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Microbiological Assessment of *Escherichia coli* Contamination in Vannamei Shrimp (*Litopenaeus vannamei*)

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Abstract

Whiteleg shrimp (*Litopenaeus vannamei*) is a globally important aquaculture commodity, including in Indonesia, due to its fast growth and salinity tolerance. Microbiological safety is crucial to protect consumer health and meet food quality standards. One key indicator in microbiological testing is the presence of *Escherichia coli* (*E. coli*), which reflects fecal contamination from poor water quality or unhygienic post-harvest handling. This study aimed to detect and characterize *E. coli* contamination in *L. vannamei* from two different pond systems with contrasting environmental conditions. Sample Q was collected from a pond relying on rainwater near a goat pen, posing a higher risk of organic contamination. Sample R originated from a pond with a controlled single water gate system and more stable water quality. Microbiological analysis was performed using the Most Probable Number (MPN) method with Lauryl Sulfate Broth (LSB) and EC broth for presumptive tests, L-EMB agar for confirmation, Gram staining, and biochemical IMViC tests (Indole, MR, VP, Citrate). Results showed higher coliform and *E. coli* contamination in sample Q, detectable up to 10^{-3} dilution, while sample R was positive only at 10^{-1} to 10^{-2} dilutions. All isolates exhibited typical *E. coli* phenotypic and biochemical characteristics, including Gram-negative short rods and IMViC pattern Indole (+), MR (+), VP (–), Citrate (–). These findings highlight the importance of water quality management and hygienic handling to control microbiological contamination in *L. vannamei*. Routine testing and strict monitoring are essential to ensure food safety and enhance the competitiveness of Indonesian aquaculture products in global.

1. Introduction

Vannamei shrimp (*Litopenaeus vannamei*) is one of the most widely cultivated shrimp species in the world, valued for its rapid growth, adaptability to various salinity levels, and high market demand (Liao and Chien, 2011; Zhang *et al.*, 2024; Elhetawy *et al.*, 2025). In Indonesia, this species contributes significantly to the national aquaculture industry, playing a key role in meeting export and domestic consumption targets (Henriksson *et al.*, 2017; Tran *et al.*, 2017; Oktopura *et al.*, 2020). However, maintaining the microbiological safety of vannamei shrimp is essential to protect consumer health and ensure compliance with both national and international food safety standards (Brito *et al.*, 2016; Knipe *et al.*, 2024).

One of the primary indicators used in the microbiological assessment of aquatic products is *Escherichia coli* (*E. coli*) (Rodrigues and Cunha, 2017; Wen *et al.*, 2020; Nowicki *et al.*, 2021). The presence of *E. coli* is generally associated with fecal contamination, which may result from poor water quality, inadequate hygiene during harvesting, or improper handling in post-harvest processes (Yohans *et al.*, 2022; Nurbaya *et al.*, 2023; Ayalew *et al.*, 2024). High levels of *E. coli* in shrimp not only pose serious risks to public health but also compromise the acceptance of these products in international markets, where regulatory standards for microbial limits are strictly enforced (Mohamed Hatha *et al.*, 2003; Alhabib and Elhadi, 2024; Onyeaka *et al.*, 2024).

Routine testing for *E. coli* serves as an essential component of quality control in aquaculture production. It allows producers, processors, and regulatory bodies to monitor potential contamination sources, implement corrective actions, and verify compliance with microbiological criteria. The accuracy and reliability of testing methods, as well as the interpretation of results, are critical for ensuring shrimp safety and protecting the reputation of aquaculture products (Walker *et al.*, 2018; Ulkhaq *et al.*, 2021; Dias *et al.*, 2024).

This study aims to analyze the testing of *E. coli* in vannamei shrimp, focusing on the procedures employed, the prevalence and concentration of contamination detected, and the implications for food safety management. The findings are expected to highlight the importance of microbiological surveillance and provide insights that support the implementation of better aquaculture practices, ultimately contributing to safer, higher-quality shrimp production.

