

Rapid and Cost-Effective Colorimetric Detection of Methicillin-Resistant *Staphylococcus aureus* in Water via Loop-Mediated Isothermal Amplification Targeting the *mecA* Gene

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Hawaii has a higher prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections compared to the national average. Environmental water is a major vehicle for the transmission of MRSA. Current MRSA detection methods rely on culture-based techniques and other biochemical tests, often requiring 2-3 days, delaying critical interventions and increasing transmission risks. Loop-mediated isothermal amplification (LAMP) offers a rapid, cost-effective alternative, enabling high-specificity DNA amplification within 30-60 minutes under isothermal conditions. Previous studies have used LAMP for MRSA detection in clinical samples, but a gap remains in its application in wastewater and other water sources. This study designed and validated a LAMP assay targeting the *mecA* gene, which confers resistance to beta-lactam antibiotics, and tested it on wastewater and water samples. Validation was performed and included MRSA isolates as positive controls and no-template negative controls. Extracted DNA from three sites across the Ala Wai Canal and three wastewater treatment plants was combined with a LAMP master mix and incubated at 65°C for 60 minutes. Positive and negative results were determined by a color change using phenol red. The LAMP assay demonstrated 100% sensitivity and specificity. All Ala Wai Canal samples and 50% of the influent wastewater samples tested positive for *mecA*, highlighting the need for public health intervention. This study successfully developed and validated a rapid, cost-effective LAMP assay for detecting MRSA in water from extracted DNA within one hour. Its accessibility and efficiency can enhance public health monitoring and improve MRSA surveillance in resource-limited settings, aiding infection prevention efforts.

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