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The Threat of Vibriosis in Aquaculture

Md Shadab Alam[1], Lipsa kuanr[2], Harshrajsinh Mahendrasinh Zankat[3], Abhay bharat Bamaniya[3] and Rajkumar M. Dabhi[4]

Abstract: -

The rapid expansion of aquaculture is accompanied by a rising number of disease outbreaks, posing significant challenges to production, profitability, and the long-term sustainability of the industry worldwide. Among the various pathogenic microorganisms affecting aquatic species, vibriosis is one of the most devastating, causing severe economic losses and leading to high mortality rates in farmed shrimp, fish, and shellfish. Traditionally, antibiotics and chemicals have been used in aquaculture to treat and prevent vibriosis. However, excessive reliance on these chemotherapeutic agents has contributed to the emergence of drug-resistant bacterial strains and has raised concerns about potential allergic reactions and toxicity in humans. As a result, alternative prophylactic strategies to enhance and stimulate immune responses in aquatic species have become increasingly necessary. This brief review explores the application of immunostimulants, vaccines, probiotics, and quorum-quenching molecules as potential approaches to disrupt Vibrio spp. communication and mitigate vibriosis in aquaculture.

Introduction

The increasing importance of aquaculture, driven by declining capture fisheries, has led to significant growth in global production. Statistics indicate that aquaculture production has been expanding at an average rate of 8.6% per year, reaching 90.4 million tonnes in 2012. This sector plays a crucial role in economic development, contributing over US$144.4 billion in farm sales and generating employment opportunities in fish processing, feed production, trade, and

Md Shadab Alam[1], Lipsa kuanr[2], Harshrajsinh Mahendrasinh Zankat[3], Abhay bharat Bamaniya[3] and Rajkumar M. Dabhi[4]

[1]M.F.Sc. Scholar, Department of Aquaculture (Fisheries Science), College of Fisheries Science, OUAT, Rangeilunda

[2]M.Sc Scholar, Department of Bioinformatics, Orissa university of Agriculture and Technology

[3]M.F.Sc. Scholar, Department of Aquaculture, College of Fisheries Science, Rajpur (Nava), Himmatnagar, Kamdhenu University

[4]Junior Research Fellow, Fisheries Research Centre, Kamdhenu University, Sikka

marketing. However, the intensification of aquaculture activities has also resulted in a rise in disease outbreaks, particularly bacterial infections, which pose a major challenge to sustainable production. Annual economic losses due to diseases in aquaculture exceed US$9 billion worldwide, with vibriosis being one of the most devastating bacterial infections. Caused by Vibrio spp., this disease has led to significant mortality in farmed fish and shrimp, resulting in substantial economic losses, especially in countries with major shrimp aquaculture industries. Vibrio pathogens have been shown to infect both saltwater and brackish water fish, with mortality rates reaching 100% under laboratory conditions. Moreover, their prevalence has been rising, particularly in coastal regions such as the North Sea. Given the growing impact of vibriosis on aquaculture, this review examines the pathogen's characteristics, socio-economic consequences, epidemiology, virulence factors, and control measures. Additionally, it highlights various prophylactic strategies designed to enhance immune responses, improve growth performance, and increase survival rates of aquatic organisms against Vibrio infections.

Vibriosis in aquaculture

Vibriosis is a significant bacterial disease affecting aquatic organisms and humans, caused by species within the genus *Vibrio*. These Gram-negative bacteria are ubiquitous in marine and estuarine environments, existing freely in the water column, within biofilms, or in association with hosts. While some *Vibrio* species are pathogenic, others, such as *Vibrio alginolyticus*, have been utilized as probiotics in shrimp farming.

