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Review

Improving the Precision of Etiological Diagnosis in Bacterial Infections Using Molecular Technologies: A Comparative Analysis of Platforms, AI Integration, and Point-of-Care Deployment

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Abstract

Bacterial infections impose a substantial global health burden, with antimicrobial resistance (AMR) further compounding the urgency of accurate and timely etiological diagnosis. Conventional culture-based methods, limited by extended turnaround times of 48–96 hours, reduced sensitivity in the presence of prior antibiotic exposure, and an inability to characterize resistomes at the molecular level, are progressively insufficient in the face of contemporary clinical demands. Molecular technologies have transformed bacteriological diagnostics by enabling rapid, sensitive, and highly specific pathogen identification directly from clinical specimens. Despite a growing body of primary evidence, no current review synthesizes these platforms under a unified comparative analytical framework that simultaneously addresses three critical dimensions: (1) the quantitative performance benchmarking of principal molecular platforms, including polymerase chain reaction (PCR) and its variants, isothermal amplification technologies, next-generation sequencing (NGS), clustered regularly interspaced short palindromic repeats (CRISPR) based diagnostics, and digital PCR (dPCR), across standardized parameters of sensitivity, specificity, limit of detection (LOD), time-to-result, and multiplexing capacity; (2) the integration of artificial intelligence and machine learning (AI/ML) algorithms into molecular diagnostic workflows for AMR prediction and clinical decision support; and (3) the translational trajectory of these technologies toward point-of-care (POC) deployment in decentralized and resource-limited settings. This review addresses this gap by providing a structured, evidence-based comparative analysis of molecular platforms applicable to bacterial infection diagnostics, critically evaluating their clinical validation status, AMR genotyping capabilities, AI augmentation potential, and readiness for POC implementation. We further delineate regulatory, health-economic, and implementation considerations, and identify key research priorities for the next generation of culture-independent precision bacteriological diagnostics.

Keywords: molecular diagnostics; bacterial infections; PCR; next-generation sequencing; CRISPR diagnostics; antimicrobial resistance; point-of-care; machine learning; comparative performance; precision microbiology

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1. Introduction

Bacterial infections represent a leading cause of morbidity and mortality worldwide. A 2022 landmark analysis estimated that 33 bacterial pathogens were directly responsible for 7.7 million deaths in 2019, positioning bacterial infections as the second leading cause of global mortality [1]. The progressive dissemination of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains — driven by inappropriate antibiotic prescribing, inadequate infection control, and global travel — has further amplified the clinical imperative for diagnostic strategies that go beyond simple pathogen identification to deliver actionable molecular characterization of antimicrobial resistance determinants [2].

Accurate and timely etiological diagnosis is a foundational determinant of clinical outcome. The diagnostic gap, the interval between clinical suspicion of infection and receipt of actionable microbiological results, is estimated at 48–96 hours under conventional culture-based workflows. During this period, empirical broad-spectrum antimicrobial therapy is initiated in the absence of microbiological data, contributing to inappropriate prescribing in 20–40% of cases, accelerated emergence of resistance, and preventable adverse outcomes [3]. In sepsis, where each hour of untargeted therapy is independently associated with increased mortality, such delays are clinically untenable [4].

Molecular diagnostic technologies, encompassing nucleic acid amplification tests (NAATs), next-generation sequencing (NGS), and emerging CRISPR-based and digital platforms, have substantially narrowed this diagnostic gap. Nevertheless, the clinical translation of these technologies into routine practice remains heterogeneous, constrained by analytical complexity, interpretive challenges, regulatory barriers, and cost-benefit considerations [5].

While previous reviews have addressed individual molecular platforms or specific infection syndromes, no existing publication provides a unified comparative framework that simultaneously benchmarks platform performance quantitatively, evaluates artificial intelligence and machine learning (AI/ML) integration as an analytical enabler, and assesses the translational readiness of each technology for point-of-care (POC) deployment. This review addresses that gap. Specifically, it makes three novel contributions: (i) a structured side-by-side comparison of all major molecular platforms across standardized analytical parameters, presented in tabular form for direct clinical reference; (ii) a synthesized analysis of AI and machine learning integration across molecular diagnostic workflows, with emphasis on antimicrobial resistance (AMR) prediction performance and limitations; and (iii) a critical evaluation of POC molecular deployment evidence, including platform readiness, WHO Target Product Profile (TPP) concordance, and real-world implementation challenges. Together, these three dimensions define the current frontier of precision etiological diagnostics for bacterial infections and constitute the primary rationale for this review.

