitted infections, and also sensitively detects the resistance markers (Koo et al., 2022; Sağıroğlu and Atalay, 2021). The GeneXpert® system is another automatic, advanced, rapid molecular diagnostic tool introduced by Cepheid, USA. GeneXpert is a real time PCR based instrument that can detect pathogens and drug resistance in a closed, disposable single use cartridge. Its faster turnaround time and rapid detection of drug resistance provide precise treatments in pulmonary and extrapulmonary TB in negative smear cases (Rimal et al., 2022). However, its sensitivity varies based on the sample type and prone for contradictory false positive and false negative results. Moreover, the higher cartridge cost can be a barrier for some low income laboratories. Furthermore, Biofire introduced FDA approved automatic genotypic AST system called Filmarray®, a fully automatic multiplex PCR system that integrates sample preparation, amplification, detection, and AST analysis. Additionally, the Verigene® system introduced by DiaSorin S.p.A. employs a microarray-based approach for rapid ID of bacterial pathogens and resistance genes directly from positive blood cultures. Although these automated genotypic AST systems offer rapid diagnosis than conventional methods, their high cost limits their utility. Additionally, these systems focus solely on detecting resistance genes associated with MDR and cannot confirm whether these genes are phenotypically expressed as functional proteins (Benkova et al., 2020). Therefore, further research is essential to eradicate these drawbacks for a rapid and efficient diagnostic workflow. The Fig. 1 depicts the different

