Sensitivity and Specificity of Specific Gene Primers for PCR-Based Detection of Salmonella typhimurium and Escherichia coli : A Systematic Review

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ABSTRACT
Foodborne disease (foodborne pathogen), commonly referred to as food poisoning, is an infection or intoxication caused by consuming food that contains hazardous materials or has been contaminated by toxic bacteria, viruses, parasites, and fungi. According to the World Health Organization (WHO), it was estimated that in 2012, 2.2 million people worldwide died from diarrheal diseases caused by various bacterial, viral, and parasitic organisms spread through contaminated water or food, with 20% of these deaths occurring among children under five years of age. The high number of casualties within a short period necessitates an accurate bacterial identification method to ensure prompt and appropriate medical treatment. The currently available simple method is the traditional culture technique, which relies on growing the bacteria on agar media. This method requires approximately 3–4 days of processing time and up to one week to confirm the specific pathogenic microorganism causing the poisoning. Therefore, it is essential to develop foodborne pathogen detection methods to overcome the limitations of the traditional culture method. This literature review aims to examine, analyze, and summarize various previous research findings regarding the sensitivity and specificity of using specific gene primer pairs to detect *Salmonella typhimurium* and *Escherichia coli* bacteria using the PCR (Polymerase Chain Reaction) method. Through a literature review approach, this article evaluates the minimum effective sample concentration detection limits and the time efficiency of PCR compared to conventional culture methods, providing a comprehensive overview of PCR's potential as a rapid and accurate instrument for the early detection.

ABSTRAK
Penyakit bawaan makanan (*Foodborne pathogen*) atau sering disebut keracunan makanan adalah infeksi atau intoksikasi yang disebabkan oleh karena mengkonsumsi makanan yang mengandung bahan berbahaya atau yang telah tekontaminasi oleh bakteri, virus, parasit dan jamur yang bersifat toksin. Menurut Organisasi Kesehatan Dunia (WHO) pada tahun 2012 diperkirakan 2,2 juta orang di seluruh dunia meninggal karena penyakit keracunan yang disebabkan oleh sejumlah organisme bakteri, virus, dan parasit, yang disebarkan oleh air atau makanan yang terkontaminasi, dan 20% dari kematian di antara anak-anak berusia kurang dari lima tahun. Banyaknya jumlah korban dalam waktu yang sangat singkat menyebabkan diperlukannya metode identifikasi bakteri yg akurat agar dapat segera dilakukan penangan medis yang tepat. Metode sederhana yang ada saat ini yaitu metode tradisional dengan metode kultur. Metode ini bergantung pada proses peng-kulturan bakteri tersebut pada media agar. Metode ini memerlukan waktu pengerjaan kira-kira 3-4 hari, dan memerlukan waktu 1 (satu) minggu untuk konfirmasi mikroorganisme pathogen spesifik penyebab keracunan tersebut. Untuk itu perlu dilakukan pengembangan metode deteksi *Foodborne Pathogen* untuk mengatasi kekurangan metode tradisional kultur. Kajian literatur ini bertujuan untuk menelaah, menganalisis, dan merangkum berbagai hasil penelitian terdahulu mengenai sensitivitas dan spesifisitas penggunaan pasangan primer gen spesifik dalam mendeteksi bakteri *Salmonella typhimurium* dan *Escherichia coli* menggunakan metode PCR. Melalui pendekatan studi pustaka, artikel ini mengevaluasi batas deteksi konsentrasi sampel terkecil yang efektif serta efisiensi waktu PCR dibandingkan dengan metode kultur konvensional, guna memberikan gambaran komprehensif mengenai potensi PCR sebagai instrumen deteksi dini *foodborne pathogen* yang cepat dan akurat.

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KEYWORDS
Foodborne pathogen, Literature Review, PCR, Specific primer.

INTRODUCTION

The high number of food poisoning cases (foodborne pathogens), particularly those caused by pathogenic bacterial infections, has become a serious problem in various countries, including both developed and developing nations like Indonesia. These food poisoning cases are often referred to as Extraordinary Events (KLB) because they occur within a short timeframe and simultaneously affect a vast number of victims, frequently involving police investigations. Based on the literature, it is known that the pathogenic bacteria causing poisoning consist of 31 identified bacterial species; several of these, such as *Salmonella enterica* species, *Campylobacter*, *Staphylococcus aureus*, *Vibrio* species, *Clostridium perfringens*, *Listeria monocytogenes*, *Toxoplasma gondii*, and *Escherichia coli* O157:H7, are the primary pathogenic bacteria responsible for the majority of frequently occurring food poisoning cases (Velusamy et al., 2010).