2. Conventional Microbiological Diagnostics: The Diagnostic Gap

The conventional diagnostic algorithm for bacterial infections, encompassing specimen collection, direct microscopy, culture-based pathogen recovery, MALDI-TOF MS identification, and phenotypic antimicrobial susceptibility testing (AST), has defined the microbiological standard of care for over a century. MALDI-TOF MS has substantially accelerated organism identification from isolated colonies, reducing identification time to minutes with species-level accuracy exceeding 95% for most clinically relevant bacteria [6]. Nevertheless, MALDI-TOF MS is contingent upon successful culture, inheriting the temporal and sensitivity limitations of the culture step.

Phenotypic AST on automated platforms (VITEK 2, MicroScan, Phoenix) requires pure culture and provides categorical susceptibility results in 8–16 hours under optimal conditions. However, these platforms are susceptible to categorical error for organisms exhibiting heteroresistance or complex resistance mechanisms, notably carbapenem-resistant Enterobacterales (CRE), where phenotypic interpretive categories may fail to reflect the clinical significance of underlying resistance genes [7].

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The cumulative diagnostic gap under conventional workflows is 48–96 hours for blood culture-based bacteremia workups and may exceed five days for fastidious organisms or specimens from patients with prior antibiotic exposure. Epidemiological studies indicate that this gap translates directly into excess mortality, longer hospitalizations, and broader antimicrobial selection pressure, forming the primary clinical justification for the adoption of molecular diagnostics [4,8].

3. Molecular Diagnostic Platforms: Overview and Analytical Performance

3.1. PCR-Based Technologies

3.1.1. Real-Time Quantitative PCR (qPCR)

Quantitative PCR (qPCR), incorporating TaqMan probe hydrolysis or SYBR Green intercalation chemistry, enables simultaneous amplification and quantification of pathogen-specific nucleic acid targets with clinical sensitivity and specificity frequently exceeding 95% and 98%, respectively, for well-validated bacterial targets [9]. For MRSA detection from clinical specimens, qPCR-based assays demonstrate pooled sensitivity of 93.5% and specificity of 97.8% across multiple meta-analytic datasets, with a time-to-result of 1–3 hours — a performance profile fundamentally superior to culture-based MRSA screening [10]. For resistance gene detection, qPCR targeting mecA/mecC, vanA/B, blaKPC, blaNDM, blaOXA-48, and blaVIM achieves analytical sensitivity in the range of 1–100 genome equivalents per reaction, enabling detection in specimens with low organism loads [11].

3.1.2. Multiplex PCR Syndromic Panels

Multiplex PCR platforms — including BioFire FilmArray (bioMérieux), Seegene Allplex, and Hologic Panther Fusion — simultaneously interrogate panels of 10 to more than 40 bacterial targets, along with resistance markers, in a single reaction. The FilmArray Blood Culture Identification Panel 2 (BCID2), encompassing 43 pathogen and resistance targets including mecA, vanA/B, blaKPC, blaNDM, and blaOXA-48-like, demonstrated 96.5% overall concordance with conventional culture-based identification in a multicenter evaluation, with a median time-to-result of 73 minutes from positive blood culture flag [12]. Prospective intervention studies demonstrated that, when panel results were coupled with structured antimicrobial stewardship programs, appropriate antimicrobial therapy was initiated 25 hours earlier, with associated reductions in 30-day mortality among bacteremic patients [13].

3.1.3. Digital PCR (dPCR)

Digital PCR partitions nucleic acid samples into thousands of individual reaction chambers, enabling absolute quantification through Poisson statistical analysis of binary readouts, with a limit of detection one to two orders of magnitude lower than conventional qPCR. Droplet digital PCR (ddPCR) has demonstrated utility in detecting low-level carbapenemase gene copies below the qPCR threshold in surveillance specimens, with direct implications for outbreak prevention [14]. Clinical applications include precise quantification of pathogen burden for therapeutic monitoring and detection of heteroresistance in specimens from patients with prior antibiotic exposure.

3.2. Isothermal Nucleic Acid Amplification

Loop-Mediated Isothermal Amplification (LAMP) employs a strand-displacing polymerase at a constant temperature (60–65 °C), yielding results in 15–60 minutes without thermocycling equipment. A systematic review and meta-analysis of LAMP for M. tuberculosis detection demonstrated a pooled sensitivity of 97.7% and a specificity of 99.1% in smear-positive specimens [15]. Colorimetric LAMP for C. difficile toxin gene detection achieved a sensitivity of 94.3% and a specificity of 97.8% compared with PCR reference in stool specimens, with a cost per test substantially below that of syndromic panel platforms [16]. Recombinase Polymerase Amplification (RPA)

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amplifies at near-physiological temperature (37–42 °C), while Transcription-Mediated Amplification (TMA) — selective for RNA targets, including ribosomal RNA — enables detection of viable organisms and discrimination from dead bacteria, a critical feature for therapeutic monitoring [17].

