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Diseases in shrimp aquaculture

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Abstract- Shrimp aquaculture in India is a major economic activity, with significant production and export values in 2019-2020. However, the industry is severely impacted by various diseases, including microbial infections and viral outbreaks. Key emerging diseases include White Faecal Syndrome (WFS), often linked to the microsporidian parasite Enterocytozoon hepatopenaei (EHP), and increased occurrences of White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV). Vibrio species are prominent bacterial pathogens causing substantial economic losses, with transmission occurring through water, mucus surfaces, and the digestive tract. Other bacterial genera such as Erythrobacteraceae, Alteromonas, and Shewanella have also been isolated from shrimp ponds. Shewanella algae is of particular concern, with little information on its biochemical profiles and infection in aquaculture.

Disease prevalence is influenced by water quality parameters like temperature, salinity, and pH, as well as factors such as increased stocking density and culture intensity. Histopathological studies show that pathogenic bacteria, such as Vibrio parahaemolyticus, rapidly target the hepatopancreas and intestinal epithelial cells of shrimp. Management strategies include the use of probiotics like Bacillus and lactic acid bacteria, which offer competitive exclusion, antiviral effects, and immune enhancement. Herbal extracts, such as those from Allium sativum and Thymus vulgaris, also demonstrate strong antibacterial properties. Other approaches include vaccination, bacteriophages, and immunostimulants. The presence of pathogenic bacteria in shrimp also poses a risk of seafood-borne illnesses to humans, underscoring the importance of proper refrigeration and monitoring from harvest to consumption.

Keywords: Shrimp diseases, Vibriosis, Aquaculture.

I. INTRODUCTION

India is cashing on demand by increasing the area under shrimp aquaculture. During the year 2019-20, the total shrimp production in India was 6,39,896 tons and shrimps of value Rs. 34152.03 crores were exported from India (Handbook of Fisheries Statistics, 2020). Shrimps are decapod crustaceans and a group of organisms with approximately 233 families and 725 genera (De Grave et al., 2009). It is one of the most popular types of seafood consumed all over the world (FAO, 2019). With the rising population, there is a huge demand for food products based on shrimps (Kobayashi et al., 2015).

Disease outbreak

Diseases have been a major source of shrimp farming setback Litopenaeus vannamei related microbial diseases and fatalities have been reported from the south-eastern part of India (Karunasagar et al., 2004; Gunalan et al., 2014). Epidemics involving microbial agents have grown in numbers over time, paralleling the growth and intensification of culturing systems. Indian cultivated shrimp production fall in 2016 due to growth retardation and spread of diseases (Salunke, 2018). Shrimp farmers all over the country have been dealing with new and emerging diseases. One of the emerging diseases namely White Faecal Syndrome (WFS), often associated with poor growth of L. vannamei has been mostly associated with a microsporidian parasite called Enterocytozoon hepatopenaei (EHP) (Otta et al., 2016). Furthermore, there has been an increase in the incidence of white spot virus (WSSV) and Infectious hypodermal and haematopoietic necrosis virus (IHHNV) outbreaks in many coastal regions of Andhra Pradesh, heavily affecting production in these regions (Balasubramanian et al., 2018). The other commonly reported diseases in Litopenaeus vannamei farms (Fig.I) in India has been identified as Vibriosis, Running Mortality Syndrome (RMS), bacterial white spots, White Muscle Syndrome (WMS), muscle cramping and White Gut Disease (WGD) (Salunke, 2018).

