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Primer Design and Optimization of Polymerase Chain Reaction Method for Detection of the *mecA* Gene in *Methicillin-Resistant Staphylococcus aureus*

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ABSCTRACT

Methicillin-Resistant Staphylococcus aureus (MRSA) is one of the main causes of nosocomial infections and exhibits resistance to beta-lactam antibiotics due to the presence of the *mecA* gene. Detection of MRSA is generally performed using traditional methods. However, these approaches have several limitations. With technological advancements, the Polymerase Chain Reaction (PCR) method has become a more effective option, as it can detect the presence of target DNA more quickly, accurately, and specifically. This study aimed to design specific primers and optimize the PCR method for detecting the *mecA* gene in MRSA. The research conducted qualitative descriptive by in silico method in primer design and in vitro method in optimization of PCR. DNA samples were obtained from MRSA ATCC 33591 isolates and extracted using the PCIA method. PCR optimization was conducted with annealing temperature variations from 47,5°C to 63,6°C. The results showed that the primer pair forward 5'-TGGCTCAGGTACTGCTATCC-'3 and reverse 5'-TGGAACTTGTTGAGCAGAGGT-'3 successfully amplified the *mecA* gene fragment of 156 bp specifically at the optimal annealing temperature of 55,5°C. It can be concluded that the primer with optimal PCR reaction produced in this study can be used as a rapid and accurate detection method for identifying the *mecA* gene in MRSA.

Keywords: Primer Design; Optimization; Polymerase Chain Reaction; *mecA* gene; *Methicillin-Resistant Staphylococcus aureus*

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INTRODUCTION

Healthcare-associated infections (HCAIs) remain a major challenge in modern healthcare, affecting millions of patients annually. The World Health Organization (WHO) reports hundreds of millions of cases worldwide, leading to prolonged hospital stays, higher costs, and increased mortality. The burden is particularly severe in low- and middle-income countries, where patients are up to three times more likely to acquire HCAIs due to limited resources, weak infection control, and inadequate antimicrobial resistance (AMR) surveillance. These challenges highlight the need for stronger prevention programs and improved diagnostic technologies for early detection.

Among the various pathogens associated with HCAIs, *Staphylococcus aureus* stands out due to its dual role as both a commensal and a pathogen, colonizing about 30% of the human population, mainly in the anterior nares and on the skin (Kurniawan et al., 2021). While often asymptomatic, it can cause diseases ranging from mild skin infections to life-threatening conditions such as pneumonia, endocarditis, osteomyelitis, bloodstream infections, and surgical site infections (Kemalaputri et al., 2017). Its adaptability and diverse virulence factors make it one of the most important pathogens in both community and healthcare settings (Haque et al., 2018).

The emergence of *Methicillin-Resistant Staphylococcus aureus* (MRSA) has become a global health concern. Since the 1960s, MRSA has spread worldwide, driven by the *mecA* gene encoding penicillin-binding protein 2a (PBP2a), which confers resistance to nearly all β-lactam antibiotics. MRSA often exhibits multidrug resistance, limiting therapeutic options and leading to worse outcomes, including increased mortality, higher costs, and prolonged hospitalization compared with *Methicillin-Susceptible Staphylococcus aureus* (MSSA) (Fitria et al., 2021).

In the Asia-Pacific region, MRSA prevalence remains high, exceeding 40% in some countries (Wong et al., 2018). In Indonesia, the National Antimicrobial Resistance Surveillance (SINAR, 2022) has documented MRSA across multiple regions—including Papua, Sumatra, Java, and Bali—isolated from critical specimens such as blood, CSF, pleural fluid, and urine. These findings emphasize the urgent need for robust surveillance and improved diagnostic methods to support timely treatment and reduce transmission in healthcare settings.

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Conventional culture and susceptibility testing for MRSA face significant limitations, as culture requires 24 – 48 hours or longer, with susceptibility testing further extending turnaround time. These delays often lead to inappropriate empiric therapy, higher risks of treatment failure, prolonged illness, increased healthcare costs, and greater transmission risk. In resource-limited settings, inadequate infrastructure and skilled personnel further widen the diagnostic gap.

