Review

Diagnostic Approaches for Methicillin-Resistant *Staphylococcus aureus* (MRSA): Accuracy, Limitations, and Future Direction

Niki Mehri , Tara Rezaei * , Boya Douho , Armin Aliyari , Richa Pandey

Posted Date: 31 March 2026

doi: 10.20944/preprints202603.2455.v1

Keywords: methicillin-resistant *Staphylococcus aureus*; MRSA; diagnostics; infectious disease

 doi:10.20944/preprints202603.2455.v1

Review

Diagnostic Approaches for Methicillin-Resistant *Staphylococcus aureus* (MRSA): Accuracy, Limitations, and Future Direction

Niki Mehri, Tara Rezaei, Boya Douho, Armin Aliyari and Richa Pandey

Department of Biomedical Engineering, University of Calgary

* Correspondence: tara.rezaei@ucalgary.ca

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global health threat responsible for significant morbidity and mortality, accounting for approximately 19,000 deaths annually in the United States. MRSA resistance is primarily mediated by the mecA and mecC genes, which are carried on the staphylococcal cassette chromosome mec (SCCmec) integrated at the OrfX locus of *Staphylococcus aureus*, resulting in reduced susceptibility to β-lactam antibiotics. Rapid and accurate diagnostic methods are therefore essential to improve clinical outcomes and limit disease transmission. This mini-review evaluates current MRSA diagnostic approaches, including polymerase chain reaction (PCR) and its variants, isothermal amplification techniques (LAMP and RPA), CRISPR-based diagnostics, and electrochemical biosensors. These methods are compared in terms of diagnostic accuracy, clinical utility, cost-effectiveness, and practical limitations. Overall, isothermal amplification demonstrated a more favorable balance in cost-effectiveness and practical limitations compared to other methods. However, when considering clinical utility and diagnostic accuracy, the results were context dependent. No single method was universally optimal, and the choice of diagnostic approach depends on the clinical context and resource availability.

Keywords: methicillin-resistant *Staphylococcus aureus*; MRSA; diagnostics; infectious disease

Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of the bacterium *Staphylococcus aureus* that causes infections in humans and is resistant to many antibiotics. MRSA is a major global health threat, contributing to roughly 19,000 deaths annually in the United States [1]. MRSA's resistance is driven by the mecA and mecC genes, which enable *Staphylococcus aureus* to survive treatment with beta-lactam antibiotics by producing modified proteins that prevent the antibiotics from effectively disrupting bacterial cell-wall formation [2]. Although historically confined to healthcare settings, MRSA emerged as a community-acquired infection in the 1990s due to genetic variations that allowed *Staphylococcus aureus* to both resist antibiotics and spread efficiently outside hospital environments [1]. The expansion of MRSA's epidemiological impact highlights the need for rapid and reliable diagnostics tools. However, timely and accurate diagnosis remains challenging. One major difficulty is distinguishing between MRSA colonization and active infection [3]. Additionally, rapid tests struggle with detecting low bacterial loads, which reduces sensitivity in early-stage infections [4]. These technical limitations are exacerbated by inconsistent screening practices across institutions, contributing to a lack of standardized diagnostic management [5].

Current MRSA diagnostics rely on both culture-based and molecular methods. Conventional culture remains the clinical gold standard but requires 24–72 hours for organism identification and susceptibility testing, which delays targeted therapy [6]. To overcome this delay, molecular assays such as PCR targeting mecA and S. aureus–specific genes (e.g., nuc) have been widely adopted. These methods significantly reduce turnaround time and improve early detection, particularly in

 doi:10.20944/preprints202603.2455.v1

bloodstream infections [6,7]. Rapid platforms such as isothermal amplification and point-of-care systems further shorten detection time to under one hour, offering practical advantages in acute settings [6]. More recently, biosensor-based technologies, including electrochemical and nanozyme-enhanced systems, have demonstrated ultra-high sensitivity and low detection limits, suggesting promising directions for rapid and portable MRSA diagnostics [8]. However, most of these remain in early validation stages.

Despite advances, significant limitations remain. Culture-based methods are slow and may yield false negatives, especially in patients receiving prior antibiotics [6,9]. Molecular assays, although rapid, may detect resistance genes without confirming active infection or phenotypic expression, potentially leading to overestimation of MRSA cases [7]. In addition, diagnostic performance is influenced by sample quality, bacterial load, and biofilm-associated infections, where organisms may evade detection [9]. Cost, equipment requirements, and the need for trained personnel also limit widespread implementation of advanced molecular platforms [7]. While rapid diagnostics have improved early detection, simultaneously achieving high sensitivity, specificity, affordability, and clinical applicability remain a major challenge.

Due to the limited treatment options, it is critical that MRSA strains are detected early to improve the clinical effectiveness of diagnostic methods and enhance patient outcomes [10]. A 2010 comparative study conducted by a Canadian agency (CADTH) evaluating the cost-effectiveness of PCR versus chromogenic agar (used as the gold standard in the experiment) showed that implementing an effective diagnostic screening procedure not only reduced pre-screening isolation time per patient but also decreased costs by $22,005 CAD per case [11]. MRSA diagnostic approaches span multiple technologies, clinical settings, and applications, and this mini review provides a comprehensive overview of current methods. Each diagnostic approach is evaluated in terms of diagnostic accuracy, clinical utility, cost-effectiveness, and practical limitations. By synthesizing and comparing existing diagnostic strategies, this mini review aims to clarify optimal approaches for MRSA detection and identify key gaps where further technological and clinical innovation is needed to improve diagnostic speed, reliability, and patient outcomes.

