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Development and Validation of a Specific PCR Primer for the Detection of the Meca Gene in MRSA

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Abstract. *Methicillin-Resistant Staphylococcus aureus (MRSA) is a major pathogen responsible for nosocomial infections with high prevalence in Indonesia. The mecA gene encodes beta-lactam antibiotic resistance, making its specific molecular detection crucial for rapid diagnosis and infection control. This study aimed to develop and validate specific PCR primers for detecting the mecA gene in MRSA as a marker of antibiotic resistance. Laboratory experimental research was conducted at the UPH Advanced Biology Laboratory in November 2025 using certified pure MRSA isolates. DNA extraction was performed with the QIAamp DNA Mini Kit and quantified using a spectrophotometer. Primer design was carried out in silico using NCBI PrimerBLAST and BioEdit, followed by conventional PCR optimization with varying annealing temperatures. Amplification results were validated through agarose gel electrophoresis, with qualitative assessment based on DNA band intensity and sharpness. The mecA primer successfully amplified an 818-bp fragment at an optimal annealing temperature of 55.5°C, producing a single, sharp band without smearing. The negative control (S. aureus) showed no amplification, confirming primer specificity. Despite low DNA concentrations (4.5–7.6 ng/µL), amplification was consistently successful, indicating that this method is capable of reliably detecting the target gene at low DNA levels. This validated method provides an accurate molecular diagnostic tool for rapid identification of resistant pathogens. It supports epidemiological surveillance, nosocomial infection control, and early detection programs within the Indonesian healthcare system. By enabling precise detection of mecA-mediated resistance, the approach strengthens laboratory capacity for monitoring MRSA and contributes to improved public health strategies.*

Keywords: *Meca Gene, MRSA, BioEdit, Health Strategies*

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INTRODUCTION

Ulcers are open wounds on the skin or mucosa that are difficult to heal and can be caused by various conditions, such as blood circulation disorders, infections, or prolonged pressure (Akbari-Lasboo et al., 2025; Bian et al., 2025; Agrawal etal., 2025). Common types of ulcers include diabetic ulcers due to complications of diabetes, arteriosum ulcers due to arterial flow disorders, venosum ulcers due to venous insufficiency, and decubitus ulcers that occur due to prolonged pressure on the skin. Ulcer management involves controlling the underlying cause, proper wound care, and preventing infection to ensure optimal healing and prevent recurrence (Boersema et al., 2021; Jiang et al., 2023; Pavitra & Kaliyaperumal, 2026).

Methicillin-Resistant Staphylococcus aureus (MRSA) is a strain of *S. aureus* that is resistant to isoxazole penicillin antibiotics such as methicillin, oxacillin, and flucloxacillin, making it difficult to treat and posing a risk of causing more widespread infection (Pasaribu et al., 2024;

Oliveira et al., 2024; Ferraz, 2024). *S. aureus* is a normal flora in the nasal cavity and on the skin but can become pathogenic when the body's immunity is weakened. About 30% of healthy adults carry this bacterium in their nose and 20% on their skin, with a higher prevalence in hospital patients and healthcare workers (Piewngam & Otto, 2024; Touaitia et al., 2025; Gaihre et al., 2025).

Irrational use of antibiotics triggers genetic mutations, causing cross-resistance to beta-lactam antibiotics and making MRSA a serious medical threat. MRSA is the leading cause of nosocomial infections, such as post-operative skin, respiratory, urinary tract, and bloodstream infections, and is commonly found in hospitals (Zardi et al., 2022; Venkataraman et al., 2023; Osagwu et al., 2024). In Indonesia, the proportion of MRSA from clinical isolates *of S. aureus* reaches about 28%, indicating a major challenge in infection control in health facilities, especially skin infections that can become serious without proper treatment (Suyasa & Mastra, 2020).

