

Microbiological Hazards in the Food Chain of the Asian Green Mussel (*Perna viridis*): A Review Focusing on *Vibrio parahaemolyticus*

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ABSTRACT

The Asian green mussel (*Perna viridis*) is an important aquaculture species in the Indo-Pacific region and, as a filter-feeding bivalve, can accumulate microorganisms from surrounding waters, including foodborne pathogens. This review synthesizes evidence on the occurrence, persistence, and control of *Vibrio parahaemolyticus* associated with *P. viridis*, with emphasis on pre-harvest environmental determinants, post-harvest survival, and processing interventions. Peer-reviewed literature and regulatory sources published between 2000 and 2026 were evaluated to characterize factors influencing pathogen prevalence and persistence and to assess control measures. Available evidence indicates that *V. parahaemolyticus* is frequently detected in filter-feeding bivalves, including populations from Southeast Asia, where strains carrying the *tdh* and *trh* virulence-associated genes have been reported. Environmental conditions, particularly temperature, influence pathogen occurrence and post-harvest persistence. Biofilm formation and the viable but non-culturable state may further contribute to pathogen persistence and complicate detection and control. Quantitative microbial risk assessment studies indicate that temperature abuse during post-harvest handling and transportation can increase the predicted risk of illness. Although seawater depuration can reduce bacterial loads under defined conditions, its efficacy may be limited by pathogen localization and physiological state. Alternative interventions, including high-pressure processing and emerging biochemical approaches such as chitooligosaccharide–polyphenol conjugates, have demonstrated potential for reducing *V. parahaemolyticus* contamination, although their effectiveness in *P. viridis* requires further validation. Overall, the evidence supports an integrated risk-management approach combining environmental monitoring, temperature control, validated post-harvest interventions, and pathogen-specific microbiological criteria. Further studies should validate emerging interventions under processing-relevant conditions and assess their feasibility for controlling *V. parahaemolyticus* in bivalve seafood.

1. INTRODUCTION

The Asian green mussel (*Perna viridis*) is a commercially significant Indo-Pacific aquaculture species, comprising 36.15% protein, 19.72% lipids, and 24.54% carbohydrates by dry weight (Nunal et al., 2023). As obligate filter feeders, these bivalves bioaccumulate marine microorganisms at substantially higher densities than the surrounding water. This physiological trait presents a direct microbial safety

hazard, frequently vectoring *Vibrio parahaemolyticus*, a halophilic marine bacterium and leading etiologic agent of seafood-borne gastroenteritis (Hikmawati et al., 2019). While the pathogen's baseline prevalence, temperature-dependent proliferation, and primary virulence mechanisms—such as the thermostable direct hemolysin (TDH)—are well established, its specific ecological interactions within the *P. viridis* matrix require further synthesis. Current research rarely integrates pre-harvest environmental determinants with post-harvest

microbial survival kinetics (Liu et al., 2025; Ong et al., 2023). Specifically, a knowledge gap exists regarding how the *P. viridis* hepatopancreas facilitates quorum-sensing-mediated biofilm formation, enabling the pathogen to persist throughout the supply chain. Because of this physiological shielding, traditional mitigation strategies, including cold-chain storage and natural seawater depuration, demonstrate limited efficacy against entrenched populations (Baker, 2016; Ndraha et al., 2022; Wang et al., 2015b). This disconnect suggests a need for updated quantitative microbiological risk assessments (QMRAs) that incorporate pathogen resilience and matrix-specific variables. Therefore, this review systematically evaluates the microbiological hazards of the *P. viridis* food chain, focusing on *V. parahaemolyticus*. The objective is to analyze the biological drivers of pathogen accumulation, molecular pathogenesis, antimicrobial resistance, and survival dynamics, ultimately assessing the efficacy of advanced post-harvest processing technologies required to achieve regulatory compliance.