2. Material and methods

2.1 Laboratory facility, sampling site, and sample collection

All laboratory analyses were conducted at a certified fishery product quality testing facility located in West Java, Indonesia. The laboratory is accredited for microbiological testing and complies with national standards for food safety and quality assurance. It is equipped with the necessary instruments and infrastructure to ensure accurate and reliable detection of microbial contaminants in aquatic food products.

Shrimp samples (*Litopenaeus vannamei*) were collected from two shrimp ponds with different water management systems. Sample Q was collected from a pond that primarily depends on rainwater as its main water source. This pond is situated adjacent to a goat pen, which poses a potential risk of organic contamination during rainfall, as runoff from the livestock area can enter the pond. Due to the reliance on rainwater, the water quality in pond Q is highly variable and unstable throughout the year, potentially affecting shrimp health and growth.

In contrast, sample R was obtained from a pond managed with a more controlled water system, equipped with a single sluice gate. This system allows for regulated water inflow and outflow, facilitating regular exchange of pond water with seawater of better quality suitable for shrimp cultivation. The sluice gate also minimizes flooding risks and prevents the entry of pests or contaminants. Consequently, pond R maintains a more stable water quality, providing a more optimal environment for shrimp growth. All samples were collected within the same season to reduce environmental variability. Shrimp were transported to the laboratory under cooled conditions for immediate processing.

2.2 Materials and Equipment

The materials used in this study included sterile plastic bags, test tubes, Durham tubes, Petri dishes, inoculating loops, and screw-cap bottles. The reagents and media used comprised Butterfield's Phosphate Buffer (BFP), Lauryl Sulfate Broth (LSB), Brilliant Green Lactose Bile (BGLB) broth, EC broth, Levine's Eosin Methylene Blue (L-EMB) agar, Plate Count Agar (PCA), tryptone broth, MR-VP broth, Simmons Citrate Agar (SCA), Kovacs reagent, methyl red indicator, and Voges-Proskauer (VP) reagents. Equipment included a Seward stomacher, vortex mixer, digital balance, incubator (35 ± 1°C), circulating water bath (45.5 ± 0.2°C), laminar airflow cabinet, light microscope, and micropipettes.

2.3 Sample Preparation

A 25 g portion of homogenized shrimp was aseptically transferred into a sterile plastic bag containing 225 mL of Butterfield's Phosphate Buffer, yielding a 1:10 dilution. The mixture was homogenized using a stomacher for 1 minute to obtain the 10^{-1} dilution, from which serial dilutions were prepared for further analysis.

2.4 Microbiological Analysis

To assess the presence of coliforms, 1 mL aliquots of appropriate serial dilutions were inoculated into tubes containing 9 mL of LSB with Durham tubes, then incubated at 35 ± 1°C for 48 ± 2 hours. Gas production indicated a presumptive positive result for coliforms. For *Escherichia coli*, positive LSB tubes were further inoculated into EC broth tubes containing Durham tubes and incubated in a water bath at 45.5 ± 0.2°C for 48 ± 2 hours. The presence of gas confirmed the presumptive presence of *E. coli*, and the most probable number (MPN) was determined using standard MPN tables. Confirmed identification of *E. coli* was conducted by streaking EC broth cultures onto L-EMB agar and incubating at 35 ± 1°C for 24 ± 2 hours. Colonies with a metallic green sheen were considered presumptive *E. coli* and subcultured on PCA slants for further testing (Kornacki *et al.*, 2015; Hossain *et al.*, 2021).

2.5 Morphological Characterization

Gram staining was performed using standard procedures. Colonies from PCA slants were smeared onto glass slides, heat-fixed, and stained sequentially with crystal violet, iodine, ethanol, and safranin. After air-drying, the preparations were examined under a light microscope. Gram-negative short rods were considered characteristic of *E. coli* (Moyes *et al.*, 2009; Paray *et al.*, 2023).

