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Comparison of DNA Extraction Results from Staphylococcus aureus Using the Boiling Method and the Combined Boiling-Centrifugation-Bead Method in Terms of DNA Quality for PCR Testing

Baiq Inges Mustika Suci[1] , Fihiruddin[2] , Ersandhi Resnhaleksmana[3]
[1,2,3] Department of Medical Laboratory, Mataram Public Health Polytechnic, Ministry of Health,
*bqingesmustikasuci@gmail.com

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ABSTRACT

DNA extraction is a critical step in molecular testing based on the Polymerase Chain Reaction (PCR). In Gram-positive bacteria such as Staphylococcus aureus, the DNA extraction process requires an effective lysis method due to their thick peptidoglycan layer. The boiling method is simple and economical; however, further development of extraction methods is still needed to improve the quality of the resulting DNA. This study aimed to compare the results of DNA extraction using the boiling method and the combined boiling-centrifugation-bead method in terms of the quality of Staphylococcus aureus DNA for PCR testing. This was a laboratory-based experimental study with a comparative design using 30 samples of Staphylococcus aureus bacterial suspensions standardized to a McFarland turbidity of 1.5. Fifteen samples were extracted using the boiling method, and 15 samples were extracted using the combined boiling-centrifugation-bead method. DNA quality was measured using a NanoDrop, including DNA concentration, DNA purity as determined by the A260/A280 ratio, and the A260/A230 ratio. The DNA's ability to serve as a template was also evaluated through PCR amplification of the nuc gene. Data analysis was performed using the Independent Samples t-test and the Mann–Whitney U test. The boiling method yielded a mean DNA concentration of 12.57 ng/µL, a mean A260/A280 ratio of 2.2433, and a median A260/A230 ratio of -2.13. The combined boiling-centrifugation-bead method yielded a mean DNA concentration of 50.19 ng/µL, a mean A260/A280 ratio of 1.4220, and a median A260/A230 ratio of 0.89. Statistical analysis revealed significant differences in DNA concentration, DNA purity as indicated by the A260/A280 ratio, and the A260/A230 ratio between the two extraction methods ($p<0.001$). PCR results showed the formation of a target DNA band from the nuc gene measuring approximately 423 bp using both DNA extraction methods. The combined boiling-centrifugation-bead method yielded a higher DNA concentration and a better A260/A230 ratio compared to the boiling method.

INTRODUCTION

Advances in molecular biology testing over the past two decades have driven rapid progress in the fields of health, microbiology, and biotechnology. One of the most commonly used molecular tests is the Polymerase Chain Reaction (PCR), a rapid, specific, and sensitive method for DNA amplification (Wainman et al., 2023). PCR has become the primary method

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for diagnosing infectious diseases, identifying microorganisms, conducting forensic analysis, and conducting genetic research due to its ability to detect and amplify target DNA in various diagnostic and scientific applications (Mcdonald et al., 2024). The success of PCR is greatly influenced by the quality of the DNA used as a template; pure, contaminant-free DNA supports optimal amplification, whereas DNA containing inhibitors can lead to reduced sensitivity or even amplification failure (Na et al., 2025).

The DNA extraction step is a crucial step prior to PCR testing. Standard methods based on silica columns or magnetic beads have been shown to consistently yield high-purity DNA, making them widely used in molecular testing and considered the gold standard. However, the relatively high cost of these kits poses a challenge, particularly for educational laboratories, small-scale research labs, and diagnostic facilities with limited resources (Li et al., 2023). As an alternative, DNA extraction using the boiling method has become increasingly popular because the procedure is brief, easy to perform, and does not require special reagents. Several studies have demonstrated the effectiveness of the boiling method for isolating DNA from various bacteria, including Staphylococcus aureus; however, DNA obtained via the boiling method is often still contaminated with proteins, lipids, and debris that can interfere with PCR (Bai et al., 2025). A modification of the boiling method—involving the addition of a centrifugation step and magnetic-bead-based purification—has been proposed to separate cellular debris from the DNA supernatant while simultaneously improving lysis efficiency, thereby potentially yielding higher DNA concentrations to support PCR amplification (Bai et al., 2025).

Although the effectiveness of both the boiling method and the combined boiling-centrifugation-bead method has been reported separately, studies directly comparing the two in terms of PCR outcome quality remain very limited, particularly in educational and diagnostic laboratories with limited resources in Indonesia. This gap is particularly relevant for Gram-positive bacteria such as S. aureus, which have thick peptidoglycan cell walls, making lysis efficiency and the quality of the resulting DNA highly dependent on the extraction method used (Galeano et al., 2025). Therefore, a systematic comparison of these two cost-effective extraction methods is needed to provide practical guidance for laboratories that must balance the cost and success of PCR testing.