In aquatic organisms, *Vibrio* infections manifest through lethargy, tissue necrosis, slow growth, body malformations, bioluminescence, muscle opacity, and melanization. Infected fish exhibit skin discoloration, red necrotic lesions in abdominal muscles, and erythema around the fins, vent, and mouth. Mussels infected with *Vibrio* display erratic movement and soft tissue deterioration. These bacteria are often opportunistic, causing disease when hosts are immunocompromised due to intensive culture practices or environmental stress. *Vibrio* species can be isolated on marine agar with incubation at 25°C for 2–7 days. Selective isolation can be performed using thiosulfate-citrate-bile salts-sucrose (TCBS) agar, incubated at 37°C for 18–24 hours. Pure colonies are typically cultured on tryptic soy agar (TSA) with 1% NaCl for further study. Long-term storage of isolates can be achieved in 30% glycerol at -80°C. Colony morphology is observed on Zobell 2116 E agar, and biochemical and physiological characterization follows the protocols of Smibert and Krieg (1994). Ongoing research into *Vibrio* biology and pathogenicity has led to the discovery of new species and a deeper understanding of long-recognized taxa. This knowledge is critical for developing effective management strategies in aquaculture and mitigating economic losses associated with vibriosis.

Virulence factors and pathology

The pathogenicity of *Vibrio* species in fish is characterized by a three-stage infection cycle: (i) pathogen entry, (ii) establishment and multiplication, and (iii) exit. These stages enable *Vibrio* to adhere to host tissues, evade immune defenses, and cause tissue damage. Virulence factors involved in these processes include motility, adhesion, host tissue degradation, iron acquisition, and immune evasion.

One of the most well-characterized virulence factors in *Vibrio anguillarum* is an efficient iron-sequestering system encoded by the 65-kb pJM1 plasmid. This system consists of a siderophore, anguibactin, and a membrane-receptor complex, which together facilitate iron uptake, a crucial factor for bacterial survival and virulence. The introduction of pJM1-encoded iron-uptake genes into avirulent *V. anguillarum* strains has been shown to enhance pathogenicity.

Other key virulence factors include:

- **Haemagglutinating activity**, which affects the intestinal tract and vascular system.
- **Leucocytolytic factors**, particularly in *Vibrio ordalii*, which disrupt immune cells.
- **Serum resistance**, allowing the pathogen to evade the bactericidal effects of non-immune serum.
- **Extracellular cytotoxins and cytolytic factors**, such as elastolytic proteases, which degrade host tissues.

The expression of virulence is influenced by interactions with the host microbiome. Recent studies suggest that *Vibrio* infections should not be considered isolated events but rather as complex interactions within microbial communities. Non-pathogenic *Vibrio* species can coexist with pathogenic strains, potentially influencing disease progression. The infection process begins with bacterial colonization of the host's skin, followed by tissue invasion. Chemotactic motility has been identified as essential for virulence. Additionally, bacteriophages play a role in transferring virulence genes through lysogenic conversion, whereby non-pathogenic strains acquire virulence traits. Phage-encoded proteins enhance bacterial invasion, immune evasion, and tissue damage. Among the most critical virulence factors are lytic enzymes, including hemolysins, proteases (such as caseinase and gelatinase), which degrade host tissues, facilitating nutrient acquisition and bacterial dissemination. Understanding these virulence mechanisms is crucial for developing effective strategies to control *Vibrio*-related diseases in aquaculture.

Control of *Vibriosis* in Aquaculture

The widespread use of antibiotics in aquaculture for controlling *Vibrio* infections has led to significant challenges, including antibiotic resistance, food safety concerns, and market restrictions. The administration of antibiotic-medicated feed and oral treatments for infected fish is a common practice. However, the overuse of these antimicrobial compounds has resulted in the emergence of drug-resistant *Vibrio* strains, reducing treatment efficacy. Beyond resistance, antibiotic residues in aquaculture products pose a serious public health risk, leading to allergic reactions and toxicity in humans. Many importing countries, including the United States, European Union, Japan, and Australia, have strict regulations against antibiotic residues in seafood. Between 2002 and 2010, U.S. authorities rejected 2,400 consignments of Vietnamese seafood due to antibiotic contamination, while the EU, Japan, and Australia rejected 422, 464, and 206 shipments, respectively. These rejections have led to significant economic losses and diminished competitiveness in the global seafood market. The prevalence of antibiotic resistance in aquaculture environments is alarming. A study by found that 74% of *Vibrio* spp. isolated from aquaculture facilities in Australia were resistant to at least one antibiotic. This highlights the urgent need for alternative strategies to manage *Vibrio* infections while ensuring sustainable aquaculture production.

