

Emerging technologies for antimicrobial resistance detection in clinical microbiology: from conventional testing to artificial intelligence

Tecnologias emergentes para a detecção da resistência antimicrobiana em microbiologia clínica: dos testes convencionais à inteligência artificial

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Abstract

Objective: to critically evaluate emerging technologies for antimicrobial resistance (AMR) detection in clinical microbiology, highlighting their applications, advantages, and limitations. **Methodology:** a systematic literature review was conducted following PRISMA guidelines. Searches were performed in the PubMed/MEDLINE and Scopus databases using terms related to antimicrobial resistance and diagnostic methods. Results: a total of 4,131 records were identified, and 38 studies published between 2020 and 2026 met the eligibility criteria and were included in the final review. The selected studies were categorized into four major groups: molecular diagnostics, genomic technologies, rapid phenotypic antimicrobial susceptibility testing (AST), and computational approaches incorporating artificial intelligence. The reviewed evidence demonstrated substantial advances in reducing diagnostic time, improving pathogen and resistance gene detection, and supporting earlier optimization of antimicrobial therapy. However, challenges related to standardization, multicenter clinical validation, result interpretation, operational costs, and workflow integration continue to limit widespread implementation. **Conclusion:** the future of AMR diagnostics will depend on the integration of molecular, phenotypic, genomic, and computational approaches capable of providing faster, more accurate, and clinically relevant information to support patient management and antimicrobial stewardship programs.

Keywords: antimicrobial resistance; clinical microbiology; molecular diagnostics; genomic sequencing; antimicrobial susceptibility testing; artificial intelligence.

Resumo

Objetivo: analisar criticamente as principais tecnologias emergentes utilizadas para a detecção da resistência antimicrobiana (RAM) em microbiologia clínica, destacando suas aplicações, vantagens e limitações. **Metodologia:** foi realizada uma revisão sistemática da literatura seguindo as diretrizes PRISMA. As buscas foram conduzidas nas bases PubMed/MEDLINE e Scopus, utilizando descritores relacionados à resistência antimicrobiana e métodos diagnósticos. **Resultados:** inicialmente, 4.131 registros foram identificados, dos quais 38 estudos publicados entre 2020 e 2026 atenderam aos critérios de elegibilidade e compuseram a amostra final. Os estudos selecionados foram agrupados em quatro categorias principais: diagnósticos moleculares, tecnologias genômicas, testes rápidos de suscetibilidade fenotípica e abordagens computacionais baseadas em inteligência artificial. As evidências demonstraram avanços significativos na redução do tempo diagnóstico, na detecção de patógenos e genes de resistência e no suporte à otimização da terapia antimicrobiana. Entretanto, desafios relacionados à padronização, à validação clínica multicêntrica, à interpretação dos resultados, aos custos operacionais e à integração aos fluxos laboratoriais ainda limitam a ampla implementação dessas tecnologias. **Conclusão:** o futuro do diagnóstico da RAM dependerá da integração de abordagens moleculares, fenotípicas, genômicas e computacionais capazes de fornecer informações mais rápidas, precisas e clinicamente relevantes para apoiar o manejo de pacientes e os programas de stewardship antimicrobiano.

Palavras chave: resistência antimicrobiana; microbiologia clínica; diagnóstico molecular; sequenciamento genômico; teste de suscetibilidade antimicrobiana; inteligência artificial.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most significant threats to global public health, compromising the effectiveness of antimicrobial therapies and increasing morbidity, mortality, healthcare costs, and the burden on healthcare systems worldwide^{1,2}. The rapid dissemination of resistant microorganisms is driven by multiple factors, including inappropriate antimicrobial use in human and veterinary medicine, inadequate infection control practices, and the widespread circulation of resistance determinants across

clinical and environmental settings.

The biological complexity of AMR is further amplified by the extensive mobility of antibiotic resistance genes (ARGs). Resistance determinants can spread through horizontal gene transfer mediated by plasmids, transposons, integrons, and other mobile genetic elements, facilitating the rapid dissemination of resistance traits among bacterial populations and accelerating the emergence of multidrug-resistant (MDR),

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extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogens³. Consequently, effective surveillance and timely detection of AMR have become essential components of modern clinical microbiology and antimicrobial stewardship programs.

Clinical microbiology laboratories play a central role in this process by providing pathogen identification and antimicrobial susceptibility testing (AST) results that guide therapeutic decision-making. Conventional culture-based methods and phenotypic susceptibility testing remain the reference standard for resistance detection due to their broad applicability and well-established standardization frameworks. However, these approaches often require prolonged incubation periods and may delay optimization of antimicrobial therapy, particularly in severe infections where rapid intervention is critical⁴.

