

Emerging *Vibrio* Infections in Fish: A Review

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ARTICLE INFO

Article History:

Received: Feb. 18, 2025

Accepted: March 3, 2025

Online: March 8, 2025

Keywords:

Vibrio infections,
Fish populations,
Aquaculture,
Pathogenic bacteria,
Sustainability

ABSTRACT

In recent years, a concerning trend has emerged in the aquatic world: *Vibrio* infections are increasingly affecting fish populations. This comprehensive review dives into the causes, effects, and implications of these infections, shedding light on their significance for fisheries and ecosystems alike. This study explored the alarming rise of these pathogenic bacteria and their impact on aquatic life. Moreover, it focused on understanding this issue since it is crucial for the health of oceans and the sustainability of fish populations. *Vibrio* species represent a group of significant aquatic pathogens that are the culprits behind vibriosis, a bacterial disease with serious implications for mariculture. This condition threatens to disrupt seafood production on a global scale, leading to extensive mortality rates in aquatic populations and substantial economic losses for those in the industry. More than 85 species within the *Vibrio* genus thrive in various natural and marine environments around the world. Fish that encounter environmental stressors—such as temperature fluctuations, pollution, or overcrowding—exhibit heightened vulnerability to *Vibrio* infections. This susceptibility presents a major challenge to achieving sustainable growth in the aquaculture sector. In these farming systems, infections caused by *Vibrio* can appear abruptly, resulting in catastrophic outcomes for entire fish populations. Furthermore, the reach of *Vibrio*-related infections is expanding to human populations, an alarming trend attributed to practices in intensive livestock farming and the broader impacts of human-induced climate change. This dual threat underscores the urgent need for better management and preventive measures within both aquaculture and public health domains.

INTRODUCTION

Aquaculture is a rapidly expanding food sector that produces one-third of the global fish supply. However, it faces a major challenge due to numerous bacterial pathogens that infect fish. Because of the elevated population densities, fish kept in aquaculture facilities are particularly susceptible to disease outbreaks, which can cause large financial losses

(Irshath *et al.*, 2023). Mariculture is a significant investment for fishermen in Egypt. However, the viability and profitability of this industry are seriously hampered by illnesses and expensive feeding costs. A valuable food item, fish is a good supply of vitamins, minerals, and high-quality protein (Abdelaziz *et al.*, 2017).

Numerous *Vibrio* species are the primary cause of vibriosis, a disease that primarily affects fish raised in aquaculture and the ocean. Fish in marine and aquaculture systems around the world are reported to die at high rates due to the disease (Hassan *et al.*, 2021). *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, and *V. vulnificus* are the main *Vibrio* species that infect marine and farmed fishes worldwide. Additionally, these species are harmful to humans, producing ear wounds and gastrointestinal infections, as well as septicemia. The most prevalent *Vibrio* species that infect fish in aquaculture environments are *V. anguillarum* and *V. vulnificus*, which are present in freshwater, marine, and estuary environments and account for over 60% of all heterotrophic bacteria. Additionally, they are a component of the typical fish flora in aquatic species that may serve as disease-transmission vectors (Salama *et al.*, 2016; ElSharaby *et al.*, 2018; Jamil *et al.*, 2023).

Review of the literature

Vibrio fish pathogen

Vibrio bacterium is a type of bacteria that thrives in high-saline (30-35ppt), warm environments. It does not require aerobic environment, enabling them to maintain the violet stain in Gram's method bacterium that can also survive in low-oxygen conditions. *Vibrio* bacterium is commonly associated with parasitic infestations and mechanical injuries. The bacteria are straight or curved rods and are able to move in water using a single, polar flagellum. They are chemoorganotrophic, meaning they obtain energy from organic compounds. These factors can impair fish immunity, and increase their tendency to vibriosis (Abdalla *et al.*, 2022).

These microorganisms are commonly found in various water environments such as estuaries, coastal areas, sediment, and aquaculture facilities. With instance for *V. cholerae* and *V. mimicus*, they thrive in salty conditions (Sanches-Fernandes *et al.*, 2022). In 1854, while investigating cholera epidemics in Florence, *V. cholerae* was the first *Vibrio* species to be recorded. Nonetheless, cholera-like illnesses have been documented historically since Hippocrates' time (460–377 BC). There are currently about 130 species of the genus *Vibrio*, divided into 14 clades that include species that live in harmony, species that benefit one another, and species that spread illness (Carboni, 2021).