The large number of victims within a very short period due to bacterial infections from food poisoning creates a pressing need for accurate bacterial identification methods so that proper medical treatment can be administered immediately. The simple method currently available is the traditional culture method. This method relies on the process of culturing the bacteria on agar media. It requires approximately 3 to 4 days of processing time and takes 1 (one) week to confirm the specific pathogenic microorganism causing the poisoning (Sharma, 2013). Therefore, it is necessary to develop foodborne pathogen detection methods to overcome the shortcomings of the traditional culture method.

Molecular biology diagnostic techniques utilizing a genotypic approach via the Polymerase Chain Reaction (PCR) method serve as one alternative for diagnosing foodborne pathogens that is currently being widely developed through various research efforts; this is because the PCR technique offers several advantages, including the ability to amplify DNA at specific target regions as desired (Sanderson et al., 2003). Previous research achieved a PCR specificity of 100% with a sensitivity 10 times better, capable of detecting 1–5 bacteria/mL of blood (Haque et al., 1999), and also demonstrated a PCR sensitivity of 63% when compared to blood culture (13.7%) and the Widal test (35.6%) (Massi et al., 2003).

Through a literature review approach, this article evaluates the minimum effective sample concentration detection limits and the time efficiency of PCR compared to conventional culture methods, providing a comprehensive overview of PCR's potential as a rapid and accurate instrument for the early detection. In addition to evaluating the detection limits and time efficiency, this article synthesizes various data from previous studies to examine the accuracy of PCR's specificity and sensitivity. Through an in-depth comparative analysis, this review is expected to provide a solid scientific foundation for medical practitioners and health authorities in adopting molecular biology techniques. Consequently, the implementation of PCR as a cutting-edge diagnostic tool will not only accelerate clinical management for food poisoning victims but also optimize mitigation strategies for managing Extraordinary Events (KLB) more effectively and efficiently.

METHODS

This paper was conducted using a literature review approach, utilizing a systematic framework that involves data collection, analysis, and synthesis of scientific articles. The selected literature was restricted based on specific inclusion criteria, focusing on studies that evaluate the detection of *Salmonella typhimurium* and *Escherichia coli* using the PCR method with specific gene primer pairs, presenting explicit data regarding the limit of detection (the smallest effective sample concentration) and time efficiency, as well as directly comparing PCR with conventional culture techniques. Data retrieved from the literature centered on analytical performance parameters such as sensitivity (%), specificity (%), limit of detection, and the total turnaround time required from sample preparation to final confirmation. This data is then presented descriptively to provide a comprehensive overview of the potential of PCR as a rapid and accurate instrument for the early detection of foodborne pathogens.

RESULTS AND DISCUSSIONS

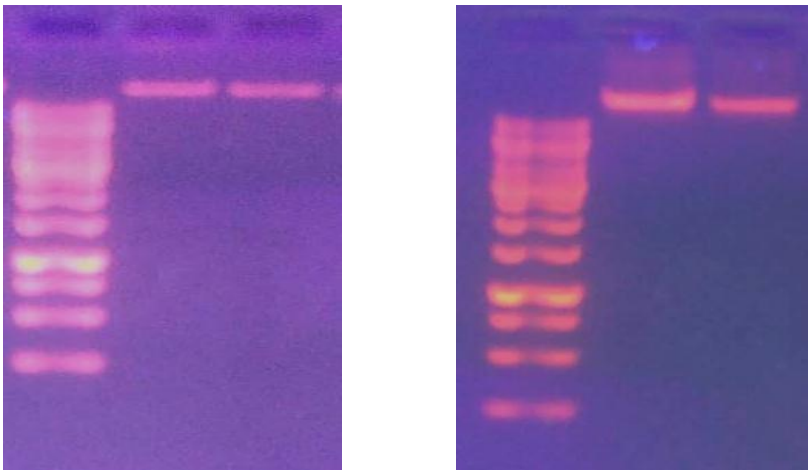
1. Sensitivity and Specificity of Specific Gene Primers for *Escherichia coli* and *Salmonella Typhimurium*

A. Isolation of Bacterial DNA from Pure Cultures and Contaminated Food Samples

Genome isolation was performed using specialized extraction kits, specifically a DNA isolation kit designed for pure bacterial cultures and a DNA isolation kit for food samples. The purpose of genome isolation in this study was to obtain pure DNA isolates to serve as templates for the PCR process.