To overcome these limitations, a wide range of diagnostic technologies has been developed over the last decade. PCR-based methods such as multiplex polymerase chain reaction (PCR) and digital PCR (ddPCR) enable rapid detection of pathogens and resistance-associated genes, substantially reducing diagnostic delays compared to conventional workflows^{5,6}. More recently, next-generation sequencing (NGS), targeted genomic approaches, and nanopore-based platforms have expanded diagnostic capabilities by enabling comprehensive characterization of microbial communities, resistance determinants, and genomic resistance mechanisms^{7,8}. In parallel, rapid phenotypic AST platforms and machine learning-based approaches have emerged as promising tools for accelerating susceptibility prediction and supporting real-time clinical decision-making⁹.

Despite these advances, significant challenges remain. These assays are frequently limited to predefined targets, genomic approaches require specialized infrastructure and bioinformatic expertise, and genotype-based predictions do not always correlate directly with phenotypic resistance expression⁸. Furthermore, differences in cost, accessibility, implementation requirements, and clinical validation continue to influence the adoption of these technologies across healthcare settings⁹.

Given the rapid expansion of diagnostic platforms and the growing complexity of resistance detection strategies, a comprehensive evaluation of their strengths, limitations, and clinical applicability is needed. Therefore, this systematic review aims to critically analyze current approaches for AMR detection in clinical microbiology, including conventional phenotypic methods, molecular diagnostics, genomic technologies, rapid susceptibility testing systems, and emerging computational approaches. Particular emphasis is placed on their diagnostic performance, implementation challenges, and potential contributions to improving AMR surveillance and clinical decision-making.

METHODS

This systematic literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. The literature search was performed between January and March 2025 using the PubMed/MEDLINE and Scopus databases.

Search strategies were developed using Medical Subject Headings (MeSH) and related keywords associated with antimicrobial resistance, laboratory diagnostic methods, and clinical microbiology. Terms were combined using the Boolean operators “AND” and “OR” and adapted to the requirements of each database.

The search strategy comprised three complementary groups of terms. The first focused specifically on bacterial resistance through the MeSH descriptor “Drug Resistance, Bacterial.” The second broadened the scope to antimicrobial resistance across microorganisms using “Drug Resistance, Microbial,” which encompasses related concepts such as antimicrobial and antibiotic resistance. The third addressed the clinical diagnostic context through the term “Clinical Microbiology,” complemented by the MeSH descriptor “Clinical Laboratory Techniques,” to retrieve studies evaluating conventional and emerging technologies for detecting antimicrobial resistance in clinical microbiology laboratories.

Studies published between 2020 and 2025 in English or Portuguese were considered eligible. Inclusion criteria comprised original research articles evaluating laboratory methods for AMR detection in clinical microbiology, including phenotypic, PCR-based, genomic, and computational approaches. Reviews, editorials, conference abstracts, letters, duplicate records, and studies not directly related to AMR diagnostics were excluded. Study selection was performed in four stages: identification of records through database searches, screening of titles, evaluation of abstracts, and full-text assessment of potentially eligible articles. Duplicate records were removed before screening. The selection process followed the PRISMA framework and is summarized in Figure 1.

Data extraction was performed using a standardized form that captured information regarding study characteristics, evaluated technologies, target microorganisms, diagnostic performance, turnaround time, and principal clinical outcomes. The selected studies were subsequently grouped according to the diagnostic approach investigated, including rapid phenotypic AST, molecular diagnostics, genomic technologies, and computational or machine learning approaches.

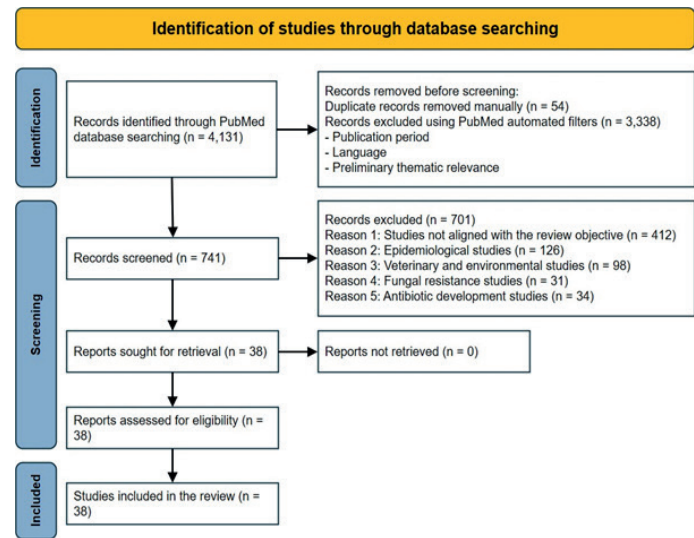
A descriptive qualitative synthesis was conducted to compare the advantages, limitations, diagnostic performance, and clinical applicability of the evaluated technologies.