Among the well-known bacterial pathogens responsible for massive economic losses in shrimp culture, Vibrios are the most dangerous and important component of the autochthonous microbiota of the marine environment. (Longyant et al., 2008). Once unfavourable environmental conditions are created, they are distributed widely in culture facilities, causing a variety of harmful infections to aquatic animals pertaining to such habitats (Moustafa et al., 2010; Elgendy et al., 2015). The mode of transmission for Vibrio infections is through water, mucus surfaces and digestive tract (Yan et al., 2007). Direct contact in populated aquaculture conditions also hastens its spread (Kanno et al., 1989). The skin of the fins and gills, whether intact or worn away, is an important site for vibrio attachment and intrusion (Spanggaard et al., 2000). Numerous Vibrio species, like V. harveyi, V. anguillarum, V. vulnificus and V. alginolyticus have long been considered among the most damaging shrimp pathogens, creating severe stock losses (Nash et al., 1992; Gopal et al., 2005; Longyant et al., 2008).

In addition to Vibrio, bacteria of genus Erythrobacteraceae, Alteromonas, Exiguobacterium, Pseudoalteromonas, Halomonas, Psychrobacter, Sulfitobacter, Salegentibacter and Shewanella were also isolated from shrimp ponds by various authors (Sombatjinda et al., 2011; Chankaew et al., 2017; Cao et al, 2018; Alfiansah et al., 2018). Among these, Shewanella putrefaciens and S. algae are bacilli that do not ferment and have a single polar flagellum. They produce 1-2-mm yellowish-brown coloured colonies on MacConkey agar after 18-24 hours of incubation. S. algae and S. putrefaciens cannot be distinguished using automated identification systems (Holt et al., 2005). Nozue et al. (1990) discovered that a large number of clinical isolates previously identified as S. putrefaciens were actually S. algae. Hence, there is little evidence on S. algae in terms of its biochemical profiles and information of infection in aquaculture to date (Holt et al., 2005).

Fig.I: Ponds with polythene lining

Fig. II: Check tray

Fig. III: Antenna cut in shrimp

Disease occurrence in shrimp farms

The major isolates from penaeid shrimps infected with the epizootic black-gill and brown-spot of shell disease syndrome were V. pelagicus and V. alginolyticus (Baticados et al., 1990). Black gill disease is also associated with protozoa Epistylis, Vorticella, Zoothamnium, together with bacteria and Fusarium fungus (Baticados et al., 1990). Gregarine disorder in shrimps is caused by Nematopsis spp. while Agmasoma sp. causes cotton shrimp disease (Baticados et al., 1990). Some virus-caused diseases stand out as highly significant among the infectious diseases of cultured shrimp. Pandemics had been reported due to the penaeid viruses TSV (Taura Syndrome), WSSV (White spot), YHV (Yellow Head virus) and IHHNV (Infectious Hypodermal and Hematopoietic Necrosis virus) (Chakraborty et al., 2002). The occurrence of WSSV (White Spot Syndrome Virus) in black tiger shrimp, Penaeus monodon, became widespread over a wide area in Tamil Nadu, from Visakhapatnam to Sirkali during 1994 (Anon, 2002).

These diseases are reported from India, Taiwan, North and South America, South East Asia, Central Asia and the Middle East (Shekar et al., 2012). Following the WSSV infection, loose shell syndrome (LSS) appeared in shrimps (Raja et al., 2015). Bacterial infections caused by V. proteolyticus, V. parahemolyticus, V. coralliilyticus and V. alginolyticus were also reported in India in 1998 (Anon, 2002). During random survey in 2006-08, shrimp farms in nine coastal states disclosed that there is loss of gross shrimp production totaled 48,717MT, worth INR 10,221 million due to shrimp diseases (Kalaimani et al., 2013). Gunalan et al. (2014) reported black gill disease caused by fungus, Fusarium sp. and muscle cramp disease. The pathogen Enterocytozoon hepatopenaei causes hepatopancreatic microsporidiosis in P. monodon and L. vannamei (Tang et al., 2016).