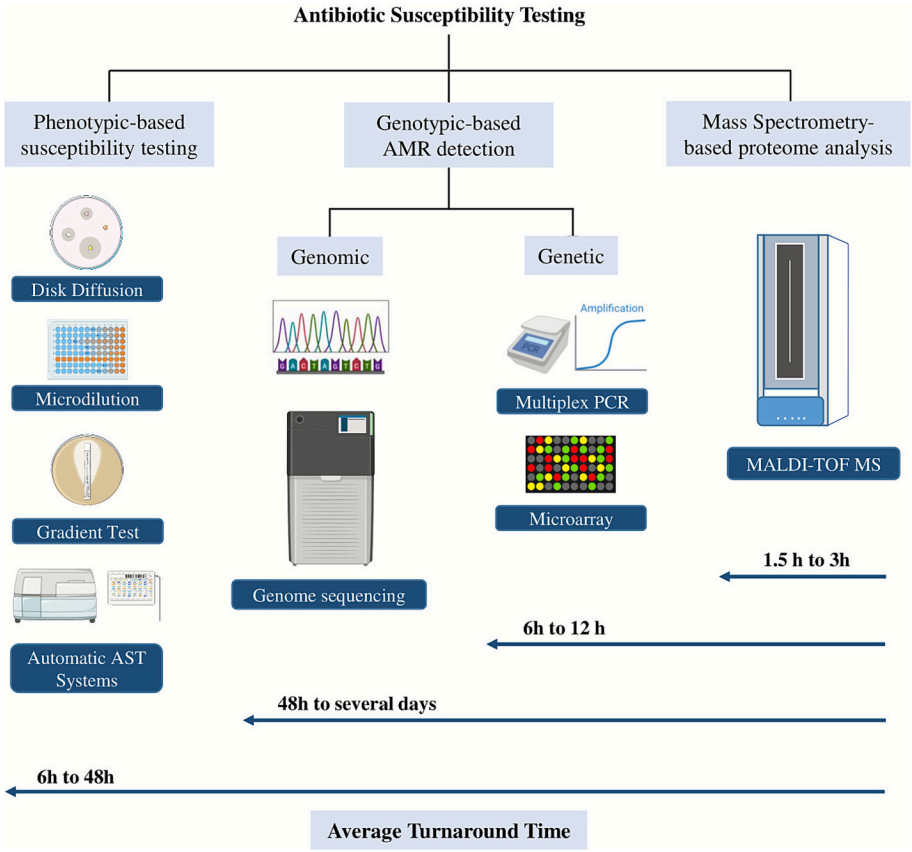

Fig. 1. AST techniques are categorized into phenotypic, genotypic and spectrometric based on the diagnostic principle. The phenotypic methods such as disk diffusion, microdilution, gradient strip testing are available in both manual and automated systems. These methods directly assessing antibiotic susceptibility profile by measuring bacterial growth, turbidity and redox reactions, requiring 6 h to 48 h. While the genotypic AST techniques directly identify the microbial resistance causing genes, with turnaround time ranging from 6 h to 12 h, while the genome sequencing takes 48 h to several days based on the genome length. Among other techniques, the mass spectrometry based proteome analysis using MALDI-TOF are rapid, sensitivity, and accurate provide AST result in 1.5 h to 3 h.

types of phenotypic, genotypic, and mass spectrometry based AST methods and their average turnaround time. Moreover, the advantages and limitations of existing AST methods were briefly demonstrated in Table.1.

5. Techniques, products, or prototypes of diagnostic AST at research level

Despite the widespread adaptation of commercial AST detection systems, the rising cases of AMR underscore their limitations in global clinical settings (Hassall et al., 2024). Emerging approaches such as rapid genomic sequencing, microfluidics, PCR-based kits, optical methods, AI-driven analysis, and biosensors aim to enhance speed, sensitivity, and early resistance detection. These recent emerging researches seek to address the gaps left by already existing systems to offer more precise, scalable point of care devices for AST detection. Ge et al. (2025) developed a wash-free technique using aggregation-induced emission luminogens to distinguish live/dead Gram-negative bacteria via fluorescence, yielding results in 6 h (Ge et al., 2025). Rojas-Andrade et al. (2024) achieved phenotypic susceptibility-resistance differentiation within 10 min using FLIM-AST to track metabolic changes via fluorescence lifetime imaging (Rojas-Andrade et al., 2024). Similarly, Sever et al. (2024) developed a system where luminescence correlates with antibiotic susceptibility testing (Sever et al., 2024). Microfluidics has emerged as a powerful tool for rapid AST by enabling the manipulation of small volumes of fluids in precisely engineered microchannels (Zhang et al., 2020). An electrochemical microfluidic device embedded with carbon screen-printed electrodes designed for rapid AST detection in spiked urine samples in 3 h, even at low bacterial density (Gopalakrishnan et al., 2025). Further, Dong and Zhao (2015) integrated ATP-bioluminescence with microfluidic antibody-coated membranes, enabling rapid pathogen identification in 20 mins and AST in 3–6 h. Similarly, a programmable manually powered microfluidic technology was invented as POCT for UTIs using spatial confinement signal enhancement to reveal AST results in 3 to 5 h. Pang et al. (2024) invented a cost effective centrifugal microfluidics for rapid AST detection to precisely determine the MIC values using an integrated mobile detection platform within 4 to 9 h (Pang et al., 2024). Recently, Whole genome sequencing was employed for rapid AST detection. Jia et al. (2024) utilized whole genome sequencing associated with artificial intelligence based neural network predictions to detect AMR (Jia et al., 2024). Additionally, machine learning and artificial intelligence (AI) has recently evolved as a powerful approach for enhanced AST data analysis. A recent study showed dynamic holographic laser speckle imaging system, along with a machine learning algorithm rapidly determines laser free AST profile during different antibiotic treatments rapidly in 2–3 h (Yang et al., 2025). In 2024, Sturm et al. reported that a machine learning assisted nanomotion technology platform demonstrated rapid and accurate determination of AST, not relying on bacterial growth. This technology directly uses positive blood culture samples to access the vibration measurements and can reveal the results in 2 to 4 h (Sturm et al., 2024). Furthermore, Gao et al. (2024) utilize machine learning assisted MALDI-TOF mass spectrometry technique for rapidly detecting vancomycin-resistance in *Enterococcus fecium* from clinical samples (Gao et al., 2024). These emerging methods promise rapid diagnosis of AST from direct clinical samples using microfluidics, lateral flow

Table 1
Diagnostic landscape of available AST methods and devices.