RESULTS AND DISCUSSION

The database search identified 4,131 records. After application of predefined filters and removal of duplicate records, 741 studies remained for title and abstract screening. Following

the eligibility assessment, 38 articles met the inclusion criteria and were selected for qualitative synthesis. The study selection process is summarized in the PRISMA flow diagram (Figure 1).

Figure 1. PRISMA flow diagram illustrating the study selection process.



The selected studies encompassed a broad range of diagnostic technologies for AMR detection in clinical microbiology. Four major categories were identified: molecular diagnostic approaches, rapid phenotypic AST, genomic technologies, and computational approaches incorporating machine learning algorithms.

Molecular diagnostics represented the largest group of studies, which included multiplex PCR assays, digital droplet PCR (ddPCR), CRISPR-based platforms, and rapid blood culture identification panels. Genomic approaches comprised targeted next-generation sequencing (tNGS), metagenomic sequencing

(mNGS), and nanopore technologies. Rapid phenotypic AST systems focused on accelerating susceptibility testing, while computational approaches explored the use of machine learning models for AMR prediction.

The main characteristics of the included studies, including the evaluated technologies and principal diagnostic findings, are summarized in Table 1. Collectively, the reviewed studies demonstrated a consistent trend toward accelerating diagnostic workflows while improving pathogen identification, antimicrobial susceptibility determination, and resistance gene detection.

Table 1. Summary of the studies selected for the systematic review: titles, authors, objectives, and main findings.

Category	Study	Technology	Main finding
Molecular diagnostics	Marxreiter et al., 2025 ¹⁰	BIOFIRE FILMARRAY AMR	Predicted AMR with 98.7% concordance and enabled earlier therapy optimization.
	Chen et al., 2024 ⁵	BioFire BCID2	Reduced time to antimicrobial de-escalation and decreased unnecessary carbapenem use.
	Agnetti et al., 2023 ¹¹	BioFire BCID	Reduced time to optimal antimicrobial therapy from 55.2 to 25.6 hours.
	Keshta et al., 2022 ¹²	MALDI-TOF + NG-Test	Achieved 100% sensitivity and specificity for direct ESBL and carbapenemase detection in less than 1 hour.
	Carranza et al., 2024 ¹³	RealCycler-Rezero-U/G PCR	Detected MDR Gram-negative bacteria with >95% sensitivity and specificity.
	Dehghani et al., 2024 ¹⁴	Real-time PCR	Outperformed culture in detecting polymicrobial and fastidious wound pathogens.

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Category	Study	Technology	Main finding
Rapid phenotypic AST	Graff et al., 2025 ¹⁵	BCID2 + stewardship	Reduced time to optimal therapy by 17.4 hours in pediatric bloodstream infections.
	Manuel et al., 2024 ¹⁶	QIAstat-Dx BCID	Predicted antimicrobial susceptibility with high negative predictive value.
	Spangler et al., 2025 ¹⁷	Multiplex PCR	Identified pathogens missed by conventional urine culture and improved antimicrobial selection.
	Bonnefoy et al., 2024 ¹⁸	Unyvero BCU	Accelerated pathogen identification in polymicrobial bloodstream infections by approximately 20 hours.
	Darie et al., 2022 ¹⁹	Unyvero HPN PCR	Enabled earlier targeted therapy in patients with pneumonia.
	Jiang et al., 2024 ⁶	ddPCR	Identified pathogens in suspected sepsis within 3 hours and improved diagnostic yield.
	Peng et al., 2025 ²⁰	Multiplex ddPCR	Achieved higher pathogen detection rates than blood culture and reduced turnaround time.
	Lou et al., 2025 ²¹	CRISPR-Cas12a assay	Improved detection of lower respiratory tract pathogens and coinfections.
	Yonetani et al., 2026 ²²	RaST-TAS	Provided rapid and accurate ESBL detection compared with conventional methods.
	Alonso-Tarrés et al., 2024 ²³	Sysmex PA-100	Provided bacteriuria detection in 15 minutes and AST results within 45 minutes.
	Olsson et al., 2026 ²⁴	QuickMIC®	Produced reliable AST results within a few hours, substantially shortening reporting time.
	Streva et al., 2025 ²⁵	Selux NGP	Generated susceptibility profiles within 5–6 hours with high agreement to reference methods.
	Osborn et al., 2025 ²⁶	Early-growth AST	Shortened AST reporting time while maintaining high agreement with conventional testing.
	Squitieri et al., 2025 ²⁷	Rapid AST platforms	Demonstrated substantial reductions in AST reporting time across different systems.
	Bui et al., 2025 ²⁸	VITEK® REVEAL™	Demonstrated substantial reductions in AST reporting time across different systems.
Sequencing-based diagnostics	Park et al., 2025 ²⁹	VITEK® REVEAL™	Delivered reliable AST results significantly faster than disk diffusion.
	Kim et al., 2025 ³⁰	VITEK 2 AST-N439	Showed suboptimal performance for some novel β -lactam/ β -lactamase inhibitor combinations.
	Caruana et al., 2026 ³¹	Nanomotion technology	Enabled rapid AST directly from Gram-negative bacteremia isolates.
	Semerci et al., 2025 ³²	Fosfomycin susceptibility testing	Identified gradient testing as a reliable alternative to agar dilution.
	Leshaba et al., 2025 ³³	Colistin resistance diagnostics	Demonstrated variable performance among commercial colistin resistance assays.
	Yu et al., 2025 ³⁴	ddPCR and mNGS	ddPCR provided the fastest results, while mNGS detected a broader range of pathogens.