Diagnostic Methods

Electrochemical Biosensors

Electrochemical biosensors detect MRSA by converting a biochemical interaction, such as the binding of a synthetic DNA probe to a target DNA sequence from the bacterium, into a measurable electrical signal [2]. The primary target is the mecA gene. This gene is significant because it encodes a modified cell-wall protein (penicillin-binding protein 2a, or PBP2a) that prevents beta-lactam antibiotics, such as methicillin and oxacillin, from binding to and disrupting the bacterial cell wall [8]. This is the mechanism by which this antibiotic class would normally kill the bacterium. Detecting mecA, therefore, confirms antibiotic resistance [2,8].

In a typical sensor design, a short synthetic DNA strand, referred to as the recognition probe, is attached to an electrode surface made of gold, glassy carbon, or screen-printed carbon [12]. This probe is engineered to bind specifically to a conserved region of mecA. When a patient DNA sample is introduced, any mecA sequences present will bind to the probe on the electrode. This process is called hybridization. The hybridization binding event physically alters conditions at the electrode surface, which is then detected using one of two electrochemical measurement techniques: differential pulse voltammetry (DPV) or electrochemical impedance spectroscopy (EIS) [13]. DPV works by applying a series of small voltage pulses to the electrode and measuring the resulting current [13]. Hybridization shifts the DPV current in proportion to how much target DNA is present [13]. EIS works differently, as it measures the resistance of the electrode surface to an alternating electrical current, which increases when DNA hybridization adds molecular bulk to the surface [13]. Both methods are highly sensitive to small changes at the electrode interface, making them well-suited to detecting trace amounts of pathogen DNA [12,13].

 doi:10.20944/preprints202603.2455.v1

A core design challenge is that bacterial DNA in clinical samples is double-stranded and is often present at very low concentrations, particularly early in an infection [14]. This makes it harder for the probe to find and bind its target. To overcome this, researchers have developed multi-signal probe (MSP) systems [14]. In MSP systems, multiple short DNA strands, each with a tagged biotin molecule, are first used to simultaneously grip the target DNA sequence at several points, forming a dense cluster [14]. This pre-assembled cluster is then captured by a specially structured receptor on the electrode surface called a DNA tetrahedron probe (TSP) [14]. The TSP holds the capture arms at fixed angles away from the electrode, preventing them from collapsing flat against the surface and becoming inaccessible [14]. This upright configuration significantly increases the number of successful capture events [14]. Once the MSP-target cluster is captured, an enzyme conjugate named streptavidin-linked horseradish peroxidase (SA-HRP) binds to the biotin tags and catalyses a chemical reaction that produces a large, measurable electrical current [14]. Because many biotin tags are present per target molecule, the current output is amplified well beyond what would result from a single binding event, enabling detection of synthetic mecA at 10 femtomolar (fM) and purified MRSA genomic DNA at a limit of detection of 57 fM [14]. These concentrations correspond to detecting just a handful of DNA molecules in a microlitre of sample, which is far below the detection threshold of conventional PCR-based assays.

Despite the analytical progress, electrochemical biosensors have not yet reached routine clinical use, which can be attributed to a number of reasons. The electrode surface used in electrochemical biosensors can be prone to fouling through contamination by proteins, lipids, or other biological molecules in blood, wounds, or swabs [12]. Probe attachment geometry is also difficult to control reproducibility across manufacturing batches [12]. Reported platforms also require purified DNA as input, necessitating a separate upstream extraction and processing steps that add time and complexity. Validation has been predominantly limited to laboratory samples rather than prospective clinical cohorts [15]. Overall, compatibility and miniaturized formats make electrochemical biosensors a strong candidate for point of care MRSA diagnostics, particularly in resource limited settings.

Polymerase Chain-Reaction (PCR)

Polymerase chain reaction (PCR) is a molecular technique that utilizes enzymatic replication to amplify short DNA sequences [16]. As previously stated, this technique is widely adopted due to its ability to accurately detect the presence of specific pathogens within a short period of time. Molecular studies on methicillin-resistant *Staphylococcus aureus* (MRSA), as well as complete genome mapping of the three types of MRSA strains, have shown that the mecA gene is located at a unique position within the chromosome, *OrfX* [17]. MRSA contains a staphylococcal cassette chromosome mec (SCCmec) that carries the mecA gene at a fixed site, and due to PCR's ability to selectively amplify specific DNA regions, the presence of this gene can be detected more rapidly and easily compared to standard culture-based methods [16].

PCR typically takes up to approximately 5 hours to detect the mecA gene, whereas culture-based methods may take 48–72 hours. Although PCR is considered the gold standard by many researchers, it has several limitations that hinder its broader adoption. Traditional PCR relies on gel electrophoresis, which requires specialized training and is not always suitable for point-of-care testing [18]. Additionally, other PCR-based techniques may involve complex technical requirements, longer processing times, suboptimal specificity and sensitivity, and the need for post-PCR processing [19]. Due to these limitations, newer methods have been developed to address and improve upon the shortcomings of PCR.