2. METHODS

2.1. Literature search strategy

A structured literature search was conducted to identify and synthesize published evidence concerning microbiological hazards associated with the Asian green mussel, with particular emphasis on *V. parahaemolyticus* throughout the food chain. The review was designed as a comprehensive narrative review to integrate microbiological, epidemiological, risk-assessment, and post-harvest processing evidence and to examine relevant regulatory frameworks. Because the objective was evidence integration across diverse study designs rather than a systematic review with a predefined meta-analytic framework, PRISMA reporting was not adopted as the primary reporting framework.

Literature published from 1 January 2000 to 1 August 2026 was searched using Scopus and Google Scholar. The search included peer-reviewed research articles, review articles, risk-assessment studies, and relevant scientific reports. The principal search strategy combined terms describing the target species, pathogen, and major microbiological characteristics as follows: ("*Perna viridis*" OR "Asian green mussel") AND ("*Vibrio parahaemolyticus*" OR "foodborne pathogens") AND ("prevalence" OR "antimicrobial resistance" OR "biofilm"). A supplementary search was conducted to identify evidence concerning risk assessment and post-harvest control using the terms ("quantitative microbial risk assessment" OR "QMRA") AND ("post-harvest processing" OR "high-pressure processing" OR "depuration"). Additional research was conducted when necessary to identify relevant studies addressing environmental determinants, pathogen persistence, virulence-associated characteristics, and emerging control technologies identified during the initial literature assessment.

2.2. Eligibility and study selection

Studies were considered eligible when they were published in English and provided microbiological, epidemiological, risk-assessment, or intervention-related evidence relevant to *V. parahaemolyticus* in *P. viridis* or, where direct evidence was limited, other bivalve matrices with relevance to the target food system. Primary studies reporting quantitative microbiological data were prioritized. Studies addressing pathogen prevalence, virulence-associated genes, antimicrobial resistance, persistence, biofilm formation, viable but non-culturable states, quantitative microbial risk assessment, depuration, thermal or non-thermal processing, and other post-harvest interventions were considered. Opinion articles and publications lacking sufficient methodological information to assess their scientific relevance were excluded.

Titles and abstracts were initially screened for relevance, followed by full-text assessment of potentially eligible publications. Study selection and data interpretation were conducted by the authors, with differences in eligibility assessment or interpretation resolved through discussion and consensus.

2.3. Data extraction and evidence synthesis

Relevant information was extracted into structured tables, including geographical location, sample type, microbiological methods, *V. parahaemolyticus* prevalence or concentration, virulence-associated gene profiles, antimicrobial resistance characteristics, environmental determinants, intervention conditions, reported microbial reductions, and quantitative risk-assessment parameters. Where reported, methodological characteristics and study limitations were also recorded.

Evidence was interpreted according to its relevance to the target food matrix. Primary empirical studies conducted directly on *P. viridis* were considered direct evidence. Findings from other bivalve species, *in vitro* experimental systems, and simulated or model-based QMRA studies were categorized as supporting evidence and were not assumed to be directly transferable to *P. viridis*. Differences in sampling strategies, analytical methods, geographical conditions, processing parameters, and outcome measures were considered when comparing findings across studies. Because of the heterogeneity of study designs and reported outcomes, the evidence was synthesized qualitatively rather than subjected to formal statistical meta-analysis.

2.4. Regulatory and microbiological criteria

In parallel with the academic literature search, relevant microbiological criteria, food-safety guidance, regulatory standards, and legislative documents were identified from official sources, including the United States Food and Drug Administration, European Commission, and relevant Indonesian governmental authorities. Regulatory documents were evaluated separately from the peer-reviewed literature because their purpose and evidentiary basis differ from those

of scientific publications. The identified regulatory requirements were subsequently compared with the microbiological hazards and post-harvest control measures reported in scientific literature to assess the extent to which current regulatory frameworks address the identified risks associated with *V. parahaemolyticus* in bivalve seafood.