Category	Study	Technology	Main finding
	Cabibbe et al., 2024 ³⁵	AmPORE TB	Identified tuberculosis and resistance mutations within 5–6 hours.
	Chang et al., 2025 ³⁶	Targeted NGS	Improved detection of urinary pathogens and resistance genes compared with culture.
	Zhang et al., 2024 ³⁷	Targeted NGS	Demonstrated superior sensitivity and resistance characterization compared with Xpert MTB/RIF.
	Lei et al., 2025 ³⁸	Targeted NGS	Improved specificity and reduced costs compared with alternative targeted sequencing workflows.
	Gillet et al., 2025 ³⁹	Target-enriched NGS	Characterized <i>H. pylori</i> virulome and resistome without bacterial culture.
	Yi et al., 2025 ⁸	mNGS and targeted NGS	Capture-based targeted NGS showed the best balance of sensitivity and diagnostic accuracy.
	Pinzauti et al., 2025 ⁴⁰	PISTE	Enabled rapid pathogen identification and resistance prediction directly from blood samples.
	Gu et al., 2025 ⁷	Nanopore sequencing	Identified pathogens and resistance genes from blood cultures within 3.5 hours.
	Tian et al., 2024 ⁴¹	mNGS	Outperformed conventional culture for diagnosis of central nervous system infections.
	Fordham et al., 2026 ⁹	MALDI-TOF + Machine Learning	Predicted AMR with AUROC values approaching 0.99.
Computational approaches	Wittenbach et al., 2026 ⁴²	Keynome gAST	Predicted susceptibility profiles directly from genomic sequences with 96.9% agreement.

Limitations of Conventional Microbiological Methods

Accurate detection of AMR remains a cornerstone of clinical microbiology and antimicrobial stewardship. Conventional culture-based identification and AST continue to serve as the reference standard for resistance determination because they provide direct phenotypic evidence of antimicrobial activity against viable pathogens and remain the basis for clinical decision-making in most healthcare settings, serving as the comparator against which molecular diagnostics, genomic technologies, and rapid phenotypic susceptibility platforms are typically validated^{5,7,28}.

Despite their established role, conventional microbiological workflows are inherently time-consuming. Several studies included in this review identified prolonged turnaround times as one of the major limitations of culture-based diagnostics. In bloodstream infections, conventional workflows frequently require more than 24–48 hours for definitive pathogen identification and susceptibility reporting, delaying optimization

of antimicrobial therapy. Studies evaluating BioFire BCID^{11,15}, BCID25, Unyvero platforms^{18,19}, and rapid PCR-based assays^{6,20} consistently demonstrated substantial reductions in diagnostic time when compared with conventional culture-based approaches.

Reduced sensitivity under antimicrobial exposure represents another important limitation. Studies evaluating metagenomic sequencing in central nervous system infections^{34,41} and bloodstream infections⁷ reported that genomic methods frequently detected pathogens that remained undetected by conventional microbiological methods, particularly in patients who had already received antimicrobial therapy. Similar findings were observed in wound infections¹⁴ and complicated urinary tract infections¹⁷, where PCR-based methods identified polymicrobial infections and fastidious organisms not recovered by routine culture (Table 2).

Table 2. Comparative overview of conventional and emerging approaches for ARM diagnostics. Technologies are compared according to turnaround time, diagnostic output, strengths, and limitations, highlighting the trade-offs between speed, analytical breadth, and clinical applicability.