Detection and Characterization of pathogenic bacterial species

Pathogenic bacteria are routinely identified using morphological and biochemical tests, which are supplemented as needed by specialised tests such as antibiotic inhibition patterns and serotyping. Culture-based methods are widely used for regulatory monitoring of pathogenic bacteria as they are low-cost, simple to use and highly standardized (Tiwari et al., 2021). The major drawbacks of these methods are the inability to distinguish between the target and other non-target endogenous microorganisms in the samples, positive results/ false negative, time-consuming, labour-intensive procedures (Fig. II) and the inability to detect viable but nonculturable (VBNC) cells (Sohier et al., 2014). As a result, molecular methods have become the standard detection techniques for pathogenic bacteria as a fast analysing tool with high accuracy and specificity.

These are classified into nucleic acid targeting methods and the protein/antigen targeting methods (Deshmukh et al., 2016). Fluorescence amplification-based methods such as polymerase chain reaction (PCR), digital PCR (dPCR), quantitative or real-time PCR (qPCR), deoxyribonucleic acid (DNA) microarray, molecular beacon, fluorescence in situ hybridization (FISH), illumina sequencing, pyrosequencing and nanopore sequencing are examples of nucleic acid targeting methods (Deshmukh et al., 2016). A traditional antibody-antigen interaction method similar to immunological methods (enzyme-linked immunosorbent assays and lateral flow tests) is included in the protein and antigen targeting method (Gilbride, 2014). Combination of paper-based and biosensor-based devices have evolved into an inexpensive, rapid and portable on-site method for pathogenic bacteria detection (Deshmukh et al., 2016).

Pathogenicity of bacterial species in shrimps

Lethal toxins has been produced by some virulent bacterial strains (e.g., haemolysins, cysteine protease) which can harm the intestinal epithelial cell lining, allowing opportunistic bacteria to infiltrate other organs and tissues of the body (Soonthornchai et al., 2010). Many ecological and epizootiological studies on the distribution of virulent strains indicate that waterborne bacteria are a major source of infection (Goarant et al., 1999). As a result, the immersion route of infection appears to be a suitable method for testing bacterial virulence experimentally.

In contrast, there have been few reports of other natural paths of infection. When animals were orally administered, de la Pena et al. (1995) were able to infect P. japonicus prawns with low amounts of V. penaeicidaisolate (103-104 CFU), whilst Lightner and Lewis (1975) discovered that adding virulent V. alginolyticus isolates to shrimp feed was ineffective in causing infection. The intramuscular route of infection between the fifth and sixth abdominal segments is also the most commonly used method (Liu et al., 1996). Haldar (2003) isolated V. cholerae and V. parahaemolyticus from P. monodon and tested them against juveniles and post larvae using intramuscular injection and bath challenge, respectively. Higher mortalities were reported in bath challenge and were more damaging than intramuscular injection.

Status of Vibriosis in shrimp ponds

The disease infected pond water contained103 to 105 CFU/mlVibrio concentrations and bottom soil had 105 to 107 CFU/g (Haldar, 2003). Alfiansah et al. (2018) discovered that the abundances of potential pathogenic Vibrio (TPPV) and cultivable heterotrophic bacteria (THB) increased with rearing time and peaked at day 60, at concentrations of 1.4 ×104 CFU mL-1 of TPPV, 3.4 × 107 CFU mL-1 of THB on 0 day and 2.65 105 CFU mL-1 of TPPV, 2.6 × 107 CFU mL-1 of THB on 60 day.

A few Vibrio species and strains found in aquatic environments, including estuaries, rivers and sea waters are infectious and pathogenic causing "vibriosis" (Kumar et al., 2017). Vibrio is also found in the water column and is part of the biofilm that forms on submerged surfaces. Pathogenic or opportunistic Vibrio bacteria infections can be disastrous, especially during the production stage of crustacean larvae (Kumar et al., 2017).

Vibriosis outbreaks frequently cause significant production losses and acute hepatopancreatic necrosis disease (Schryver et al., 2014). According to reports, AHPND caused a $44 billion loss in production in Malaysia, China, Thailand, Vietnam and Mexico, between 2010 to 2016 (Tang and Bondad-Reantaso, 2019). Vibriosis illnesses in crustaceans have also been observed to include luminescent and hatchery vibriosis, tail necrosis, shell disease syndrome, limp lobster disease,summer syndrome, red body disease and Vibrio-caused bacteremia (Radhakrishnan and Kizhakudan, 2019).