2.6 Biochemical Confirmation

Indole production was tested by inoculating PCA-grown colonies into tryptone broth, incubated at 35 ± 0.5°C for 24 ± 2 hours, followed by the addition of Kovacs reagent. The formation of a red ring indicated a positive reaction. The Voges-Proskauer (VP) test was performed by inoculating MR-VP broth, incubating for 48 ± 2 hours, and adding alpha-naphthol and potassium hydroxide to the culture; a red coloration after shaking indicated a positive result. The methyl red test was carried out by adding methyl red indicator to MR-VP broth; red color indicated a positive result, while yellow indicated negative. For citrate utilization, isolates were streaked on Simmons Citrate Agar and incubated for up to 96 hours; a color change from green to blue indicated positive utilization. Gas production from lactose was confirmed by inoculating LSB tubes with Durham tubes and observing gas formation after 48 ± 2 hours at 35 ± 0.5°C (BSN, 2015).

2.7 Biotyping and Enumeration of *Escherichia coli*

The identification and biotyping of Escherichia coli were performed based on biochemical characteristics following the protocol outlined by the National Standardization Agency of Indonesia (BSN, 2015). Gas production in Lauryl Tryptose Broth (LTB) and indole production were initially assessed. Further differentiation between biotype 1 and biotype 2 was determined based on the Methyl Red (MR) test, Voges-Proskauer (VP) test, and citrate utilization test. Morphological examination was also conducted through Gram staining to confirm that the isolates were Gram-negative, short rod-shaped, and non-spore-forming bacteria.

The Most Probable Number (MPN) of *E. coli* was determined by using a three-tube serial dilution method. The number of positive EC tubes in three consecutive dilutions was recorded to calculate the MPN value. Results were expressed as MPN/g for non-shellfish fishery products and as MPN/100 g for shellfish samples, following international microbiological standards.

Table 1. Biochemical and Morphological Characteristics of Two *E. coli* Biotypes

Biochemical Test	**Biotype 1**	**Biotype 2**
Gas in LTB	+	+
Indole	+	+
Methyl Red (MR)	+	–
Voges-Proskauer (VP)	–	+
Citrate	–	+
Morphology	Gram-negative, short rod, non-sporulated	Gram-negative, short rod, non-sporulated

3. Results

3.1 Detection of *Escherichia coli* in Shrimp Samples

The presence of *Escherichia coli* in *Litopenaeus vannamei* shrimp samples was examined through a series of microbiological and biochemical tests, including presumptive coliform and *E. coli* tests, confirmation on selective media, Gram staining, and IMViC biochemical assays. These sequential steps were performed to ensure accurate identification of *E. coli* contamination in shrimp muscle.

3.1.1 Presumptive Test for Coliforms

In the presumptive coliform test, homogenized shrimp samples were serially diluted and inoculated into Lauryl Sulfate Broth (LSB), then incubated at 35 °C for 48 h. The presence of turbidity and gas production in Durham tubes indicated coliform activity (Figure 1). Both Vaname Q and Vaname R samples showed positive results at 10^{-1} and 10^{-2} dilutions, confirming the initial presence of coliform bacteria (Table 2).

Table 2. Presumptive Coliform Detection in Vannamei Shrimp Samples at Different Dilution Levels

Sample Code	Dilution	Coliform Groups		
		A	B	C
Vaname Q	10^{-1}	+	+	+
	10^{-2}	+	+	+
	10^{-3}	+	+	-
Vaname R	10^{-1}	+	+	+
	10^{-2}	+	+	-
	10^{-3}	-	-	-

Base on Table 2, Presumptive coliform tests indicated positive (+) gas formation in Lauryl Sulfate Broth (LSB) tubes, suggestive of coliform presence in both Vannamei shrimp samples. Sample Vaname Q exhibited consistent positive reactions across all three groups (A, B, C) at 10^{-1} and 10^{-2} dilutions, with only one group showing negative at 10^{-3}. Meanwhile, sample Vaname R showed a similar trend at higher concentrations (10^{-1} and 10^{-2}), but all groups tested negative at 10^{-3} dilution, indicating a lower coliform concentration relative to sample Q. These results suggest a higher presumptive coliform load in Vaname Q compared to Vaname R.