3. RESULTS AND DISCUSSION

3.1. Prevalence and Distribution of *V. parahaemolyticus* in the Green Mussel Supply Chain

Environmental conditions, particularly water temperature and salinity, largely govern the distribution of *V. parahaemolyticus* in bivalve harvesting areas (Froelich & Noble, 2016). Predictive risk assessment models indicate a linear relationship between water temperature and the mean logarithmic density of total *V. parahaemolyticus*, while salinity follows a quadratic relationship with optimal conditions modeled near 22 parts per thousand (FAO/WHO, 2011). As an

obligate halophile, the population density of this mesophilic bacterium increases during seasonal warming, particularly when ambient water temperatures exceed 15°C (Froelich & Noble, 2016). Predictive models suggest that coastal warming may expand the geographic distribution of pathogenic *Vibrio* species (Koutsoumanis et al., 2024).

Driven by these ecological parameters, global seafood databases document regional variability in *V. parahaemolyticus* prevalence, with high contamination rates frequently reported in Southeast Asia (Table 1). A meta-analysis of 5,811 seafood samples across 24 countries reported an overall isolation rate of 47.5% of this microorganism, while the category encompassing mussels, scallops, and periwinkles demonstrated a pooled global prevalence of 28.0% (Odeyemi, 2016). In Southeast Asian coastal environments, the obligate filter-feeding mechanism of the Asian green mussel (*P. viridis*) facilitates the bioaccumulation of these marine microorganisms at densities higher than the surrounding water (Hikmawati et al., 2019).

Table 1. Prevalence and concentration metrics of *V. parahaemolyticus* in the *P. viridis* supply chain.

Supply Chain Node/Region	Sample Matrix	Key Epidemiological Finding	Reference
Pre-Harvest (Singapore)	Coastal farm water	100% detection rate (1.0 – 2.9 log CFU/g)	(Ong et al., 2023)
Harvest (Singapore)	Farmed <i>P. viridis</i>	68.9% prevalence (Mean: 3.0 log CFU/g)	(Ong et al., 2023)
Retail (Singapore)	Hypermarket <i>P. viridis</i>	91.1% prevalence (Mean: 5.9 log CFU/g)	(Ong et al., 2023)
Retail (Malaysia)	Marketed mollusks	44.6% contamination rate	(Odeyemi et al., 2023)
Bivalve Isolates (Thailand)	<i>P. viridis</i> isolates	12% carriage of <i>tdh</i> virulence gene	(Palamae et al., 2022)
Global Meta-Analysis	Mussels/scallops	28.0% pooled global prevalence	(Odeyemi, 2016)

Quantitative assessments tracking green mussels from harvest to retail confirm this bioaccumulation and document progressive contamination. Evaluations of farmed green mussels in Singapore detected *V. parahaemolyticus* in 100% of the surrounding farm water samples, with concentrations ranging from 1.0 to 2.9 log CFU/g (Ong et al., 2023). Directly at the harvest point, the farmed mussels exhibited a 68.9% positive isolation rate and a mean pathogenic concentration of 3.0 log CFU/g (Ong et al., 2023). As the green mussels transited to retail hypermarkets, the isolation rate reached 91.1%, and mean bacterial concentrations increased to 5.9 log CFU/g (Ong et al., 2023). This progressive contamination aligns with systematic reviews of retail seafood in Malaysia, which found

that 44.6% of investigated mollusk samples were contaminated, predominantly by *Vibrio* species (Odeyemi et al., 2023).

Beyond baseline prevalence, the microbiological hazard is characterized by specific molecular virulence markers. While molecular identification utilizes the *tlh* (thermolabile hemolysin) gene as a species-specific diagnostic target exhibiting a 100% carriage rate in confirmed isolates, human illness is primarily associated with the *tdh* and *trh* genes (Bechlars et al., 2015; Wang et al., 2015a, 2015b). Targeted sampling of *P. viridis* revealed that 12% of the isolated *V. parahaemolyticus* strains carried the *tdh* gene (*tdh*+/ *trh*-) and could induce the Kanagawa phenomenon, presenting a potential hazard to consumers (Palamae et al., 2022).

