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Cas12a/Guide RNA-Based Platforms for Rapidly and Accurately Identifying *Staphylococcus aureus* and Methicillin-Resistant *S. aureus*

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ABSTRACT

In order to ensure the prevention and control of methicillin-resistant *Staphylococcus aureus* (MRSA) infection, rapid and accurate detection of pathogens and their resistance phenotypes is a must. Therefore, this study aimed to develop a fast and precise nucleic acid detection platform for identifying *S. aureus* and MRSA. We initially constructed a CRISPR-Cas12a detection system by designing single guide RNAs (sgRNAs) specifically targeting the thermonuclease (*nuc*) and *mecA* genes. To increase the sensitivity of the CRISPR-Cas12a system, we incorporated PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA). Subsequently, we compared the sensitivity and specificity of the three amplification methods paired with the CRISPR-Cas12a system. Finally, the clinical performance of the methods was tested by analyzing the fluorescence readout of 111 clinical isolates. In order to visualize the results, lateral-flow test strip technology, which enables point-of-care testing, was also utilized. After comparing the sensitivity and specificity of three different methods, we determined that the *nuc*-LAMP-Cas12a and *mecA*-LAMP-Cas12a methods were the optimal detection methods. The *nuc*-LAMP-Cas12a platform showed a limit of detection (LOD) of 10 aM (~6 copies μL^{-1}), while the *mecA*-LAMP-Cas12a platform demonstrated a LOD of 1 aM (~1 c μL^{-1}). The LOD of both platforms reached 4×10^3 fg/ μL of genomic DNA. Critical evaluation of their efficiencies on 111 clinical bacterial isolates showed that they were 100% specific and 100% sensitive with both the fluorescence readout and the lateral-flow readout. Total d



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