Molecular methods, particularly *Polymerase Chain Reaction* (PCR), address these challenges by enabling rapid, sensitive, and specific detection of the *mecA* gene within hours. This accelerates appropriate therapy, improves outcomes, and supports antimicrobial stewardship by minimizing unnecessary broad-spectrum antibiotic use. Detection of the conserved *mecA* gene remains the gold standard for MRSA identification (Pristianingrum et al., 2021), and bioinformatics-based primer design enhances assay specificity for accurate MRSA detection (Koentjoro et al., 2024).

Numerous studies have validated the effectiveness of PCR in detecting MRSA. For example, Arbefeville et al. (2011) emphasized the utility of molecular methods but also acknowledged potential pitfalls such as false positives from *mecA* homologues or false negatives due to mutations within primer binding regions. Syamsidi et al. (2021) highlighted the challenges in optimizing PCR conditions, particularly regarding primer stability, while Melati et al. (2022) reported the importance of avoiding primer-dimer formation and secondary structures such as hairpins that compromise amplification efficiency.

Careful primer design and optimization are essential in PCR-based MRSA detection. Factors such as primer specificity, length, GC content, and avoidance of self-complementarity must be considered to prevent nonspecific amplification and primer-dimer formation. Optimizing annealing temperature is also crucial, as temperatures that are too low may reduce specificity, while excessively high temperatures may lower amplification efficiency.

The rationale of this study stems from the persistent global and regional burden of MRSA and the diagnostic challenges in its detection, particularly in resource-limited settings such as Indonesia. Despite advances in molecular diagnostics, reliable, cost-effective, and optimized assays remain limited. This research addresses this gap by designing specific *mecA*

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primers and systematically optimizing annealing temperature, aiming to establish a validated PCR method for MRSA detection suitable for clinical laboratories.

The novelty of this study lies in three aspects: integration of bioinformatics for rational primer design, systematic annealing temperature optimization to enhance assay performance, and contextual relevance to Indonesian healthcare needs. By combining these approaches, the study contributes both scientifically and practically to improving timely MRSA diagnosis and supporting antimicrobial resistance surveillance.

In conclusion, MRSA continues to pose a major threat to global healthcare systems, with particularly high prevalence in the Asia-Pacific region including Indonesia. Conventional diagnostic methods are inadequate for timely and effective detection, underscoring the need for reliable molecular approaches. PCR targeting the *mecA* gene represents a powerful tool, but requires careful primer design and optimization to achieve optimal performance. Through its focus on rational primer design, annealing temperature optimization, and clinical applicability, this study seeks to advance MRSA diagnostics and contribute to improved patient outcomes, infection control, and antimicrobial resistance surveillance in Indonesia and beyond.

METHOD

TIME AND PLACE OF STUDY

This study was conducted from March to April 2025 at the Microbiology and Molecular Biology Laboratory, Health Polytechnic of the Ministry of Health, Denpasar, Indonesia.

TARGET AND PARTICIPANTS

The study focused on *Methicilllin-Resistant Staphylococcus aureus* (MRSA). MRSA ATCC 33591 bacterial isolate obtained from the Department of Microbiology, Faculty of Medicine, Udayana University, and sub-cultured at the Integrated Bacteriology and Molecular Biology Laboratory, Health Polytechnic of the Ministry of Health, Denpasar. The inclusion criteria were bacterial isolates previously confirmed as MRSA using standard microbiological and susceptibility testing methods.

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SAMPLING TECHNIQUE

Purposive sampling was applied, where isolates meeting inclusion criteria were selected as the study sample.

PROCEDURES

The research was designed as an experimental laboratory study. DNA extraction from MRSA isolates was performed using a standard bacterial genomic DNA isolation kit. Primer design targeting the *mecA* gene was carried out with bioinformatics tools (NCBI BLAST, NetPrimer, and Benchling) to ensure specificity, optimal GC content, appropriate melting temperature, and absence of hairpin or dimer structures. PCR amplification was conducted with varying annealing temperatures (47,5°C – 55,6°C) to determine optimal amplification conditions. The reaction mix contained DNA template, forward and reverse primers, dNTPs, Taq DNA polymerase, $MgCl_2$, buffer, and nuclease-free water. Amplification was performed in a thermal cycler with the following steps: denaturation, annealing, and extension. PCR products were subjected to electrophoresis using 1.5% agarose gel stained with ethidium bromide. Amplified fragments were visualized under UV light, and the presence of specific *mecA* bands (156 bp) was recorded.