***Vibrio* infection in aquaculture**

Of all the heterotrophic bacteria in aquatic species are *Vibrio* bacteria. They are a normal component of aquatic animals' microbial communities. In addition to fish, *Vibrio* bacteria are frequently found in zooplankton, shellfish, crabs, and aquatic invertebrates such as leech, corals, and sea moss. In nature, *Vibrio* and its hosts may have a mutually beneficial relationship, merely coexist, or even be pathogenic (Liu *et al.*, 2016). Warm temperatures generally promote the growth of *Vibrio* species, which may facilitate their shift from commensal to harmful behavior. It has also been proposed that environmental variables, such as warming, weaken fish immunity and make them more vulnerable to vibriosis (Manchanayake *et al.*, 2023).

Effect of stress on pathogenicity of *Vibrio* spp.

Aquatic animals are more susceptible to microbial infections on being under stress. Vibriosis in fish is often linked to various stressors such as sewage pollution, hypersaline water from desalination plants, shipping operations, and landfilling (Arafa *et al.*, 2021). Certain strains, notably *V. anguillarum*, *V. ordalii*, and *V. salmonicida*, are highly infectious primary pathogens. Vibrios are more prevalent in regions with temperate or tropical climates (Toranzo *et al.*, 2017). Salinity, particularly sodium salt (Na⁺), is essential in the environment for the distribution of vibrios. This bacterium is usually found in pelagic ecosystems, and outbreaks of disease occur when fish are exposed to stress factors (Hu *et al.*, 2007).

Pathogenicity of vibriosis

The pathogenesis of vibriosis is a multifaceted interaction between the host, pathogen, and environment. *Vibrio* spp. exhibit diverse survival strategies and virulence factors that facilitate their persistence and pathogenicity. Their survival outside the host, especially in aquatic environments, plays a critical role in transmission and infection. *Vibrio* spp. can thrive in a range of environmental conditions, with their persistence often linked to sediment and biofilm formation, which further aids in their survival and pathogenic potential (Huang *et al.*, 2022).

The initial phase of infection involves the adherence and colonization of the host. *Vibrio* spp. utilize various mechanisms, including cell-surface hydrophobicity, flagella-mediated motility, and the production of biofilms, to establish a foothold on the host's mucosal surfaces. The complexity of fish mucous and its antimicrobial components can hinder and sometimes inadvertently support bacterial adherence (Lamari *et al.*, 2018).

The entry routes of *Vibrio* spp. are predominantly through the mouth, gills, or skin lesions, exploiting the host's vulnerabilities. Once established, these pathogens can overcome host defenses, leading to infection and potential disease development.

Overall, understanding these interactions and the factors influencing *Vibrio* spp. pathogenicity is crucial for managing and mitigating the impact of vibriosis in aquatic

environments. Subsequent discussions will delve deeper into the specific virulence aspects of *Vibrio* spp. and the impact of surroundings stressors in disease dynamics.

Numerous virulence factors that are expressed by virulence genes aid in the pathogenicity of *Vibrio* strains. Generally speaking, virulence factors facilitate the adhesion and admission of pathogens, their establishment and growth, their evasion of host defenses, the damage they inflict on to the host, and ultimately their get away from the infected host. *Vibrio* sp. includes 5 main virulence aspects: flagella, lipopolysaccharides, cytotoxins, adhesion factors, and capsular polysaccharides. Moreover, with innate (typical) virulence genes, bacteria also gain new (atypical) virulence genes, subsequently improving horizontal gene transfer (HGT) allowing pathogenic bacteria to get lyaseence genes from other bacteria or the environment (**Deng *et al.*, 2020**).

Vibriosis a global human health concern

Humans are able to contract severe vibriosis by consuming raw or undercooked seafood and tainted water (**Mancini *et al.*, 2023**). In immunocompromised people, the bacterium has been linked to gastroenteritis, which can result in extra-intestinal illnesses, wound infections, cellulitis, seawater-related otitis media, soft tissue infections, and septicaemia in 2022 (**Sampaio *et al.*, 2022**).