3.3. Next-Generation Sequencing (NGS)

3.3.1. Whole-Genome Sequencing (WGS)

WGS using Illumina short-read or Oxford Nanopore long-read platforms provides comprehensive genomic characterization of bacterial isolates — encompassing species identification, multi-locus sequence typing (MLST), whole-genome phylogenetics, complete characterization of the resistome and virulome, and determination of plasmid architecture [18]. Meta-analytic data demonstrate WGS resistome-phenotype concordance exceeding 95% for major antibiotic classes in Staphylococcus aureus, Enterococcus spp., and Enterobacterales [19]. WGS has been established as the cornerstone of national bacterial surveillance programs in multiple European countries and the United States, providing near-real-time phylogeographic tracking of priority pathogens [20].

3.3.2. Metagenomic NGS (mNGS)

Metagenomic NGS enables untargeted, culture-independent pathogen identification from clinical specimens by aligning all sequenced nucleic acids to comprehensive microbial reference databases. A prospective multicenter study of patients with unexplained sepsis demonstrated that mNGS pathogen identification was achieved in 52.9% of culture-negative cases, with a direct impact on clinical management in 29.4% [21]. For central nervous system infections — the application where mNGS evidence is most robust — Wilson et al. demonstrated CSF mNGS identified the causative agent in 13% of cases with negative conventional diagnostics, including rare and unexpected pathogens [22]. However, mNGS faces implementation barriers: the low fraction of microbial reads in blood (<0.01%), background contamination, complex interpretive requirements, and current per-test costs of USD 250–2000 limit routine deployment to specialized settings [23].

3.3.3. Nanopore-Based Rapid Sequencing

Oxford Nanopore Technologies (ONT) MinION enables real-time, long-read sequencing with clinical bacteremia turnaround — from positive blood culture to comprehensive genomic report — of as little as 6 hours in optimized workflows. A prospective nanopore-targeted sequencing (NTS) study analyzing 387 blood samples demonstrated high concordance with mNGS and an 80.6% genotype-phenotype correlation for resistance genes in bloodstream infections, with species identification achievable within 30 minutes of sequencing initiation [24].

3.4. CRISPR-Based Diagnostic Platforms

The clustered regularly interspaced short palindromic repeats (CRISPR)- based diagnostics exploit the programmable target recognition of guide RNAs, combined with the trans-cleavage (collateral nuclease) activity of Cas12 and Cas13 effectors, to generate sequence-specific signals. The SHERLOCK platform (Cas13a-mediated RNA detection with lateral flow readout) achieves attomolar sensitivity and enables single-nucleotide variant discrimination without high-resolution melting curve analysis, enabling differentiation of resistance alleles [25]. The DETECTR system (Cas12a with lateral flow) achieved 100% concordance with PCR for the detection of the C. difficile toxin gene in a prospective clinical evaluation [26]. Recent advances include CRISPR-Cas12a microfluidic chip systems that simultaneously identify eight pathogens in 45 minutes, with 99.2% concordance with qPCR, and are currently undergoing CE marking validation [27].

For AMR detection specifically, CRISPR-based assays targeting blaKPC, blaNDM, and mecA variants have demonstrated single-nucleotide discriminatory capacity that PCR-based platforms cannot achieve without supplementary high-resolution melt analysis [28]. A CRISPR/Cas13a-based

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simultaneous amplification and testing platform (SATCAS), integrating one-pot RNA detection and SNP discrimination, was clinically validated in 2024 [29].

4. Comparative Performance Analysis of Molecular Platforms

Table 1 provides a structured side-by-side comparison of major molecular diagnostic platforms for etiological diagnosis of bacterial infections across seven standardized analytical parameters. This comparative framework, absent from existing reviews, enables direct clinical and laboratory decision-making regarding platform selection based on context-specific performance requirements.

Table 1. Comparative analytical performance of major molecular diagnostic platforms for bacteriological diagnosis. Data synthesized from [9–29].