Real-Time PCR

Real-time PCR, also known as quantitative PCR (qPCR), is a PCR-based method that utilizes gene amplification along with the simultaneous quantification of specific DNA sequences in real time. It can quantify the substrates present in the initial mixture before amplification and detect sequences

 doi:10.20944/preprints202603.2455.v1

as they accumulate during the amplification process [20,21]. It offers several advantages over traditional PCR, including quantitative measurement capabilities, lower contamination rates, increased sensitivity and specificity, and easier standardization [20]. The technique utilizes fluorescent dyes and probes to detect DNA, such as the non-specific dye SYBR Green, which binds to any double-stranded DNA [21].

As previously stated, traditional PCR requires several hours to reach the endpoint (plateau), at which point detection occurs; real-time PCR eliminates this waiting period by enabling detection during the amplification process. By doing so, real-time PCR increases the dynamic range of detection and significantly reduces turnaround time compared to traditional PCR, from approximately 4–5 hours to about 2 hours [16,20]. It also does not require post-PCR processing and can detect twofold changes in DNA concentration. In a study evaluating the sensitivity of real-time PCR, it was found that "real-time PCR assays were at least 10-fold more sensitive than conventional PCR methods"; however, it should be noted that this study examined anthrax spores in spiked soil rather than MRSA [20].

There are various types of real-time PCR devices, but in the United States and Canada, only about 3–4 automated, commercially available systems are approved for MRSA detection [16], including the BD GeneOhm MRSA Assay, Xpert MRSA Assay, and Roche LightCycler MRSA Advanced. Although qPCR offers clear advantages over traditional PCR, it still has some drawbacks. It is more expensive due to the need for specialized equipment and reagents, and it remains somewhat prone to false positives if not properly validated.

Multiplex PCR

Multiplex PCR is a molecular diagnostic technique that allows for the simultaneous amplification of multiple DNA target sequences in a single reaction [22,23]. It can be used to identify either multiple pathogens or specific subtypes of a similar agent in samples such as blood, feces, and other tissue lesions [22]. To set up multiplex PCR, the first step is primer selection. There are various software tools that can be used to design and analyze PCR primers; Oligo is commonly used for general primer design, while Primer-BLAST is a more robust tool that can design target-specific primers and utilizes global databases, such as GenBank, to check for specificity across a wide range of organisms [22].

The next step is reaction setup, where all primers, DNA samples, buffers, and other necessary components are combined. To ensure efficient amplification without interference, all primers should have similar melting temperatures. Multiplex PCR offers high sensitivity and specificity, while also saving time and reducing costs in the detection of infectious organisms in patients. In the context of MRSA detection, multiplex PCR has been used in studies involving samples containing MRSA and less pathogenic coagulase-negative Staphylococci (CoNS), both of which may carry the mecA gene [24].

CRISPR-Based Diagnostics

CRISPR-based diagnostic technologies have recently gained attention as a powerful strategy for detecting MRSA by targeting key antimicrobial resistance genes, particularly *mecA*. These systems rely on CRISPR-associated enzymes, such as Cas12a, which are guided by programmable RNA sequences to identify specific DNA targets. Upon successful target recognition, the activated enzyme initiates nonspecific cleavage of nearby reporter molecules, producing a measurable signal such as fluorescence or a colorimetric response [25,26].

Typically, the diagnostic workflow begins with extraction of bacterial nucleic acids, followed by an optional amplification step using isothermal methods such as recombinase polymerase amplification (RPA) to enhance sensitivity. The amplified DNA is then introduced into the CRISPR reaction mixture, where sequence-specific recognition leads to rapid signal generation. Recent developments have enabled integrated "one-pot" systems that combine sample processing, amplification, and detection in a single step, achieving detection limits as low as 10 CFU/mL within

 doi:10.20944/preprints202603.2455.v1

approximately one hour [26]. Furthermore, amplification-free CRISPR platforms have been explored to reduce contamination risk while maintaining high sensitivity [27].

Compared to conventional molecular diagnostics, CRISPR-based approaches offer several advantages, including exceptional specificity due to precise sequence targeting, rapid turnaround time, and compatibility with portable and point-of-care platforms. In addition, their programmability allows rapid adaptation to emerging resistance mechanisms. However, several limitations remain, including dependence on sample preparation, lack of standardized clinical protocols, and limited large-scale validation. As most CRISPR-based MRSA diagnostic systems are still in early development stages, further optimization is required before widespread clinical implementation [25]. Nonetheless, these technologies represent a promising direction for next-generation MRSA diagnostics and antimicrobial resistance surveillance [28].

Isothermal Amplification

Isothermal amplification techniques have emerged as powerful alternatives to conventional polymerase chain reaction (PCR) for the detection of bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA). Unlike PCR, which requires repeated thermal cycling, these methods operate at a constant temperature, eliminating the need for complex instrumentation and reducing assay time. This makes isothermal amplification particularly suitable for point-of-care testing (POCT) and use in resource-limited settings. Among the available approaches, loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) are the most widely applied techniques due to their rapid amplification, high sensitivity, and ease of operation [29].

LAMP

Loop-Mediated Isothermal Amplification (LAMP) is a highly specific nucleic acid amplification method that utilizes four to six primers targeting multiple regions of the DNA sequence, enabling efficient amplification under isothermal conditions, typically between 60 and 65 °C [29]. This multi-primer design enhances specificity and allows amplification to be completed within 30–40 minutes. LAMP has demonstrated diagnostic performance comparable to PCR, with reported sensitivity and specificity approaching 99%, indicating strong reliability for MRSA detection [29].

