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Occurrence of *Vibrio cholerae* in Whiteleg Shrimp: Microbiological and Biochemical Analysis

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Abstract

Vibrio cholerae is a pathogenic bacterium known to contaminate aquaculture products, including whiteleg shrimp (*Litopenaeus vannamei*), and thereby represents a potential threat to public health through the food chain. The present study aimed to investigate the occurrence of *V. cholerae* in shrimp obtained from two aquaculture ponds (designated P and Q) located in West Java, Indonesia. Shrimp samples were subjected to standard microbiological and biochemical procedures for the detection and confirmation of the pathogen. The results revealed that samples from both ponds were positive for *V. cholerae*, despite the differences in culture conditions applied at each site. This finding provides preliminary evidence of *V. cholerae* contamination in shrimp aquaculture systems in Indonesia and highlights the critical importance of implementing rigorous biosecurity measures and maintaining effective water quality management practices to mitigate the potential risks of contamination in aquaculture production.

1. Introduction

Vaname shrimp (*Litopenaeus vannamei*) is a major aquaculture commodity in Indonesia, vital for both domestic consumption and export (Achmad *et al.*, 2020; Amelia *et al.*, 2021). As one of the world's largest shrimp producers, Indonesia must maintain both production quantity and product quality to meet increasing global demand and address challenges from disease outbreaks (Mustafa *et al.*, 2023). A major challenge and serious threat in the shrimp production chain is contamination by pathogenic bacteria, particularly Vibrio species, which are well-known pathogens in shrimp aquaculture and have been frequently associated with disease outbreaks and significant production losses. (Amatul-Samanah *et al.*, 2020; Zhou *et al.*, 2012). Of particular concern is *Vibrio cholerae*, the causative agent of human cholera, whose contamination in fishery products directly threatens public health and may contribute to cholera outbreaks, while also affecting global market competitiveness (Haque *et al.*, 2023; Maheshwari *et al.*, 2011).

The presence of *V. cholerae* in farmed shrimp can be attributed to various environmental and pond management factors (Tey *et al.*, 2015). This bacterium thrives in water with poor sanitation, high organic matter content, and inadequate circulation (Lara *et al.*, 2011). Feed waste, shrimp feces, and the inflow of contaminated water can enrich nutrients that support the growth of Vibrio (Iber & Kasan, 2021). Moreover, warm water temperatures in tropical regions such as Indonesia provide favorable conditions for this pathogen to proliferate (Lusiastuti *et al.*, 2024). Which increases the country's susceptibility to pathogen proliferation, potentially leading to more frequent and severe disease outbreaks in aquaculture systems. Suboptimal biosecurity practices, including overcrowding, inadequate sanitation, and insufficient disease monitoring, exacerbate the risk of pathogen contamination in shrimp ponds and highlighting current gaps in pond management (Au-Yeung *et al.*, 2025).

Although vannamei shrimp have high economic and nutritional value, data on the prevalence of *Vibrio cholerae* in shrimp aquaculture in Indonesia remain limited. Most studies on *V. cholerae* have focused primarily on food products or human health, whereas research in aquaculture has generally targeted the organisms themselves rather than pond management practices (Praja *et al.*, 2021).This raises concerns regarding food safety and the effectiveness of

disease control in aquaculture ponds. This study aims to detect the presence of *V. cholerae* in vannamei shrimp from two farming locations in Cirebon, West Java, through isolation on selective media and biochemical identification. The results of the pathogen analysis are expected to provide a basis for strengthening food safety surveillance programs, improving aquaculture management practices, and enhancing the competitiveness of Indonesia's shrimp industry in both domestic and international markets (Martins *et al.,* 2022; Bauer *et al.,* 2021).

2. Material and methods

2.1 Sample and Sampling Method

Shrimp samples of vannamei (*Litopenaeus vannamei*) were collected from two ponds in the Cirebon region, West Java, Indonesia. The ponds differed in environmental conditions and management practices. The first sample (Sample Q) was obtained from a pond that relied entirely on rainwater as its water source. The pond had no water circulation system and was located near residential areas and goat pens.

The second sample (Sample R) was obtained from a pond equipped with a water circulation system directly connected to the sea. The pond underwent regular seawater replacement and was located far from residential areas and livestock farms.