Platform	Sensitivity	Specificity	LOD	Time-to-Result	Multiplex Capacity	AMR Genotyping	POC Feasibility
qPCR / RT-PCR	93–98%	97–99%	1–100 copies/rxn	1–3 h	Low (1–5 targets)	Targeted genes (mecA, vanA/B,blaKPC)	Moderate (GeneXpert)
Multiplex PCR Panel	90–97%	97–99%	10–100 copies/rxn	1–2 h	High (20–43 targets)	Panel-specific genes	Moderate (FilmArray)
Digital PCR (ddPCR)	98–99%	98–99%	0.1–1 copy/rxn	3–5 h	Low (1–3 targets)	High sensitivity variants	Low
LAMP	90–98%	97–99%	10–100 copies/rxn	15–60 min	Low (1–6 targets)	Selected resistance genes	High (colorimetric)
RPA	88–96%	95–99%	1–10 copies/rxn	10–30 min	Low	Selected targets	High
WGS (Illumina)	99%+	99%+	Requires culture	24–72 h	Complete genome	Full resistome, MLST, plasmids	Very Low
mNGS	65–85% (clinical)	85–95%	Variable	12–48 h	Unlimited (hypothesis-free)	Full resistome (culture-free)	Very Low
Nanopore Sequencing	90–99% (targeted)	95–99%	~10 copies/rxn	1–6 h	High to complete	Full resistome, plasmid context	Low-Moderate
CRISPR (SHERLOCK/ DETECTR)	94–100%	98–100%	1–100 aM	30–60 min	Moderate (multiplex emerging)	SNV-level AMR variants	High (lateral flow)

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Platform	Sensitivity	Specificity	LOD	Time-to-Result	Multiplex Capacity	AMR Genotyping	POC Feasibility
CRISPR-Microfluidic	96–99%	99%+	1–10 copies/µL	30–45 min	High (8+ simultaneous)	AMR genes (emerging)	High (integrated chip)

LOD: limit of detection; AMR: antimicrobial resistance; MLST: multi-locus sequence typing; SNV: single-nucleotide variant; POC: point-of-care; aM: attomolar; rxn: reaction.

Several key observations emerge from this comparative analysis. First, there is an inherent performance trade-off between analytical comprehensiveness and time-to-result: WGS and mNGS provide the most complete pathogen characterization but require 12–72 hours and are currently confined to centralized laboratories. LAMP, RPA, and CRISPR-based platforms offer the shortest turnaround times (15–60 minutes) and highest POC feasibility, but at the cost of reduced multiplexing breadth. Second, digital PCR occupies a distinct niche defined by unmatched quantitative precision and sensitivity, but is not suited to broad syndromic diagnostics or POC contexts. Third, CRISPR-based platforms are unique in combining attomolar sensitivity with single-nucleotide discrimination and POC-compatible lateral-flow readout, positioning them as the most likely candidates for next-generation decentralized precision AMR diagnostics.

The selection of the optimal platform in clinical practice is therefore context-dependent, and a tiered diagnostic algorithm — deploying rapid POC platforms for initial triage and reserving NGS-based approaches for complex, culture-negative, or epidemiologically significant cases — represents the most clinically and economically rational strategy [5,30].

5. Molecular AMR Genotyping: Current Capabilities and Limitations

Molecular AMR genotyping — the detection of resistance determinants at the nucleic acid level — provides actionable resistance information within timeframes compatible with clinical decision-making, independent of the culture-based AST workflows that currently impose the critical diagnostic delay in bacteremia management. The principal platforms for AMR genotyping span a spectrum from targeted single-gene assays to comprehensive whole-genome resistome profiling.

Targeted PCR-based AMR detection panels — encompassing mecA/mecC for MRSA, vanA/B for VRE, blaKPC, blaNDM, blaOXA-48-like, blaVIM, and blaIMP for carbapenemase-producing organisms (CPOs) — represent the most clinically mature and widely deployed molecular AMR tools. A prospective multicenter evaluation of Xpert Carba-R for carbapenemase gene detection from rectal surveillance swabs demonstrated a sensitivity of 98.1% and specificity of 99.5% compared with culture and confirmatory PCR, with a time-to-result of 75 minutes [31]. These performance parameters are clinically sufficient for infection control decision-making and have been incorporated into national CPO surveillance guidelines in multiple European countries.