Detection in LAMP assays is commonly achieved through colorimetric or fluorescent methods. Colorimetric detection relies on pH-sensitive dyes or metal indicators, allowing results to be interpreted visually without the need for specialized equipment. However, these approaches are often qualitative and may produce ambiguous results at low target concentrations. To overcome this limitation, advanced systems such as electrochemical LAMP (E-LAMP) have been developed, which convert biochemical signals into measurable electrical outputs, enabling more accurate and quantitative detection [30].

In addition to improvements in detection strategies, LAMP has been successfully integrated into portable diagnostic platforms, including microfluidic chips and paper-based devices. These systems often incorporate pre-loaded reagents and require minimal sample preparation, enabling rapid, low-cost, and user-friendly testing [31]. Furthermore, paper-based LAMP devices have demonstrated high sensitivity and strong agreement with qPCR while maintaining portability and ease of use [32].

Despite these advantages, LAMP is associated with certain limitations. The use of multiple primers increases the risk of primer-dimer formation, which can lead to nonspecific amplification and false-positive results. Additionally, its high sensitivity makes the method particularly susceptible to contamination, highlighting the need for improved assay design and validation strategies [33].

RPA

Recombinase Polymerase Amplification (RPA) is another widely used isothermal amplification technique that operates at lower temperatures, typically between 37 and 42 °C, and enables rapid amplification without thermal cycling [34]. This method relies on recombinase enzymes to facilitate

 doi:10.20944/preprints202603.2455.v1

primer binding to target DNA, followed by strand displacement synthesis. RPA is characterized by fast reaction kinetics, often producing detectable results within 15–30 minutes, making it highly suitable for rapid and point-of-care diagnostics.

While RPA offers advantages in speed and simplicity, it is frequently combined with additional detection systems to improve specificity and signal clarity. One of the most significant advancements in this area is the integration of RPA with CRISPR-based detection systems. In these hybrid approaches, RPA serves as the initial amplification step, while CRISPR-associated enzymes such as Cas12a or Cas13a provide sequence-specific recognition and signal amplification through collateral cleavage activity, significantly enhancing sensitivity and specificity [35].

To further improve usability and reduce contamination risks, one-pot and single-tube RPA–CRISPR systems have been developed. These platforms integrate amplification and detection within a closed reaction environment, eliminating the need for intermediate handling steps and reducing the risk of aerosol contamination [34]. Advanced designs, such as light-controlled systems, enable temporal separation of amplification and detection within a single reaction, improving system efficiency and minimizing interference between components [36].

In addition, visual detection methods such as lateral flow strips and colorimetric readouts have been incorporated into RPA-based systems, allowing rapid and instrument-free result interpretation. These systems are particularly suitable for field applications and decentralized testing environments [22]. However, RPA-based platforms can be more complex due to the involvement of multiple enzymatic components, and their performance may be influenced by enzyme stability and storage conditions [34].

Overall, isothermal amplification techniques, particularly LAMP and RPA, provide rapid, sensitive, and portable alternatives to traditional PCR-based methods for MRSA detection. LAMP offers simplicity and strong diagnostic performance, while RPA enables faster reactions and facilitates integration with advanced detection technologies such as CRISPR. Despite challenges related to specificity, contamination, and system complexity, ongoing advancements in assay design and platform integration continue to improve the reliability and applicability of these methods. As a result, isothermal amplification is expected to play an increasingly important role in the development of rapid and accessible diagnostic tools.

Conclusions

Diagnostic Accuracy and Clinical Utility

PCR-based methods remain the most clinically validated approach for MRSA detection due to their high analytical sensitivity and specificity in targeting resistance-associated genes such as *mecA* [7,12]. Real-time PCR (qPCR) further enhances diagnostic accuracy by enabling quantitative and real-time detection, significantly reducing turnaround time to approximately 1–2 hours while maintaining robust sensitivity and specificity [13,15]. In addition, multiplex PCR improves clinical efficiency by allowing simultaneous detection of multiple genetic targets, which is particularly advantageous in polymicrobial infections or in distinguishing MRSA from coagulase-negative staphylococci [20]. Despite these advantages, PCR-based methods are primarily confined to centralized laboratory settings due to their dependence on specialized instrumentation, trained personnel, and relatively high operational costs [7].

Isothermal amplification techniques, including loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), offer comparable diagnostic sensitivity with substantially improved speed and operational simplicity. These methods operate under constant temperature conditions and can deliver results within 15–40 minutes, making them highly suitable for point-of-care and resource-limited settings [29,32]. LAMP has demonstrated diagnostic performance approaching that of PCR, with reported sensitivity and specificity near 99% [29,30], while RPA enables rapid amplification at lower temperatures, facilitating portable and field-deployable testing platforms [31]. However, these methods are more susceptible to non-specific

 doi:10.20944/preprints202603.2455.v1

amplification and contamination, which can compromise diagnostic accuracy if assay conditions are not carefully controlled [31].

