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Occurrence of *Escherichia coli* in Fresh and Frozen Frog Legs: Implications for Quality Assurance of Export-Oriented Fishery Products

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Abstract

Background: Frog legs are an important Indonesian export commodity that must meet strict food safety standards. Among microbiological hazards, *Escherichia coli* is widely recognized as an indicator of faecal contamination and poor hygienic practices during processing.
Aim: This study aimed to determine the occurrence of *E. coli* in fresh and frozen frog leg products and evaluate the effectiveness of quality assurance measures applied throughout the production chain.
Methods: The study was conducted at the Fishery Product Quality Control and Inspection Agency (BPPMHKP) Palembang, Indonesia, from April to May 2025. Detection of *E. coli* was performed using the Most Probable Number (MPN) method according to Indonesian National Standard SNI 2332.1:2015. The procedure included enrichment in Lauryl Tryptose Broth (LTB), presumptive testing in EC Broth, and confirmation on Eosin Methylene Blue (EMB) Agar.
Results: Several samples showed positive reactions during the enrichment and presumptive stages, suggesting the presence of coliform bacteria. However, no characteristic metallic-green colonies of *E. coli* were observed on EMB Agar during confirmation. All fresh and frozen frog leg samples had contamination levels below 3 MPN/100 mL. No significant difference in *E. coli* contamination was detected between the two product categories.
Conclusion: Fresh and frozen frog legs complied with microbiological safety requirements and were free from confirmed *E. coli* contamination. The findings indicate that quality assurance systems, including certified raw material sourcing, Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), and cold-chain management, effectively maintained product safety and quality for export purposes.

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INTRODUCTION

Food safety has become a critical issue in international fisheries trade due to increasingly stringent regulations regarding product quality and consumer protection. Exported fishery products must comply with microbiological, chemical, and physical safety requirements to maintain market access and ensure consumer confidence (Bintsis, 2018). Consequently, the implementation of effective quality assurance systems has become an essential component of fishery product processing and export activities.((EFSA), 2024)

Frog legs are among Indonesia's export-oriented fishery commodities and contribute to national foreign exchange earnings. The commodity is marketed to several international destinations where compliance with food safety standards is mandatory. Nevertheless, export rejections associated with microbiological contamination continue to occur and may negatively affect the competitiveness of Indonesian fishery products in global markets (Irawati et al., 2019). Therefore,

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maintaining microbiological quality throughout the production chain is essential to support sustainable export performance.((EFSA), 2024) ; (Vieco-Saiz et al., 2019)

Among foodborne microorganisms, *Escherichia coli* is widely recognized as an indicator of fecal contamination and inadequate hygienic handling. Although most strains are harmless commensal bacteria inhabiting the intestinal tract of humans and animals, pathogenic strains can cause serious illnesses, including diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (Kim et al., 2005 ; Alhadlaq et al., 2024). The presence of *E. coli* in food products may therefore indicate deficiencies in sanitation practices during harvesting, handling, processing, transportation, or storage.(Mia et al., 2025; Plakantara & Karakitsiou, 2025)

Contamination of aquatic food products by *E. coli* may originate from polluted water sources, poor personal hygiene, inadequate sanitation procedures, cross-contamination during processing, or failures in temperature control systems (Bintsis, 2018 ; Click or tap here to enter text.Novoslavskij et al., 2016). To minimize these risks, microbiological monitoring programs are routinely implemented as part of food safety management systems. In Indonesia, the determination of coliform bacteria and *E. coli* in fishery products is standardized through SNI 2332.1:2015 using the Most Probable Number (MPN) method (BSN, 2015).