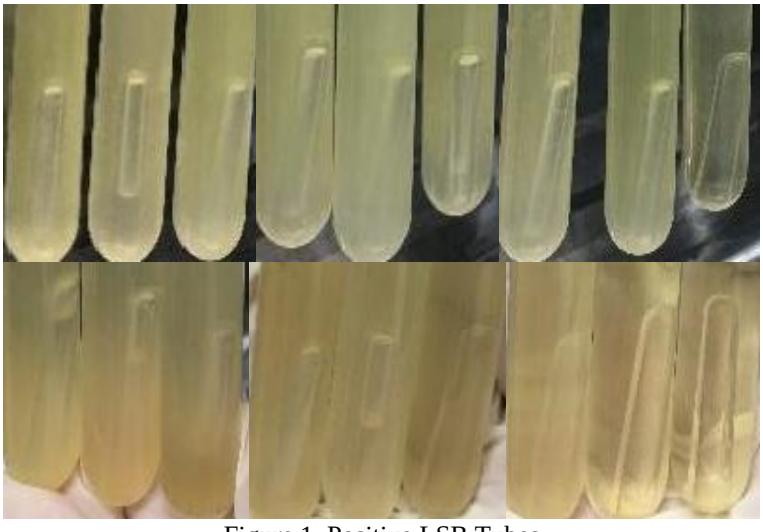

Figure 1. Positive LSB Tubes

3.1.2 Presumptive Test for *E. coli*

Samples positive in LSB were further subjected to the presumptive *E. coli* test using EC Broth, incubated at 45.5°C for 48 hours. Gas formation and discoloration in the EC Broth confirmed the presence of thermotolerant *E. coli* (Figure 2). Both shrimp samples showed positive results up to 10^{-2} dilution, with Vaname Q also positive at 10^{-3} dilution (Table 3).

Base on Table 3, The presumptive coliform test results varied across dilution levels and sample codes. Sample Vaname Q showed consistent coliform presence (+) across all groups at 10^{-1} and 10^{-2} dilutions, and partially at 10^{-3} dilution (Groups A and B positive; Group C not detected). This indicates a relatively high coliform load in the sample. In contrast, Vaname R showed a more limited presence of coliforms. Only Group A was positive at 10^{-1} and 10^{-2}, while Groups B and C were negative or not detected. At the 10^{-3} dilution, no coliforms were detected in any group, suggesting a lower coliform concentration in this sample compared to Vaname Q. These findings suggest that

Vaname Q had a higher level of coliform contamination, with persistence even at higher dilutions, whereas Vaname R exhibited coliform presence only at the lowest dilution.

Table 3. Presumptive *E. coli* Detection in Vannamei Shrimp Samples at Serial Dilutions

Sample Code	Dilution	Coliform Groups		
		A	B	C
Vaname Q	10^{-1}	+	+	+
	10^{-2}	+	+	+
	10^{-3}	+	+	ND[1]
Vaname R	10^{-1}	+	-	-
	10^{-2}	+	-	ND[1]
	10^{-3}	ND[1]	ND[1]	ND[1]

[1]Not Determined – negative in presumptive test

Figure 2. Positive EC Broth Tubes

3.1.3 *E. coli* Confirmatory Test

For confirmation, EC Broth cultures were streaked onto Eosin Methylene Blue (EMB) agar and incubated at 35°C for 24 hours. The appearance of colonies with metallic green sheen was indicative of *E. coli* (Figure 3). Vaname Q showed positive colonies at 10^{-3} dilution, whereas Vaname R was positive up to 10^{-2} dilution (Table 4).

Table 4. Detection of *E. coli* in Vannamei Shrimp Samples at Serial Dilutions

Sample Code	Dilution	*E. coli* Groups		
		A	B	C
Vaname Q	10^{-1}	-	-	-
	10^{-2}	-	-	-
	10^{-3}	+	+	ND[1]
Vaname R	10^{-1}	+	ND[1]	ND[1]
	10^{-2}	+	ND[1]	ND[1]
	10^{-3}	ND[1]	ND[1]	ND[1]

[1]Not Determined – negative in presumptive test

Base on Table 4, The detection of *Escherichia coli* in vannamei shrimp samples revealed differing contamination profiles between the two samples. For Vaname Q, no *E. coli* was detected at the higher concentrations (10^{-1} and 10^{-2} dilutions) across all groups. However, at the 10^{-3} dilution, *E. coli* was present in Groups A and B, while Group C showed no detection, indicating low levels of *E. coli* contamination only at higher dilution. In contrast, Vaname R demonstrated *E. coli* presence in Group A at the 10^{-1} and 10^{-2} dilutions but was not detected in Groups B and C at any dilution. No *E. coli* was detected at the 10^{-3} dilution for any group in Vaname R. These results suggest that *E. coli* contamination was detectable in specific groups and dilutions, with Vaname R showing *E. coli* presence at higher concentrations only in Group A, and Vaname Q showing low-level contamination at a higher dilution.