WGS-based comprehensive resistome profiling, interrogating curated databases including Comprehensive Antibiotic Resistance Database (CARD), ResFinder, ARG-ANNOT, and NCBI AMRFinder Plus, provides genotype-phenotype concordance exceeding 95% for major antibiotic classes in Enterobacterales and S. aureus [19]. However, significant concordance gaps persist for organisms with novel or chromosomally mediated resistance mechanisms, for which phenotypic reference databases may lag behind the emergence of clinical resistance. The 2021 WHO catalog of M. tuberculosis mutations associated with drug resistance, integrating data from 38,215 isolates, established the most comprehensive genotype-phenotype framework to date for a single pathogen, achieving sensitivity of 94.3% and specificity of 99.4% for rifampicin resistance prediction [32].

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A critical limitation of genotypic AMR prediction is the inherent database dependency: resistance genes or mutations not represented in the reference database will not be detected, creating a systematic blind spot for novel mechanisms. Furthermore, the detection of a resistance gene does not invariably predict phenotypic resistance, as expression-level regulation, gene copy number variation, and epistatic interactions between resistance determinants may attenuate or enhance phenotypic expression beyond what genotype alone can predict [33].

6. Artificial Intelligence and Machine Learning in Molecular Bacteriological Diagnostics

The integration of artificial intelligence and machine learning into molecular diagnostic workflows represents one of the most consequential developments in contemporary clinical microbiology. AI/ML applications address critical bottlenecks in the interpretation of data-rich molecular platforms, particularly WGS, mNGS, and multiplex panels, where the volume and complexity of generated data preclude efficient manual analysis at clinical throughput scales [34].

6.1. ML-Based AMR Phenotype Prediction from WGS Data

Supervised machine learning models trained on curated WGS datasets have demonstrated performance competitive with or superior to EUCAST/CLSI phenotypic breakpoints for genotype-to-phenotype AMR prediction in priority pathogens. A 2024 systematic review evaluating 26 studies integrating WGS and ML for AMR prediction in critical pathogens, encompassing 104,141 microbial samples, identified Random Forest (RF), XGBoost, and logistic regression as the highest-performing algorithms, with mean AUC values of 0.89, 0.87, and 0.87, respectively [35]. RF demonstrated superior performance with AUC values ranging from 0.66 to 0.97 across species-antibiotic combinations.

For Acinetobacter baumannii, one of the WHO critical priority pathogens, a 2024 study applying RF, SVM, and XGBoost with k-mer features from WGS data of 339 isolates predicted MICs for 13 antimicrobials, with category agreement exceeding 95.29% and essential agreement above 90.90%, demonstrating that ML-based AMR prediction can achieve interpretive agreement competitive with conventional AST [36]. For Pseudomonas aeruginosa, BioWeka and RF classifiers trained on WGS data achieved mean classification accuracy of 98% and 96%, respectively, across 12 antimicrobial classes [37].

Deep learning architectures have extended AMR prediction beyond resistance gene catalogs to capture complex genotype-phenotype relationships mediated by regulatory mutations, promoter polymorphisms, and epistatic interactions. A deep learning framework employing CNNs and denoising autoencoders for M. tuberculosis drug resistance prediction achieved a sensitivity of 0.90 and a specificity of 0.87 across first- and second-line antituberculars, and the model was made publicly accessible as TB-DROP [38]. The 2024 assessment of four state-of-the-art ML methods for AMR prediction across 78 species-antibiotic datasets highlighted that no single algorithm universally outperforms others, underscoring the need for pathogen- and antibiotic-specific model selection and validation [39].

6.2. AI-Assisted mNGS Interpretation

The clinical interpretation of mNGS data, distinguishing the infecting pathogen from colonizers, contaminants, or bystanders, remains a critical, unresolved challenge that AI approaches are beginning to address. Convolutional neural network and transformer-based models that integrate read counts, genome coverage breadth, phylogenetic signal, and patient clinical metadata have been developed to generate probabilistic pathogen likelihood scores that outperform abundance thresholding alone in discriminating clinically significant organisms from background [40]. The Karius Test, an FDA-authorized plasma mNGS platform, incorporates a proprietary AI interpretation module providing clinician-facing reports with pathogen-specific significance scoring, representing the most advanced commercially deployed AI-assisted mNGS diagnostic tool [41].

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Importantly, AI models trained on single-center or geographically restricted datasets may exhibit significant performance degradation when applied to external populations with different pathogen epidemiology, microbiome composition, or specimen processing protocols — a generalizability challenge directly analogous to those documented in AI-based radiology and pathology applications. Multi-center AI model development and prospective external validation across diverse clinical settings are essential prerequisites for clinical translation [42].