Diagnostic approach	Typical turnaround time	Main information provided	Major advantages	Major limitations
Conventional culture and AST	24–72 h (or longer)	Pathogen identification and phenotypic susceptibility	Reference standard; direct phenotypic confirmation; broad applicability	Long diagnostic time; reduced sensitivity after antimicrobial exposure; limited detection of fastidious and polymicrobial infections
Molecular diagnostics (PCR, multiplex PCR, ddPCR)	1–6 h	Pathogen identification and resistance genes	Rapid detection; high sensitivity; supports early therapeutic decisions	Limited to predefined targets; genotype does not always predict phenotype
Sequencing-based approaches (tNGS, mNGS, Nanopore)	3–24 h	Pathogens, resistance genes, virulence factors, genomic information	Broad diagnostic coverage; detection of unexpected pathogens; comprehensive resistome characterization	Higher cost; bioinformatic complexity; interpretation challenges
Rapid phenotypic AST	3–8 h	Direct antimicrobial susceptibility	Phenotypic confirmation with rapid reporting	Limited validation for some species and antimicrobials; instrumentation costs
Computational prediction (AI/ML)	Minutes after data acquisition	Predicted antimicrobial susceptibility	High scalability; integration with genomic and proteomic data; rapid interpretation	Requires large training datasets; limited clinical validation; model transparency concerns

The reviewed literature also highlights the difficulty of conventional methods in rapidly characterizing antimicrobial susceptibility. Multiple studies evaluating rapid phenotypic AST systems—including Sysmex PA-100²³, QuickMIC²⁴, VITEK REVEAL^{28,29}, Selux NGP²⁵, and RaST-TAS²²—were specifically developed to address delays associated with traditional susceptibility testing workflows. The consistent objective across these technologies is the generation of clinically actionable susceptibility information within hours rather than days.

Collectively, these findings demonstrate that the principal challenge facing conventional microbiology is not necessarily diagnostic accuracy, but the time required to generate actionable results. This limitation has stimulated the development of advanced diagnostic approaches designed to accelerate ARM detection while maintaining clinical reliability^{5,6,9,28}.

Molecular Diagnostic Approaches

Molecular diagnostic technologies have emerged as one of the most important strategies for reducing the time required for pathogen identification and ARM detection. Unlike conventional culture-based workflows, these methods target microbial nucleic acids directly, enabling identification of pathogens and resistance determinants within a few hours after sample collection or blood culture positivity^{5,6,11}.

Among the PCR-based approaches evaluated in this review, multiplex PCR platforms represented the most extensively studied group. Systems such as BioFire BCID and BCID2 demonstrated substantial reductions in time to organism identification and antimicrobial optimization. These platforms

enabled earlier de-escalation of broad-spectrum therapy and reduced unnecessary carbapenem exposure, highlighting their potential contribution to antimicrobial stewardship programs^{5,11,15}.

Similarly, Unyvero-based systems evaluated in bloodstream and respiratory infections^{18,19} accelerated pathogen detection in comparison with conventional microbiology and showed particular value in polymicrobial infections, where culture-based identification may be more challenging. Other targeted PCR approaches, including RealCycler-Rezero-U/G¹³, also demonstrated high sensitivity and specificity for the detection of MDR Gram-negative bacteria, reinforcing the value of targeted PCR-based assays for rapid identification of clinically relevant resistance mechanisms.

Digital droplet PCR (ddPCR) represents a further evolution of molecular diagnostics. Pathogen identification within approximately three hours, together with higher detection rates than conventional blood cultures, has been reported for ddPCR-based approaches, suggesting particular value in severe infections where rapid therapeutic decisions are critical^{16,20}.

Recent technological advances have also expanded molecular diagnostics beyond traditional PCR. CRISPR-Cas12a-based platforms have enabled rapid detection of respiratory pathogens and co-infections, illustrating the growing potential of programmable nucleic-acid recognition systems in clinical microbiology²¹. Similarly, the combination of MALDI-TOF and rapid immunochromatographic assays, including NG-Test CTX-M and Carba 5, has achieved highly accurate detection of clinically relevant resistance mechanisms in less than one hour¹² (Table 3).

Table 3. Comparative characteristics of molecular diagnostic technologies used for ARM detection. Methods are compared according to turnaround time, diagnostic targets, principal strengths, and major limitations relevant to clinical microbiology applications.

Technology	Typical turnaround time	Main target	Principal strengths	Main limitations
Multiplex PCR	1–6 h	Pathogens and selected resistance genes	Rapid identification; high sensitivity; suitable for routine diagnostics	Restricted to predefined targets
Digital droplet PCR (ddPCR)	~3 h	Pathogens and resistance genes	High analytical sensitivity; absolute quantification; rapid results	Limited target panel; higher technical complexity
BioFire BCID / BCID2	~1 h after positive blood culture	Bloodstream pathogens and resistance genes	Rapid identification; supports antimicrobial stewardship; easy workflow	Requires positive blood culture; restricted panel
Unyvero platforms	4–6 h	Respiratory and bloodstream pathogens	Broad multiplex detection; useful for polymicrobial infections	Limited to included targets; higher operational costs
RealCycler multiplex PCR	3–6 h	MDR Gram-negative bacteria	High sensitivity and specificity for targeted resistance mechanisms	Restricted diagnostic scope
NG-Test CTX-M / Carba 5	<1 h	ESBL and carbapenemase enzymes	Simple workflow; excellent performance for key resistance mechanisms	Detects only selected enzymes
CRISPR-Cas12a assays	1–4 h	Pathogens and specific resistance markers	High analytical specificity; programmable detection	Limited clinical validation; target-dependent design