Live shrimp were collected on-site with clean nets. The samples were placed in sterile plastic bags, transported on ice in insulated containers, and delivered to the laboratory the same day. Samples were processed aseptically prior to analysis.

2.2 Materials and Equipment

In this study, preliminary isolation and identification employed Alkaline Peptone Water (APW), Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS), Triple Sugar Iron Agar (TSI), Kligler Iron Agar (KIA), oxidative–fermentative (O/F) media, oxidase test kits, Trypticase Soy Broth (TSB), O/129 sensitivity discs (10 µg and 150 µg), and ONPG discs. Further characterization included salinity tolerance tests (0%, 3%, and 6% NaCl in tryptone), carbohydrate fermentation assays (sucrose, lactose, D-cellobiose, arabinose, D-mannose, and D-mannitol), amino acid decarboxylase tests (arginine, lysine, and ornithine), and urea broth. All media and reagents were sterilized and handled aseptically according to standard microbiological procedures.

2.3 Sample Preparation and Selective Agar

The method was performed on the basis of the ISO 21872-1 standard method (ISO 21872-1, 2017). A total of 25 g of homogenized shrimp was aseptically combined with 225 mL of Alkaline Peptone Water (APW) to obtain the initial 10^0 suspension. Serial tenfold dilutions (10^{-1} to 10^{-3}) were subsequently prepared in APW and incubated at 36 ± 1 °C for 6–8 h, followed by streaking of turbid cultures onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar and incubation for 24 h to obtain selective isolates.

2.5 Bacterial Purification

Vibrio colonies were distinguished based on sucrose fermentation, where *Vibrio cholerae* typically forms shiny yellow color colonies (Ebob, 2020; Senoh *et al.,* 2014). Three to five yellow colonies are selected and transferred to Tryptic Soy Agar with NaCl (TSAN) for purification and long-term storage.

2.6 Morphological Characterization

Bacterial colonies grown on Tryptic Soy Agar with NaCl (TSAN) were subjected to Gram staining following standard protocols, and cell morphology was observed under a microscope. *Vibrio cholerae* isolates were identified based on their Gram-negative, curved, and comma-shaped rod morphology (Xu *et al.,* 2025; Sultana *et al.,* 2022).

2.7 Biochemical Confirmation

Biochemical confirmation of isolates was performed using categorized tests, following the standard methods described in ISO 21872-1:2017 with slight modifications. The confirmation of isolates was performed using preliminary analyses included oxidase activity, antimicrobial sensitivity, sugar fermentation, oxidative–fermentative (OF) metabolism, and growth at elevated temperature. Advanced tests assessed urease activity, amino acid utilization, carbohydrate fermentation, and salt tolerance. Serological identification was conducted using agglutination assays.

3. Results

3.1 Preliminary and Morphological Test

Bacterial contamination was detected in shrimp samples Q and R, as evidenced by turbidity in APW following 24 h of incubation. Subsequent culture on TCBS agar yielded medium-sized, bright yellow colonies in both samples (Figure 1a), consistent with the morphology of presumptive *Vibrio cholerae*. Purification on TSAN further supported bacterial growth and enabled the isolation of colonies for subsequent confirmatory analyses.

Table 1. Preliminary Test Result of Two Sample

Sample Code	Dilution	APW	TCBS	TSI	KIA	Of Medium	Oxsidase Test	Growth test 42°C	Sensitivity 10ug (0/129)	Sensitivity 150ug (0/129)	ONPG
Q	10^{-1}	+	+	A/A	K/A	+	+	+	S	S	+
	10^{-2}	+	+								
	10^{-3}	+	+								
R	10^{-1}	+	+	A/A	K/A	+	+	+	S	S	+
	10^{-2}	+	+								
	10^{-3}	+	+								

The oxidase test yielded positive results, as indicated by the rapid development of a purple–blue coloration on oxidase paper (Figure 2g). Sensitivity testing with the vibriostatic agent O/129 (10 µg and 150 µg discs) also produced clear inhibition zones, with the larger zone observed for the higher concentration (Figure 1b), consistent with the characteristics of presumptive *Vibrio cholerae*.

The Triple Sugar Iron (TSI) test produced a yellow/yellow reaction in both the slant and the butt (Figure 2c). The Kligler Iron Agar (KIA) test yielded a red slant and yellow butt, while no hydrogen sulfide (H_2S) production was detected (Figure 2c). The Oxidative–Fermentative (OF) test produced positive results, with both sealed and unsealed tubes showing a yellow color change (Figure 2f). The ortho-

Nitrophenyl-β-galactoside (ONPG) disc test also produced a yellow color change (Figure 2e).