S. aureus is a normal bacterium on human skin and in the nose, but it can become an opportunistic pathogen when the body's resistance is low. Under certain conditions, such as inappropriate antibiotic use, this bacterium can mutate into a resistant strain known as MRSA, which is resistant to various beta-lactam antibiotics such as methicillin, oxacillin, and flucloxacillin (Rahman et al., 2023; Hardefeldt & Prescott, 2024; Sakoulas & Nizet, 2024). MRSA is a major cause of nosocomial and community infections, such as abscesses, cellulitis, impetigo, and post- operative wounds. In Indonesia, its prevalence reaches 28% of clinical *S. aureus* isolates, with a detection rate of 11.5% in skin specimens at Dr. H. Abdul Moeloek General Hospital during January–March 2023.

MRSA has limited sensitivity to last-line antibiotics such as linezolid, tigecycline, and quinupristin/dalfopristin (Ningsih et al., 2024; Zhang et al., 2025; Akenten & Thye, 2026). Therefore, MRSA poses a serious threat in healthcare facilities and the community due to skin infections that are difficult to cure and easily spread without early detection and appropriate treatment. Fast and accurate detection methods are essential for effective diagnosis and treatment. PCR (Polymerase Chain Reaction) is an effective molecular technique for detecting MRSA through the identification of the *mecA* gene, the main marker of resistance to methicillin antibiotics. The *mecA* gene mediates the formation of a protein with low affinity for beta-lactam antibiotics, allowing MRSA to survive and form cell walls even in the presence of antibiotics (Khairullah et al., 2022; Lade & Kim, 2023; Abebe & Birhanu, 2023).

PCR enables the amplification of MRSA-specific DNA in a single reaction, which is more efficient than conventional methods such as culture and sensitivity testing. In diabetic ulcer patients, PCR detects the *mecA* gene with 533 bp DNA band visualization, indicating MRSA infection. The advantages of PCR lie in its high specificity and sensitivity, which are clinically useful in preventing antibiotic errors and speeding up patient treatment (Sugireng & Rosdarni, 2020; Duan et al., 2025; Serapide et al., 2025). This study aims to develop and test PCR primers specific to genetic mutations in *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria, particularly those related to the *mecA* gene as the main marker of antibiotic resistance. The primer design process will be carried out using bioinformatics software such as Primer3Plus, Primer-BLAST, and OligoAnalyzer to optimize specificity, melting temperature, and thermodynamic compatibility. In addition, this study will also evaluate the effectiveness of primers in detecting genetic variants or mutations that occur in MRSA through *in vitro* tests, to ensure that the primers developed can be used accurately in clinical detection and antimicrobial resistance surveillance.

METHODS

This research is a laboratory based experimental study aimed at developing and validating specific PCR primers for the detection of the *mecA* gene in *methicillin resistant Staphylococcus aureus* (MRSA). All stages of the research were conducted at the Mochtar Riady Institute of Nanotechnology, Pelita Harapan University (MRIN UPH), which is equipped with molecular biology laboratory facilities. The test material used was the MRSA reference strain

Staphylococcus aureus ATCC 33591, obtained from Thermo Fisher Scientific–Remel Inc. (Lot 114675). This strain is a certified laboratory strain from the American Type Culture Collection (ATCC) containing more than 10^4 CFU per loop. The *Staphylococcus aureus* strain was used as a comparative control in the primer amplification evaluation. The research process began with genomic DNA extraction using the QIAamp DNA Mini Kit according to the manufacturer's protocol. The concentration and purity of the extracted DNA were then measured using a spectrophotometer before it was used as a template in the PCR reaction. The next step was to retrieve the *mecA* gene sequence from the NCBI GenBank database, followed by multiple sequence alignment using BioEdit Sequence Alignment Editor to identify suitable conserved regions as targets for primer design. Primer design was performed using NCBI Primer BLAST, while primer characteristics were evaluated using OligoAnalyzer to ensure the absence of secondary structures, such as hairpins, self dimers, or cross dimers.