Table 1. Purity and concentration of genomic DNA isolated from two bacterial samples

	Bacterial	A260/280	A260/230	ng/µl
	Eschericia coli	2,01	2,01	24,5
S	Salmonella thypimurium	1,99	0,98	18,0



A B

Figure 1. Electropherogram results of isolates with a 1 kb DNA ladder; A) Pure culture sample and B) DNA isolate from a contaminated food sample.

Based on the research results by Pratiwi et al. (2017), the figure shows the isolated genomic DNA, as indicated by the appearance of DNA bands on the gel. The DNA bands are located higher than the first line of the DNA marker, corresponding to a size greater than 1.5 kb. Data analysis from various literatures reveals that the genomic DNA size of *S. Typhimurium* is approximately 4.86 Mb, while that of *E. coli* is around 4.8 Mb (Parkhill et al., 2001; Sabbagh et al., 2010; Oshima et al., 2008).

B. DNA amplification of food samples contaminated with *Salmonella typhimurium* and *Escherichia coli* using the Polymerase Chain Reaction method

The sensitivity results of the specific primer pairs for *Salmonella Typhimurium* and *Escherichia coli* can be determined through the DNA amplification process using the Polymerase Chain Reaction (PCR) method. During the template DNA amplification stage, several components are required to support the reaction process, including template DNA isolated from the sample, a master mix containing PCR buffer, $MgCl_2$, and DNA polymerase enzyme, as well as crucial specific forward and reverse primer pairs. The pair of primers serves as the starting point for DNA amplification.

According to the research results by Winda (2016), the *E. coli* primer pair successfully amplified the template DNA from food samples contaminated with *E. coli*, producing an amplicon size of 121 bp. This is evidenced by the appearance of a DNA band located above 100 bp, which corresponds to the primer design that yields a 121 bp amplicon when tested on pure bacterial cultures. Meanwhile, the *S. Typhimurium* primer pair successfully amplified the template DNA from food samples contaminated with *S. Typhimurium* at an amplicon size of 139 bp. This can be observed from the resulting DNA band, which is positioned higher than the *E. coli* amplicon band, consistent with the primer design intended to produce a 139 bp amplicon.

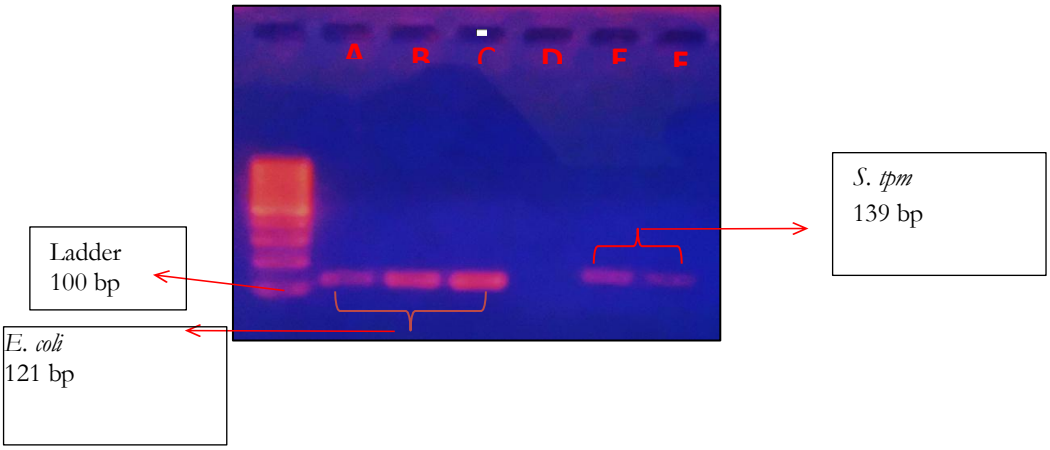


Figure 2. Amplification results of contaminated food samples

Based on the literature, the figure demonstrates that both *E. coli* and *S. Typhimurium* primers successfully amplified the template DNA from the food samples (Winda, 2016).