DATA COLLECTION TECHNIQUES

Data were collected from the visualization of electrophoresis results. The presence, intensity, and specificity of amplified bands at different annealing temperatures were documented with gel documentation equipment.

INSTRUMENTS

The instruments used included a thermal cycler, micropipettes, electrophoresis apparatus, UV transilluminator, and gel documentation system. Software tools (NCBI BLAST, NetPrimer, and Benchling) were used for primer design.

DATA ANALYSIS

The data obtained were analysed qualitatively by comparing the specificity and clarity of the DNA bands produced at various annealing temperatures. The optimal annealing temperature was determined based on the sharpest, most specific band without nonspecific amplification. The results were interpreted in relation to the research objectives and compared with previous studies to identify potential gaps and improvements.

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RESULTS

PRIMER DESIGN FOR THE MECA GENE OF MRSA

Primer design was carried out *in silico* using the National Center for Biotechnology Information (NCBI) Primer-BLAST tool, resulting in 10 pairs of candidate primers as presented in Table 1.

Table 1. Primer Design Results from NCBI Primer-BLAST

Candidate	Primer	Base Length	Tm	%GC	Product Size
Primer 1	TCCACCCTCAAACAGGTGAA	20 bp	58,78° C	50,00%	757 bp
	TCTGCAGTACCGGATTTGCC	20 bp	60,35° C	50,00%	
Primer 2	TGCTATCCACCCTCAAACAGG	21 bp	59,72° C	52,38%	761 bp
	CTGCAGTACCGGATTTGCCA	20 bp	60,68° C	55,00%	
Primer 3	TTGGCCAATACAGGAACAGCA	21 bp	60,20° C	47,62%	571 bp
	GGTGGATAGCAGTACCTGAGC	21 bp	59,93° C	57,14%	
Primer 4	GCTATCCACCCTCAAACAGGT	21 bp	59,72° C	52,38%	285 bp
	AACGTTGTAACCACCCCAAGA	21 bp	59,79° C	47,62%	
Primer 5	ACGGTAACATTGATCGCAACG	21 bp	59,61° C	47,62%	598 bp
	TCGTCAACGATTGTGACACG	20 bp	58,87° C	50,00%	
Primer 6	TCAGGTACTGCTATCCACCCT	21 bp	59,71° C	52,38%	816 bp
	AAACCACCCAATTTGTCTGCC	21 bp	59,58° C	47,62%	
Primer 7	TTGGCCAATACAGGAACAGC	20 bp	58,46° C	50,00%	422 bp
	ACGATTGTGACACGATAGCCA	21 bp	59,80° C	47,62%	
Primer 8	TGGCTCAGGTACTGCTATCCA	21 bp	60,34° C	52,38%	297 bp
	ACGTTGTAACCACCCCAAGAT	21 bp	59,58° C	47,62%	

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Primer 9	GGCTCAGGTACTGCTATCCA	20 bp	58,59° C	55,00%	300 bp
	TGTAACGTTGTAACCACCCCA	21 bp	59,51° C	47,62%	
Primer 10	TGGCTCAGGTACTGCTATCC	20 bp	58,59° C	55,00%	156 bp
	TGGAACTTGTTGAGCAGAGGT	21 bp	59,51° C	47,62%	

Subsequently, an evaluation of the secondary structural characteristics of the ten candidate primer pairs was performed using the NetPrimer platform, with the results presented in Table 2.

Table 2. Secondary Structure Characteristics of Candidate Primers using NetPrimer