After eating improperly cooked or raw seafood, *V. parahaemolyticus* is the most prevalent food-borne pathogen globally. It produces severe stomach discomfort and dysentery, which can lead to watery stools, a high temperature, shivers, nausea, vomiting, and a sharp drop in blood pressure, which can cause shock (**El-Gazzar *et al.*, 2020**). In extreme situations, patients lose consciousness, experience frequent seizures, turn pale or cyanotic, and ultimately pass away. The most popular methods for treating *V. parahaemolyticus* infections include oral rehydration and antibiotic treatment (**Tan *et al.*, 2021**).

Among the foodborne diseases categorized between Biosafety Levels 3 and 4, such as anthrax, bubonic plague, Ebola, and Marburg virus, *V. vulnificus* has the greatest death rate and is responsible for more than 95% of seafood-related deaths in the United States (**Baker-Austin & Oliver, 2018**). It can infect individuals through an open cut or bruise. In severe situations, it may lead to necrotizing fasciitis, limb removal, and lethal septicemia in at-risk individuals (**Haftel & Sharman, 2023**). Fishermen who handle contaminated fish and seafood, especially shellfish, and who engage in aquatic activities like swimming are at increased risk of contracting cellulitis and septicemia from this bacterium. This bacterium causes outbreaks in seabream, which can be consumed by humans (**Williams *et al.*, 2014; Hamdan *et al.*, 2016**). When *Vibrio* spp. are associated with conditions such as liver disease, diabetes, and immunosuppressed, the consequences can be more severe (**Baker-Austin & Oliver, 2018**). Studies show that excess of 50% of cases of reported septicemia end in mortality during the first 72 hours of hospital

admission (Yun & Kim, 2018). Consequently, whenever a *V. vulnificus* infection is identified, it is imperative to provide timely and suitable antibiotic and surgical therapies. The public health sector must recognize the increasing risk of human wound infections caused by *Vibrio* spp., as recent reports indicate infections in individuals from *V. harveyi*. Additionally, *Vibrio*-related illnesses are expected to become more common in the future owing to global climate change (Brehm *et al.*, 2020). Hygiene measures are effective in preparing and processing seafood for human consumption. However, the development of new, sustainable, and environmentally friendly methods to prevent bacterial diseases in large-scale fishery shows pledge in reducing the industry's environmental impact and the associated hazards to human health (Wang *et al.*, 2015).

Common thermal-based processing of foods procedures, like a cold freezing at -18°C or -24°C and elevated temperatures over 55°C for 10 minutes, are used to prevent severe illness in humans from consuming seafood contaminated with *Vibrio* species.

Diagnosis of *vibrio* bacteria

It is essential to have readily deployable detection techniques that can provide simultaneous and real-time identification of several disease markers. Technologies that identify pathogens quickly, accurately, and sensitively can aid in the instant inhibition of *Vibrio* infestation and can offer prompt timely care in medical institutions. Furthermore, readily available detection techniques can be very helpful in identifying these bacteria. Furthermore, rapid diagnostic techniques are essential for disease monitoring and verification, ensuring that preventative and protective measures are implemented promptly to safeguard the community from an outbreak (Loo *et al.*, 2022).

Phenotypic and biochemical characteristics of *Vibrio* spp.

The gold standard for identifying the pathogens responsible for an issue typically involves conventional procedures. Horizontal approaches are primarily used for detecting *Vibrio* species in food and aquaculture. After enriching samples in alkaline peptone water, both techniques utilize a selective-medium called TCBS (Thiosulfate–Citrate–Bile Salts–Sucrose Agar). Biochemical assays are performed to confirm the species of isolated vibrios. ISO/TS 21872-2:2007 (International Organization for Standardization, 2007a), ISO/TS 21872-1:2007 (International Organization for Standardization, 2007b)

Rapid detection methods

Rapid detection techniques have proven to be very successful in controlling outbreaks of infectious illnesses caused by *Vibrio* species. Quickly identifying and detecting these pathogens is essential for initiating a timely and efficient response to treatment. Compared to traditional methods, rapid detection techniques such as polymerase-chain-reactions (PCR), loop-mediated-isothermal-amplification (LAMP), and immunoassays offer greater accuracy, reliability, specificity, and efficiency, making them less time-consuming (Baker-Austin & Martinez-Urtaza, 2023).