C. Sensitivity testing of pef gene-specific primers for *Salmonella Typhimurium* and fimC gene-specific primers for *Escherichia coli*

Based on the research results by Pertiwi et al. (2017), DNA bands appeared within the concentration range of 122.5 ng/5 µL to 1.225 ng/5 µL. No DNA bands were observed at concentrations of 0.1225 ng/µL and 0.01225 ng/µL, indicating that the *E. coli* primers could no longer amplify DNA at these concentration levels. Thus, it can be concluded that the *E. coli* primers used are sensitive and capable of amplifying template DNA down to a concentration of 1.225 ng/µL. Regarding the *Salmonella Typhimurium* primers, the electropherogram results in Figure 4 show that among the six tested concentrations, DNA bands appeared at 99 ng/5.5 µL, 9.9 ng/5.5 µL, and 0.99 ng/5.5 µL, while a very faint yet visible band appeared at 0.099 ng/5.5 µL. No DNA bands were observed at concentrations of 0.0099 ng/5.5 µL and 0.00099 ng/5.5 µL, demonstrating that the *S. Typhimurium* primers were unable to amplify DNA from food samples contaminated with *S. Typhimurium* at those concentrations. Therefore, it can be concluded that the *S. Typhimurium* primers used are sensitive and capable of amplifying down to a template DNA concentration of 0.099 ng/5.5 µL

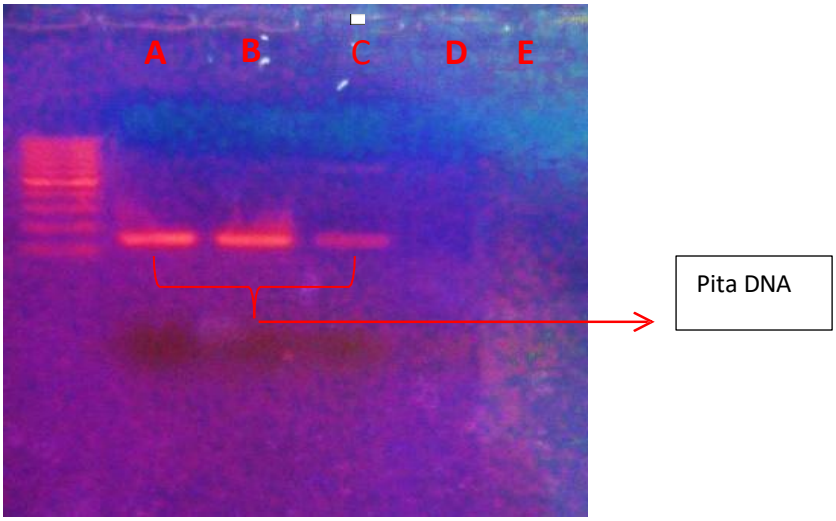


Figure 3. Results of E.coli primer sensitivity test with template DNA. Food samples contaminated with E.coli. DNA leadder 100 bp; concentration A) 122.5 ng/5µ; B) 12.25 ng/5µ; C) 1.225 ng/5µ ; D) 0.1225 ng/5µ; E) 0.01225 ng/5µ