Previous studies have reported the occurrence of microbiological contamination in various fishery commodities and highlighted the importance of implementing Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), and quality control measures to improve food safety performance (Acthur et al., 2021 ; Sulistiani & Hafiludin, 2022). However, most available studies focus on fish and seafood products, whereas scientific information regarding microbiological quality in frog leg products, particularly those intended for export markets, remains relatively limited. Furthermore, comparative information concerning *E. coli* contamination in fresh and frozen frog legs under industrial processing conditions is still scarce. (Ahmadi et al., 2025; Gonzales et al., 2022)

Considering the economic importance of frog leg exports and the limited availability of microbiological data on this commodity, further investigation is necessary to evaluate product safety and verify the effectiveness of quality assurance systems implemented throughout the production chain. Such information is important for supporting export compliance and strengthening consumer confidence in Indonesian fishery products. (Fauziah et al., 2025; Karyani et al., 2024)

Therefore, this study aimed to determine the occurrence of *Escherichia coli* in fresh and frozen frog leg products analyzed at the Fishery Product Quality Control and Inspection Agency (BPPMHKP) Palembang using the MPN method according to SNI 2332.1:2015. In addition, the study evaluated the role of integrated quality assurance practices, including certified raw material procurement, Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), and cold-chain management, in maintaining microbiological safety in export-oriented frog leg products.

METHOD

Research Design

This study employed a descriptive observational laboratory design to evaluate the occurrence of *Escherichia coli* contamination in fresh and frozen frog leg products intended for export markets. Laboratory analyses were conducted at the Fishery Product Quality Control and Inspection Agency (BPPMHKP) Palembang, South Sumatra, Indonesia, between April and May 2025. The study followed the analytical procedures outlined in the Indonesian National Standard SNI 2332.1:2015 for the detection and enumeration of coliform bacteria and *Escherichia coli* in fishery products (BSN, 2015).

Population and Sampling Method

The study population consisted of frog leg products processed for export purposes. Samples included both fresh frog legs as raw materials and frozen frog legs as finished products.

Four representative samples were selected based on routine quality control submissions received by BPPMHKP Palembang. The sample codes were S/05/05/25/28, S/05/05/25/29, S/05/05/25/30, and S/05/05/25/31. (Jiang et al., 2023; Rejeb et al., 2025)

Participants, Population, and Sampling Method

The study population consisted of frog leg products processed by export-oriented fish processing units operating under the supervision of BPPMHKP Palembang. The analytical samples included both fresh frog legs (raw material stage) and frozen frog legs (final export product stage).
A purposive sampling approach was applied because the samples were selected based on their relevance to export quality monitoring activities. Four laboratory sample codes were examined: S/05/05/25/28, S/05/05/25/29, S/05/05/25/30, and S/05/05/25/31. The selected samples represented products routinely subjected to microbiological quality assessment within the national fishery quality assurance system.

Instruments and Materials

The laboratory equipment used in this study included an autoclave, incubator, water bath, stomacher, analytical balance, sterile Petri dishes, sterile test tubes, measuring cylinders, forceps, scissors, personal protective equipment, and standard microbiological laboratory glassware.
The microbiological media consisted of Lauryl Tryptose Broth (LTB) for enrichment, EC Broth for confirmation of fecal coliform bacteria, and Eosin Methylene Blue (EMB) Agar for final verification of *E. coli*. Sterile dilution solution and distilled water were used throughout the analytical procedures.
The analytical method employed was the Most Probable Number (MPN) technique, which is widely recognized for detecting low concentrations of coliform bacteria and *E. coli* in food products (Wardhana et al., 2021)

Procedures

Sample Preparation

Twenty-five grams of each frog leg sample were aseptically weighed and transferred into sterile stomacher bags containing 225 mL of sterile diluent. The mixture was homogenized for approximately two minutes using a stomacher to obtain a representative suspension. Serial ten-fold dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were subsequently prepared.

Enrichment Test

Aliquots from each dilution were inoculated into Lauryl Tryptose Broth tubes containing Durham tubes. The inoculated media were incubated at 35°C for 48 h. Gas formation and turbidity were interpreted as presumptive evidence of coliform growth (BSN, 2015).