Candidate	Primer	Self Dimer	Hairpin	Repeat (# of pairs)	Run (# of bases)	Rating
Primer 1	TCCACCCTCAAACAGGTGAA	-7,37	-3,27	0	3	82
	TCTGCAGTACCGGATTTGCC	-10,24	-1,79	0	3	79
Primer 2	TGCTATCCACCCTCAAACAGG	-5,67	-1,27	0	3	88
	CTGCAGTACCGGATTTGCCA	-10,24	-1,79	0	3	79
Primer 3	TTGGCCAATACAGGAACAGCA	-17,07	0	0	0	69
	GGTGGATAGCAGTACCTGAGC	-4,55	-0,11	0	0	91
Primer 4	GCTATCCACCCTCAAACAGGT	-5,67	-1,31	0	3	87
	AACGTTGTAACCACCCCAAGA	-10,17	-0,7	0	4	80
Primer 5	ACGGTAACATTGATCGCAACG	-4,9	-0,4	0	0	90
	TCGTCAACGATTGTGACACG	-6,53	-5,19	0	0	80
Primer 6	TCAGGTACTGCTATCCACCCT	-5,67	-1,11	0	3	88
	AAACCACCCAATTTGTCTGCC	-5,36	0	0	3	90
Primer 7	TTGGCCAATACAGGAACAGC	-17,07	0	0	0	69
	ACGATTGTGACACGATAGCCA	0	0	0	0	100
Primer 8	TGGCTCAGGTACTGCTATCCA	-6,02	-0,22	0	0	88
	ACGTTGTAACCACCCCAAGAT	-6,3	-0,7	0	4	87
Primer 9	GGCTCAGGTACTGCTATCCA	-3,65	0	0	0	93
	TGTAACGTTGTAACCACCCCA	-10,18	0	0	4	81
Primer 10	TGGCTCAGGTACTGCTATCC	-3,65	0	0	0	93
	TGGAACTTGTTGAGCAGAGGT	0	0	0	0	100

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The secondary structure analysis of all primer pairs using the NetPrimer platform showed variable results. Primer pair 10 met the ideal criteria, with a forward self-dimer of –3.65 kcal/mol, no reverse self-dimer, no hairpin formation, and no run or repeat motifs. This pair, consisting of forward primer 5'-TGGCTCAGGTACTGCTATCC-3' and reverse primer 5'-TGGAACTTGTTGAGCAGAGGT-3', produced a 156 bp product and was identified as the most optimal primer. The selected primer was further validated using the Benchling platform, with results summarized in Table 3.

Table 3. Primer Characteristics of the *mecA* Gene

Primer Criteria	Standards	Primer Characteristics		Suitability
		Forward Primer	Reverse Primer	
Base length	18 – 30 bp	20 bp	21 bp	Suitable
%GC	40 – 60%	55,00%	47,62%	Suitable
Temperature melting (Tm)	50 – 65°C	58,59°C	59,51°C	Suitable
Repeat	Max. 4	0	0	Suitable
Run	Max. 3	0	0	Suitable
Hairpin	≥ -3 kcal/mol	0 kcal/mol	0 kcal/mol	Suitable
Self dimer	≥ -6 kcal/mol	-3,65 kcal/mol	0 kcal/mol	Suitable
ΔG homodimer	≥ -6 kcal/mol	-2,49 kcal/mol	-2,86 kcal/mol	Suitable
ΔG heterodimer	≥ -6 kcal/mol	-4,21 kcal/mol		Suitable

Based on Table 3, the *mecA* primer pair met all ideal primer characteristics. *In silico* specificity testing using NCBI BLAST showed 100% identity of both the forward primer (5'-TGGCTCAGGTACTGCTATCC-3') and reverse primer (5'-TGGAACTTGTTGAGCAGAGGT-3') with the *mecA* gene of MRSA across multiple accession numbers. These results confirmed the primers' specificity, and PCR optimization was subsequently performed using the designed pair.

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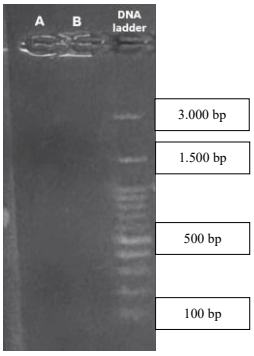
PCR OPTIMIZATION AND *MECA* GENE IDENTIFICATION RESULTS

Identification of the *mecA* gene was carried out on DNA samples from MRSA ATCC 33591 bacterial colonies obtained from the Department of Microbiology, Faculty of Medicine, Udayana University, and sub cultured at the Integrated Biology and Molecular Laboratory, Health Polytechnic of the Ministry of Health, Denpasar. The process was performed using optimized PCR conditions with specific primers. DNA extraction of MRSA was conducted using the Phenol-Chloroform Isoamyl Alcohol (PCIA) method. Two extraction results, labeled A and B, were obtained to provide sufficient DNA template for duplicate PCR experiments. The extracted DNA was then qualitatively analyzed using gel electrophoresis. The electrophoresis results are presented in Picture 1.