6.3. Federated Learning and Data Governance

Federated learning — enabling ML model training across distributed healthcare datasets without centralized data sharing — offers a compelling solution to the data governance and patient privacy constraints that have historically limited multicenter AI development in clinical microbiology. Early implementations of federated AMR prediction models across multiple university hospitals have demonstrated model performance equivalent to centrally trained counterparts while maintaining full patient data sovereignty [43]. This approach is particularly relevant in the European regulatory context governed by the GDPR, where centralized aggregation of genomic data across institutions poses significant legal and ethical challenges.

6.4. AI-POC Convergence: Edge Computing and Smartphone-Integrated Diagnostics

The convergence of AI with POC molecular platforms — through edge-computing algorithms embedded in diagnostic devices and smartphone-connected cloud-based analytics — is creating a new class of 'smart' POC diagnostics capable of automated result interpretation, real-time quality control, and integration with national AMR surveillance systems. The POC diagnostics market was valued at approximately USD 53 billion in 2024, with AI integration projected to substantially penetrate molecular, biosensor, and microfluidic subsegments by 2034 [44]. Deep learning-assisted predictive diagnostics integrating time-series AI architectures have demonstrated a reduction of assay interpretation time to 1–2 minutes in prototype infectious disease POC systems [45] (Table 2).

Table 2. Representative ML models for AMR prediction from WGS and phenotypic data. Data synthesized from [35–39,46].

Pathogen / Target	Algorithm	AUC / Accuracy	AMR Classes Predicted	Dataset Size	Reference
A. baumannii (WGS)	RF + XGBoost + k-mer	CA >95.3%, EA >90.9%	13 antimicrobials	339 isolates	[36]
P. aeruginosa (WGS)	Random Forest / BioWeka	98% / 96% accuracy	12 classes	NR	[37]
Critical pathogens (WGS+AST)	RF, XGBoost, LR	AUC 0.89 / 0.87 / 0.87	Multiple	104,141 samples	[35]
M. tuberculosis (WGS+DL)	CNN + Denoising AE	Sens. 0.90, Spec. 0.87	First + second line	>38,000 genomes	[38]
AMR (phenotypic data)	XGBoost	AUC 0.96	Multiple organisms	917,049 isolates	[46]
E. coli / Salmonella WGS	Random Forest + k-mer	91–98% accuracy	Selected classes	Large-scale PATRIC	[39]

CA: category agreement; EA: essential agreement; AUC: area under the receiver operating characteristic curve; NR: not reported; DL: deep learning; AE: autoencoder.

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7. Point-of-Care Molecular Diagnostics: From Laboratory to Bedside

The translation of molecular diagnostic capability to the point of care — the site of patient encounter without reliance on centralized laboratory infrastructure — is a strategic priority for infectious disease management, AMR stewardship, and pandemic preparedness [44]. The global POC infectious disease diagnostics market was estimated at USD 12–15 billion in 2024, with a compound annual growth rate of 8–9% projected through the late 2020s, driven by infectious disease assays and accelerated by innovations in molecular amplification, biosensors, microfluidics, and AI [44].

7.1. Commercially Available POC Molecular Platforms

The Cepheid GeneXpert system, the most extensively validated commercial POC molecular platform, encompasses cartridge-based assays for more than 20 bacterial and viral targets, including MRSA/SA, C. difficile, N. gonorrhoeae/C. trachomatis, M. tuberculosis/rifampin resistance, and Streptococcus agalactiae, validated across emergency departments, intensive care units, and resource-limited settings [47]. The study conducted by Emilio Bouza and colleagues demonstrates that integrating a rapid molecular diagnostic test such as GeneXpert MRSA/SA SSTI into a structured SSTI management program, combined with antimicrobial stewardship principles, significantly optimizes antimicrobial therapy. This approach enables the rapid identification of causative pathogens—particularly Staphylococcus aureus and MRSA—thereby facilitating early initiation of targeted treatment, reducing unnecessary use of broad-spectrum antibiotics, and ultimately helping to limit antimicrobial resistance. Furthermore, the implementation of such a "fast-track" program improves clinical workflow efficiency and may reduce hospital length of stay and overall healthcare costs [48]. The Abbott ID NOW platform employs isothermal amplification to deliver results in 5–13 minutes, while bioMérieux's SpotFire MDx system is a next-generation POC platform that leverages novel approaches for broader pathogen target coverage [49].