Confirmation Test

Cultures showing positive reactions in LTB were transferred to EC Broth and incubated at 45. 5°C for 48 h using a water bath. Gas production within Durham tubes was considered indicative of fecal coliform bacteria and potential *E. coli* contamination.

Final Verification Test

Positive EC Broth cultures were streaked onto EMB Agar plates and incubated at 35°C for 24–48 h. Colonies exhibiting a metallic green sheen were identified as presumptive *E. coli*, whereas colonies lacking these characteristics were classified as non-*E. coli* microorganisms (Cappuccino & Sherman, 2008 ; BSN, 2015).

Time Frame

All laboratory analyses, data collection, and interpretation activities were conducted during the April until May 2025 observation period.

Analysis Plan

The data were analyzed descriptively. Results from enrichment, confirmation, and verification stages were interpreted based on gas production, turbidity, colony morphology, and MPN values.

Comparisons between fresh and frozen frog leg samples were performed qualitatively to determine whether differences existed in *E. coli* contamination levels. The laboratory findings were subsequently interpreted in relation to quality assurance practices implemented by fish processing

units, including Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), supplier certification under Good Fish Handling Practices (CPIB), and cold-chain management systems (Acthur et al., 2021 ; Valeria Siregar et al., 2021).

Scope and Limitations

This study focused exclusively on the detection of *Escherichia coli* contamination in selected fresh and frozen frog leg samples submitted to BPPMHKP Palembang. The investigation did not include molecular confirmation techniques, such as polymerase chain reaction (PCR), nor did it evaluate other foodborne pathogens, including *Salmonella spp.*, *Listeria monocytogenes*, or *Vibrio* species.

Furthermore, the limited number of samples analyzed restricts the generalization of the findings to all frog leg products produced in Indonesia. Nevertheless, the study provides valuable information regarding the effectiveness of microbiological quality control measures applied within export-oriented processing systems.

RESULTS AND DISCUSSION

Results

Enrichment Test (Lauryl Tryptose Broth)

The enrichment stage was performed using Lauryl Tryptose Broth (LTB) to detect presumptive coliform bacteria. Gas production in Durham tubes together with medium turbidity was considered a positive reaction indicating the possible presence of coliform microorganisms.

Table 1. Results of the enrichment test using Lauryl Tryptose Broth (LTB).

Sample code	10^{-1}	10^{-2}	10^{-3}	Interpretation
A1	+ – –	– – –	– – –	Positive
A2	+ – –	– – –	– – –	Positive
A3	– – –	– – –	– – –	Negative
A4	– – –	– – –	– – –	Negative
A5	– – –	– – –	– – –	Negative
B	+ + +	– – –	– – –	Positive
C1	+ + +	– – –	– – –	Positive
C2	+ – –	– – –	– – –	Positive
C3	– – –	– – –	– – –	Negative
C4	– – –	– – –	– – –	Negative
C5	– – –	– – –	– – –	Negative
D	– – –	– – –	– – –	Negative

Positive reactions were observed in samples A1, A2, B, C1, and C2, primarily at the first dilution (10^{-1}). Gas formation and turbidity indicated the presence of presumptive coliform bacteria. Samples showing positive reactions were subsequently subjected to confirmation testing using EC Broth in accordance with SNI 2332.1:2015 (BSN, 2015). Similar enrichment procedures have been widely adopted for detecting coliform bacteria in aquatic food products because of their high sensitivity during the preliminary screening stage (Cappuccino & Sherman, 2008 ; Fardiaz, 1993).

Confirmation Test (EC Broth)

Positive LTB cultures were transferred to EC Broth and incubated at 45.5°C to selectively detect thermotolerant coliforms. The calculated contamination level for both positive EC Broth samples was <3 MPN/100 mL, indicating that *E. coli* was not detected at a significant level according to SNI 2332.1:2015 (BSN, 2015).

Table 2. Results of the confirmation test using EC Broth.