Emerging diagnostic technologies, particularly CRISPR-based systems and electrochemical biosensors, have demonstrated exceptional analytical sensitivity and specificity, with detection limits in some cases surpassing those of conventional PCR-based assays [8,23]. CRISPR-based diagnostics provide highly specific sequence recognition through programmable guide RNA and rapid signal amplification, often achieving detection within one hour and showing strong potential for integration with isothermal amplification platforms [21,34]. Electrochemical biosensors enable label-free detection with ultra-low limits of detection and offer strong potential for miniaturized, portable diagnostic devices suitable for decentralized testing [8]. Nevertheless, both technologies remain in early stages of clinical validation and lack standardized protocols, limiting their current applicability in routine clinical practice.

Overall, PCR-based methods continue to serve as the clinical benchmark due to their reliability, standardization, and widespread validation. In contrast, isothermal amplification and emerging diagnostic technologies provide significant advantages in speed, portability, and accessibility, making them highly promising for point-of-care applications. The optimal diagnostic approach therefore depends on the clinical context, with centralized healthcare settings favouring highly validated molecular techniques, while decentralized and resource-limited environments benefit from rapid, simplified diagnostic platforms.

Cost-Effectiveness & Practical Limitations

The cost-effectiveness of MRSA diagnostic methods is closely linked to their turnaround time, infrastructure requirements, and impact on clinical decision-making. Conventional culture-based methods, while relatively low in cost, require 24–72 hours to produce results, which can delay targeted treatment and increase hospital stays and associated healthcare costs [6]. In contrast, molecular techniques such as PCR significantly reduce diagnostic time, enabling earlier intervention and improved infection control. A comparative study demonstrated that implementing PCR-based screening reduced pre-emptive isolation time and lowered healthcare costs by approximately $22,005 CAD per case, highlighting the economic benefit of rapid diagnostics despite higher initial testing costs [11]. However, advanced molecular diagnostics are associated with significant practical limitations. PCR and real-time PCR require specialized laboratory infrastructure, expensive reagents, and trained personnel, which restrict their use in decentralized or resource-limited settings [7]. In addition, these methods may detect resistance genes such as mecA without confirming phenotypic expression, potentially leading to overdiagnosis and unnecessary treatment [7,9].

Isothermal amplification methods, including LAMP and RPA, offer a more cost-effective and accessible alternative due to their minimal equipment requirements and rapid turnaround times. These techniques are particularly advantageous for point-of-care testing and low-resource environments. However, their high sensitivity increases susceptibility to contamination and non-specific amplification, which can compromise diagnostic accuracy if proper controls are not implemented [32].

Emerging diagnostic technologies, such as CRISPR-based systems and electrochemical biosensors, demonstrate strong potential for improving both sensitivity and portability while reducing long-term costs. Nevertheless, these technologies currently face challenges related to standardization, large-scale clinical validation, and integration into existing healthcare systems, limiting their immediate cost-effectiveness and practical deployment [8,21].

Overall, while rapid molecular and emerging diagnostic methods offer clear clinical and economic advantages through earlier detection and improved patient management, their widespread implementation remains constrained by cost, infrastructure requirements, and the need for standardized validation. A balanced approach that considers both economic feasibility and diagnostic performance is essential for optimizing MRSA detection strategies across diverse healthcare settings.

 doi:10.20944/preprints202603.2455.v1

These findings highlight that no single diagnostic method is universally superior; rather, each approach reflects a trade-off between speed, accuracy, cost, and accessibility. PCR-based methods remain the most reliable in centralized clinical settings due to their validation and standardization, whereas isothermal amplification offers a more practical solution for decentralized and resource-limited environments. Emerging technologies such as CRISPR-based diagnostics and biosensors further demonstrate that future diagnostic strategies will likely depend on the integration of complementary methods rather than reliance on a single platform.

Future Outlook

Future diagnostic development should prioritize bridging the gap between analytical performance and clinical actionability. This requires moving beyond optimizing detection sensitivity in isolation toward validating platforms in real clinical workflows, diverse patient populations, and low resource settings. Standardization of screening protocols across institutions should be considered alongside technological advancement. Longer term, the convergence of rapid isothermal amplification, CRISPR-based specificity and miniaturized readout systems into closed, single-step platforms offer a realistic path toward diagnostics that are fast, accurate, and deployable. Routine clinical use will depend on rigorous large-scale validation and cost reduction alongside sensitivity gains.

MRSA remains a significant clinical burden, and no single diagnostic method currently satisfies all the requirements of speed, sensitivity, and portability simultaneously. Culture based methods remain the clinical reference standard but they are too slow for timely intervention.

PCR-based approaches, particularly real time and multiplex variants, offer high accuracy and have seen broad adoption. However, they remain constrained by cost and laboratory infrastructure. Isothermal amplification methods, especially LAMP and RPA, address the portability and speed gap and are well-positioned for point of care deployment but carry contamination and non-specific amplification risks. CRISPR-based diagnostics and electrochemical biosensors represent the most analytically sensitive platforms currently reported, with detection limits surpassing conventional PCR, but both lack large-scale clinical validation and standardized protocols necessary for routine use. Additionally, detecting mecA confirms that the genetic potential for resistance exists but does not verify active expression of PBP2a or distinguish colonization from active infection, which is a distinction with direct consequences for treatment decisions [3,7]. This remains an open problem that next generation diagnostics will need to explicitly address rather than optimizing solely for detection sensitivity.