Status of Shewanella in shrimp ponds

Shewanella spp. are gram-negative rods, able to colonise both in anoxic and oxygenated environments all over the world, ranging from freshwater (Fig. III) and marine ecosystems to sediments and soils due to their remarkable physiological versatility and diversity (Hau and Gralnick, 2007). Shewanella spp. are found in the gut microbiota of many marine, freshwater animals and their presence is also recorded in human microbiome studies (Flemer et al., 2017). They are increasingly being reported to cause infections with S. algae accounting for more than 80 percent of the cases (Janda and Abbott 2007). Four species have been identified as S. algae, S. haliotis, S. xiamenensis and S. putrefaciens as human pathogens (Mart'n-Rodr'guez et al., 2017).

Shewanella species have been linked to huge mortality in Sciaenops ocellatus (Zhang et al., 2013), Carrasius auratus (Altun et al., 2014), Babylonia and Cynoglossus semilaevis (Li et al., 2015; Han et al., 2017). However, there is little information on S. algae isolates as potential pathogens for P. vannamei grown in brackish water. Enzymatic activity, cytotoxin secretion, adhesion ability, lipopolysaccharide and the presence of siderophores are all involved in the pathogenesis of Shewanella infections (Padzior, 2016). The production of these virulent factors is usually associated with diseases caused by Shewanella species in aquaculture. When challenged with a concentration of 2.65×105 CFU/mL, S. algae (SFH3) caused LD50 mortality in healthy P. vannamei (Cao et al., 2018).

Relationship between pathogenic bacteria and Water quality parameters

Temperature, salinity, nutrients and pH in the water column have an impact on the existence of pathogenic bacteria such as Vibrio which are seasonal and are most commonly found during the summer months, when temperature is higher and rainfall is lower (Cheng et al., 2005). Bacterial cells are commonly found in a non-culturable stage during the winter months (Radhakrishnan and Kizhakudan, 2019).

Other Factors contributing to disease occurrence

The unused nutrients lead to a change in dissolved oxygen, pH in the water column, pond sediment, proliferation of plankton, bacteria, an increase of particulate organic matter and eutrophication (Avnimelech et al., 1994; Martin et al., 1998). Furthermore, Kautsky et al. (2000) found that the risk of shrimp diseases increased with increased stocking density and culture intensity.

Effect of aeration on shrimp culture

Aquaculture employs a variety of mechanical aerators, including paddlewheel, vertical turbine, long arm, venturi aerators and diffuser (Boyd et al., 2018). According to Boyd et al., (2018), the total aeration capacity of these aerators ranged from 22.5 hp/ha to 89.6 hp/ha. Long-arm aerators provided the majority of installed aeration on all shrimp farms. Shrimp yields ranged from 6.9 to 25.0 tons/ h/ crop. 234 to 476 kg/hp was the range of installed aerator capacity to shrimp yield ratio. Cordova et al. (1997), on the other hand, conducted a study in northwest Mexico to assess the effect of aeration rate on the survival, growth, and yield of Penaeus vannamei. Aeration rates of 0, 6, 12, and 24 hours per day were testedbut no significant differences in growth rates were found. Ponds with 12 and 24 hours of aeration had significantly higher yield and survival.

The type of aerator used is also determined by the production system. Aerotube units used in biofloc provided micro-size bubbles and created uniform circulation and water movement to speed up the biofloc formation process (Chaignon et al., 2002). In contrast, air stone units used in biofloc created a vigorous and disproportionate movement of water, collapsed the suspended biofloc and generated anaerobic condition at the bottom of the tank (Choo and Caipang, 2015) resulting in deterioration of water quality and survival of organisms.