Figure 3. Positive L-EMB Agar Colonies

3.1.4 Gram staining

Isolated colonies from EMB agar were further purified on Plate Count Agar (PCA) slants and incubated at 35°C for 24 hours. Gram staining revealed short, pink rods characteristic of Gram-negative bacteria, supporting the identification of *E. coli* (Figure 4). The detection of *E. coli* in vannamei shrimp samples across different dilutions is presented in Table 5.

Table 5. Detection of *E. coli* in Vannamei Shrimp Samples

Sample Code	Dilution	*E. coli* Groups		
		A	B	C
Vaname Q	10^{-1}	ND[1]	ND[1]	ND[1]
	10^{-2}	ND[1]	ND[1]	ND[1]
	10^{-3}	-	-	ND[1]
Vaname R	10^{-1}	-	ND[1]	ND[1]
	10^{-2}	-	ND[1]	ND[1]
	10^{-3}	-	-	-

[1]Not Determined – negative in presumptive test

The analysis for *Escherichia coli* presence in vannamei shrimp samples indicated no detection (ND) in most groups at higher dilutions (10^{-1} and 10^{-2}) for both samples Vaname Q and Vaname R, suggesting a negative result in the presumptive test. For Vaname Q, *E. coli* was not detected in Groups A and B at the lowest dilution (10^{-3}), while Group C was consistently negative or not determined at all dilutions. Vaname R showed negative results across all groups at the 10^{-3} dilution and no *E. coli* detection in Groups B and C at higher dilutions. Only Group A showed negative results at higher dilutions, confirming absence of *E. coli*. Overall, the results suggest that *E. coli* contamination was absent or below detectable levels in the tested vannamei shrimp samples.

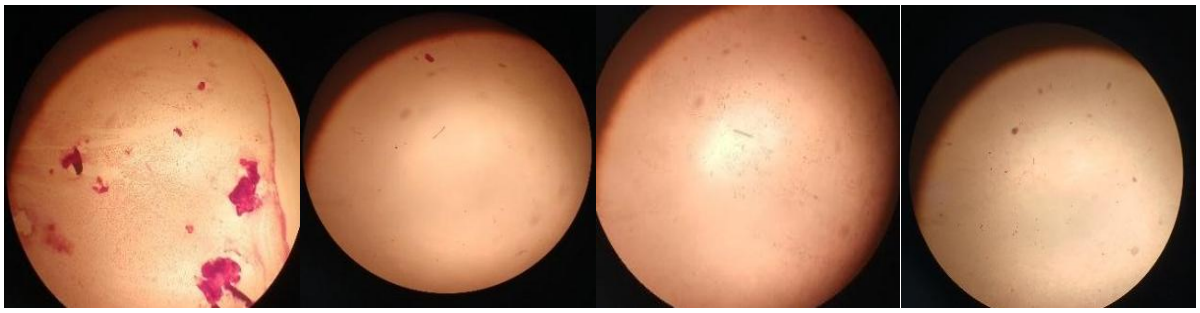

Figure 4. Gram Staining Result

3.1.5 Biochemical Tests

Biochemical confirmation was conducted using the IMViC test series (Figure 5). The Indole test yielded a positive result, indicated by the formation of a red ring after the addition of Kovac's reagent (Figure 5a). The Methyl Red (MR) test also showed a positive result, confirmed by a red color change, while the Voges–Proskauer (VP) test was negative, as no color change occurred following the addition of reagents (Figure 5b). The Citrate test was negative, with the medium remaining green, consistent with the inability of *E. coli* to utilize citrate as the sole carbon source (Figure 5c). In addition, gas production in LSB medium confirmed lactose fermentation activity, which is characteristic of *E. coli* (Figure 5d). The biochemical test results, including IMViC profiles and lactose fermentation for *E. coli* isolates from vannamei shrimp samples, are summarized in Table 6.