Growth testing on Tryptic Soy Broth (TSB) at 42 °C for 24 h showed positive turbidity (Figure 2d), while subsequent Gram staining of isolates from TSAN slants revealed reddish, comma-shaped rods (Figure 3).

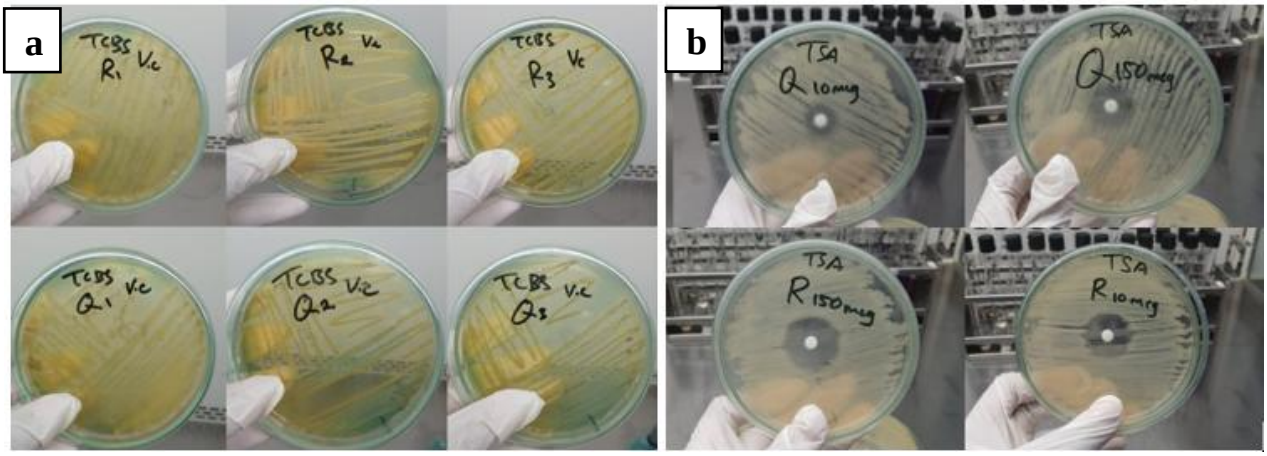

Figure 1. Vibrio Colonies on Thiosulfate Citrate Bile Salts Sucrose (TCBS) (a), Sensitivity test (b)

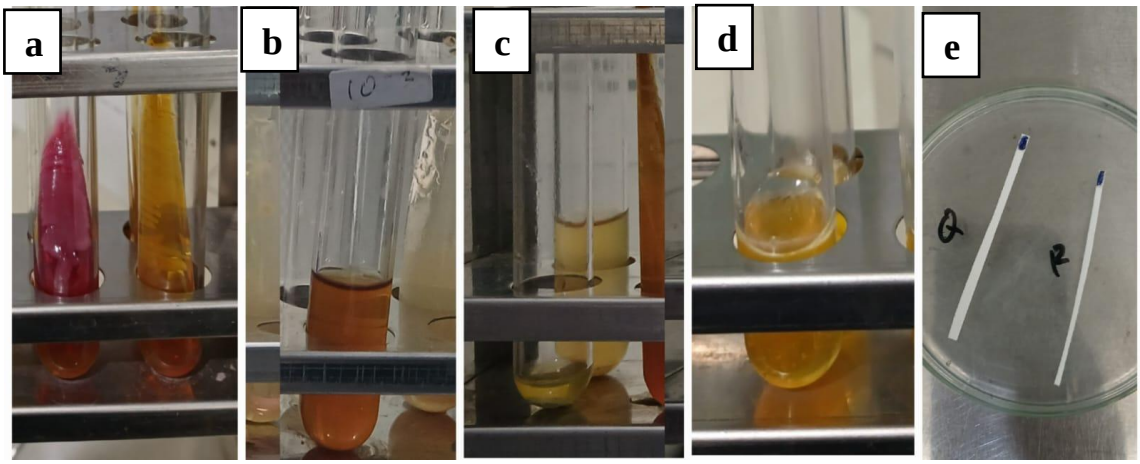

Figure 2. Preliminary Result: TSI-KIA Test (a), Growth at 42°C Test (b), ONPG Test (c), Oxidative-Fermentative Test (d), and Oxidase Test (e)