Immunological techniques

Successful detection of *Vibrio* bacteria can be enhanced by using antibodies that target specific antigens present in the various strains for bacterial detection. Immunological-based techniques allow for the identification, measurement, and depiction of *Vibrio* cells *in-situ*. Compared to traditional methods, these techniques are faster, easier to use, and offer higher accuracy and precision in detecting bacteria. Examples of immunological-based-techniques include the enzyme-linked-immunosorbent-assay (ELISA) and lateral-flow-immunoassays, such as dipsticks and strips (**Ebob , 2020**).

Molecular analysis of *Vibrio* spp.

Since molecular strategies are quick and affordable, they offer an advantage over conventional microbiological culture-based techniques. Candida gene-based multiplex PCR is regarded as a significant molecular technique because of its effectiveness in differentiating related species and isolates, as well as its affordability and time constraints. Heat-shock proteins (HSPs), groESL, tRNA gene intergenic spacer region (ISR), 16S rRNA, and groESL are examples of candidate genes that have been thoroughly characterized for variety. Any vibriosis strain has genetic differences due to insertions, deletions, mutations, etc. These differences show the relationship between the bacteria's pathogenicity and evolution (**Mishra *et al.*, 2024**).

MALDI-TOF-MS (matrix-assisted-laser-desorption/ionization-time-of-flight-mass-spectrometry)

It may be challenging to routinely use multiple gene amplicon sequencing techniques for diagnosing every different disease. In such cases, using MALDI-TOF MS for identifying *Vibrio* species could be advantageous compared to PCR-based phylogenetic marker gene detection. MALDI-TOF MS is suitable for quickly and automatically processing many samples at a low cost, and it does not require highly skilled operators or extensive labor (**Li *et al.*, 2018; Mougín *et al.*, 2020**).

When compared to molecular biology or classical phenotypic methods, MALDI-TOF-MS is a quick, precise, and inexpensive identification tool. It has significantly shortened the time to identification (30 minutes) by analyzing whole microorganisms with minimal sample processing. Following clinical, veterinary, soil, plant, food, and water microbiology, MALDI-TOF has been employed more recently for aquatic infections (**Gökdağ & Çağatay 2024**). Ionizing and measuring the mass-to-charge ratios (m/z) of microorganisms' ribosomal proteins produces a mass spectrum with a distinct fingerprint, which is the fundamental mechanism of the MALDI-TOF MS approach. Identification is done by comparing the acquired microbial peptide mass fingerprinting with the mass-spectral-library-database of previously used reference-samples (**Moussa *et al.*, 2021**).

Methods based on biosensors

Biosensors are made up of a transducer and a bioreceptor, making them practical analytical instruments. Bioreceptors can be biomimetic (such as synthetic catalysts and imprinted polymers), biological (including antibodies, enzymes, and nucleic acids), or biologically derived (like aptamers and recombinant-antibodies). Biosensors can be classified into mass-based biosensors, optical biosensors, and electrochemical biosensors. They enable the identification of multiple pathogens and real-time sample analysis. Additionally, they are susceptible, cost-effective, portable, and user-friendly. Consequently, during *Vibrio* outbreaks, biosensors can be used to precisely identify the infected organism (Da-Silva *et al.*, 2018).

Control of *Vibrio* spp. infection

1-Antibiotic treatment

Antibiotics are frequently combined with feed for medicinal purposes or used in water treatments. Vibriosis has been treated with antibiotics, including oxytetracycline, tetracycline, trimethoprim, sarafloxacin, flumequine, quinolones, nitrofurans, potentiated sulfonamides, and oxolinic acid. Rising local temperatures can alter physiological functions of bacterial cells and can encourage genetic mutations, allowing for the early emergence of drug resistance genetic changes. Thus, new study suggests that the usage of antimicrobial products may not be the only factor causing antibiotic-resistant bacteria to appear in the environment (MacFadden *et al.*, 2018; Reverter *et al.*, 2020). Although the extensive and regular use of antimicrobial agents in aqua farming in the past has led to the development of antibiotic resistance in microorganisms.

The transfer of antibiotic-resistant bacteria to consumers can affect public health and treatment efficacy due to the establishment of resistant genes and bacteria (Kah Sem *et al.*, 2023). Numerous resistance genes have been discovered in the environment and in microbes. Genes that provide resistance to antibiotics can be transmitted vertically to the following generation or horizontally to other bacteria. Tetracycline resistance genes (*tatA-tatE*, *tatG*, *tatH*, *tatJ*, *tatY*, and *tatZ*) are among the resistance genes, along with penicillin resistance genes (*penA* and *blaTEM-1*). Ampicillin, penicillin, and tetracycline are the most frequently found antibiotic resistance in *Vibrio* species (Letchumanan *et al.*, 2015).