Looking forward, the most promising direction is platform integration. Platform integration refers to combining the rapid amplification of RPA or LAMP with the specificity of CRISPR-based detection and the miniaturization potential of electrochemical or paper-based readouts in a single closed system. Deployable architectures that minimize contamination risk and operator burden are likely to define the next generation of MRSA diagnostics. Broader clinical validation studies, standardization of protocols across institutions, and cost reduction will be critical for implementing these technologies. Ultimately, a tiered diagnostic strategy combining rapid POC screening with confirmatory molecular testing may offer the most pragmatic path to improving MRSA detection at scale.

TOC Graphic

 doi:10.20944/preprints202603.2455.v1

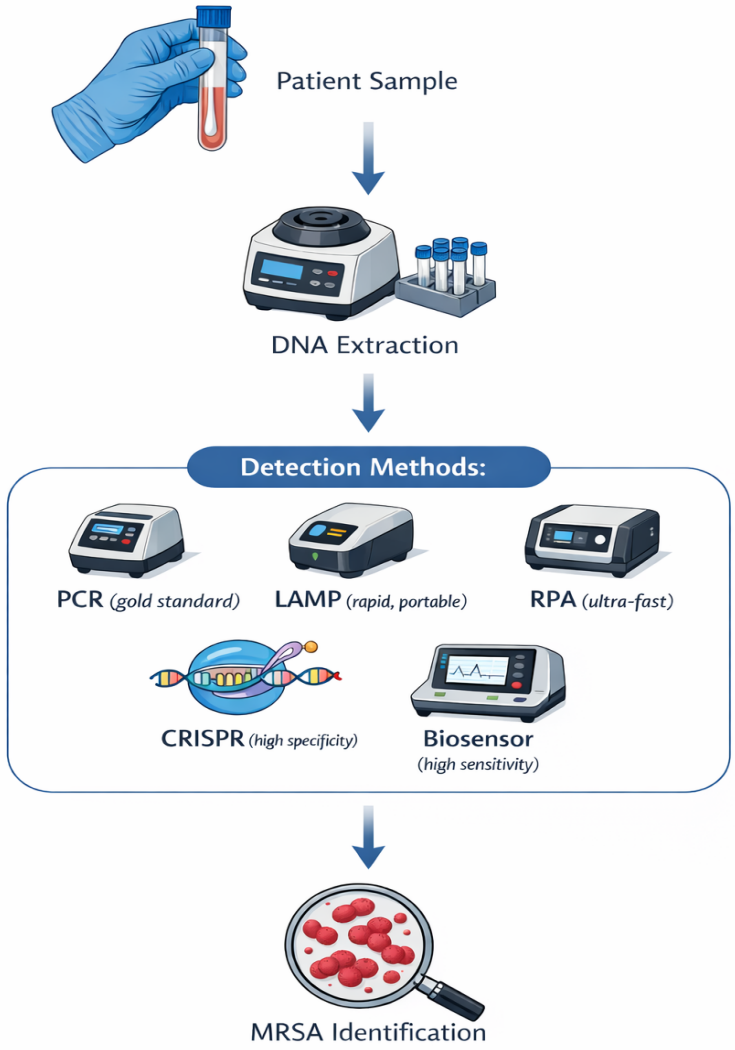

Figure 1. Overview of MRSA diagnostic workflow.

Method	Time	Accuracy / Sensitivity	Cost / Equipment	Limitations
PCR	2–5 hours	High	High (lab-based)	Expensive, requires infrastructure
LAMP	30–40 min	~99%	Low / portable	Contamination, false positives
RPA	15–30 min	High	Low / portable	Enzyme complexity, stability issues
CRISPR	~1 hour	Very high specificity	Moderate	Limited clinical validation
Biosensor	Not specified	Ultra-sensitive	Moderate	Not clinically validated

Figure 2. Comparative overview of MRSA diagnostic methods based on reported performance characteristics.

Authorship Credits: T.R. contributed to the isothermal amplification section (LAMP and RPA). A.A. contributed to CRISPR-based diagnostics. B.D. contributed to PCR-based methods. N.M. contributed to electrochemical biosensors. All authors contributed to literature review, manuscript writing and editing, approved the final version, and agreed to be accountable for the content.