Immunostimulation

Immunostimulants are chemical compounds that enhance the immune response of aquatic organisms, making them more resistant to infections caused by bacteria, viruses, fungi, and parasites in aquaculture environments. These compounds work by activating leukocytes (white blood cells), thereby strengthening both innate and adaptive immunity.

According to Raa (1996), immunostimulants can be classified into several categories, including:

1. **Structural elements of bacteria**, such as lipopolysaccharides, capsular glycoproteins, and muramyl peptides.
2. **β-1,3 glucans** derived from bacteria and mycelial fungi.
3. **β-1,3/1,6-glucans** from yeast cell walls.
4. **Glycans** from various biological structures.
5. **Peptides** with immune-boosting properties.

6. **Nucleotides** that support immune function.
7. **Synthetic immunostimulants** developed for targeted immune enhancement.

The use of immunostimulants has been widely studied for their effectiveness in enhancing disease resistance in aquaculture species. Research has shown their ability to improve immune function in shrimp and other aquatic organisms. Specifically, various commercial immunostimulants have been successfully used to enhance fish resistance against vibriosis.

However, the effectiveness of immune stimulants can vary. Bridle et al. (2005) reported that dietary administration of β-glucan to Atlantic salmon (*Salmo salar*) increased respiratory burst activity (RBA) in head kidney macrophages *in vitro*. However, *in vivo* challenge tests showed a negative effect on RBA, serum lysozyme production, and disease resistance to *Vibrio anguillarum* and *Dicentrarchus labrax* against amoebic gill disease. These findings highlight the importance of optimizing immunostimulant dosage and application methods to achieve consistent and effective disease resistance in aquaculture.

Probiotics

The use of probiotics as a prophylactic approach in aquaculture has gained significant interest in recent years. Probiotic strains can be isolated from both indigenous and exogenous microbiota of aquatic animals. Studies in penaeid aquaculture ponds have demonstrated that adding selected strains of *Bacillus* to pond water reduces *Vibrio harveyi*-induced mortality.

While probiotics offer a promising alternative to antibiotic treatments, their application has limitations. The need for regular supplementation to maintain high concentrations of beneficial bacteria is a major challenge. This ongoing requirement makes probiotic techniques less efficient and cost-effective compared to other disease management strategies.

Further research is needed to develop more sustainable probiotic delivery systems, such as encapsulated probiotics or biofilm-based applications, to enhance their long-term effectiveness in aquaculture.

Quorum sensing

Quorum sensing (QS), as defined by Defoirdt et al. (1997), is a communication mechanism that allows bacteria to regulate the expression of specific genes in response to the concentration of small signaling molecules. In Gram-negative bacteria like *Vibrio*, QS operates through either a single-channel or multi-channel system. The single-channel system relies solely on Acylated Homoserine Lactones (AHLs) as signaling molecules.

According to Miller and Bassler (2001), AHLs are synthesized by the LuxI protein and detected by the LuxR protein. Once the signal reaches a critical concentration, LuxR binds to the autoinducer, triggering the transcription of the *luxICDABE* operon, which encodes luminescence-related genes. Exposure to AHLs has been linked to high mortality in various aquaculture species, likely due to the activation of QS-controlled bacterial virulence factors. To counteract QS, researchers have been developing non-bacteriostatic compounds that interfere with bacterial communication, thereby reducing pathogen virulence and allowing the host to better control infections. In the context of vibriosis, halogenated furanones, particularly *4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone*, have been shown to inhibit QS-regulated gene expression in *Vibrio harveyi* by reducing the DNA-binding activity of its QS response regulator. Additionally, various aquatic organisms—including microalgae, macroalgae, invertebrates, and certain bacteria—have demonstrated the ability to disrupt QS through mechanisms such as enzymatic degradation of signals, chemical inactivation, and antagonistic or agonistic interactions. Utilizing natural resources to disrupt bacterial communication presents a promising, sustainable strategy for managing *Vibrio*-induced infections in aquaculture.

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