However, it has been demonstrated that antibiotic pollution affects the composition of natural bacterial populations and water-quality; high dosages of antibiotics may result in bioaccumulated residues at high levels in food chains, which may ultimately cause secondary harm to non-target species. Antibiotic residues may be consumed by wild fish, compromising the safety of aquatic foods (Milijasevic *et al.*, 2024).

Consumers have experienced allergic reactions and toxicological problems due to consuming aquatic items that include antibiotic residues. When residues bioaccumulate in humans, they cause chronic-toxicity, which injures different body parts. Workers in the aquaculture industry who lack immunity may have toxicity issues and antibiotic allergies

because they run the risk of being exposed to high concentrations of antibiotics when the drugs are spread all over ponds after being mixed with feed. It is frequently recognized that contact dermatitis can result from exposure to a number of medications including sulphonamides (**Santos & Ramos, 2018**).

2-Vaccination

Vaccination is a crucial method for protecting fish from harmful diseases. It plays a growingly important role in aquaculture by reducing disease-related losses and lowering the need for antibiotics. Numerous vaccinations have been created to combat vibriosis, which include inactivated, live attenuated, and DNA-based vaccines. The most often utilized bacterial vaccines in the aquaculture sector are inactivated bacterial pathogen vaccines because of their high efficacy in fish (**Silvaraj *et al.*, 2020; Abdellatief & Alkalamawey, 2024**). Although vaccines are anticipated to produce long-lasting, antibody-mediated immune protection, little is understood about the fundamental defence mechanisms that fish vaccination elicits. A vaccine's effectiveness should be assessed based on its capacity to generate humoral and cellular immune responses to the targeted antigen rather than just its level of protection (**Rathor & Swain, 2024**).

In aquaculture, vaccination is a less hazardous and more efficient method of preventing and controlling vibriosis than antibiotics. In addition to lowering the need for antibiotics, vaccination can also elicit a potent immune response. The drawbacks of inactivated vaccines include a high vaccination dosage, a brief protection interval, and a mono vaccination route that necessitates several doses (**Kumar *et al.*, 2024**). Mutations in live attenuated vaccines may increase virulence, and they are linked to an increased risk of infection in those with compromised immune systems. Vaccinations have several issues, including challenging administration, the requirement for a large workforce, and increased expenses due to the need for numerous doses to maintain host protection. Fish are typically vaccinated against *Vibrio* species through intraperitoneal injection. Only fish with appropriate body sizes can receive these injections, and trained individuals are required to administer the injection with the least amount of stress. Otherwise, fish may experience stress from the injection, increasing their vulnerability to infection (**Mohd-Aris *et al.*, 2019**). Thus, the generation of oral or immersion vaccinations against *Vibrio* spp. may represent a novel approach to vibriosis control. There is controversy around the use of immunizations in invertebrates, despite their beneficial effects. Theoretically, because invertebrates do not have the same specialized and adaptive immune system as vertebrates, they cannot react to vaccines in a selective way (**Mohamad *et al.*, 2022**).

3. Live microbial feed additives (Probiotics)

Live microbial feed additives known as probiotics aid in preserving the host's intestinal microbial equilibrium (**Anee *et al.*, 2021**). They can be added to fish diet or water as supplements to enhance their health, growth, immunity against disease, and control of the stress response. Probiotics include various types of bacteria such as Gram-positive (e.g.,

Bacillus, Lactococcus, Lactobacillus, Bifidobacterium, Clostridium), Gram-negative (e.g., Aeromonas, Phaeobacter, Pseudoalteromonas, Roseobacter), microalgae (e.g. Spirulina, Alteromonas) (**Abdellatif et al., 2019**), and yeast (e.g., Rhodosporidium, Streptomyces) (**Islam et al., 2021**). These microbial substances have been shown to be beneficial in aquaculture feed due to their special and advantageous qualities. One of the most popular probiotics, *Bacillus* species, is extensively used in aquaculture to enhance immunity and antioxidant capacity while reducing toxicity symptoms (**Diwan et al., 2023**). Nevertheless, the application of probiotics in aquatic environment is limited as the result of obstacles such as the incapacity to mass-produce aquatic different animals on a large scale, insufficient readiness for the industry, and limited knowledge among fish farmers. Further research on probiotics' mechanism of action, impact on microbial communities, and the cooperation between microbiologists, fish nutritionists, and aquaculturists is needed to overcome these limitations. Other obstacles include difficulty in isolating and verifying probiotics, their interaction with culture media, and the need to evaluate strain candidates according to global standards. Further research is urgently needed to recognize the ecological dangers associated with microbial communities and the possible use of probiotics against them (**Rahayu et al., 2024**).