Effect of microalgae addition on shrimp culture

Microalgae are very well recognised factor which play an important role in shrimp larval nutrition. Some reports suggested that microalgae are prevalent in shrimp intestines due to inadvertently ingestion with bait by shrimps (Martnez-C'ordova and Pena-Messina, 2005; Kent et al., 2011).

Microalgae are also a good source of nutrients (essential amino acids and minerals, lipids, essential fatty acids) for crustaceans, abalone and cultured fish in their early stages of life (Conceiçao et al., 2010; Courtois de Viçose et al., 2012) and for farmed bivalves throughout their lifecycle (Aji, 2011). Godoy et al. (2011) proved that diatom supplementation during L. vannamei nursery rearing not only enhanced shrimp survival, but increased the weight gain and feed conversion efficiency. The inclusion of microalgae in the rearing system could produce anti-bacterial substances (e.g., tropodithietic acid) that are harmful to Vibrio spp. and inhibit the fast growth of pathogenic microbes (D'Alvise et al., 2012). Microalgae are effective at removing nutrients, efficiently treating aquaculture wastewater and reducing pollution in nearby areas (Han et al., 2019).

Histopathological studies in bacterial infected shrimps

According to Khimmakthong and Sukkarun (2017),V. parahaemolyticus spreaded rapidly by using hepatopancreas as a target organ. At 1 minute after exposure, histopathology indicated an absence of B (vesicular), F (fibrillar) and R (reabsorptive)cells in the hepatopancreatic tubule and shrimp epithelial cells, cellular inflammatory infiltration of submucosa and sloughing of tunica mucosa in the shrimp intestines. At 6 hours after exposure, histopathology revealed degeneration of shrimp basement membrane, severe sloughing of haepatopancreatic tubule epithelial cells and the absence of B, F and R cells of tubule epithelial cells in the shrimp hepatopancreas. Hemocytic infiltration of the intertubular space and sloughing of shrimp haepatopacreatic tubule epithelial cells 12 hours after exposure was also recorded (Rendón et al., 2020).

Antibacterial efficacy of probiotics

Probiotics, described as microbes which, when administered in sufficient amounts, impart a health benefit on the host (Hill et al., 2014), are becoming prominent antibiotic alternatives in shrimp aquaculture to enhance growth and reduce disease. To date, roughly 20 bacteria genera were seen having a probiotic effect in shrimps, however the majority of research has concentrated on Bacillus and lactic acid bacteria, such as Lactobacillus, due to their widespread use and success as probiotics in poultry and mammals. There is strong evidence that they can accord probiotic effects via competitive exclusion of pathogenic bacteria, antiviral effects, enhancement of the shrimp immune response, enzymatic and nutrient contribution to shrimp digestion (Ring, 2020). Probiotics can be given orally with feed (Immanuel, 2016), directly into water as spores or purified cultures (Ring, 2020) or within a fermented growth medium, such as Bacillus subtilis (E20) fermented soybean meal (Wang et al., 2019).

Antibacterial activity of herbal extracts in infected shrimps

Herbal remedies do not have side effects that synthetic drugs cause and they have a high therapeutic potential for treating a variety of infectious diseases in shrimps (Aminzare et al., 2015). They have strong antibacterial properties and play an important role in the treatment of various illnesses around the world (Aminzare et al., 2017). The most important plants with anti-vibrio activity are Allium sativum, Camellia sinensis, Cuminum cyminum, Eucalyptus globules, Mentha spicata, Punica granatum,Rosmarinus officinalis, Satureja bachtiarica, Syzygium aromaticum, Thymus vulgaris, Zataria multiflora, and Zingiber officinale(Aminzare et al., 2018). Phytochemical studies showed that T. vulgaris (thyme) extract and its essential oils contain antimicrobial components such as thymol, linalool, carvacrol, terpineol and geraniol (Borugă et al., 2014).

