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ANALYSIS THE INFECTION ASSOCIATED WITH VIBRIOS OF SPECIES OF SHRIMP

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ABSTRACT

When it comes to raising shrimp commercially in WestBengal,India vibriosis is a problem. A few of the traditional brackish water ponds remain after disease epidemics wiped out the shrimp farming sector in Tajpur of West Bengal. The goal of this study was to catalogue the many Vibrio species responsible for shrimp of vibriosis in saltwater pools, as seen in their natural environments. Using an exploratory method and selecting participants at random, this study uncovered new information. Twenty-four shrimp, most likely ill with vibriosis, remained composed from binary region areas on the southern shore of Tajpur, WestBengal in the months of July and September of 2018. Bacteria linked to shrimp vibriosis were cultivated in TCBS medium from the shrimp's inner hepatopancreas and telson. To determine which microbial stressors are associated with shrimp vibriosis, we used rep-PCR (replicate-polymerase chain reaction). Following analysis of the rep-PCR data, five strains were selected as good representatives for use in subsequent studies. The 16S rDNA sequences of the JKP19, JKP05, JKP03, JKM06, and JKM01isolates were most similar to those of Vibrio diabolicus, Shewanella algae, Vibrio parahaemolyticus, Vibrio rotiferianus, and Vibrio rotiferianus. Vibriosis-causing vibriosa were found in shrimp, and they came in many different forms. These results suggest vibriosis may be an issue with current methods of shrimp farming. It is important to develop vaccines, probiotics, and immunostimulant formulations to prevent and control the spread of shrimp vibriosis in traditional saltwater ponds.

Keywords: Shrimp, Shewanella algae, Vibrio diabolicus, Vibrio rotiferianus, Vibrio parahaemolyticus, Vibrio alginolyticus.

1. INTRODUCTION

Due to its proteinaceous composition, export potential is raised, and profit yield is considerable, making shrimp farming a prominent player in the aquaculture business worldwide. [1] Since the 1980s, shrimp farming for the penaeid species has become a major industry in the world's tropical and subtropical regions. Shrimp output climbed from 1,564,563 metric tonnes in 2017 to 2,002,449 metric tonnes in 2019. To be specific, the black tiger shrimp, *Penaeus monodon*, Commercially significant members of the Penaeidae family include the Fenneropenaeus indicus (Indian white shrimp) and the *Litopenaeus vannamei* (Pacific white shrimp).[3] *F. indicus* is significant to commercial fisheries throughout India's east and west coasts because it thrives in both marine and estuarine conditions. [2] The United States, Europe, and Japan have all relied on shrimp from India as a reliable source. A variety of shrimp species are farmed in India's coastal regions, including the Pacific white shrimp (L. vannamei), the white shrimp *(P. penicillatus*), Indian white shrimp *(F. indicus*), green tiger shrimp *(P. semisulcatus)*, the black tiger shrimp *(P. monodon*), and the banana shrimp *(P. merguiensis*).[4] There have been significant episodic dead owing to illness of the cultivated shrimp, despite the fact that production of cultured shrimp has increased. Viruses were responsible for 50% of disease-related losses, whereas bacteria were responsible for roughly 22%, according to a survey conducted by the Global Aquaculture Alliance (GAA) [5]. Shrimp production losses on a global scale have prompted studies into the prevention and treatment of infectious illnesses, which are vital to the continued success of the aquaculture sector. The GOAL (Global Outlook in Aquaculture Leadership) GOAL conference was seized in Chennai, India in October, and the forecast for 2019 was a decrease in production in the Indian aquaculture business. GOAL predictions that India will maintain a level of production of around 600,000 tonnes in 2019 and 2020, down from as much as[6].

Each and every aquatic crustacean is at risk for infection with Vibrio and the diseases it causes. Vibriosis outbreaks are a regular cause of significant production losses, and diseases like AHPND (acute hepatopancreatic necrosis disease) have posed a danger to the sustainability of shrimp farming. Between 2010 and 2016, AHPND was estimated to have cost India, Malaysia, China, Mexico, Vietnam and Thailand a combined US$44 billion in lost production. In addition to hatchery and luminous vibriosis, other vibriosis disorders affecting crustaceans include case illness syndrome, limp lobster sickness tail necrosis, bacteremia red body disease, induced by Vibrio, and seasonal syndrome[7]. Vibrio has a significant negative impact on global aquaculture productivity, leading to substantial societal and economic losses and posing a threat to international food security. A species of Vibrio responsible for economically significant vibriosis infections in crustaceans, and a thorough assessment of both the Vibrio species and their typical host species, are identified. [8] Vibriosis can harm a wide variety of aquatic crustacean species; therefore, it is important to learn about Vibrio's biocomplexity, disease features, epidemiology, microbial ecology, and methods for mitigating its effects. No systematic study of Vibrio and the vibriosis diseases they cause that are important to decapod crustacean aquaculture has been conducted to date, despite the fact that these organisms have been the subject of a great deal of research. This review aims to fill that information gap by concentrating on economically significant crustaceans, including shrimp, lobsters, and crabs. Cecilia de Souza Valente (2021). Bacteria belonging to the type Vibrio are native to water ecosystems and can be found virtually anywhere where aquaculture is practised. Many Vibrio species are completely harmless and are found naturally in healthy aquacultured animals. Unfortunately, a number of diseases fall under the umbrella term "vibriosis," which is caused by pathogenic Vibrio species and strains. Shrimp, lobsters, and crabs are all susceptible to these infections, which can have a devastating effect on animal productivity. Because of this, vibriosis can threaten global food security and the ability to keep up with rising demand for food. Vibriosis is preventable with proper preventative measures. Among these are the provision of adequate nutrition, the implementation of preventative and curative measures, and the implementation of an effective health management plan. Prebiotics, probiotics, quorum sensing disruption, synbiotics, biofloc, immunological priming, green water, and bacteriophages could be used to prevent and treat vibriosis. This article aims to give readers with an in-depth summary of the existing knowledge around Vibrio and vibriosis in farmed crustaceans (i.e., shrimp, lobster, and crabs). Furthermore, the review will point out any holes in the current literature, laying the groundwork for where future research should go.[8]