Picture 1. Results of DNA Extraction

Description:

A: DNA extraction result of MRSA ATCC 33591 I

B: DNA extraction result of MRSA ATCC 33591 II

Based on the qualitative analysis of the extraction results shown in Figure 3, fluorescent bands were observed in wells A and B. To confirm that the observed fluorescence bands represented bacterial DNA, an internal control PCR was performed using 16S rRNA gene primers. The internal control PCR was considered positive if a band appeared at the size of 1,550 bp. The internal control PCR products were analysed qualitatively using electrophoresis, as shown in Picture 2.

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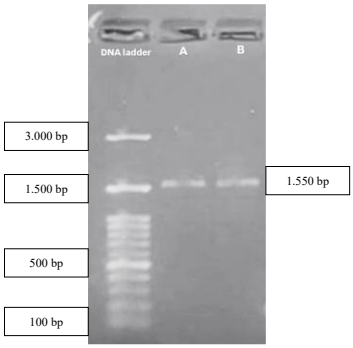

Picture 2. Results of Internal Control PCR

Description:

A: Internal control PCR result of MRSA ATCC 33591 DNA I

B: Internal control PCR result of MRSA ATCC 33591 DNA II

The internal control PCR results showed the presence of a 1,550 bp band in both DNA extractions, confirming that the extracted samples contained bacterial DNA. The study was then continued with PCR optimization using the designed *mecA* primers. PCR optimization was performed twice using a gradient PCR approach. In the first optimization, DNA template from extraction A was used with pre-denaturation at 95°C, denaturation at 95°C, annealing at 51.0°C with a gradient increase of 1.0°C producing a temperature range of 47.5°C to 55.6°C, extension at 72°C, and final extension at 72°C for 30 cycles. In the second optimization, DNA template from extraction B was used with pre-denaturation at 95°C, denaturation at 95°C, annealing at 59.0°C with a gradient increase of 1.0°C producing a temperature range of 55.5°C to 63.6°C, extension at 72°C, and final extension at 72°C for 30 cycles. After completion of the PCR process, the results of PCR optimization were qualitatively analysed using gel electrophoresis. The analysis results are shown in Picture 3.

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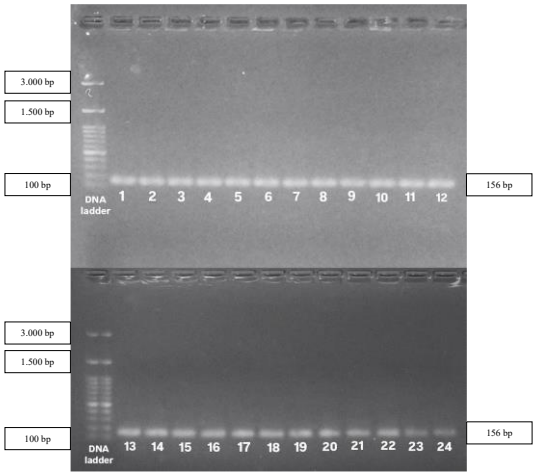

Picture 3. Results of PCR Optimization

Based on Picture 3, the results showed the presence of a 156 bp band with varying DNA band intensities. This indicated that the *mecA* forward primer (5'-TGGCTCAGGTACTGCTATCC-3') and reverse primer (5'-TGGAACTTGTTGAGCAGAGGT-3') successfully amplified the *mecA* gene in the MRSA ATCC 33591 isolate. Subsequently, DNA band analysis was performed to determine the most optimal annealing temperature. The results are presented in Table 6.

Table 6. Analysis of *mecA* Gene DNA Bands

Sample Code	Annealing Temperature	Result
1	47,5°C	Smear band
2	47,7°C	Smear band
3	48,2°C	Smear band
4	49,0°C	Smear band
5	50,0°C	Smear band
6	51,0°C	Smear band
7	52,1°C	Smear band
8	53,1°C	Smear band
9	54,1°C	Smear band
10	54,9°C	Smear band
11	55,4°C	Smear band
12	55,6°C	Smear band
13	55,5°C	Sharp band
14	55,7°C	Smear band
15	56,2°C	Faint band
16	57,0°C	Faint band
17	58,0°C	Smear band

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18	59,0°C	Dim band
19	60,1°C	Smear band
20	61,1°C	Smear band
21	62,1°C	Dim band
22	62,9°C	Faint band
23	63,4°C	Dim band
24	63,6°C	Dim band

Based on Table 10, the annealing temperature of 55.5°C produced a DNA band with the sharpest intensity compared to other temperatures. Therefore, the most optimal annealing temperature for the *mecA* primer was determined to be 55.5°C.