Management of bacterial diseases in shrimp ponds

Vaccination is the practise of administering pathogenic bacteria that have been weakened or killed in order to provide long-term protection via immunological memory (Gudding et al., 1999). Many strategies are being used to enhance shrimps adaptive immune systems. Bacteriophages (phages) have been proposed as potential aquaculture therapeutics (Nakai, 2002). As bacteria's natural enemies, they could reduce pathogenic bacteria in a safe, effective and environmentally friendly manner. Immunostimulants used to treat vibriosis in shrimp improved survival rates (Marques et al., 2005).

Antibiotics are promising molecules for the first-line treatment of bacterial diseases (Sorum, 2006). Antibiotic-based post-infection therapy continues to remain the preferred method for many farmers (Selvin and Lipton, 2009). Herbs also act as antibiotics in the aquaculture sector, reducing or controlling pathogen infection and increasing organism survival rates during management of disease outbreak. In the aquaculture sector, a significant proportion of herbs act as antibiotics, anti-pathogenic agents to enhance organisms' immune systems and protect them from disease.

Role of bacterial infected shrimpsin sea food borne illness

Contaminated seafood consumption is a leading cause of death and hospitalisation, particularly in developing and poor countries. Seafoods, like shrimps, are not immune to food-borne pathogens and there are several risks associated with consumption of infected shrimps. Vibrio spp., Listeria monocytogenes, Salmonella spp., Clostridium botulinum, Staphylococcus aureus, Aeromonas spp. and Shigella spp., are considered microbiological hazards for seafood (Marques et al., 2005). These bacteria could pollute seafood products from farm to table (Marques et al., 2005). Intoxications that are food-borne can be avoided by appropriately refrigerating seafood and continuously monitoring the chill chain throughout the entire production process from harvest to consumption (Ali et al., 2020).

CONCLUSION

This review highlights the significant impact of diseases on shrimp aquaculture, a sector crucial to India's economy. The intensification of culturing systems has unfortunately paralleled an increase in microbial epidemics, leading to substantial production losses. Notably, White Faecal Syndrome (WFS), often linked to Enterocytozoon hepatopenaei (EHP), and outbreaks of White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) have severely affected shrimp production in coastal regions of Andhra Pradesh. Other prevalent diseases in Litopenaeus vannamei farms in India include Vibriosis, Running Mortality Syndrome (RMS), bacterial white spots, White Muscle Syndrome (WMS), muscle cramping, and White Gut Disease (WGD).

Among bacterial pathogens, Vibrios are particularly dangerous, causing massive economic losses and spreading through water, mucus surfaces, and the digestive tract. Unfavorable environmental conditions facilitate their wide distribution in culture facilities. Beyond Vibrio, other bacterial genera like Erythrobacteraceae, Alteromonas, and Shewanella have been isolated from shrimp ponds. Shewanella algae and Shewanella putrefaciens are bacilli that produce yellowish-brown colonies and have been associated with significant mortality in various aquatic animals.

Effective disease management is paramount. While traditional culture-based methods for detecting pathogenic bacteria are low-cost and standardized, they suffer from limitations such as being time-consuming and unable to detect viable but nonculturable cells. This has led to the adoption of rapid and accurate molecular methods, including PCR, qPCR, and DNA microarray, for pathogen detection.

Prevention and treatment strategies include the use of probiotics, which enhance shrimp growth and reduce disease by competitively excluding pathogens, boosting immune responses, and contributing to digestion. Herbal extracts also show promise due to their strong antibacterial properties and lack of side effects associated with synthetic drugs. Plants like Allium sativum and Thymus vulgaris exhibit anti-vibrio activity. Furthermore, vaccination and bacteriophages are emerging as potential therapeutics, offering long-term protection and environmentally friendly pathogen reduction. Maintaining optimal water quality parameters like temperature, salinity, and pH is crucial, as these factors influence the prevalence of pathogenic bacteria. High stocking density and culture intensity also increase disease risk. Finally, the review underscores the importance of proper refrigeration and continuous monitoring of the chill chain to prevent seafood-borne illnesses from contaminated shrimp.

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