Table 2. Distribution of *V. parahaemolyticus* virulence markers in Southeast Asia.

Southeast Asian Nation	Detection Rate of <i>tdh</i> (%)	Detection Rate of <i>trh</i> (%)	Target Matrix	Reference
Vietnam	17.9	8.0	Marketed molluscan shellfish	(Nakaguchi, 2013)
Malaysia	11.1	16.7	Marketed molluscan shellfish	(Nakaguchi, 2013)
Indonesia	9.1	13.6	Marketed molluscan shellfish	(Nakaguchi, 2013)
Thailand (Southern)	12.0 (<i>tdh</i> +/ <i>trh</i> -)	Not Assessed	<i>P. viridis</i> specific isolates	(Palamae et al., 2022)

3.2. Molecular Determinants of Virulence and Biofilm-Mediated Persistence

Following ingestion by the filter-feeding bivalve, microorganisms primarily localize within the hepatopancreas, an organ that functions as a biological sink for environmental contaminants. Generalized bivalve proteomic analyses suggest that virulent *V. parahaemolyticus* strains actively suppress bivalve cellular immunity, establishing the hepatopancreas as a primary niche for long-term persistence. The clinical hazard posed by these persisting strains is mostly determined by their specific molecular virulence markers, notably the *tdh* (thermostable direct hemolysin) and *trh* (TDH-related hemolysin) genes (Li et al., 2019). While the *tlh* (thermolabile hemolysin) gene serves as a ubiquitous species marker, pathogenicity is closely tied to the expression of TDH and TRH proteins, which form pores in host cells and disrupt cellular ion homeostasis. Specifically distinguishing this generalized mechanism within the target matrix, quantitative phenotypic assessments of *V. parahaemolyticus* isolates recovered directly from Asian green mussels indicate that 12% carried the *tdh* gene and demonstrated positive β -hemolytic activity (the Kanagawa phenomenon) on Wagatsuma blood agar, confirming their capacity to induce human gastroenteritis (Table 2).

Bacterial survival and persistence within the bivalve host are further facilitated by the construction of biofilms within an extracellular polymeric substance (EPS) matrix. The transition from a vulnerable planktonic state to a resilient sessile state is regulated by quorum-sensing mechanisms and facilitated by initial attachment proteins such as Multivalent Adhesion Molecules. Phenotypic evaluations highlight the ubiquity of this survival strategy; in vitro assays of *P. viridis* isolates demonstrated that 100% of the tested *V. parahaemolyticus* strains possessed biofilm-producing capabilities, supported by strong swimming and swarming motilities required for surface colonization (Palamae et al., 2022). Complementary analyses of hemolytic *Vibrio* species isolated from bivalves, including green mussels, categorized specific isolates as strong biofilm formers, recording biofilm formation indices (BFI) greater than 1.10 (Sharma et al., 2024). This robust structural encapsulation protects the bacterial cells from physical clearance and environmental stressors.

Because quorum-sensing-mediated biofilms mechanically shield *V. parahaemolyticus* within the bivalve digestive tract, traditional depuration utilizing sterilized seawater exhibits limited efficacy for complete pathogen eradication. Consequently, integrating targeted anti-biofilm interventions into post-harvest processing frameworks warrants further evaluation. Future mitigation strategies should evaluate the implementation of biochemical treatments capable of disrupting the EPS matrix and neutralizing virulence factors without inducing further antimicrobial resistance. For instance, *in vitro* applications of marine-derived chitooligosaccharide (COS) and tea polyphenol conjugates have demonstrated membrane disruption and bactericidal effects against *Vibrio* isolates in laboratory models (Palamae et al., 2022; Sharma et al., 2024). Exploring the incorporation of such functional bio-compounds into commercial depuration phases could facilitate the breakdown of structural biofilms, thereby enhancing the clearance of entrenched pathogens prior to retail distribution.