 doi:10.20944/preprints202603.2455.v1

References

1. E. M. Marlowe and M. J. Bankowski, "Conventional and Molecular Methods for the Detection of Methicillin-Resistant *Staphylococcus aureus*," *J. Clin. Microbiol.*, vol. 49, no. 9 Suppl, pp. S53–S56, Sep. 2011, doi: 10.1128/JCM.00791-11.
2. E. H. Tobin, P. Jogu, and J. Koirala, "Methicillin-Resistant *Staphylococcus aureus*," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 21, 2026. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK482221/
3. B. Y. Ng, A. O'Reilly, G. Pill, and L. Dunsmure, "MRSA: diagnosis, screening and management," The Pharmaceutical Journal. Accessed: Feb. 21, 2026. [Online]. Available: https://pharmaceutical-journal.com/article/ld/mrsa-diagnosis-screening-and-management
4. E. Bulliard, B. Grandbastien, L. Senn, G. Greub, and D. S. Blanc, "Evaluation of three consecutive versions of a commercial rapid PCR test to screen for methicillin-resistant *Staphylococcus aureus*," *Clin. Microbiol. Infect.*, vol. 25, no. 11, p. 1430.e1-1430.e4, Nov. 2019, doi: 10.1016/j.cmi.2019.03.029.
5. K. L. McGinigle, M. L. Gourlay, and I. B. Buchanan, "The Use of Active Surveillance Cultures in Adult Intensive Care Units to Reduce Methicillin-Resistant *Staphylococcus aureus*-Related Morbidity, Mortality, and Costs: A Systematic Review," *Clin. Infect. Dis.*, vol. 46, no. 11, pp. 1717–1725, Jun. 2008, doi: 10.1086/587901.
6. L. Zhao et al., "A point-of-care test device for MRSA rapid detection," *J. Pharm. Biomed. Anal.*, vol. 209, p. 114464, Feb. 2022, doi: 10.1016/j.jpba.2021.114464.
7. Y. X. Sim, Q. W. Lee, A. Abushelaibi, K.-S. Lai, S. H. E. Lim, and S. Maran, "Current molecular approach for diagnosis of MRSA: a meta-narrative review," *Drug Target Insights*, vol. 16, no. 1, pp. 88–96, Dec. 2022, doi: 10.33393/dti.2022.2522.
8. Y. Xing et al., "Ultra-sensitive electrochemical biosensor integrated with nanozyme-enhanced signal amplification for MRSA detection using a dual-target recognition strategy," *J. Environ. Chem. Eng.*, vol. 13, no. 5, p. 118522, Oct. 2025, doi: 10.1016/j.jece.2025.118522.
9. C. L. Jenkins and H. D. Bean, "Current Limitations of Staph Infection Diagnostics, and the Role for VOCs in Achieving Culture-Independent Detection," *Pathogens*, vol. 12, no. 2, p. 181, Feb. 2023, doi: 10.3390/pathogens12020181.
10. M. Aghamali, M. Rahbar, H. Samadi Kafil, A. Esmailkhani, and A. Zahedi bialvaei, "Laboratory methods for identification of methicillin-resistant *Staphylococcus aureus*," *Rev. Res. Med. Microbiol.*, vol. 28, no. 4, p. 140, Oct. 2017, doi: 10.1097/MRM.0000000000000116.
11. "Supplement: PCR Tests for MRSA in Hospitalized Patients—Clinical and Cost-Effectiveness Analysis," Jan. 2011, Accessed: Feb. 19, 2026. [Online]. Available: https://canadacommons-ca.ezproxy.lib.ucalgary.ca/artifacts/1187387/supplement-to-polymerase-chain-reaction-tests-for-methicillin-resistant-staphylococcus-aureus-in-hospitalized-patients/1740513/
12. D. K. Corrigan et al., "Development of a PCR-free electrochemical point of care test for clinical detection of methicillin resistant *Staphylococcus aureus* (MRSA)," *Analyst*, vol. 138, no. 22, pp. 6997–7005, Oct. 2013, doi: 10.1039/C3AN01319G.
13. G. Dai et al., "Simultaneous electrochemical determination of nuc and mecA genes for identification of methicillin-resistant *Staphylococcus aureus* using N-doped porous carbon and DNA-modified MOF," *Microchim. Acta*, vol. 188, no. 2, p. 39, Jan. 2021, doi: 10.1007/s00604-020-04698-6.
14. L. Xu et al., "An ultrasensitive electrochemical biosensor for the detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus*," *Biosens. Bioelectron.*, vol. 99, pp. 424–430, Jan. 2018, doi: 10.1016/j.bios.2017.08.014.
15. A. Shamsabadi, T. Haghighi, S. Carvalho, L. C. Frenette, and M. M. Stevens, "The Nanozyme Revolution: Enhancing the Performance of Medical Biosensing Platforms," *Adv. Mater.*, vol. 36, no. 10, p. 2300184, 2024, doi: 10.1002/adma.202300184.
16. J. Polisena, S. Chen, K. Cimon, S. McGill, K. Forward, and M. Gardam, "Clinical effectiveness of rapid tests for methicillin resistant *Staphylococcus aureus*(MRSA) in hospitalized patients: a systematic review," *BMC Infect. Dis.*, vol. 11, no. 1, p. 336, Dec. 2011, doi: 10.1186/1471-2334-11-336.