The primers obtained have the following sequences: forward 5'-GGCTCAGGTACTGCTATCCAC-3' and reverse 5'-AACCACCCAATTTGTCTGCC-3'. The forward primer is 21 bp, while the reverse primer is 20 bp, with an expected amplicon size of 818 bp. The GC content of the forward primer is 57% and that of the reverse primer is 50%, with melting temperatures (Tm) of 59.7°C and 60.0°C, respectively. In silico analysis results indicate that neither primer contains runs, repeats, or secondary structures that could potentially interfere with the amplification process, thus meeting the criteria for specific primers. The PCR reaction was performed in a total volume of 12.5 µL, consisting of 6.25 µL PCR Master Mix, 0.5 µL forward primer, 0.5 µL reverse primer, and 5.25 µL template DNA with a DNA concentration of 7.6 ng. Amplification was performed using a thermal cycler with annealing temperature optimization via the gradient PCR method. The annealing temperatures tested included 55,5°C, 55,6°C, and 56,0°C for 30 seconds per cycle, with a total of 25 amplification cycles. The optimal annealing temperature was determined based on the formation of a single specific DNA band at the expected target size without any nonspecific bands or smears, as well as producing a consistent amplification pattern upon repeat testing.

The PCR products were analyzed using 1.5% agarose gel electrophoresis prepared with Tris Acetate EDTA (TAE) buffer. A total of 5 µL of the PCR product was mixed with 1 µL of loading dye and then loaded into the gel wells. Electrophoresis was performed at 110 volts for 25 minutes. After electrophoresis was complete, the gel was soaked in GelRed solution for 20 minutes, then visualized using a UV transilluminator. The size of the DNA fragments was determined using a DNA ladder as a molecular marker. The success of the amplification was determined based on the electrophoresis results. Amplification was considered valid if a single DNA band formed at the target size corresponding to the primer design and the DNA marker used, without the presence of nonspecific bands or smears indicating DNA degradation or nonspecific amplification. The intensity of the band must be sufficient to be consistently detected on the gel visualization system; however, it should not be used as the sole indicator of success, as the thickness or brightness of the band can be influenced by DNA concentration, the amount of PCR product, the staining process, or lighting conditions during visualization.

RESULT AND DISCUSSION

The results of DNA concentration and purity measurements using the NanoDrop, presented in Table 1, show that MRSA 1 sample has a DNA concentration of 7.6 ng/µL, while MRSA 2 has a concentration of 4.8 ng/µL. The non-MRSA *Staphylococcus aureus* samples used as negative controls had DNA concentrations of 5.5 ng/µL and 2.3 ng/µL, respectively. Although the DNA concentrations obtained were below the range commonly used in conventional PCR approximately 10–50 ng/µL DNA with lower concentrations can still be used if it meets minimum quality standards based on DNA purity parameters. DNA purity in this study was assessed based on the A260/A280 absorbance ratio using a NanoDrop. The measurement results showed a DNA purity ratio of 1.9, which falls within the optimal range of 1.8–2.0. This value indicates that the DNA is relatively free of protein contamination and meets the minimum quality standards for use as a PCR template. DNA concentration is not the only factor determining amplification success, as

PCR success is also greatly influenced by DNA purity, primer specificity, and amplification reaction conditions. DNA with a low concentration can still produce good PCR products if it has adequate quality and purity. Variations in DNA concentration among samples can be caused by several factors, such as the initial cell count, the efficiency of the bacterial cell wall lysis process, and DNA loss during the extraction process. This is of particular concern for Gram-positive bacteria such as *Staphylococcus aureus*, which have a thick peptidoglycan layer, making DNA release more difficult compared to Gram-negative bacteria. Previous studies have shown that the concentration of DNA extracted from MRSA can vary depending on the extraction method, sample conditions, and the effectiveness of the cell lysis process (Hanina & Humaryanto, 2022; Wu et al., 2023; Choi et al., 2024). Therefore, DNA quality as determined by purity plays a more significant role in ensuring successful amplification than simply considering the amount of DNA obtained.