Sample code	10^{-1}	10^{-2}	10^{-3}	Interpretation
A1	– – –	– – –	– – –	Negative
A2	– – –	– – –	– – –	Negative
B	+ + +	– – –	– – –	Positive
C1	+ + +	– – –	– – –	Positive
C2	– – –	– – –	– – –	Negative

Only samples B and C1 remained positive after incubation in EC Broth, while samples A1, A2, and C2 produced no gas and were considered negative. The positive EC Broth reactions suggested the possible presence of thermotolerant coliform bacteria. However, confirmation using selective agar media remained necessary because EC Broth may also permit the growth of several non-Escherichia coli coliform species (Ray & Bhunia, 2013 ; Wardhana et al., 2021).

Final Confirmation on EMB Agar

Samples producing positive EC Broth reactions were streaked onto EMB Agar to verify the presence of Escherichia coli.

Table 3. Final confirmation of Escherichia coli on EMB Agar.

Sample code	EMB colony characteristics	Final MPN
B	No characteristic metallic-green colonies	<3 MPN/100 mL
C1	No characteristic metallic-green colonies	<3 MPN/100 mL

No colonies exhibiting the characteristic metallic green sheen typical of E. coli were observed on EMB Agar. Consequently, all tested frog leg samples were interpreted as negative for confirmed E. coli contamination, with bacterial levels below 3 MPN/100 mL, satisfying the microbiological requirements specified in SNI 2332.1:2015 (BSN, 2015).

The positive reactions observed during enrichment and EC Broth testing were therefore attributed to other coliform bacteria rather than E. coli. This finding agrees with previous reports indicating that presumptive and confirmation media may detect non-E. coli thermotolerant coliforms, making selective plating on EMB Agar an essential step for accurate identification (Cappuccino & Sherman, 2008 ; Madigan et al., 2019).

Discussion

The absence of confirmed *Escherichia coli* contamination in both fresh and frozen frog leg products indicates that the processing and handling systems applied by the fish processing units were effective in controlling microbiological hazards. Although several samples showed positive reactions during enrichment and presumptive testing, the confirmation stage demonstrated that these microorganisms were not *E. coli*. Similar observations have been reported in microbiological food safety studies where non-*E. coli* coliform bacteria may produce positive reactions in selective enrichment media but fail to exhibit typical colony morphology on EMB Agar (Cappuccino & Sherman, 2008 ; Madigan et al., 2019).

The findings are consistent with previous reports indicating that microbiological contamination in fishery products can be effectively minimized through appropriate sanitation practices and quality assurance systems (Bintsis, 2018 ; Novoslavskij et al., 2016). The absence of *E. coli* is particularly important because this microorganism is widely recognized as an indicator of fecal contamination and inadequate hygienic handling (Kim et al., 2005 ; Alhadlaq et al., 2024).

The results also demonstrate the effectiveness of integrated preventive control measures implemented throughout the supply chain. Certified suppliers operating under Good Fish Handling Practices (CPIB) contribute to the procurement of raw materials with reduced contamination risk. Furthermore, the application of Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP) provides systematic control of sanitation, personnel hygiene, equipment cleanliness, and production environments (Acthur et al., 2021).

Cold-chain management may have further contributed to the low microbial load observed in this study. Maintaining low temperatures during transportation, storage, and processing is essential for suppressing bacterial growth and preserving product quality (Valeria Siregar et al., 2021). The absence of detectable *E. coli* contamination in frozen products suggests that temperature control measures were effectively maintained throughout processing and distribution.
These findings support previous observations by Sulistiani & Hafiludin (2022), who reported that microbiological quality assurance plays a critical role in preventing export rejection of fishery products. Given that international markets increasingly emphasize food safety compliance, continuous monitoring remains essential to maintain competitiveness and consumer confidence.