The prevalence of antimicrobial resistance (AMR) in aquaculture is corroborated by localized phenotypic profiling specifically targeting the *P. viridis* matrix (Table 3). In Singaporean retail markets, *V. parahaemolyticus* isolated from farmed green mussels demonstrated resistance frequencies against tetracycline (89%), penicillin G (76%), and ampicillin (67%) (Ong et al., 2023). Similarly, retail mollusk sampling in Malaysia found that *Vibrio* species isolated from these matrices were resistant to 94% of tested antibiotics, with ampicillin resistance reaching 88% (Odeyemi et al., 2023). While environmental isolates of *V. parahaemolyticus* from natural Asian green mussel habitats often exhibit broader susceptibility to antibiotics—with only isolated instances of intermediate resistance to therapeutics like cefuroxime—resistance profiles become highly complex at the point of clinical infection (Palamae et al., 2022). Outbreak-associated *V. parahaemolyticus* clinical strains demonstrate a multiple antibiotic resistance (MAR) index of 0.64 (Palamae et al., 2022). These clinical strains exhibit resistance to nine specific therapeutics, including amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefuroxime, and meropenem, highlighting a substantial limitation for pharmacological interventions during the management of seafood-borne gastroenteritis (Palamae et al., 2022).

Table 3. Antimicrobial resistance profiles and MAR indices of *Vibrio* isolates associated with *P. viridis*.

Matrix/ Parameter	Key Quantitative Finding	Reference
Retail <i>P. viridis</i> (Singapore)	Resistance to tetracycline (89%), penicillin G (76%), and ampicillin (67%)	(Ong et al., 2023)
Retail mollusks (Malaysia)	88% ampicillin resistance; resistance to 94% of tested antibiotic classes	(Odeyemi et al., 2023)
General aquaculture isolates	Up to 80% exhibit ampicillin resistance globally	(Odeyemi et al., 2023)
Clinical outbreak strains	Multiple antibiotic resistance (MAR) index of 0.64	(Palamae et al., 2022)
<i>P. viridis</i> microbiome	Presence of MDR <i>V. cidicii</i> (resistant to cefotaxime, cefuroxime) posing horizontal gene transfer risk	(Sharma et al., 2024)

The molecular basis for this resistance primarily involves the upregulation of multidrug efflux pumps, enzymatic degradation of antimicrobial compounds, and structural alterations in outer membrane permeability concerning the OmpA protein (Destoumieux-Garzón et al., 2014; Fernández & Hancock, 2012). These genetic resistance determinants are frequently situated on mobile genetic elements, such as plasmids, integrons, and transposons (Destoumieux-Garzón et al., 2014; Sharma et al., 2024). Consequently, the presence of multidrug-resistant (MDR) *V. parahaemolyticus* is complicated by the broader "resistome" co-habituating within the *P. viridis* hepatopancreas. For example, microbiological assessments of bivalve mollusks from Southern Thailand successfully isolated pathogenic, β -hemolytic *Vibrio* *cidicii* directly from Asian green mussels, which demonstrated multidrug resistance against human therapeutics, including cefotaxime and cefuroxime (Sharma et al., 2024). Because resistance genes can be readily transferred among Gram-negative bacteria sharing the same microenvironment via mobile elements, the co-habitation of *V. parahaemolyticus* with resistant species like *V. cidicii* in the green mussel matrix creates a risk for horizontal gene transfer, accelerating the emergence of resistant pathogenic strains (Sharma et al., 2024).