Table 1. Nanodrop result

Sample	Concentration (ng/µL)	Purity
MRSA 1	7.6	1.9
MRSA 2	4.8	1.5
Staphylococcus aureus 1	5.5	1.9
Staphylococcus aureus 2	2.3	1.3

Table 2. Electrophoresis result

Electrophoresis results	DNA Ladder
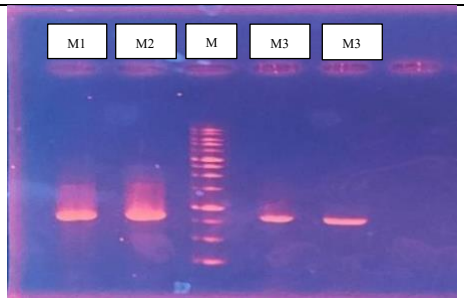	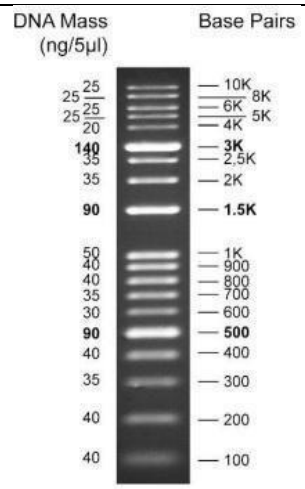
Figure 1. Electrophoresis Results of *Methicillin-resistant Staphylococcus aureus* (MRSA). M1(56,0°C), M2 (55,6°C), M3 (55,5°C), M (Marker). *Source: Author's documentation*	**Figure 2. *Methicillin-resistant Staphylococcus aureus* (MRSA) DNA *Ladder*** *Source: https://www.abcam.cn/products/reagents/1-kb- dna-ladder-ab286887*

Figure 3. Electrophoresis Results of *Staphylococcus aureus* as Control. M (Marker), K (Control).

Source: Author's documentation

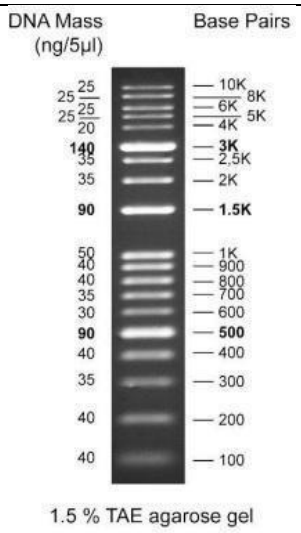

Figure 4. *Staphylococcus aureus* DNA *Ladder*

Source: https://www.abcam.cn/products/reagents/1-kb- dna-ladder-ab286887

The mecA specific primers used in this study were designed based on the target gene sequence obtained from the NCBI GenBank database and analyzed using the NCBI Primer BLAST, BioEdit, and OligoAnalyzer software. The forward primer used had the sequence 5'-GGCTCAGGTACTGCTATCCAC-3' with a length of 21 bp, while the reverse primer had the sequence 5'-AACCACCCAATTTGTCTGCC-3' with a length of 20 bp. The forward primer has a GC content of 57% with a melting temperature (Tm) of 59.7°C, while the reverse primer has a GC content of 50% with a Tm of 60.0°C. The results of the in silico analysis showed that the primers did not contain any hairpin, self-dimer, cross-dimer, run, or repeat structures that could inhibit the amplification process. Based on these primer designs, the expected amplicon size is 818 bp. Differences in mecA amplicon sizes reported in other studies such as 154 bp, 310 bp, 533 bp, or 571 bp do not indicate any inconsistency, as the size of the PCR product is highly dependent on the binding sites of the primers on the target gene. Each primer pair will produce fragments of different lengths depending on the position of the amplified target region (Syamsidi et al., 2021; McCabe et al., 2024; Zeng et al., 2025). Therefore, the 818 bp size in this study is specific to the primer design developed and is used as a reference in interpreting the electrophoresis results.