S. No	AST Method	Detection type (Genotypic/ Phenotypic)	Sample Type	Turnaround Time	Advantages	Disadvantages
	Disk Diffusion (Kirby-Bauer)	Phenotypic	Pure bacterial lawn culture	16–24 h	Cost-effective, simple, flexible antibiotic selection	Time consuming, No MIC determination, Labor intensive, Variations in antibiotic efficacy
	Agar dilution		Pure bacterial culture	16–18 h	Quantitative MIC determination	Labor-intensive, Limited antibiotic concentrations
	Antibiotic gradient test		Pure bacterial culture	16–24 h	Easy MIC reading, flexible	Expensive compared to disk diffusion, relatively long turnaround time
	Chromogenic agar media		Bacterial isolate	24–48 h	Differentiation of multiple MDR pathogens	Not efficient for a complete AST profile
	Broth microdilution		Pure bacterial culture	16–24 h	Quantitative MIC, reproducible, Easy commercialization	Manual setup, error prone
	Broth microdilution based colorimetric test		Pure bacterial culture	12–24 h	Quantitative MIC, Less turnaround time compared to turbidity method	Complex manual handling, Labor intensive
	Broth microdilution based automated systems		Pure bacterial culture or positive blood culture bottles	5 to 6 h	Rapid, automated, Less error prone	Expensive, Not affordable by low resource laboratories, Requires skilled labor
	MALDI-TOF MS based systems		Bacterial isolate	2–3 h	Rapid, high throughput, Low sample quantity, Low cost analysis	Expensive instrument cost, Indirect AST, No MIC determination
	Isothermal microcalorimetry		Pure bacterial culture	6–48 h	Label free, Real-time detection of metabolic growth	Longer turnaround time,
	Lateral flow based AST		Bacterial isolate	15–30 min	Rapid, simple, minimal expertise required	Only detects specific resistances not all new variants
	ATP bioluminescence		Bacterial isolate	3 h	Simple, metabolic-based, affordable	May overestimate resistance, No MIC value, Requires challenging optimized conditions
	Flow cytometry		Bacterial isolate	1–3 h	Quantitative, rapidly detects cell changes	High setup cost, limited validated methods
	PCR based genotypic ASTs	Genotypic	Direct clinical sample or bacterial isolate	6–12 h	Highly specific, rapid, sensitive	Only detects known genes, High cost, Requires skilled labor
	Loop-mediated Isothermal Amplification (LAMP)		Direct clinical sample or bacterial isolate	2–4 h	Rapid, isothermal mechanism (no thermos cycler needed)	Limited to known targets, complex primer design, prone for false positives
	DNA Microarray		Bacterial isolate or Extracted sample DNA	6–12 h	High throughput resistance profile, Customizable panels, Detection of polymicrobial cultures	High initial setup cost, complex data analysis and poor reproducibility
	DNA sequencing		Bacterial isolate or Extracted sample DNA	8–16 h	Comprehensive resistance profiling, reduced reliance on culture	Expensive, requires expertise in data analysis and limited clinical utility
	Fluorescence in situ hybridization (FISH)		Direct clinical sample	7 h	Rapid, specific detection of mutations in resistance genes	Requires expensive fluorescence equipment and technical expertise
	CRISPR based ASTs		Direct clinical sample or bacterial isolate	0.5–1 h	Ultra-specific, rapid, potential for point-of-care	Still experimental, immunogenicity, and lack of regulatory framework

