Chapter 1

Introduction

1.1 Background

Food safety remains one of the most significant public health concerns worldwide, particularly in developing countries such as Indonesia. The World Health Organization (WHO) estimates 600 million people suffer from unsafe food each year, with approximately 420,000 deaths reported annually (WHO, 2024). Milk and red meat, including beef, are among the most widely consumed food products globally due to their accessibility and nutritional value (Sanosi et al., 2025; Warsewicz et al., 2019). Additionally, individuals around the globe are prone to consuming beef without the meat being fully cooked; a notable cultural example would be the consumption of *yukhoe*, a food item originating from Korea (Choi & Lee, 2023). Hence, a lack of proper sterilization and handling during processing or distribution can lead to microbial contamination by foodborne pathogens, posing significant health risks to consumers.

In Indonesia, food safety concerns have gained attention following the implementation of the *Makan Bergizi Gratis* (MBG) program issued by the government. Data from the *Badan Pengawas Obat dan Makanan* (BPOM) recorded 9,089 food poisoning cases across 28 provinces associated with the program, with laboratory tests confirming bacterial contamination involving *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. (Arlinta, 2025; Sahal, 2025). Among these, *S. aureus* infections are of major concern due to their characteristic property to express heat-stable enterotoxins. These toxins remain active even after application of heat treatment, e.g., cooking and Ultra-High Temperature (UHT) processing, resulting in symptoms such as nausea, vomiting, and diarrhea (Necidová et al., 2019; Yuliza et al., 2024). On the other hand, *E. coli* persists as one of the most common foodborne pathogenic outbreak sources. Among the numerous strains of *E. coli*, Enterohemorrhagic *E. coli* (EHEC), of which *E. coli* O157:H7 is the most well-known *E. coli* strain to

cause outbreaks linked to fecal-oral transmission due to poor handling techniques between the handler and the food item (Schlundt, 2017; Werner, 2016).

Traditional methods for detecting foodborne bacteria, including culture-based identification and biochemical testing, remain the gold standard for detecting foodborne pathogens. However, these methods are often time-consuming, require multiple procedural steps for confirmation, and may be limited by their low sensitivity (Law et al., 2015). Another widely accepted and reliable method of detection is Polymerase Chain Reaction (PCR). PCR is a multi-step nucleic acid amplification (NAA) that offers exceptional sensitivity and faster turnaround times by utilizing precise thermal cycling to target genetic markers, such as the *nuc* gene for *S. aureus* and *fimH* gene for *E. coli*. (Tran et al., 2024).

Nevertheless, Indonesia faces a shortage of food testing laboratories that comply with the National Agency for Drug and Food Control (NADFC) standards due to limited facilities, equipment, testing capacity, and trained personnel (Putri, 2022). While PCR overcomes the sensitivity and time limitations of culture methods, its reliance on expensive thermocyclers, specialized facilities, and stable power supplies restricts its utility in resource-limited or on-site monitoring environments (Khehra et al., 2023). Therefore, evaluating and establishing the baseline Limit of Detection (LoD) performance of both conventional culture and PCR within complex food matrices is critical for optimizing food safety monitoring systems.

Concurrently, to address the constraints associated with traditional testing and PCR thermocycling, alternative isothermal platforms like Loop-Mediated Isothermal Amplification (LAMP) have emerged as promising low-resource diagnostic tools. Thus, the primary objective of this study is to evaluate and compare the baseline Limit of Detection (LoD) of the culture method and conventional PCR for *S. aureus* and *E. coli* in milk and beef matrices, while establishing a conceptual framework for an *in silico*

LAMP evaluation through primer design targeting the *nuc* and *fimH* genes, respectively, for future low-resource detection alternatives.

1.2 Objective

This research aims to evaluate and compare the Limit of Detection (LoD) of the culture method and conventional PCR for the detection of *Staphylococcus aureus* and *Escherichia coli* in milk and beef. Specifically, the objectives of this research are:

- To determine the optimal conditions for the sterilization method of raw beef samples to eliminate background microbiota prior to artificial spiking.
- To design and validate conventional PCR primers targeting the *nuc* gene for *S. aureus* detection and the *fimH* gene for *E. coli* detection.
- To determine and compare the Limit of Detection (LoD) of the culture method and conventional PCR through serial dilutions of *S. aureus* and *E. coli* in artificially spiked milk and beef samples.
- To design LAMP primers targeting the *nuc* gene for *S. aureus* detection and the *fimH* gene of *E. coli* as a conceptual framework for future low-resource detection alternatives.

1.3 Hypothesis

H_0 : There is no significant difference in the sensitivity or Limit of Detection (LoD) between the conventional culture method and PCR for detecting *Staphylococcus aureus* and *Escherichia coli* in milk and beef samples.

H_1 : Conventional PCR exhibits a significantly lower Limit of Detection (LoD) and higher sensitivity compared to the culture method for detecting *Staphylococcus aureus* and *Escherichia coli* in milk and beef samples.