Abstract

Foodborne pathogens such as *Staphylococcus aureus* and *Escherichia coli* remain major public health concerns in Indonesia, particularly following food poisoning outbreaks associated with the *Makan Bergizi Gratis* (MBG) program. Rapid and sensitive detection methods are required to improve food safety monitoring. This study evaluated the Limit of Detection (LoD) of culture and conventional polymerase chain reaction (PCR) methods for detecting *S. aureus* and *E. coli* in beef and milk matrices while establishing an *in silico* framework for loop-mediated isothermal amplification (LAMP) assay development. Raw beef sterilization was optimized prior to artificial spiking with bacterial dilutions ranging from 10^1-10^7 CFU/mL. Culture-based detection, genomic DNA extraction, conventional PCR targeting the *nuc* and *fimH* genes, and *in silico* primer design for PCR and LAMP were performed. A 60-minute treatment with 70% ethanol was identified as the optimal beef sterilization protocol. Culture methods achieved LoDs of 10^2 CFU/mL in beef and 10^1 CFU/mL in milk for both bacteria. PCR successfully amplified the *fimH* gene of *E. coli* with a LoD of 10^5 CFU/mL but failed to amplify the *nuc* gene of *S. aureus*, likely due to inefficient genomic DNA extraction associated with the bacterium's resistant cell wall. Overall, culture methods exhibited greater sensitivity than PCR under the tested conditions. This study establishes a foundation for future optimization of molecular diagnostics, including LAMP assays, to strengthen food safety monitoring in Indonesia.

Keywords: food safety monitoring, *Staphylococcus aureus*, *Escherichia coli*, foodborne pathogen detection, LoD