Implications
The findings have practical implications for export-oriented fishery industries. The study demonstrates that comprehensive implementation of supplier certification, GMP, SSOP, and cold-chain management can effectively prevent microbiological contamination in frog leg products. Maintaining these systems is essential to comply with international food safety regulations and reduce the risk of export rejection.
Furthermore, the results support the adoption of preventive food safety management rather than relying solely on end-product testing. Strengthening control measures throughout the production chain may improve product consistency and enhance market acceptance.

Research Contribution
This study contributes to the limited scientific information regarding the microbiological quality of export-oriented frog leg products in Indonesia. The research provides empirical evidence that fresh and frozen frog legs processed under controlled conditions can meet microbiological safety requirements for *Escherichia coli*.
Additionally, the study proposes an integrated quality assurance framework combining CPIB-certified suppliers, GMP implementation, SSOP application, and cold-chain management as a practical approach for strengthening food safety systems in amphibian-derived export commodities.

Limitations
Several limitations should be acknowledged. First, the study was conducted using a limited number of samples obtained during a specific observation period. Second, the investigation focused exclusively on *Escherichia coli* and did not evaluate other important foodborne pathogens such as *Salmonella spp.*, *Listeria monocytogenes*, or *Vibrio* species. Third, the study relied on conventional culture-based methods and did not employ molecular confirmation techniques such as Polymerase Chain Reaction (PCR), which may provide higher analytical sensitivity (Zhao et al., 2014).

Suggestions
Future studies should include larger sample sizes and broader sampling periods to improve the representativeness of microbiological assessments. The incorporation of molecular detection methods, including PCR and quantitative PCR, is also recommended to enhance pathogen identification accuracy and reduce analytical turnaround time (Zhao et al., 2014).
In addition, further research should investigate other microbiological hazards relevant to export products, including *Salmonella spp.*, *Listeria monocytogenes*, and pathogenic strains of *E. coli*. The development of digital food safety monitoring systems utilizing Internet of Things (IoT)-based technologies may also provide valuable opportunities for strengthening real-time quality assurance in export-oriented fishery processing facilities.

CONCLUSION

This study successfully evaluated the occurrence of *Escherichia coli* in fresh and frozen frog leg products processed for export and assessed the effectiveness of quality assurance practices implemented throughout the production chain. Microbiological analysis conducted in accordance with SNI 2332.1:2015 demonstrated that all tested samples contained *E. coli* levels below 3 MPN/100

mL, indicating compliance with microbiological safety requirements for export-oriented fishery products.

No significant difference was observed between fresh and frozen frog leg products with respect to *E. coli* contamination. Although several samples showed positive reactions during the enrichment and presumptive stages, the absence of characteristic metallic-green colonies on EMB Agar confirmed that *E. coli* was not present in the analyzed samples. These findings indicate that the tested products were microbiologically safe and suitable for international market requirements.

The results further demonstrate that integrated quality assurance measures, including the use of CPIB-certified suppliers, implementation of Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), and continuous cold-chain management, were effective in minimizing microbiological contamination risks. Therefore, strengthening preventive food safety systems remains essential for maintaining the competitiveness and sustainability of Indonesian frog leg exports.

Future studies should incorporate larger sample sizes, longer monitoring periods, and molecular detection techniques such as Polymerase Chain Reaction (PCR) to improve pathogen detection sensitivity. In addition, the integration of digital monitoring technologies, including IoT-based traceability systems and real-time sanitation control, may further enhance food safety management and support the development of more resilient export-oriented fishery supply chains.

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AUTHOR CONTRIBUTION STATEMENT

MS conducted the laboratory work, collected and analyzed the data, and prepared the initial manuscript draft. MRS and WP contributed to data interpretation, literature review, manuscript preparation, and critical revision of the scientific content. RLNF conceived and supervised the study, designed the research methodology, validated the findings, critically revised the manuscript, and served as the corresponding author. IK contributed to manuscript editing, scientific writing, language refinement, and critical review of the final version. All authors read and approved the final manuscript.

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