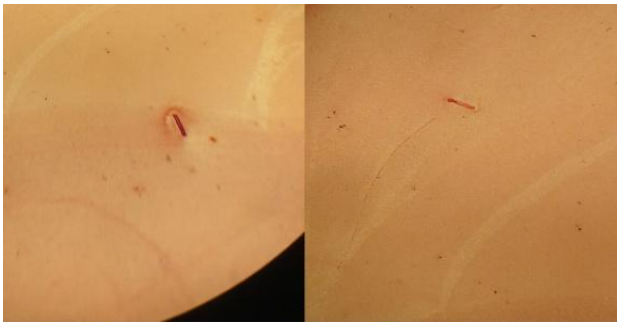

Figure 3. Gram Staining Test Result

3.2 Advanced Biochemical Tests

Several advanced biochemical assays were performed, and the results are summarized (Table 2). The urea hydrolysis test yielded negative results with no observable color change in the medium (Figure 4i). In the amino acid decarboxylation tests, positive reactions were recorded for lysine and ornithine, with a color change from purple to yellowish (Figure 4j).

Table 2. Subsequets Test Result of Two Samples (Q and R)

Sample Code	Salinity test			Carbohydrate fermentation tests			Amino Acid test				Urease	Serology
	T1 N0	T1 N3	T1 N6	Sucrose	Lactose	Arabinose	D-Mannos	Arginin dihydrolase	Ornithin dihydrolase	Lysine dihydrolase		
Q	+	+	-	+	-	-	+	-	+	+	-	+
R	+	+	-	+	-	-	+	-	+	+	-	+

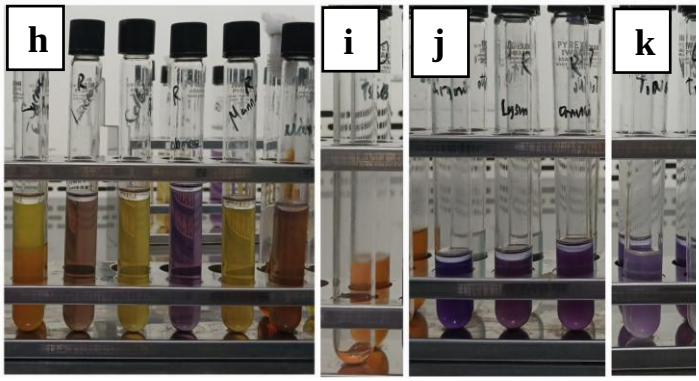

Figure 4. Subsequent Test Result: Carbohydrate Fermentation Test (h), Urease Test (i), Amino Acid Test (j), Salinity Test (k)

In addition to the amino acid decarboxylation assays, further confirmatory tests were conducted. No change was observed in the arginine dihydrolase test (Figure 4j). Both samples Q and R showed positive turbidity in the salt tolerance test at 0% and 3% NaCl (TIN0 and TIN3) after incubation at 36 ± 1 °C for 18–24 hours (Figure 4k). No growth was observed on TIN6 medium. Carbohydrate fermentation tests with six sugars (sucrose, lactose, D-cellobiose, arabinose, D-mannose, and D-mannitol) showed positive reactions for sucrose, D-mannose, and D-mannitol, indicated by a yellow color change in the medium. No reaction was observed for lactose, D-cellobiose, and arabinose, and the medium remained purple (Figure 4h). Serological testing with Hikojima Inaba–Ogawa polyvalent antiserum produced positive agglutination in the culture suspension.

Table 3. Conclusions of the Two Samples

Sample Code	Result of Vibrio Cholerae Contamination
Q	**+**
R	**+**

Taken together, the results of preliminary tests, Gram staining, and subsequent biochemical assays consistently demonstrated that both samples Q and R were positive for nearly all diagnostic parameters. These findings included colony growth with characteristic morphology on selective media, specific enzymatic activities, carbohydrate fermentation patterns, and positive serological reactions. The consistency of these results supports the identification of *V. cholerae* in both samples.