Given the increasing prevalence of MDR strains and the associated risk of horizontal gene transfer within the bivalve microbiome, it is necessary to reduce reliance on conventional antibiotics in coastal aquaculture environments (Destoumieux-Garzón et al., 2014; Sharma et al., 2024). Mitigating this selective pressure highlights the need to explore alternative, non-antibiotic intervention strategies to manage bacterial loads. Future research and industry practices should explore the efficacy of advanced biochemical treatments. For instance, the application of chitooligosaccharide-polyphenol conjugates offers a potential pathway to disrupt established biofilms and neutralize resistant *Vibrio* populations without contributing to further antimicrobial resistance (Palamae et al., 2022). Implementing such biochemical controls during the post-harvest phase could help ensure that public health standards are met prior to market distribution.

3.3. Risk Characterization and Post-Harvest Vulnerabilities

Quantitative Microbiological Risk Assessment (QMRA) provides a structured framework for evaluating the public health risks associated with *V. parahaemolyticus* in the *P. viridis* supply chain (FAO/WHO, 2011). Within this framework, hazard characterization employs the Beta-Poisson dose-response model to estimate the probability of human illness following consumption (FAO/WHO, 2011). While the maximum-likelihood estimation parameters adopted by international authorities ($\alpha = 0.6$ and $\beta = 1.31 \times 10^6$) were initially derived from oyster consumption data, this mathematical framework has been adapted to evaluate the specific risk profile of green mussels (Ong et al., 2023; US FDA, 2005). For instance, an Antimicrobial Resistance Risk Assessment (ARRA) evaluating farmed *P. viridis* in Singapore

estimated an average probability of illness per serving from hemolytic *V. parahaemolyticus* at 5.7×10^{-3} for farm-to-home chains, and 1.3×10^{-2} for retail-to-home chains (Ong et al., 2023).

However, these quantitative values are context-dependent; risk estimates rely on the variables of the respective food chain, including regional ambient temperatures, transit durations, and local consumption practices (Ong et al., 2023). Because the Beta-Poisson model indicates that the probability of illness scales with the ingested dose, generalized models may not fully capture local dynamics (FAO/WHO, 2011). Nevertheless, existing models demonstrated that temperature control during post-harvest transit is a primary variable. Exposure to ambient temperatures could lead to logarithmic bacterial proliferation, which has been shown to increase initial farm-level loads of 1.0–2.9 log CFU/g to retail concentrations reaching 1.6–5.9 log CFU/g in green mussels (Ong et al., 2023).

While cold-chain logistics are a standard industry practice to limit this proliferation, they present specific microbiological limitations. Refrigeration at 4°C and freezing at -20°C are fundamentally bacteriostatic; they restrict active bacterial growth but do not consistently achieve absolute microbial inactivation (Di Salvo et al., 2023). Under thermal stress, *Vibrio* species can enter a viable but non-culturable (VBNC) state, retain pathogenicity while becoming undetectable by standard culture-based methods (Di Salvo et al., 2023; Liu et al., 2025). Demonstrating this physiological characteristic, viable *V. parahaemolyticus* organisms have been recovered from oyster meat stored at -20°C for up to 12 weeks (Di Salvo et al., 2023; Liu et al., 2025). Because the duration required for complete bacterial inactivation via freezing generally exceeds the commercial shelf-life of raw bivalve products, cold storage functions primarily as a preventive measure against proliferation rather than an absolute bactericidal intervention (Liu et al., 2025).

Consequently, relying solely on temperature control and conventional cold-chain logistics may not completely ensure the microbiological safety of *P. viridis* (Liu et al., 2025). It may be beneficial to implement supplementary post-harvest processing interventions capable of addressing both actively proliferating and VBNC bacterial states. To achieve the logarithmic pathogen reductions modeled by QMRA, the seafood industry could explore advanced non-thermal technologies. Methods such as high-pressure processing (HPP) or targeted biochemical treatments utilizing chitooligosaccharide-polyphenol conjugates offer potential approaches to inactivate *V. parahaemolyticus* populations within the bivalve tissue, while aiming to maintain the sensory and nutritional qualities of raw bivalve products (Palamae et al., 2022; Puértolas et al., 2023). Furthermore, additional QMRA studies tailored to green mussel supply chains are encouraged to better inform regional risk management strategies (Ong et al., 2023).