DISCUSSION

DESIGN OF *MECA* GENE PRIMERS FOR MRSA

Primer design is the initial step that determines the amplification performance in the PCR assay. Primer design was carried out using an *in silico* approach on bioinformatics platforms, beginning with the retrieval of the full genome of MRSA from the NCBI GeneBank database. The FASTA sequence of the MRSA full genome (KU194301.1) was used to design primers using NCBI Primer-BLAST, resulting in 10 candidate primer pairs. The success of primer design for PCR is strongly influenced by the primer characteristics. Important factors to consider when designing primers include primer length, the percentage of guanine and cytosine (%GC), melting temperature (Tm), and other criteria such as low values of self-dimer, hairpin, repeat, and run (Saraswati et al., 2019).

The selected *mecA* primers consisted of a forward primer (5'-TGGCTCAGGTACTGCTATCC-3', 20 bases) and a reverse primer (5'-TGGAACTTGTTGAGCAGAGGT-3', 21 bases), producing a 156 bp amplicon. Both primers met the ideal criteria for length (18–30 bases), GC content (40 – 60%), melting temperature (forward 58.59°C, reverse 59.51°C), and showed no problematic runs, repeats, hairpins, or dimers. Free energy (ΔG) values for self-dimers, homodimers, and heterodimers were all within tolerable limits (> –6 kcal/mol).

Primers that are too short risk mispriming, while overly long primers increase hybridization potential and hinder amplification (Praja & Rosalina, 2021). Optimal GC content (40–60%) ensures stable binding; too low reduces efficiency, too high hampers strand

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separation (Maitriani et al., 2015).The ideal melting temperature (Tm) is 50–65°C (Anika et al., 2019), with forward and reverse primers differing by no more than 5°C to maintain efficiency (Praja & Rosalina, 2021). Runs (>4 identical bases) and repeats (>3 short motifs) should be avoided to prevent false priming (Sasmitha et al., 2018). Hairpins are undesirable as they inhibit amplification; acceptable free energy is $\Delta G > -3$ kcal/mol (Budiyanti Putri et al., 2021). Self-dimers, homodimers, and heterodimers are tolerated if $\Delta G > -6$ kcal/mol, ensuring they do not significantly disrupt PCR (Handoyo & Rudiretna, 2001).

The amplicon size (156 bp) was also optimal for conventional PCR, allowing efficient and specific amplification. The ideal PCR product size is 100–1000 base pairs (bp), as this range allows efficient reactions, rapid and specific amplification, and reduces the risk of secondary structure formation, such as loops or hairpins, that may hinder the process (Green & Sambrook, 2012). This short fragment length is highly suitable for PCR as it facilitates rapid denaturation, annealing, and extension. PCR products under 300 bp are generally more efficiently amplified using standard Taq DNA polymerase without requiring special enzymes or conditions. Longer products (>1000 bp) slow down amplification, increase extension time requirements, and often require proofreading enzymes. Moreover, long fragments are more prone to degradation and amplification errors, complicating gel electrophoresis interpretation (Mackay, 2007).

Specificity testing with NCBI BLAST confirmed 100% identity with *mecA* genes from multiple MRSA strains. Thus, the *mecA* primers fulfilled all ideal characteristics and were suitable for further PCR optimization. Primers designed *in silico* ideally require further laboratory optimization to determine the optimal conditions. Optimization typically focuses on the annealing temperature (Ta) to achieve the most effective PCR results (Sasmitha et al., 2018). Therefore, further PCR optimization was conducted using the designed *mecA* primers.