Despite their advantages, molecular diagnostics share important limitations. Most assays are restricted to predefined targets and therefore cannot detect organisms or resistance determinants that are not represented within the diagnostic panel^{5,11,18}. Furthermore, detection of a resistance gene does not necessarily indicate phenotypic resistance, as gene expression, regulatory mechanisms, and host-pathogen interactions may influence the final susceptibility profile^{12,13}. Consequently, these approaches should be viewed as complementary tools that accelerate clinical decision-making while still requiring integration with phenotypic and epidemiological information^{5,6,20}.

Sequencing-Based Technologies

Genomic technologies have expanded the diagnostic scope of AMR detection by enabling broader characterization of pathogens, resistance genes, and genomic features associated with therapeutic response. Unlike targeted PCR panels, NGS approaches can provide comprehensive information regarding microbial identity, resistance determinants, virulence factors, and strain-level relationships^{7,36,41}.

mNGS has proven particularly valuable when conventional culture shows limited sensitivity or when infections involve fastidious, rare, polymicrobial, or previously treated pathogens. Studies evaluating central nervous system, bloodstream, and lower respiratory tract infections consistently reported improved pathogen detection and broader diagnostic coverage compared with routine culture^{7,8,34,38,41}.

tNGS represents an intermediate strategy between multiplex

PCR and unrestricted metagenomics. By enriching predefined pathogen or resistance-associated targets, tNGS can improve analytical sensitivity and reduce costs while maintaining a focused and clinically interpretable workflow. Applications in urinary tract infections, tuberculosis, lower respiratory tract infections, and *Helicobacter pylori* infection demonstrated reliable detection of pathogens and resistance-associated mutations^{7,35,37,39}.

Nanopore sequencing has further increased the clinical appeal of genomic diagnostics by enabling real-time data generation and faster reporting. Studies involving blood cultures, tuberculosis, and sepsis demonstrated rapid pathogen identification and resistance gene detection, supporting the potential of portable sequencing platforms for early clinical decision-making^{7,35,40}.

A recurring theme across genomic studies is the trade-off between breadth, speed, cost, and interpretability. Metagenomic approaches provide the broadest diagnostic coverage but require specialized bioinformatic infrastructure, whereas targeted strategies are generally more affordable and easier to interpret. Comparative evaluations indicate that no single sequencing strategy is universally superior, with performance largely dependent on the clinical setting, urgency of results, and available laboratory infrastructure^{8,38}.

Despite their promise, genomic technologies still face important barriers to routine implementation, including contamination control, human DNA background, interpretation of low-abundance organisms, and the need for standardized analytical pipelines^{8,38}. Moreover, detection of resistance genes or

mutations does not always predict phenotypic susceptibility^{35,39}. Consequently, genomic analysis should be viewed as a high-resolution diagnostic layer that complements rather than replaces culture and phenotypic susceptibility testing^{7,41}.

Rapid Phenotypic AST

Although PCR-based and genomic approaches have substantially accelerated pathogen identification and resistance gene detection, antimicrobial therapy ultimately depends on reliable susceptibility information. Because the presence of a resistance determinant does not necessarily translate into phenotypic resistance^{7,8}, rapid phenotypic AST has emerged as an important strategy for bridging the gap between conventional culture-based methods and rapid molecular diagnostics^{24,28}.

The studies included in this review demonstrate a growing effort to reduce AST reporting times from days to hours while maintaining agreement with reference methods. Platforms such as QuickMIC²⁴, VITEK REVEAL^{28,29}, Selux NGP²⁵, Sysmex PA-10023, and early-growth AST approaches²⁶ consistently generated susceptibility results within a few hours using different analytical principles, including automated microscopy, growth kinetics monitoring, microfluidics, and image-based bacterial growth assessment. Despite methodological differences, all aim to provide clinically relevant susceptibility information before conventional AST results become available^{23,24,28}.

Particularly noteworthy are technologies capable of delivering results directly from positive blood cultures, where even modest reductions in reporting time may influence antimicrobial optimization in sepsis and bloodstream infections^{24,25,28}. The reviewed literature also highlights targeted rapid phenotypic approaches focused on clinically relevant resistance mechanisms. RaST-TAS enabled rapid detection of extended-spectrum β -lactamase (ESBL) producers²², while studies evaluating colistin resistance assays and fosfomycin susceptibility testing illustrated both the opportunities and challenges associated with rapid characterization of difficult-to-test resistance phenotypes^{32,33}.