3.4. Advanced Mitigation Technologies and Global Regulatory Disparities

Given the limited efficacy of standard depuration against entrenched biofilms (Martinez-Albores et al., 2020), ensuring product safety necessitates advanced physical and biochemical interventions. Consequently, ensuring product safety requires the implementation of advanced physical and biochemical interventions. Mild thermal processing offers a highly effective physical approach; decimal reduction time (D-value) kinetics for *Vibrio* species demonstrate rapid inactivation at elevated temperatures, such as 2.7 minutes at 50°C (Baker, 2016). Building on these kinetics, commercial microwave processing targeted at 50°C can achieve the requisite 3-log reduction while mitigating thermal degradation to the meat (Ulusoy et al., 2019). However, to preserve the sensory attributes of strictly raw products, non-thermal technologies are often preferred (Puértolas et al., 2023). High-pressure processing (HPP) at isostatic pressures ≥ 400 MPa rapidly disrupts bacterial cell membranes and denatures intracellular proteins and has demonstrated the capacity to significantly reduce Gram-negative *V. parahaemolyticus* populations without compromising the raw bivalve tissue matrix (Hwang et al., 2023; Puértolas et al., 2023). Alternatively, non-antibiotic biochemical controls offer targeted cellular disruption. For instance, chitoooligosaccharide-catechin (COS-CAT) conjugates exhibit potent bactericidal activity; exposure to a 2x minimum inhibitory concentration (0.256 mg/L) induced severe membrane disruption, reducing viable *V. parahaemolyticus* cells by 84% in shucked green mussels within six hours (Palamae et al., 2022).

The implementation of these advanced technologies is closely tied to regulatory compliance; however, global harmonization specifically targeting *V. parahaemolyticus* remains inconsistent. While traditional frameworks often rely on generic fecal indicators like *E. coli*, these do not correlate with the presence of natural marine vibrios (Sharp et al., 2021). Consequently, international legislative bodies approach *V. parahaemolyticus* management differently. The European Union, for instance, currently lacks specific legislative limits for *Vibrio* species in seafood, relying instead on broader hygiene criteria (Commission Regulation, 2005; Koutsoumanis et al., 2024). In contrast, the United States Food and Drug Administration (FDA) establishes a specific guidance action level of 1.0×10^4 CFU/g or MPN/g under the National Shellfish Sanitation Program (US FDA, 2023). Regional producers in Southeast Asia navigate even stricter barriers; in Indonesia, a primary producer of *P. viridis*, BPOM Regulation No. 13 of 2019 mandates a rigorous maximum limit of < 3 MPN/g for *V. parahaemolyticus*, enforced through a multi-tiered sampling plan ($n=5$, $c=0$) (Indonesian National Agency of Drug and Food Control, 2019). When comparing these frameworks, it is important to emphasize that direct numerical equivalence is complex; the specific action levels, mandated sampling plans, and regulatory scopes (e.g., harvesting guidelines versus processed food limits) are not directly comparable on a one-to-one basis. These profound regulatory disparities underscore the critical need for advanced, validated processing technologies capable of uniformly satisfying both domestic public health mandates and stringent international trade parameters.

Table 4. International regulatory criteria specifically for *V. parahaemolyticus* in seafood and bivalves.

Regulatory Authority	Maximum Limit/Safety Criterion	Application Context	Reference
Japan (Ministry of Health, Labour and Welfare)	< 100 MPN/g	Seafood intended for raw consumption	(Hara-Kudo et al., 2012)
United States (FDA / NSSP)	$< 1.0 - 10^4$ CFU/g or MPN/g	Guidance Action Level for bivalve molluscan shellfish	(US FDA, 2023)
European Union (EC No 2073/2005)	No specific limit established	Currently governed under general seafood hygiene standards	(Commission Regulation, 2005)
Indonesia (BPOM No. 13 of 2019)	< 3 MPN / g	Processed bivalve mollusks; standardized sampling plan ($n=5$, $c=0$)	(Indonesian National Agency of Drug and Food Control, 2019)