This study aims to compare the results of DNA extraction using the boiling method and the combined boiling-centrifugation-bead method in terms of the quality of Staphylococcus aureus DNA, as evaluated by DNA concentration, purity ratio, and the success of nucleic acid gene amplification via PCR.

MATERIALS/METHODS

This study employed a laboratory experimental design with a comparative design and was conducted at the Biomolecular Laboratory of the Mataram Public Health Polytechnic (Poltekkes Kemenkes Mataram) in May 2026. The study population consisted of pure Staphylococcus aureus isolates from the Microbiology Laboratory collection at the Poltekkes Kemenkes Mataram. A total of 30 pure colonies that met the inclusion and exclusion criteria were used as samples and divided into two treatment groups of 15 samples each: extraction using the boiling method and extraction using a combined boiling-centrifugation-bead method.

Before use, Staphylococcus aureus isolates were revived on Nutrient Agar and incubated at 37°C for 18–24 hours; a single, apparently pure colony was then selected for the preparation of a bacterial suspension. The bacterial suspension was prepared by suspending the pure colony in sterile 0.85% physiological NaCl solution and adjusting the turbidity to a McFarland standard of 1.5 ($\pm$ 4.5 $\times$ 10^8 CFU/mL). In the boiling method, 1 mL

of the suspension was centrifuged at 14,000 rpm for 5 minutes, the supernatant was discarded, and then 200 µL of TE buffer solution (Tris-EDTA, pH 8.0) was added; the tube is heated at 95°C for 10 minutes, cooled on ice for 2 minutes, and centrifuged again at 14,000 rpm for 5 minutes, after which 50 µL of the clear supernatant containing DNA is collected. The combined boiling-centrifugation-bead method follows the same initial centrifugation and TE buffer addition steps, accompanied by the addition of magnetic beads, followed by five repeated centrifugation cycles (14,000 rpm, 5 minutes) interspersed with vortexing and brief freezing in a freezer (30 seconds–1 minute) to enhance the efficiency of mechanical lysis of the cell walls. The sample is then heated at 95°C for approximately 10 minutes to completely lyse the cells, centrifuged again at 14,000 rpm for 5 minutes to separate cell debris, and 50 µL of the clear supernatant containing DNA is collected as the extraction product. The DNA extracted using both methods was stored at 4°C for immediate use or at −20°C for long-term storage.

DNA concentration and purity (A260/A280 and A260/A230 ratios) were measured using a NanoDrop spectrophotometer in dsDNA mode after blanking with the same solvent as the sample. The suitability of the extracted DNA as a PCR template was evaluated through amplification of the S. aureus nuc gene. Each 25 µL reaction consisted of 12.5 µL PCR Master Mix, 2 µL forward primer, 2 µL reverse primer, 2 µL DNA template, and 6.5 µL nuclease-free water, along with a confirmed positive control and a negative control without DNA template. Amplification was performed with a pre-denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C (30 seconds), annealing at 55°C (30 seconds), and extension at 72°C (45 seconds), as well as a final extension at 72°C for 5 minutes. PCR products were visualized via 1% agarose gel electrophoresis at 110 V for 30 minutes alongside a 100 bp DNA ladder, then observed under UV light; a band at approximately 423 bp was considered a positive result for the nuc gene.

Data normality was tested using the Shapiro-Wilk test. Normally distributed data (DNA concentration and A260/A280 ratio) were analyzed using an independent samples t-test, while non-normally distributed data (A260/A230 ratio in the boiling method) were analyzed using the Mann-Whitney U test, at a significance level of 0.05.

RESULTS AND DISCUSSION

A total of 30 samples of Staphylococcus aureus suspension standardized to McFarland 1.5 were extracted using the boiling method (n = 15) and the combined boiling-centrifugation-bead method (n = 15). Table 1 summarizes the DNA concentrations and purity obtained from each method.

Table 1. Comparison of DNA concentration and purity between the boiling method and the combined boiling-centrifugation-bead method

Parameter	Boiling Method	Combined Boiling-Centrifugation-Bead Method Centrifugation-Bead Method	p-value
DNA Concentration (ng/µL), mean ± SD	12.57 ± 1.84	50.19 ± 5.00	<0.001
A260/A280 ratio, mean ± SD	2.2433 ± 0.1555	1.4220 ± 0.0246	<0.001
A260/A230 ratio, median (IQR)	−2.13 (2.34)	0.89 (0.06)	<0.001

The combined boiling-centrifugation-bead method yielded a significantly higher average DNA concentration (50.19 ± 5.00 ng/µL) compared to the boiling method (12.57 ±

1.84 ng/µL) ($p < 0.001$). These findings are consistent with those of Fujimoto et al. (2004), who reported that adding a bead-beating step to S. aureus DNA extraction increased DNA yield compared to extraction without mechanical treatment, as bead impact helps disrupt the thick peptidoglycan layer characteristic of Gram-positive bacteria (Zeden & Grundling, 2023). Thermal lysis alone in the boiling method appears limited in completely disrupting the cell wall, whereas repeated centrifugation, vortexing, freeze-thaw cycles, and bead treatment in the combined method are more effective at breaking down the cell wall, resulting in more DNA being released into the supernatant.