Table 6. Biochemical Test Results (IMViC and Lactose Fermentation) of *E. coli* Isolates from Vannamei Shrimp Samples

Sample Code	Dilution	IMVC.A				IMVC.B				IMVC.C				LSB		
		I	MR	VP	C	I	MR	VP	C	I	MR	VP	C	A	B	C
Vaname Q	10^{-1}	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	10^{-2}	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	10^{-3}	+	+	-	-	+	+	-	+	ND[1]	ND[1]	ND[1]	ND[1]	+	+	
Vaname R	10^{-1}	+	+	-	+	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	+	ND[1]	ND[1]
	10^{-2}	+	+	-	-	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	+	ND[1]	ND[1]
	10^{-3}	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]

[1]Not Determined – negative in presumptive test

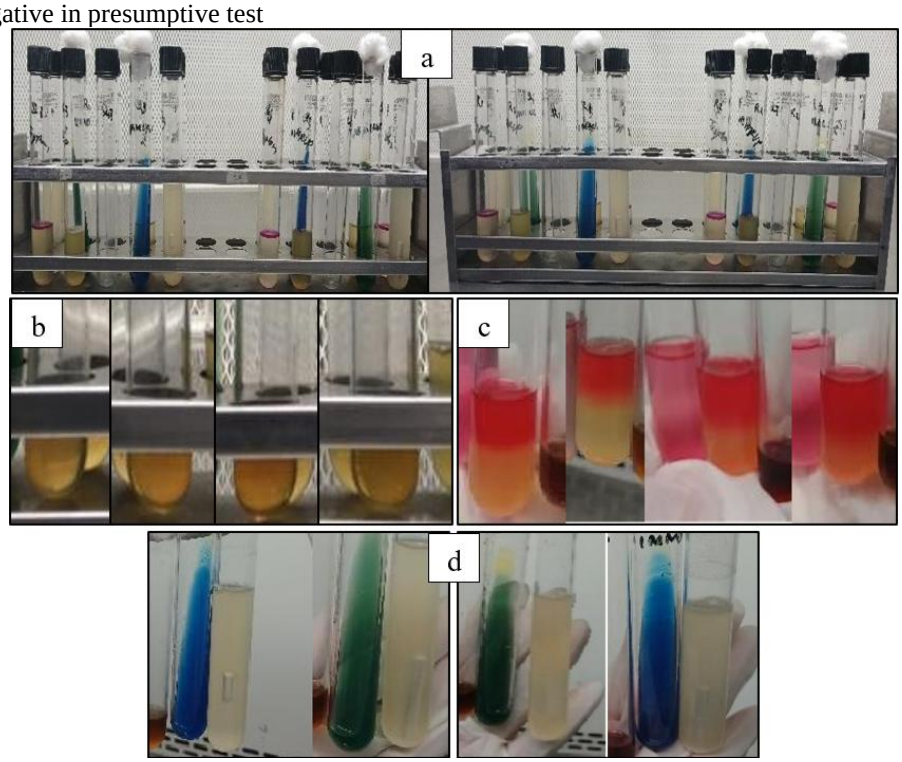

Figure 5. Biochemical confirmation using IMViC tests: (a) Indole test, (b) Voges–Proskauer test, (c) Methyl Red test, (d) Citrate utilization and gas production from lactose.

Table 6 presents the biochemical characterization results of presumptive *Escherichia coli* isolates obtained from two vannamei shrimp samples (Vaname Q and Vaname R), tested across three serial dilutions (10^{-1} to 10^{-3}). The isolates were assessed using the IMViC test series (Indole, Methyl Red (MR), Voges–Proskauer (VP), and Citrate) as well as the Lactose Broth (LSB) test for lactose fermentation. A typical *E. coli* biochemical profile is indicated by positive results for Indole and Methyl Red, and negative results for Voges–Proskauer and Citrate. Gas formation in LSB further supports the identification of *E. coli* through evidence of lactose fermentation. Results marked as ND[1] refer to tests that were not determined due to negative outcomes in the initial presumptive screening.