*MPN = most probable number

Following the synthesis of current technological capabilities and regulatory landscapes, it becomes relevant to identify subsequent actionable steps. Establishing harmonized, pathogen-specific international safety criteria for *V. parahaemolyticus* is a primary consideration to reconcile existing regulatory disparities. This alignment is necessary because inconsistent legislative limits create potential trade

barriers and uneven public health protections across different geographical regions (Liu et al., 2025). To facilitate this regulatory alignment, international food safety authorities must collaborate to formulate updated quantitative microbiological risk assessments (QMRAs) explicitly tailored to bivalve supply chains and informed by contemporary climate and antimicrobial resistance data. Concurrently, at the

industrial level, the bivalve aquaculture sector must transition toward the phased integration of validated non-thermal and hurdle technologies—such as high-pressure processing (HPP) and targeted biochemical applications—into standard operating procedures. Initiating regional pilot programs to evaluate the cost-effectiveness, scalability, and sensory retention of these advanced interventions (Puértolas et al., 2023) will incentivize broader commercial adoption. Ultimately, modernizing these processing frameworks represents a critical pathway to help producers consistently satisfy strict global microbial safety thresholds and secure the *P. viridis* supply chain

4. CONCLUSION

The biological and ecological characteristics of the Asian green mussel (*P. viridis*) facilitate the bioaccumulation of marine pathogens, notably *V. parahaemolyticus*. Synthesized epidemiological and molecular data indicate that this pathogen presents a persistent food safety consideration, particularly within Southeast Asia. The capacity of the bacteria to evade bivalve cellular immunity and establish quorum-sensing-mediated biofilms within the hepatopancreas renders conventional post-harvest mitigation strategies, such as sterilized seawater depuration, largely ineffective. Additionally, reliance on cold storage alone demonstrates limitations, as it frequently induces a VBNC state rather than achieving absolute microbial inactivation. The public health risk, as quantified by Beta-Poisson dose-response models, is amplified by post-harvest temperature variations and the documented presence of multidrug-resistant *Vibrio* isolates in coastal aquaculture systems. Furthermore, current international regulatory harmonization is lacking, with frameworks ranging from reliance on generic fecal indicators to the imposition of strict pathogen-specific limits, which creates trade vulnerabilities and uneven public health outcomes.

Consequently, it is relevant to consider subsequent actions to address these systemic vulnerabilities in the *P. viridis* supply chain. Transitioning beyond traditional processing methods is recommended to better protect consumers and maintain supply chain viability. This shift is needed because conventional approaches do not reliably eliminate entrenched, biofilm-protected, or multidrug-resistant *Vibrio* populations. To achieve these safety objectives, the bivalve industry could integrate validated, non-antibiotic interventions, such as high-pressure processing (HPP) or biochemical treatments utilizing chitooligosaccharide-polyphenol conjugates, which have shown potential to achieve logarithmic microbial reductions while preserving raw product quality. Concurrently, technological advancements should be supported by legislative updates. International food safety authorities are encouraged to collaboratively establish unified, pathogen-specific microbiological criteria for *V. parahaemolyticus*, which would provide standardized safety benchmarks and facilitate more consistent global trade practices.

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AUTHOR CONTRIBUTIONS

Fenny Crista Anastasia Panjaitan: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Resti Nurmala Dewi:** Writing – review & editing. **Nodali Ndraha:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

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The authors declare no competing interests.

DECLARATION OF AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the author(s) used Google Gemini Pro and Trink AI (Enago) solely to improve the readability, grammar, and language clarity of the text. These tools were not used to generate original research ideas, interpret data, or draw scientific conclusions. Following the use of these tools, the author(s) reviewed and edited all suggestions to ensure scientific accuracy and take full responsibility for the content and integrity of the final publication.

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