4. Nanoparticles

Nonetheless, the industry views environmental degradation and disease prevalence as crucial problems. New technical approaches have been developed in this area to effectively address these problems. As a novel and cutting-edge method, nanoparticles offer a lot of uses and enormous promise in aquaculture (**Fajardo et al., 2022**). It has recently been found that nanoparticles may be able to stop the growth of microorganisms. With little chance of bacterial resistance, treatment with nanoparticles has been demonstrated to eliminate the majority of bacterial illnesses effectively. One of the most popular applications for nanoparticles is their antimicrobial qualities (**Hetta et al., 2023**). **Saad et al. (2024)** demonstrated that silver nanoparticles AgNPs-H₂O₂ has a bactericidal effect. Additionally, aerA, exoU, and trh genes were noticeably down-regulated in *A. hydrophila*, *P. aeruginosa*, and *Vibrio* spp., respectively. According to **Anand et al. (2022)**, different zinc oxide nanoparticles ZnONP dosages showed distinct growth suppression zones against *V. harveyi*. According to **Korni et al. (2021)**, the use of ginger nanoparticles (GNPs) and *Saccharomyces cerevisiae* as feed additives is recommended for the prevention of *V. alginolyticus* infections in *Dicentrarchus labrax* farms.

Even though nanoparticles were discovered to overcome *Vibrio* spp., assessing their hazard to aquatic creatures is important. There is currently insufficient research on nanoparticles in aquatic animals. The risk of accumulating in animals and the viability of massive production and use from an economic standpoint will require a lot of investigation (**Sarkar et al., 2022**).

5. Phages

Phages, or bacteriophages are viruses that invade cells of bacteria. Bacteria lyse due to lytic phages interfering with their metabolism. Initially identified in the early 1900s, they were considered a potential way to treat bacterial illnesses. However, after the discovery of antibiotics, interest declined. Recently, phage treatment has regained attention due to the rise in genetic resistance to antibiotics. They are the most prevalent microbes in the world, especially in freshwater and marine habitats. In freshwater, they can survive for a few weeks, and in marine conditions, for more than five to seven months. The distribution of marine phage species in the water's interior is similar to that of their hosts, and they can be found in deep benthic settings, including the deep-sea floor, as well as near the surface. Despite minor localized variations, genetically identical phages can spread over great distances for decades (**Letchumanan *et al.*, 2015**).

The survival of bacteriophages is generally unaffected by temperature, salinity, pH, and the presence of organic materials. Bacteriophages can either exist as replicons, like *Vibrio* species, or as prophages that become integrated into the host's DNA. The association of prophages with lysogeny may vary and depends on the depth and geographic location of the marine species. Phages infect numerous bacterial pathogen species in fish (**Bondad-Reantaso *et al.*, 2023**).

CONCLUSION

The identification of *Vibrio* species from various sources has greatly benefited from the revolutionary discoveries and advancements in microbiology. Continuous advancements in molecular detection have made it possible to conduct higher throughput analyses with increased sensitivity and accuracy. This has resulted in reduced cost per sample and the ability to process a large number of samples for pathogen detection. These fast identification approaches use molecular processes that drastically reduce the time required to obtain results and have the ability to be automated and customized. However, these methods have some disadvantages, such as challenges related to pathogen quantity and nucleic acid integrity, including degradation and the existence of PCR hindrance. For example, samples that have extremely low bacteria loads may require a pre-enrichment step for detection, which can increase the risk of additional contamination. Proper staff training and sample handling procedures can help mitigate this risk. Therefore, the benefits and drawbacks of each rapid detection technique should be carefully considered when selecting the best approach for monitoring isolates of *Vibrio* species, based on the specific conditions and sample type. Additionally, studying the ecology and epidemiology of disease-causing organisms can benefit from the combination of conventional and molecular detection approaches.

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