Based on the A260/A280 ratio, the boiling method yielded an average of 2.2433 ± 0.1555 and the combined method 1.4220 ± 0.0246 ($p < 0.001$); both values fall outside the ideal range of 1.8–2.0, which indicates minimal protein or RNA contamination (Lucena-Aguilar et al., 2016). A boiling method ratio value exceeding the ideal range indicates the presence of RNA contamination that was not fully removed during extraction, whereas a combined method ratio value below the ideal range indicates the presence of protein or other compound contamination (Congiu et al., 2024). Thus, neither method has achieved optimal purity for this parameter, although the DNA extracted by both methods remains suitable for use as a PCR template.

The assessment of contamination by organic compounds and salts using the A260/A230 ratio showed a median of -2.13 (IQR 2.34) for the boiling method and 0.89 (IQR 0.06) for the combined method ($p < 0.001$), compared to the ideal range of 2.0–2.2 (Waris & Pradana, 2026). The negative median for the boiling method indicates unstable absorbance readings associated with low concentrations of extracted DNA, reflecting suboptimal removal of contaminants, whereas the less negative and positive values for the combined method indicate relatively better removal of organic contaminants and salts, although not yet ideal.

PCR amplification of the nuc gene produced a specific band measuring approximately 423 bp in representative samples examined from both extraction methods (Figure 1), indicating that DNA extracted using both the boiling method and the combined boiling-centrifugation-bead method can still be used as a template for amplification, even though the purity ratio is not yet optimal. This is consistent with the findings of Oliveira et al. (2014), who found that coagulase-negative Staphylococcus isolates extracted using various methods could still be amplified by PCR, even though the quality and quantity of DNA obtained differed among the methods. Band intensity appeared to correlate with DNA concentration, with samples having higher concentrations tending to produce brighter bands; however, all samples still produced detectable bands at the target size. These results indicate that the success of PCR amplification is determined not only by DNA purity but also by the availability of target DNA and the sensitivity of the PCR method itself.

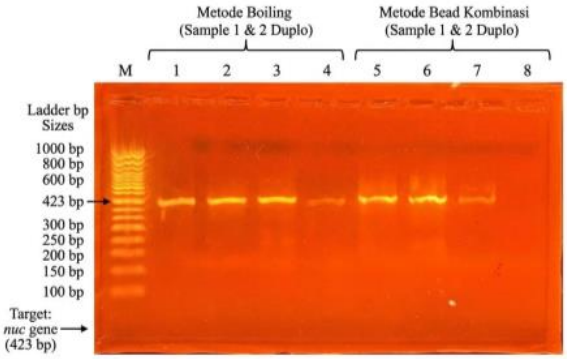

Figure1 . Results of PCR electrophoresis for the nuc gene of Staphylococcus aureus. M: DNA ladder; lanes 1–4: representative samples from the boiling method (isolates 1 and 2, duplicates); lanes 5–8: representative samples from the combined boiling-centrifugation-bead method (isolates 1 and 2, duplicates); target band size 423 bp.

Overall, the combined boiling-centrifugation-bead method yielded significantly higher DNA concentrations and a relatively better A260/A230 ratio compared to the boiling method, while both methods still produced DNA capable of supporting successful amplification of the nuc gene

CONCLUSIONS

The boiling method yielded an average DNA concentration of 12.57 ± 1.84 ng/µL, an average A260/A280 ratio of 2.2433 ± 0.1555, and a median A260/A230 ratio of -2.13 (IQR 2.34), whereas the combined boiling-centrifugation-bead method yielded a mean DNA concentration of 50.19 ± 5.00 ng/µL, a mean A260/A280 ratio of 1.4220 ± 0.0246, and a median A260/A230 ratio of 0.89 (IQR 0.06), with significant differences between the two methods for all three parameters ($p < 0.001$). The DNA extracted using both methods successfully amplified the S. aureus nuc gene, producing a 423-bp band as expected, indicating that the DNA from both extraction methods can be used as PCR templates. With higher DNA concentrations and a relatively better A260/A230 ratio, the combined boiling-centrifugation-bead method is recommended as a practical and economical alternative to commercial extraction kits for S. aureus PCR testing in laboratories with limited resources. Future research is encouraged to include a purification step—such as using proteinase K—or to compare these two methods with the phenol-chloroform extraction method to further improve DNA purity.

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