immunoassay, integrated biosensors, or metagenomics sequencing and machine learning capabilities. They offered improved turnaround time and affordability, which are not attainable with existing conventional methods. However, the low microbial yield can affect sensitivity and can be counteracted by using pre-cultured samples. The pre-cultured samples in growth medium can be utilized in various microfluidic technologies, fluorescence quenching, bioluminescence, and CRISPR based genome editing techniques for improved sensitivity and accuracy. Even with a pre-culture step, the overall process will significantly reduce the turnaround time of traditional AST methods (Fig. 2). Importantly, these research stage emerging methods are expected to focus on enhancing the automation, miniaturization, and integration with digital health platforms enabling their use in point of care testing and making real-time decisions. Furthermore, the artificial intelligence and machine learning platforms are evolved to be utilized in interpretation of complex datasets and high-throughput image processing. These technologies or prototypes should be ensured to meet all the requirements for efficient transition from research to clinical application and must meet the requirements for clinical efficacy, scalability, and regulatory compliance. In addition, the proven prototypes should demonstrate the potential for large scale batch manufacturing, reproducibility and adherence to established quality standards, which are crucial for commercial viability and widespread clinical adaptation.

6. Current practical approaches & their challenges

The clinical specimens commonly used for AST diagnosis are urine, blood, wound swabs, pus, sputum, throat swabs, cerebrospinal fluid (CSF), and body fluid discharges. Among them, urine and blood samples were widely tested for clinical ID and AST diagnosis. Approximately 40 % of clinical laboratory cultures are from urine samples and considered an ideal specimen for easier collection in sufficient amount (Bartlett, 2004; Tuuminen, 2012). According to US Centers for Disease Control and Prevention (CDC), UTIs are among the most prevalent infections worldwide and account for 20–30 % of HAI diagnosed in clinical care settings. Catheter associated urinary tract infection (CAUTI) in particular, majorly responsible for UTI and also reported as a secondary cause of bloodstream infections, accompanying with the increased mortality in patients staying long term in health care systems (Chant et al., 2011; Werneburg, 2022).

Likewise, bloodstream infections (BSI) or sepsis are reported as the most frequent cause of mortality worldwide with 48.9 million cases and