3.2 Summary of *E. coli* Detection in Shrimp

The detection outcomes for each dilution of shrimp samples are summarized in Table 7.

Table 7. Detection of *E. coli* and Estimated MPN per Gram in Vannamei Shrimp Samples

Sample Code	Dilution (tubes positive)			Positive Tube Pattern	Estimated MPN (MPN/g)
	10^{-1}	10^{-2}	10^{-3}		
Vaname Q	3/3	3/3	2/3	3-3-2	93
Vaname R	2/3	2/3	0/3	2-2-0	6.1

Sample Vaname Q exhibited the highest *Escherichia coli* contamination, with three positive tubes observed at both the 10^{-1} and 10^{-2} dilutions, and two positive tubes at the 10^{-3} dilution. This pattern corresponds to an estimated Most Probable Number (MPN) of 93 per gram, indicating a relatively high presence of *E. coli*. In contrast, Vaname R showed a lower contamination level, with only two positive tubes at both the 10^{-1} and 10^{-2} dilutions, and none at 10^{-3}, resulting in an MPN of 6.1 per gram, which suggests moderate contamination. These findings point to potential microbial quality concerns, particularly in the Vaname Q sample, and emphasize the critical importance of proper hygiene and handling practices during shrimp processing to ensure food safety.

4. Discussion

The present study demonstrates the presence and quantification of *Escherichia coli* contamination in *Litopenaeus vannamei* samples obtained from two different sources, Vaname Q and Vaname R. The application of the Most Probable Number (MPN) method combined with selective culture on Eosin Methylene Blue (EMB) agar, Gram staining, and IMViC biochemical tests allowed for a reliable confirmation and estimation of *E. coli* levels in both samples.

Initial screening using Lauryl Sulfate Broth (LSB) indicated that both shrimp samples harbored coliform bacteria, with Vaname Q showing higher positivity and growth at greater dilutions than Vaname R. This suggests exposure to fecal contamination, as coliforms are widely recognized as indicators of such contamination in aquatic environments (McCrady, 1943; Holcomb and Stewart, 2020; Some *et al.*, 2021). The subsequent confirmation of thermotolerant coliforms, particularly *E. coli*, using EC Broth at 45.5°C further substantiates the presence of fecal pollution. The higher MPN values observed in Vaname Q (approximately 93 MPN/g) compared to Vaname R (6.1 MPN/g) indicate a significantly greater bacterial load in Vaname Q. This quantitative difference is crucial as it reflects varying hygienic conditions related to the shrimp's source and handling.

The selective growth of characteristic metallic green colonies on EMB agar reinforced the identification of *E. coli*, with Vaname Q showing colonies at the 10^{-3} dilution, while Vaname R showed growth only up to the 10^{-2} dilution. These findings corroborate the MPN data and align with accepted thresholds for *E. coli* contamination in seafood. According to international food safety guidelines, *E. coli* counts exceeding recommended limits signify a potential health hazard (Roberts *et al.*, 2005; Hazards *et al.*, 2017). Gram staining revealed Gram-negative rods consistent with *E. coli* morphology, and the IMViC biochemical profile (positive for Indole and Methyl Red tests, negative for Voges-Proskauer and Citrate tests) matched classical *E. coli* characteristics (Bhutia *et al.*, 2020; Saimin *et al.*, 2020). The combined use of biochemical tests alongside culture methods ensured accuracy and minimized false positives.

The detection of *E. coli* in these shrimp samples carries important food safety and public health implications. *E. coli* serves as a reliable indicator of fecal contamination, and certain pathogenic strains pose risks of foodborne illness, including gastroenteritis and more severe infections (Barbosa *et al.*, 2016; Chandraval and Chandan, 2016; Bintsis, 2017). The elevated *E. coli* counts in Vaname Q exceed acceptable levels, suggesting insufficient sanitary control either in the cultivation environment or during processing. Water quality in aquaculture systems plays a pivotal role, as contaminated pond water can introduce fecal bacteria into shrimp populations (Iber and Kasan, 2021). Additionally, inadequate sanitation during harvesting and post-harvest handling can exacerbate contamination levels (Ahmed *et al.*, 2001; Dao *et al.*, 2018). The disparity between Vaname Q and Vaname R underscores the need for stringent hygiene protocols and water quality monitoring.