Despite these advances, rapid phenotypic AST remains associated with important limitations. Many platforms have been validated primarily for bloodstream infections and a restricted range of bacterial species^{24,25,28}, while performance may vary according to organism, antimicrobial class, inoculum characteristics, and sample preparation protocols^{23,26}. Instrument costs, workflow integration, and laboratory standardization also remain barriers to widespread implementation^{25,28}.

Nevertheless, rapid phenotypic AST offers a unique advantage over PCR-based and genomic approaches: direct measurement of antimicrobial susceptibility. By assessing bacterial responses to antimicrobial exposure rather than inferring resistance from genetic markers, these technologies reduce uncertainty associated with genotype–phenotype discrepancies^{23,24,28}. Consequently, rapid AST systems are likely to become

increasingly important components of integrated diagnostic workflows^{22,25}.

Computational Prediction of ARM

Recent advances in computational biology and artificial intelligence have introduced new possibilities for ARM prediction that extend beyond traditional laboratory workflows. Rather than directly measuring microbial growth or detecting specific resistance determinants, these approaches seek to infer antimicrobial susceptibility from complex biological datasets, including genomic sequences, proteomic profiles, and large-scale clinical microbiology databases^{9,42}.

The integration of machine learning algorithms with microbiological data has emerged as a particularly promising area of research. Computational models can identify patterns associated with ARM that may not be readily apparent through conventional analytical approaches. By leveraging large training datasets, these systems can generate susceptibility predictions within minutes once the underlying biological data have been acquired^{9,42}.

Among the studies included in this review, machine learning approaches demonstrated encouraging predictive performance. Analysis of MALDI-TOF mass spectrometry data combined with artificial intelligence algorithms achieved high discriminatory power for resistance prediction across several clinically relevant pathogens, with some models reporting area under the receiver operating characteristic curve (AUROC) values approaching 0.999. These findings suggest that routinely generated laboratory data may contain substantial untapped information that can be exploited for resistance prediction without requiring additional experimental procedures⁹.

Similarly, genome-based AST has emerged as an alternative to conventional phenotypic testing. Machine learning models trained on whole-genome sequence data have shown high categorical agreement with reference susceptibility testing and, in some scenarios, have outperformed conventional resistance gene-based prediction approaches⁴². Such findings underscore the ability of computational methods to capture complex genomic signatures associated with ARM that extend beyond the simple presence or absence of individual resistance determinants.

A major advantage of computational prediction lies in its scalability. Once developed and validated, predictive algorithms can be integrated into existing laboratory infrastructures and applied to large numbers of samples with minimal additional laboratory costs. Furthermore, computational approaches may facilitate real-time interpretation of genomic and proteomic data, supporting earlier clinical decision-making and antimicrobial stewardship interventions^{9,42}.

Despite these advantages, several challenges currently limit routine implementation. Machine learning models are highly

dependent on the quality, diversity, and representativeness of training datasets. Predictive performance may decline when models are applied to geographic regions, bacterial populations, or resistance mechanisms that were underrepresented during model development. In addition, algorithm transparency and interpretability remain important concerns, particularly in clinical settings where therapeutic decisions require robust and explainable evidence^{9,42}.

Another limitation is that computational prediction remains fundamentally dependent on the quality of the biological data used as input. Errors in genome assembly, incomplete resistance databases, variability in MALDI-TOF spectra, and differences in laboratory workflows may all influence predictive accuracy^{9,42}. Consequently, computational approaches should currently be viewed as complementary tools capable of supporting, rather than replacing, established diagnostic methods.

Nevertheless, the convergence of artificial intelligence, genomics, proteomics, and clinical microbiology represents one of the most significant emerging trends in ARM diagnostics. As sequencing costs continue to decline and computational infrastructure becomes more accessible, predictive models may increasingly contribute to the development of integrated diagnostic workflows capable of delivering rapid and clinically actionable susceptibility information^{9,42}.

Challenges, Clinical Integration, and Future Perspectives

The studies analyzed in this review demonstrate a clear shift toward faster and more comprehensive AMR diagnostics. PCR-based assays, genomic technologies, rapid phenotypic AST systems, and computational models all seek to reduce the time between sample collection and clinically relevant results, although important barriers to routine implementation remain^{5,7,9,24}.

PCR-based assays provide rapid detection of predefined pathogens and resistance determinants but remain limited to targets included in the diagnostic panel^{5,11}. In contrast, genomic technologies offer broader pathogen and resistance characterization but require specialized infrastructure, bioinformatic expertise, and more complex data interpretation^{8,38}. Rapid phenotypic AST systems provide direct susceptibility information, although instrumentation costs, workflow requirements, and species-specific validation constraints may limit implementation^{25,28}.