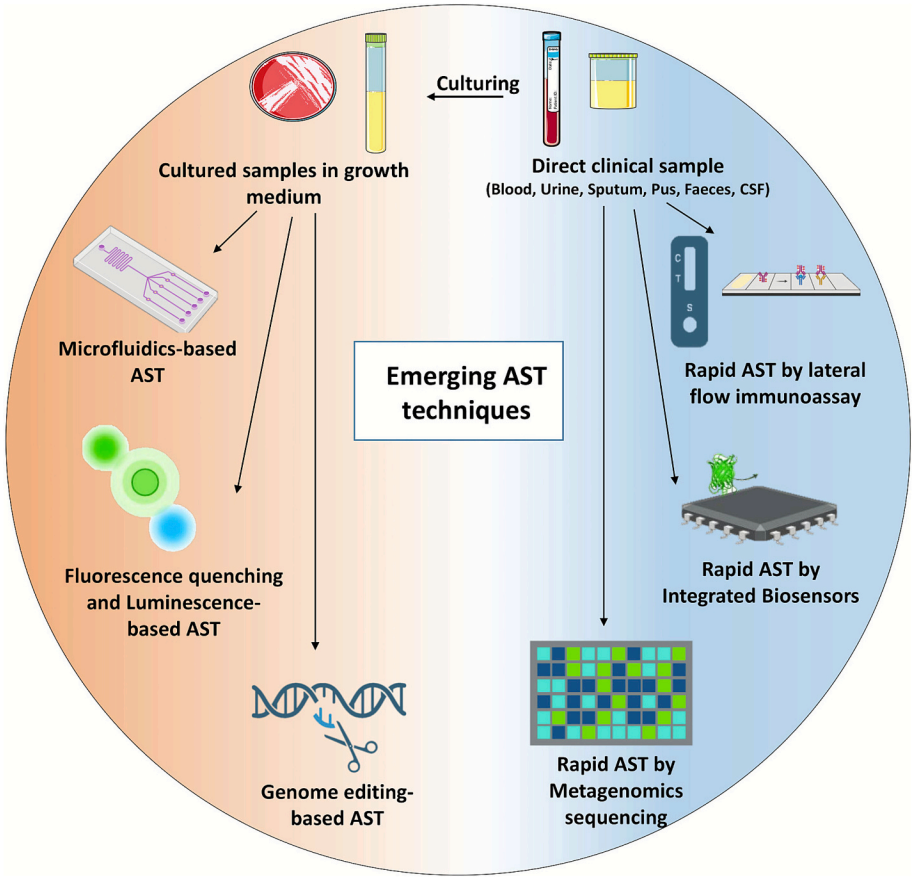

Fig. 2. Emerging AST techniques are designed for POCT and clinical translational purposes. These techniques are optimized to use either direct clinical specimens (blood, urine, sputum, pus, faeces, CSF) or cultured inoculum. The direct clinical samples enable rapid testing via lateral flow mediated immunoassay, integrated biosensors and metagenomics sequencing. Whereas the cultured samples are used in microfluidics, fluorescence quenching, bioluminescence, and CRISPR based genome editing techniques. These techniques would enhance the diagnostic sensitivity with shorter turnaround time compared with traditional AST techniques.

11 million sepsis-related deaths (Rudd et al., 2020). The symptomatic patient's blood sample can be manually tested with blood culture agar plates or in dedicated blood culture bottles in automated blood culture systems, to confirm the presence of infections. Further, these automated blood culture systems subsequently facilitate ID and AST in integrated total automation systems, or the positive blood samples are manually incorporated in separate ID/AST systems for diagnosis. Other specimens, such as wound swabs, pus, sputum, and CSF, require careful handling and proper culture to isolate pure colonies before AST. Sputum and throat swabs are frequently contaminated with normal flora, complicating AST interpretation. CSF samples, used in suspected meningitis cases, typically contain low pathogen yield and may require molecular methods for detection (Gomes, 2022).

Primarily, urine or blood samples should be tested as soon as possible after collection. Due to limited access, not all the laboratories have sophisticated automated blood culture systems, ID/AST devices or integrated total automation systems, which are often limited to ICUs and tertiary care centers. Diagnosing UTIs require midstream clean catch urine collection in a sterile container and can be stored in the refrigerator at 4 °C for 24 h without significant alterations in the bacterial growth and further transported to centralized laboratories. However, the urine containers kept at room temperature or improperly stored during transportation for more than 4 h result in overgrowth of microorganisms, ultimately leads to false positives (LaRocco et al., 2016). Similarly, the blood culture bottles must be transported within 2 h of sample collection and a maximum 4 h at room temperature, considered stable. Whenever, the transit time beyond the limit, it significantly influences the microbial growth, especially fastidious organisms (De Plato et al., 2019). These critical pre-analytical barriers such as high diagnostic cost, and transportation difficulties, demand the underprivileged laboratories to perform manual AST methods, instead transporting or keeping the samples overtime in the collection containers. Around the globe, most of the clinical microbiology laboratories still uses disk diffusion manual method for susceptibility testing and is still being accepted as one of the gold standard protocols for comparing with other techniques, despite its long turnaround time and limitations.

In critical clinical scenarios such as UTI, sepsis or severe pneumonia, this delay in obtaining targeted therapy can lead to the overuse of broad-spectrum antibiotics, driving the escalation of AMR (Ramasco et al., 2024). To date, there are limited randomized controlled trials available for evaluating the clinical impact of existing automated AST methods. While antimicrobial stewardship programs have reduced inappropriate antibiotic use by up to 45 %, no significant improvements have been observed in overall treatment duration, length of hospital stay, or mortality (Darie et al., 2022; Shrestha et al., 2023). Thus, more robust multicenter randomized controlled trials are required to determine the rapid AST kits clinical impact, especially regarding AMR targeted therapy. To overcome the limitations of traditional systems, a variety of innovative AST technologies have been explored. Point of care testing using microfluidics has greatly emerged as a promising tool for AST detection and is often considered lab on a chip platform. In recent days, microfluidic systems uphold some advantages of miniaturization, automation, and cost effective rapid AST detection at low bacterial concentration (Li et al., 2017; Pang et al., 2024). Advances in artificial intelligence and machine learning further enhance AST prediction by analyzing large resistance datasets. These innovative emerging

technologies will ultimately reduce the transportation time and prevent the cross contamination possibilities by poor storage conditions in low resource laboratory settings. However, a key barrier to adoption remains in clinical integration and IVD market entry. New systems must deliver accurate, rapid results while aligning with standard international guidelines (CLSI, EUCAST, & CDSCO), supporting antimicrobial stewardship programs, and allowing easy operation by minimally trained staff.