PCR OPTIMIZATION AND MECA GENE IDENTIFICATION RESULTS

Identification of the *mecA* gene in MRSA ATCC 33591 was carried out using an optimized PCR method with specifically designed primers. The bacterial isolate was sub-cultured in Tryptic Soy Broth (TSB), and DNA was extracted using the Phenol-Chloroform Isoamyl Alcohol (PCIA) method, considered a gold standard for obtaining high-purity DNA. DNA quality was assessed using agarose gel electrophoresis, and the presence of bacterial

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DNA was confirmed by internal control PCR with 16S rRNA primers, producing the expected 1,550 bp band.

The PCR process consisted of denaturation, annealing, and extension cycles, with optimization focused on the annealing temperature. In this study, PCR optimization was carried out by varying annealing temperatures to determine the most efficient amplification of the *mecA* gene fragment (156 bp). Two gradient PCR optimizations were performed. In the first, annealing temperature was set at 51.0°C with a 1.0°C gradient increase, producing variations at 47.5°C to 55.6°C. In the second, annealing temperature was set at 59.0°C with a 1.0°C gradient increase, producing variations at 55.5°C to 63.6°C.

PCR optimization results were analysed using 1.5% agarose gel electrophoresis at 77 volts for 60 minutes. As shown in Figure 5, the *mecA* primers (forward: 5'-TGGCTCAGGTACTGCTATCC-'3, reverse: 5'-TGGAACTTGTTGAGCAGAGGT-'3) successfully amplified the *mecA* gene from MRSA ATCC 33591, producing the expected 156 bp fragment consistent with in silico analysis. Electrophoresis results showed bands at the expected size in all samples, but with varying intensities depending on annealing temperature.

The sharpest and most intense band was observed at 55.5°C, which corresponded to the theoretical optimal annealing temperature range of 54 – 56°C based on primer melting temperatures. Lower annealing temperatures resulted in smeared bands due to nonspecific binding, while higher temperatures reduced amplification efficiency. Thus, the optimal PCR condition for detecting the *mecA* gene in MRSA was determined to be an annealing temperature of 55.5°C.

Previous studies support these findings. Mindhumalid, (2018) successfully identified *mecA* in MRSA using PCR, showing that three clinical isolates produced 533 bp bands while one isolate and negative control (*S. aureus* ATCC 25923) did not, confirming the presence of *mecA* in MRSA but not in MSSA. Another study by Syamsidi et al., (2021) designed specific *mecA* primers (forward: 5'-GTGAAGCAACCATCGTTAC-'3, reverse: 5'-CCTTCTACACCTCCATATCAC-'3) also designed *mecA* primers that met length requirements but produced a 2008 bp amplicon, exceeding the optimal 100 – 1000 bp range, making amplification inefficient compared with the 156 bp amplicon achieved in this study.

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Such long fragments slow amplification, increase extension time, and raise risks of degradation and misamplification (Mackay, 2007).

Oligoanalyzer results of Syamsidi et al.'s primers showed acceptable GC content but low Tm values, which risked mismatches at optimal annealing temperatures. Structural analysis revealed hairpins and dimer formations, which, though within tolerance, reduce PCR efficiency. Moreover, their primers were not validated experimentally, leaving specificity and sensitivity unconfirmed, unlike in this study where in vitro optimization verified both.

In conclusion, PCR method in this study successfully detected the *mecA* gene in MRSA with primers designed to meet all ideal characteristics, producing a 156 bp product under optimal annealing temperature (55.5°C). Thus, PCR with specific primer design can be applied as a reliable method for *mecA* gene detection in MRSA.

CONCLUSION(S)

Based on the results of the study, the following conclusions can be drawn:

1. The primer design that meets the requirements of primer characteristics and is specific to the *mecA* gene of MRSA is primer pair candidate 10, consisting of the forward primer 5'-TGGCTCAGGTACTGCTATCC-'3 and the reverse primer 5'-TGGAACTTGTTGAGCAGAGGT-'3, which successfully amplified the *mecA* gene in the MRSA ATCC 33591 isolate, producing a 156 bp product consistent with the in silico analysis.
2. The PCR reaction with the specific primer design was optimal at a pre-denaturation temperature of 95°C, denaturation at 95°C, annealing at 55.5°C, extension at 72°C, and final extension at 72°C, with 30 amplification cycles. This resulted in sharp and specific DNA bands. The optimized PCR reaction using the specific primer design can be applied for the detection of the *mecA* gene in MRSA.

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Conflict of Interest

The author(s) declare that they have no conflict of interest.

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