The Frontiers 2026 synthesis of POC molecular technologies identified isothermal NAATs, rapid PCR, CRISPR-based detection, nanomaterials-enabled biosensors, and microfluidic platforms as the five principal technology classes advancing toward POC deployment, with all five achieving sub-30-minute time-to-result in optimized formats [50]. Multiplex POC panels currently provide pathogen identification and resistance profiles in 45–90 minutes, enabling rapid, evidence-based therapy and minimizing unnecessary antimicrobial use — particularly valuable for MDR TB, drug-resistant N. gonorrhoeae, MRSA, and CRE [50].

7.2. Emerging POC Technologies: LAMP-on-Chip and CRISPR-POC

LAMP-based POC biosensors have seen substantial innovation in the 2020–2025 period. Colorimetric LAMP with pH-sensitive dyes enables visual interpretation of results without instrumentation, with LODs of 1–100 copies per reaction for bacterial targets [51]. Paper-based LAMP devices combining RNA extraction, isothermal amplification, and visual readout have achieved LODs of 10^3 copies/mL with a time-to-result of 30 minutes [51]. Digital LAMP — partitioning reactions into thousands of microchambers for absolute quantification — extends LAMP's analytical envelope toward the performance levels of ddPCR while preserving isothermal operation.

CRISPR-based POC diagnostics represent the technology with the strongest transformative potential for decentralized AMR diagnostics. CRISPR infrastructure investment requirements are estimated to be 90% lower than those for NGS, using lateral flow test strips or handheld devices [27]. CRISPR-Cas12a-integrated electrochemical biosensors (E-CRISPR) and isotropic electrophoresis technologies provide instrument-free POC solutions without requiring fluorescent labeling [52]. A portable Dragonfly LAMP-based platform demonstrated power-free nucleic acid extraction in under 5 minutes and colorimetric results in 30 minutes, with an LOD of 100 genome copies per reaction, and was validated across multiple pathogen targets in resource-limited settings [53].

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7.3. WHO Target Product Profile Concordance and Implementation Gaps

The WHO Target Product Profile (TPP) for POC diagnostics in priority bacterial infections specifies minimum performance requirements of sensitivity ≥80%, specificity ≥95%, time-to-result <30 minutes, and cost per test <USD 5 for low- and middle-income country (LMIC) applicability [54,55]. Current commercial platforms meet sensitivity and specificity thresholds but substantially exceed cost thresholds, with most cartridge-based assays priced at USD 15–50 per test. Lyophilized LAMP reagents and CRISPR lateral flow assays are the most realistic near-term candidates for meeting WHO TPP cost requirements, with prototype costs of USD 2–5 per test in research-grade manufacturing contexts [27].

The implementation of POC molecular diagnostics in LMICs faces multidimensional barriers beyond reagent costs: cold-chain requirements for reagent stability, inconsistent electrical infrastructure, limited trained personnel, lack of connectivity for result reporting, and absence of post-market surveillance frameworks. Cloud-connected POC platforms with smartphone-based readout and AI-assisted interpretation are beginning to address connectivity and interpretive barriers, with digital health integration enabling near-real-time AMR surveillance linkage between clinical diagnostics and public health intelligence [50] (Table 3).

Table 3. Comparative overview of POC molecular platforms for bacteriological diagnosis and AMR detection. Data synthesized from [44–54].

Platform	Technology	Time-to-Result	Sensitivity	Specificity	AMR Detection	LMIC Feasibility	Regulatory Status
GeneXpert (Cepheid)	Cartridge PCR	45–90 min	92–99%	97–99%	Yes (targeted genes)	Moderate	FDA/CE cleared
ID NOW (Abbott)	Isothermal (NEAR)	5–13 min	85–96%	98–100%	Limited	Moderate	FDA cleared
FilmArray (BioFire)	Multiplex PCR	45–90 min	93–97%	97–99%	Yes (panel-based)	Low (cost)	FDA/CE cleared
Colorimetric LAMP	Isothermal (LAMP)	15–45 min	90–98%	97–99%	Selected genes	High	CE (select)
CRISPR lateral flow	CRISPR-Cas12/13	30–60 min	94–100%	98–100%	SNV-level AMR	High (emerging)	CE pending
CRISPR-Microfluidic chip	CRISPR + microfluidics	30–45 min	96–99%	99%	AMR genes (emerging)	Moderate	CE validation
Nanopore POC (MinION)	Nanopore sequencing	30 min – 6 h	90–99%	95–99%	Full resistome	Low	Research use

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E-CRISPR biosensor	Electrochemical CRISPR	< 45 min	95–99%	98–100%	AMR variants	High (emerging)	Pre-commercial

NEAR: nicking enzyme amplification reaction; LMIC: low- and middle-income country; SNV: single-nucleotide variant.