PCR optimization was performed using the gradient PCR method with annealing temperatures of 55,5°C, 55,6°C, and 56,0°C. The PCR reaction was performed in a total volume of 12.5 µL, consisting of 6.25 µL PCR Master Mix, 0.5 µL forward primer, 0.5 µL reverse primer, and 5.25 µL template DNA at a concentration of 7.6 ng. Amplification was performed for 25 cycles with annealing temperatures varied for 30 seconds at each condition. The optimal annealing temperature was determined based on objective criteria, namely the formation of a single DNA band at the target size of 818 bp without the presence of nonspecific bands or smears. In addition, the amplification results must show a consistent pattern upon repeated testing. Based on the optimization results, a temperature of 55.5°C produced a single specific DNA band at the target size and was therefore selected as the optimal condition for mecA gene amplification. Based on the mecA primer reported in the latest study, it has a length of 18–25 bases, a GC content of 40–60%, and a Tm of approximately 55–65°C, making it suitable for both conventional and multiplex PCR (Kashef & Helmy, 2022). A study conducted by (Duraiswamy, et al., 2023) showed that the use of mecA primers in PCR provides high sensitivity and specific results without cross-amplification (Duraiswamy et al., 2023). Conversely, the presence of double bands or smears may

indicate non-specific amplification, primer dimers, or DNA degradation. Size interpretation is performed by comparing the migration distance of the sample band to the ladder bands using the semi-log curve principle recommended in the electrophoresis protocol (Black Jr, 2021).

The PCR products were analyzed using 1.5% agarose gel electrophoresis with TAE buffer. A 5 µL PCR sample was mixed with 1 µL of loading dye and then loaded into a gel well. Electrophoresis was performed at 110 volts for 25 minutes. After the separation process was complete, the gel was soaked in GelRed for 20 minutes and visualized using a UV transilluminator. The size of the DNA fragments was determined by comparing the migration positions of the sample bands to a 1 kb DNA ladder used as a molecular size standard (Syahputra, 2022; Parks & Torres, 2023; Allen et al., 2023). The electrophoresis results were interpreted qualitatively based on the presence of DNA bands at sizes corresponding to the primer design. The electrophoresis results showed that lanes 4 and 5 produced a single DNA band at approximately 818 bp, while lanes 1 and 2 exhibited a suboptimal banding pattern in the form of a smear. The single band in lanes 4 and 5 indicated that amplification occurred specifically according to the primer design targets. The criteria for valid amplification in this study were determined based on the formation of a single DNA band at the target size corresponding to the DNA marker, the absence of nonspecific bands, and the absence of smears indicating DNA degradation or nonspecific amplification. Band intensity was not used as a quantitative parameter because this study did not perform densitometric analysis. Interpretation was based solely on the presence, position, and size correspondence of the bands relative to the DNA ladder.

Testing using non-MRSA *Staphylococcus aureus* as a negative control showed no DNA band formation after amplification using mecA specific primers, whereas MRSA samples exhibited a DNA band at the target size. These results indicate that the primers demonstrate preliminary ability to distinguish samples carrying the mecA gene from those that do not. However, the results of this study are still categorized as preliminary evidence of the primers specificity and cannot yet be considered a comprehensive validation of their specificity. More comprehensive specificity testing requires the use of various non target bacterial controls, particularly closely related *Staphylococcus* species and other clinically relevant bacteria. Thus, further research is needed to ensure that no cross reactions occur with other organisms.

This study has several limitations. The analysis of PCR results was still performed qualitatively based on the visualization of DNA bands on an agarose gel and was not supported by densitometric analysis to objectively measure band intensity. In addition, this study did not use a no template control (NTC), so the possibility of contamination during the PCR process could not be directly evaluated. The use of an NTC is crucial in PCR based testing because it ensures that the amplified bands truly originate from the target DNA and are not the result of reagent or environmental contamination. Therefore, future studies are advised to include an NTC, expand the panel of negative controls, and perform additional quantitative analyses to strengthen primer validation.

CONCLUSION

This study successfully amplified the mecA gene from MRSA isolates using PCR with DNA concentrations of 7.6 and 4.8 ng/µL. Electrophoresis results showed a sharp single DNA band at 818 bp at the optimal annealing temperature of 55.5°C, confirming the specificity of the mecA primers. The *S. aureus* negative control did not produce a band, proving that there was no cross-amplification. For further development, it is recommended to optimize the DNA extraction method to increase yield, test more clinical samples, validate using the multiplex PCR method for simultaneous detection of other resistance genes, and explore real-time PCR techniques for more accurate and rapid quantification.

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