4. Discussion

The detection of *Vibrio cholerae* in both shrimp samples (Q and R) demonstrates that bacterial contamination can occur under diverse aquaculture management conditions. Despite differences in pond infrastructure and environmental control, the pathogen was consistently identified, highlighting the need to consider both farm-level practices and broader ecological determinants of pathogen persistence.

Differences in pond management and environmental characteristics influenced pathogen risk. Sample Q originated from a pond relying solely on rainwater without adequate circulation, resulting in stagnant conditions and accumulation of organic matter (Herbeck *et al.*, 2013). Such conditions create a nutrient-rich environment that promotes the growth and persistence of pathogenic Vibrio, as stagnant, organic matter–rich waters are known to facilitate bacterial proliferation (Ginn *et al.*, 2021; Neogi *et al.*, 2014; Kegler *et al.*, 2018). Proximity to residential areas and livestock pens further increased the likelihood of runoff and organic enrichment. By comparison, sample R had regular water turnover via direct seawater exchange, supporting more stable water quality and lower organic loads. Nonetheless, the persistence of *V. cholerae* in R indicates that natural marine waters can serve as reservoirs, and even well-managed systems remain vulnerable to contamination (Vezzulli *et al.*, 2009; Sampaio *et al.*, 2022).

Seasonal and climatic variations, including temperature fluctuations, salinity changes, and rainfall patterns, may affect bacterial growth rates and survival of pathogenic strains. Host-related factors, such as shrimp immunity and health status, also likely influence the ability of *V. cholerae* to colonize and persist within pond ecosystems. Although host immunity was not evaluated in this study, incorporating host-level assessments in future research could provide a more comprehensive understanding of pathogen dynamics (Li & Chen, 2008).

From a public health perspective, the presence of *V. cholerae* in shrimp intended for consumption emphasizes the importance of strict biosecurity measures and effective water quality management. For ponds with conditions similar to Q, short-term interventions—such as mechanical aeration, recirculating culture systems, sedimentation ponds or biofilters, and runoff control—can reduce organic loading and limit bacterial proliferation (Boyd *et al.*, 2019). In better-managed farms such as R, maintaining infrastructure hygiene, treating incoming seawater, and monitoring microbial loads remain critical to prevent pathogen entry and persistence (Moss *et al.*, 2012; Turkmen & Toksen, 2014).

Building upon local interventions, internationally recognized standards further support disease prevention. Guidelines from the FAO Code of Conduct for Responsible Fisheries and the OIE Aquatic Animal Health Code recommend safe and treated water sources, maintenance of optimal dissolved oxygen and organic matter levels, risk analyses, sludge removal, disease diagnostics, and routine microbiological monitoring (Palić *et al.*, 2015). This is intended to provide information on domestic disease control programs and/or supply relevant disease occurrence data that trading partners can use for both qualitative and quantitative risk assessments (Bondad-reantaso *et al.*, 2021). Hygienic post-harvest handling—such as rapid cooling, clean equipment, and sanitary packaging—can additionally reduce bacterial proliferation.

This study has several limitations. Only two ponds were sampled, which may limit the generalizability of findings. Molecular confirmation of *V. cholerae* was not performed, and seasonal sampling was not included, potentially overlooking temporal variations in bacterial prevalence. Acknowledging these constraints is important for contextualizing results and guiding future investigations.

The contrasting environmental conditions of farms Q and R demonstrate how aquaculture management directly shapes bacterial ecology and pathogen risk. While complete elimination of *V. cholerae* from coastal waters is unlikely, implementing international biosecurity and water quality protocols can significantly reduce risks to both aquaculture productivity and public health

5. Conclusions

Vibrio cholerae was detected in shrimp from both farms, demonstrating that contamination can occur under diverse aquaculture conditions and highlighting the importance of pond management, water quality, and

biosecurity. Although complete elimination is unlikely, targeted farm-level interventions and internationally recognized protocols can reduce contamination risks. This study was limited by small sample size, lack of molecular confirmation, and absence of seasonal data, warranting further research with molecular and host-level assessments. Ensuring shrimp safety is critical for public health, aquaculture productivity, and maintaining compliance with international seafood trade standards.

Ethics approval

No permits were required. No permits were required for marine biota sampling. The study was conducted following the relevant institutional and national regulations on the use of aquatic organisms in research.

Data availability statement

T All data supporting the findings of this study are contained within the article.

Author contributions

The author confirms sole responsibility for all aspects of the manuscript.

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Declaration of competing Interest

None

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