Conversely, IVD industries targeting AST manufacturing should be concerned about the dual challenge of developing high-performance sensitive systems, and also ensuring affordability and regulatory compliance. In the high income clinical market, manufacturers often focus on fully automated systems that offer rapid results, require minimal manual intervention, support direct clinical sample testing, and computerized interpretations. These features align with the requirements of well-resourced hospitals, centralized laboratories that demand high-throughput and rapid diagnosis. Whereas in low income clinical markets, a different strategy of scalable, semi-automatic, and user friendly systems is warranted with minimal user training and infrastructure. Market success in these settings often depends on collaboration with public health agencies and regional dealers to subsidize equipment costs and streamline distribution to the end user. Importantly, alongside their regulatory compliances and economic feasibilities, emerging AST technologies are strengthened to play a critical role in advancing precision medicine, which are the major gaps that existing classic AST technologies failed to fill. Integration of host biomarker profiling, genomic data, phenotypic colorimetric changes, or AI/machine learning based analytics can tailor treatment decisions to the unique characteristics of individual patients. This innovative system can be strategically marketed in rural, underdeveloped healthcare settings to improve diagnostic feasibility with affordable test cost, especially in developing and fast growing countries. Consequently, this approach can improve patient outcomes by optimizing the antibiotic choices, dosage, and treatment duration, thereby preventing the risk of resistance development, and guides clinicians to personalize antibiotics based on their susceptibility profile. Therefore, the growing innovations must focus on developing cost effective semi-automatic AST systems integrated with emerging technologies like microfluidics and machine learning tools, which are not currently available in the IVD market.

Leveraging simple detection techniques, such as enhanced colorimetry combined with low-cost optical readers or image capturing digital cameras, along with basic machine learning tools for quick interpretations, can create systems that are both cost effective and efficient. Their user-friendly workflows, featuring automated data processing and actionable outputs, minimize manual workload and reduce human handling errors. Despite these advantages, some anticipated challenges and future direction must be addressed, including compact instrument design, development of image processing software, early stage validation with pathogenic clinical samples, ensuring shelf life and stability, late stage validation, multicentric trials, performance evaluation reporting, pre-commercialization, large-scale manufacturing, market launch, and post market surveillance. Further, these semi-automated systems should obtain approval by international regulatory bodies and comply with the recent, updated AMR/AST guidelines and ISO standards to ensure that the innovations translate effectively to improve patient care.

7. Conclusion

AST diagnosis has witnessed exciting advancements in its technologies through both phenotypic and genotypic innovations, still some challenges persist. Phenotypic methods remain time-consuming, and genotypic approaches are limited to detecting only known resistance genes. The commercial automated systems may be beneficial for rapid diagnosis, but are often expensive and limited to centralized laboratories. Research-stage innovations show great promise for a rapid AST method, their technical complexity and lack of large scale validations face substantial obstacles for clinical translation. Therefore, future efforts must focus on developing affordable, semi-automatic AST platforms integrated with emerging technologies like microfluidics and AI/machine learning-mediated tools. By combining the precision and speed of advanced diagnostics with affordable and user-friendly designs, these systems will be widely adopted across diverse clinical settings. Subsequently, eliminates the need for performing time consuming, error prone manual AST methods or sample transportation to well-resourced healthcare settings. The successful bridging of these gaps would transform AST into a more accessible and impactful tool to significantly improve the potential of precision-guided antimicrobial therapy and rapidly combat antimicrobial resistance.

CRediT authorship contribution statement

Pradhapsingh Bharathiraja: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Elavarasan Tamilmani:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no conflicts of interest to disclose.

Data availability

No data set associated with this manuscript.

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