Standardization and clinical validation remain major challenges. While several technologies have demonstrated excellent performance under controlled study conditions, multicenter validation remains limited for many platforms^{9,24,42}. Differences in patient populations, pathogen prevalence, laboratory workflows, and AMR epidemiology may significantly influence diagnostic performance, highlighting the need for broader validation studies before widespread adoption.

Interpretation of diagnostic results also remains critical. Detection of resistance genes does not necessarily predict phenotypic resistance^{7,13}; genomic approaches may identify organisms or genetic determinants of uncertain clinical significance^{38,41}, and machine learning models often lack full interpretability despite high predictive performance^{9,42}. Consequently, microbiological expertise remains essential for appropriate result interpretation.

Economic considerations represent an additional barrier, particularly in resource-limited settings where investment in instrumentation, reagents, computational infrastructure, and specialized personnel may be challenging^{11,28}. Nevertheless, the reviewed studies consistently indicate that future diagnostic workflows will integrate complementary technologies rather than rely on a single method^{5,7,9,24} (Table 4).

Table 4. Clinical roles, implementation challenges, and future perspectives of emerging AMR diagnostic technologies.

Technology category	Primary clinical application	Main contribution	Major implementation challenges	Future perspective
Molecular diagnostics	Rapid pathogen and resistance gene detection	Early therapeutic optimization and antimicrobial stewardship	Restricted target panels; genotype–phenotype discrepancies	Expanded multiplex panels and point-of-care applications
Sequencing-based technologies	Comprehensive pathogen and resistome characterization	Broad detection spectrum and genomic epidemiology	Cost, bioinformatics infrastructure, data interpretation	Real-time sequencing and routine genomic surveillance
Rapid phenotypic AST	Accelerated susceptibility determination	Direct measurement of antimicrobial susceptibility within hours	Instrumentation costs; limited validation for some organisms	Integration into routine bloodstream infection workflows

Technology category	Primary clinical application	Main contribution	Major implementation challenges	Future perspective
Computational prediction and AI	Resistance prediction from genomic or proteomic data	Rapid interpretation of complex datasets and susceptibility forecasting	Model transparency, dataset bias, regulatory validation	Clinical decision-support systems and predictive microbiology
Integrated diagnostic workflows	Combination of molecular, phenotypic, genomic, and computational approaches	Improved diagnostic accuracy and faster clinical decision-making	Workflow harmonization and interoperability	Personalized and real-time infectious disease management

PCR-based assays may provide rapid pathogen identification, genomic platforms may deliver high-resolution characterization of resistance determinants, rapid phenotypic systems may confirm susceptibility profiles, and computational tools may support data interpretation. Future research should prioritize multicenter validation, workflow standardization, cost-effectiveness analyses, and integration with clinical decision-support systems^{9,24,42}. Continued advances in artificial intelligence, nanopore sequencing, and rapid phenotypic testing are expected to further improve diagnostic speed and predictive accuracy^{7,9}.

Ultimately, the future of AMR diagnostics will depend on the convergence of genomics, computational science, and clinical microbiology.

CONCLUSION

ARM continues to challenge clinical microbiology by increasing the need for rapid and accurate diagnostic strategies capable of supporting timely therapeutic decisions. Although conventional culture-based identification and AST remain the reference standard for resistance determination, their prolonged reporting times represent a significant limitation in the management of severe infections.

The studies analyzed in this review demonstrate that substantial progress has been achieved through the development of molecular diagnostics, genomic technologies, rapid phenotypic AST systems, and computational prediction models. Each of these approaches addresses different limitations of conventional microbiology and contributes unique advantages to ARM detection. Molecular platforms enable rapid identification of pathogens and resistance determinants, genomic technologies provide comprehensive pathogen and resistance characterization, rapid phenotypic systems accelerate

susceptibility determination, and artificial intelligence-based approaches offer new possibilities for predictive resistance assessment.

However, no single technology currently provides a complete solution. Trade-offs between speed, diagnostic breadth, cost, infrastructure requirements, and interpretability remain important considerations for routine implementation. Consequently, the reviewed evidence suggests that the future of ARM diagnostics will likely depend on integrated workflows that combine complementary technologies rather than replacing conventional microbiology with a single alternative approach.

Future efforts should prioritize multicenter validation studies, standardization of analytical workflows, cost-effectiveness evaluations, and the integration of diagnostic outputs into antimicrobial stewardship programs and clinical decision-support systems. Continued advances in sequencing technologies, rapid phenotypic testing, and artificial intelligence are expected to further shorten diagnostic timelines while improving predictive accuracy and clinical applicability.

Ultimately, the evolution of ARM diagnostics reflects a broader transition in clinical microbiology toward faster, more data-driven, and increasingly integrated approaches. The successful implementation of these technologies has the potential to improve antimicrobial stewardship, optimize patient management, and strengthen global efforts to combat ARM.

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