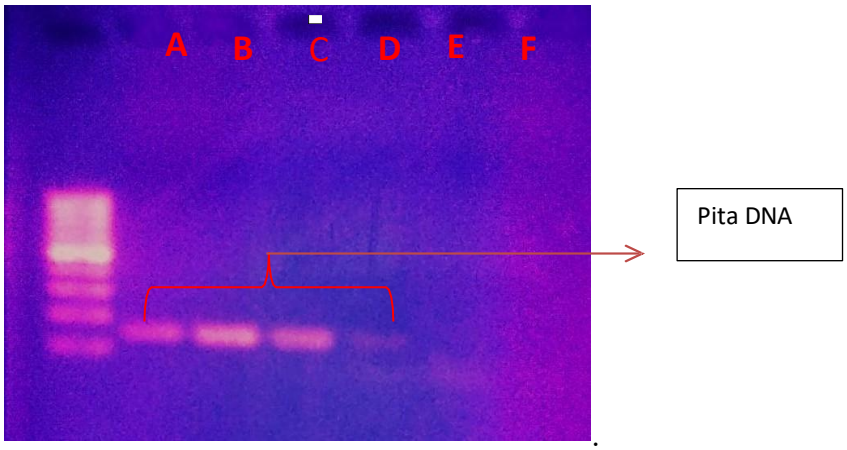


Figure 4. Results of primary sensitivity test for S. typhimurium with DNA template Food samples contaminated with S. thypimurium. DNA leadder 100 bp Concentration ; A) 99 ng/5.5 µl; B) 9.9 ng/5.5 µl; C) 0.99 ng/5.5 µl; D) 0.099 ng/5.5 µl; E) 0.00099 ng/5.5 µl; F) 0.000099 ng/5.5 µl

2. Time Comparison Between Conventional Culture Method and PCR Method

The comparison of time efficiency between the conventional culture method and the Polymerase Chain Reaction (PCR) is fundamentally based on different analytical mechanisms. The culture method relies on a relatively long

incubation time to grow microorganisms until they form macroscopic colonies that can be visually identified. Conversely, the PCR method specifically detects the target pathogen's genetic material (DNA/RNA), thereby bypassing the time-consuming solid culture and biochemical test stages. The detailed comparison of duration and testing stages between the two methods is presented in Table 2 below.

Table 2. Comparison of time and analytical stages between conventional culture method and PCR method

Analytical Stages	Conventional Culture Method	PCR Method	References
Pre-enrichment	18 – 24 hours	8 – 20 hours	ISO 6579-1:2017 / FDA BAM Ch. 5
Selective Enrichment	18 – 24 hours	-	ISO 11290-1:2017
Selective Media Isolation (Agar)	24 – 48 hours	-	FDA BAM Ch. 10 / ISO 21528-2
DNA Extraction & Amplification	-	1,5 – 3 hours	ISO 22174:2005 / AOAC RI PTM
Confirmation TesT	24 – 48 hours	Directly integrated	FDA BAM Ch. 5 / ISO 16140-2
Total Turnaround Time (TAT)	3 – 7 Days (72 – 168 hours)	12 – 24 hours	Mandrell et al. (2012) / Law et al. (2014)

CONCLUSIONS

Based on this study literature, it can be concluded that the DNA of S. typhimurium and E. coli in food samples was successfully amplified using Salmonella typhimurium pef gene primers and Escherichia coli fimC gene primers. Characterization results showed that, in the food samples, the S. typhimurium primers amplified a pef gene fragment yielding an amplicon length of 139 bp, while the E. coli primers amplified a fimC gene fragment yielding an amplicon length of 121 bp. Sensitivity test results indicated that the S. typhimurium* primers were sensitive, capable of amplification down to a food sample DNA template concentration of 0.099 ng/5.5 µL; meanwhile, the E. coli primers were sensitive but only capable of amplification down to a food sample DNA template concentration of 1.225 ng/5 µL. In the culture method, detection can only be observed once bacterial cells have biologically proliferated and formed dense, macroscopic colonies on the surface of the agar mediuma growth process requiring a step-by step incubation period of 3 to 4 days for initial screening, and up to a full week to obtain final biochemical confirmation.

Time acceleration and testing efficiency serve as the primary differentiators when comparing the performance of these two detection methodologies. The PCR method bypasses the lengthy cellular growth process by directly targeting and amplifying the genetic material from food samples *in vitro* within just a few hours, whether derived from intact bacterial cells or lysed cell fragments. Conversely, conventional culture is limited by its capacity to only detect the presence of viable, actively growing bacterial cells. Consequently, the ability of the PCR method to identify specific genetic codes from the *pef* gene ($139\ bp$) and the *fimC* gene ($121\ bp$) at extremely low concentrations not only offers an accurate diagnosis, but also establishes this molecular technique as a far superior, responsive, and adaptive rapid testing instrument for mitigating foodborne pathogen contamination compared to traditional conventional culture methods.

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