Comparing these findings with previous research, similar contamination levels have been documented in farmed shrimp, underscoring the influence of environmental and hygienic practices during production and handling. For example, at the farm level in Bangladesh, *E. coli* contamination was detected in 62.5% of water samples, 43.7% of pond scum, 60% of shrimp, and 60% of basket samples. In depot environments, *E. coli* was found in 53.3% of shrimp, 37.5% of basket surfaces, and 92.3% of mats. Additionally, nearly all samples from seafood processing facilities—except those from a single plant—tested positive for *E. coli*, reflecting a systemic issue in post-harvest handling and sanitation within the shrimp industry (Faridullah *et al.*, 2016).

To address these issues, aquaculture operations must implement comprehensive control strategies. Continuous microbiological surveillance of pond water and shrimp products can provide early warnings of contamination. Employing water treatment technologies such as biofiltration, UV sterilization, or ozonation has proven effective in reducing microbial loads in aquatic environments (Sharrer and Summerfelt, 2007; Ali *et al.*, 2025). The implementation of UV disinfection systems significantly reduced *E. coli* contamination in household water, with a risk difference of −28.0% between intervention

and control groups and up to −38.6% in pre- and post-intervention comparisons (Reygadas *et al.*, 2015). Furthermore, educating farm workers and processors on good aquaculture and hygiene practices is essential to prevent cross-contamination. Proper sanitation of equipment, storage at appropriate temperatures, and avoiding contact between contaminated and clean materials are fundamental steps to ensure product safety (WHO, 2015; Bhagwat, 2019; Sheng and Wang, 2021; Ovissipour *et al.*, 2024). Advancements in molecular diagnostic methods, such as quantitative PCR targeting *E. coli* virulence genes, can further enhance detection sensitivity and specificity, facilitating timely corrective actions (Liu *et al.*, 2023; Ndraha *et al.*, 2023; Yamin *et al.*, 2023; Alsharksi *et al.*, 2024).

Despite the valuable insights provided, this study has limitations. Identification relied on conventional microbiological and biochemical methods, which do not allow differentiation between pathogenic and non-pathogenic *E. coli* strains. Future studies incorporating molecular techniques for virulence gene detection and strain typing would offer a more precise assessment of health risks. Additionally, longitudinal studies tracking contamination sources throughout the shrimp production chain could identify critical control points for intervention. Investigating the effectiveness of various water treatment and hygienic interventions in reducing *E. coli* contamination would be beneficial for developing best management practices.

The detection of *Escherichia coli* in *Litopenaeus vannamei* samples from both tested sources highlights ongoing challenges in maintaining microbiological safety in shrimp aquaculture. The significantly higher contamination levels in Vaname Q suggest varying degrees of hygienic management and environmental quality. These findings underscore the necessity of rigorous water quality monitoring, strict hygiene protocols, and adoption of effective microbial control strategies to reduce contamination risks. Ensuring the microbial safety of shrimp products not only protects consumer health but also enhances marketability and sustainability of shrimp aquaculture operations.

5. Conclusions

The study successfully confirmed the presence of *Escherichia coli* contamination in *Litopenaeus vannamei* samples from two different sources using MPN, selective media, and biochemical tests. Vaname Q showed significantly higher *E. coli* levels than Vaname R, indicating differences in hygienic conditions and potential fecal contamination risks. These results highlight the urgent need for improved water quality management, hygiene practices, and continuous microbiological monitoring in shrimp aquaculture to ensure product safety and protect public health.

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

RAF is responsible for formal analysis, data curation, project administration, funding acquisition, and responsible for writing the original draft. IP contributed to investigation, conceptualization, resource acquisition, methodology, writing – review & editing.

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Declaration of competing Interest

None

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