 doi:10.20944/preprints202603.2455.v1

17. R. Hagen, "Development of a real-time PCR assay for rapid identification of methicillin-resistant from clinical samples," *Int. J. Med. Microbiol.*, vol. 295, no. 2, pp. 77–86, Jun. 2005, doi: 10.1016/j.ijmm.2004.12.008.
18. Y. Liu et al., "One-tube RPA-CRISPR Cas12a/Cas13a rapid detection of methicillin-resistant *Staphylococcus aureus*," *Anal. Chim. Acta*, vol. 1278, p. 341757, Oct. 2023, doi: 10.1016/j.aca.2023.341757.
19. A. Dubouix-Bourandy et al., "Direct detection of Staphylococcus osteoarticular infections by use of Xpert MRSA/SA SSTI real-time PCR," *J. Clin. Microbiol.*, vol. 49, no. 12, pp. 4225–4230, Dec. 2011, doi: 10.1128/JCM.00334-11.
20. N. Jain, S. Merwyn, G. P. Rai, and G. S. Agarwal, "Real-time polymerase chain reaction assay for rapid and sensitive detection of anthrax spores in spiked soil and talcum powder," *Folia Microbiol. (Praha)*, vol. 57, no. 3, pp. 237–242, May 2012, doi: 10.1007/s12223-012-0117-6.
21. U. Jamnikar Ciglenečki and I. Toplak, "Development of a real-time RT-PCR assay with TaqMan probe for specific detection of acute bee paralysis virus," *J. Virol. Methods*, vol. 184, no. 1, pp. 63–68, Sep. 2012, doi: 10.1016/j.jviromet.2012.05.010.
22. W. Yan, "Multiplex PCR Primer Design for Simultaneous Detection of Multiple Pathogens," in *PCR Primer Design*, C. Basu, Ed., New York, NY: Springer, 2015, pp. 91–101. doi: 10.1007/978-1-4939-2365-6_6.
23. Y. Xu et al., "A fully sealed plastic chip for multiplex PCR and its application in bacteria identification," *Lab. Chip*, vol. 15, no. 13, pp. 2826–2834, Jun. 2015, doi: 10.1039/C5LC00244C.
24. C. Wu, M. Ling, Q. Chen, H. Chai, and H. Chen, "Multiplex Digital PCR-Based Development and discussion of the Detection of Genetic Association Between *Staphylococcus aureus* and mecA," *Infect. Drug Resist.*, vol. 17, pp. 2031–2041, May 2024, doi: 10.2147/IDR.S464213.
25. Y. Gao and J. Chen, "Fast but accurate: a systematic review and meta-analysis on diagnostic performance of MRSA detection in clinical samples by using CRISPR-based rapid molecular methods," *Front. Microbiol.*, vol. 16, Nov. 2025, doi: 10.3389/fmicb.2025.1703247.
26. Y. Sun et al., "Direct visual detection for methicillin-resistant *Staphylococcus aureus* in milk based on the RPA-Cas12a-LFS method," *Food Control*, vol. 173, p. 111209, Jul. 2025, doi: 10.1016/j.foodcont.2025.111209.
27. L. Wei, Z. Wang, L. Wu, and Y. Chen, "CRISPR/Cas12a-based magnetic relaxation switching biosensor for nucleic acid amplification-free and ultrasensitive detection of methicillin-resistant *Staphylococcus aureus*," *Biosens. Bioelectron.*, vol. 222, p. 114984, Feb. 2023, doi: 10.1016/j.bios.2022.114984.
28. M. M. Ahmed et al., "CRISPR-Cas Systems in the Fight Against Antimicrobial Resistance: Current Status, Potentials, and Future Directions," *Infect. Drug Resist.*, vol. 17, pp. 5229–5245, Nov. 2024, doi: 10.2147/IDR.S494327.
29. L.-J. Long et al., "Evaluation of the loop-mediated isothermal amplification assay for *Staphylococcus aureus* detection: a systematic review and meta-analysis," *Ann. Clin. Microbiol. Antimicrob.*, vol. 21, no. 1, p. 27, Jun. 2022, doi: 10.1186/s12941-022-00522-6.
30. L. F. de Lima et al., "3D-printed electrochemical loop-mediated isothermal amplification (E-LAMP): A miniaturized and portable platform to detect methicillin-resistant *Staphylococcus aureus* (MRSA)," *Anal. Chim. Acta*, vol. 1372, p. 344455, Oct. 2025, doi: 10.1016/j.aca.2025.344455.
31. L. Zhao et al., "A point-of-care test device for MRSA rapid detection," *J. Pharm. Biomed. Anal.*, vol. 209, p. 114464, Feb. 2022, doi: 10.1016/j.jpba.2021.114464.
32. I. Choopara et al., "Fluorometric Paper-Based, Loop-Mediated Isothermal Amplification Devices for Quantitative Point-of-Care Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)," *ACS Sens.*, vol. 6, no. 3, pp. 742–751, Mar. 2021, doi: 10.1021/acssensors.0c01405.
33. S.-H. Kim, S.-Y. Lee, U. Kim, and S.-W. Oh, "Diverse methods of reducing and confirming false-positive results of loop-mediated isothermal amplification assays: A review," *Anal. Chim. Acta*, vol. 1280, p. 341693, Nov. 2023, doi: 10.1016/j.aca.2023.341693.
34. Y. Liu et al., "One-tube RPA-CRISPR Cas12a/Cas13a rapid detection of methicillin-resistant *Staphylococcus aureus*," *Anal. Chim. Acta*, vol. 1278, p. 341757, Oct. 2023, doi: 10.1016/j.aca.2023.341757.
35. X. Wu, D. Jin, W. Ni, F. Wang, Y. Zhang, and G.-J. Zhang, "CRISPR/Cas12a coupling with RPA and MNPs for rapid and visualized identification of methicillin-resistant *Staphylococcus aureus*," *Sens. Actuators B Chem.*, vol. 382, p. 133546, May 2023, doi: 10.1016/j.snb.2023.133546.

 doi:10.20944/preprints202603.2455.v1

36. Z. Yu, Y. Man, W. Li, C. Shi, P. Xiao, and Q. Zhang, "Light-controlled one-pot RPA-CRISPR/Cas12a biosensor for MRSA detection based on tailed crRNA," *Sens. Actuators B Chem.*, vol. 448, p. 138925, Feb. 2026, doi: 10.1016/j.snb.2025.138925.