8. Regulatory Frameworks and Health-Economic Considerations

The clinical implementation of molecular diagnostics is governed by jurisdiction-specific regulatory frameworks: FDA 510(k) or de novo authorization in the United States, CE-IVD marking under EU IVDR (Regulation 2017/746), and equivalent national frameworks in other jurisdictions. The EU IVDR — fully applicable from May 2022 with transition periods extending to 2027 — has substantially raised the regulatory bar for in vitro diagnostics, requiring clinical evidence packages that many legacy molecular assays are only beginning to assemble [56].

Health-economic analyses of syndromic molecular panels for bloodstream infections have consistently demonstrated favorable cost-effectiveness when structured antimicrobial stewardship programs are co-implemented. A cost-effectiveness model of FilmArray BCID-guided stewardship in septic patients demonstrated an incremental cost-effectiveness ratio of USD 4,200 per quality-adjusted life year gained versus conventional diagnostics plus standard stewardship — well within accepted willingness-to-pay thresholds [57]. For mNGS, a targeted deployment strategy — applied to patients with confirmed infection syndrome and culture-negative results after 48–72 hours — achieves the highest cost-benefit ratio by concentrating diagnostic utility where incremental yield is greatest [58].

External quality assurance (EQA) participation through programs such as NEQAS Bacteriology, QCMD, and ECDC-coordinated AMR surveillance schemes is an essential component of infrastructure for maintaining molecular diagnostic quality standards across healthcare networks. Standardization of bioinformatic pipelines for WGS-based surveillance — actively promoted by ECDC through standardized core-genome MLST schemes and shared sequence repositories — remains a critical prerequisite for cross-border epidemiological comparisons and outbreak investigation [59].

9. Conclusions

This review provides a structured, evidence-based synthesis of molecular technologies for etiological diagnosis of bacterial infections, organized around three analytical dimensions that have not been previously addressed in combination: quantitative platform comparison, AI/ML integration, and POC translational readiness.

The comparative analysis (Table 1) reveals that no single platform is superior across all performance dimensions: qPCR-based multiplex panels offer the optimal balance of sensitivity, breadth, and clinical validation for acute infection management; mNGS provides unique value for culture-negative and diagnostically challenging cases; CRISPR-based platforms uniquely combine attomolar sensitivity, SNV-level discriminatory power, and POC-compatible formats; and WGS delivers unmatched genomic resolution for outbreak investigation and surveillance.

The AI/ML synthesis (Table 2) demonstrates that machine learning — particularly Random Forest and XGBoost algorithms applied to WGS data — achieves AMR prediction performance competitive with conventional AST for multiple priority pathogens, with meta-analytic AUC values of 0.87–0.89 across species-antibiotic combinations. However, generalizability across institutions and geographies remains the primary translational barrier, addressable through federated learning architectures and prospective multicenter validation.

The POC analysis (Table 3) identifies colorimetric LAMP and CRISPR lateral flow assays as the technologies with the highest near-term potential for achieving WHO TPP cost and simplicity thresholds in LMIC settings, while commercially cleared cartridge-based platforms (GeneXpert,

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FilmArray) represent the current clinical standard for decentralized molecular testing in well-resourced settings. The convergence of AI-assisted interpretation with POC platforms, enabled by edge computing and smartphone integration, is creating a new class of 'smart' POC diagnostics with real-time AMR surveillance linkage.

Critical research priorities identified in this review include: (1) prospective multicenter RCTs establishing the patient outcome impact of mNGS versus conventional diagnostics plus targeted molecular panels across specific infection syndromes; (2) federated AI model development for AMR prediction with prospective external validation across diverse clinical and geographical settings; (3) head-to-head comparative evaluations of CRISPR platforms against qPCR for AMR genotyping in priority organism-antibiotic combinations; (4) health-economic modeling incorporating full diagnostic cascade effects and AMR stewardship program co-implementation; and (5) coordinated regulatory pathway development for next-generation CRISPR-POC and AI-assisted diagnostic platforms under IVDR and FDA frameworks.

The realization of precision etiological diagnostics for bacterial infections — delivering the right pathogen identification, with complete resistance characterization, for the right patient, in the right setting, within clinically actionable timeframes — requires sustained interdisciplinary collaboration among clinical microbiologists, infectious disease clinicians, bioinformaticians, regulatory scientists, and health technology assessment bodies. The technological foundations are demonstrably mature across multiple platform classes; the imperative now lies in their rigorous clinical validation, equitable deployment, and integration into precision antimicrobial stewardship frameworks.

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