The Septicemic Effects of Bacterial Pathogens (Vibrio disease)

In shrimp species like *P. monodon* and *P. vannamei*, the bacterium Vibrio parahaemolytics causes a severe systemic disease known as AHPND (acute hepatopancreatic necrosis disease). [9] Due to chromatophores, the periopods of infected animals turn a bright red, and other symptoms include damaged gills and black blisters on the carapace and belly. Severe bacterial infections in shrimp are also possible. Vibrio spp., including Vibrio alginolyticus, Vibrio anguillarum, and Vibrio parahaemolyticus, contribute to the pathophysiology of this disease. [5]

Luminous bacterial diseases

In addition to wreaking havoc on the economy and reducing production rates, this bacteria also poses a significant threat to aquaculture operations. Shrimp infected with these luminescent bacteria may seem fluorescent or luminescent in the dark. Virulent Vibrio harveyi is a primary causal agent of this issue in breeding facilities. Using Zobell's Marine Agar, the luminous bacteria could be separated, and then their morphological and biochemical features could be studied.[10]

Spots of brown coloration (rust disease or Shell disease)

The chitinolytic bacteria Flavobacterium spp Vibrio spp., and Aeromonas spp. may be responsible for the black and brown corrosions seen on the skin and appendages of infected animals. [15] The Fourth Order of Arthropods Confirmation of a diagnosis based on more easy findings, including physical symptoms, was obtained by further testing, which included isolating bacteria from the infection site using Zobell's Marine Agar and identifying the pathogen.

Appendageal necrosis
Shrimp with this condition develop necrosis at the tips of their swimmerets, uropods, and walking legs, turning them a dark brown colour. [13] Oftentimes, the antennae, setae, and other attachments are mangled and darkened by the time we see them. The gross symptoms in infected shrimps were caused by epibiotic bacteria like *Flavobacterium sp., Pseudomonas sp., Vibrio sp.,* and *Aeromonas sp.*

Vibrio in developing shrimp embryos
Appendage necrosis, enlarged chromatophores, an empty gut, no faecal strands, and poor feeding are all symptoms observed in infected larvae. [16] In just a few days, the overall death toll might be in the 80-percent range. Infections with Vibrio alginolyticus, Vibrio parahaemolyticus, and Vibrio anguillarum led to this illness. [12]

Spiral-shaped bacterial infection
Fouling of the setae, gills, body surface and appendages is observed in the affected shrimp larvae. [18] The shrimp are unable to successfully moult and may perish as a result of low oxygen levels. This illness is caused by filamentous bacteria like Leucothrix mucor. Fouling of the body surface and appendages by filamentous bacteria can be seen under the microscope, confirming a diagnosis of filamentous bacterial illness in shrimp larvae that was initially based on gross symptoms.[11]

2. MATERIALS AND METHODS

Shrimps Sampling
This research employed an exploratory approach and used a purposive sample. Traditional brackish water samples were taken from the Tajpur in west Bengal where the presence of vibriosis is suspected. Before bringing them to the lab for bacterial isolation, the samples were kept cool in an enclosed container. Measurements of the shrimp samples were used to create categories (16.9–17.2 cm). From there, we randomly selected three *L. vannamei* (*Pacific white shrimp*) and three *P. monodon* (*black tiger shrimp*) from each site. *Pacific white shrimp* (n = 13) and *Black tiger shrimp* (n = 11) transported the entire amount of specimens used in this study to 24.

The Isolation of Bacteria
In order to isolate the bacteria, the telson and the inner section of the hepatopancreas were scraped away using a sterile scalpel. Sterile water was used to form a paste from the tissues, and then it was diluted by a factor of ten, one, three, and five. Then, a 50 L aliquot to each watering was placed on agar TCBS (England, Oxoid) and allowed to grow at area malaise for 48 hours. Then, by repeatedly streaking single colonies on agar plates, we were able to isolate and purify colonies with distinct morphological characteristics.

rep-PCR (Repetitive-Polymerase Chain Reaction)
DNA Bacterial was isolated from a soup values of isolated straining grown for 24 hours using a modified version of the chelex method. The Vibrio sp. strains were typed using an adapted version of the rep-PCR technique. We used three oligonucleotide primers for rep-PCR in this study: BOXA1R (5′ CTACGGCAAGGCCTGACG3′), REP1RI (5′ IIIICGICGICATCI GGC3′), and REP2I (5′ IIICGNCGNCATCNGGC3′).We used GoTaq® Green Master Mix (Promega) and its recommended methodology to purify the PCR product. Following a 5-minute denaturation at 95 degrees Celsius, the reaction mixtures were amplified for 60 seconds at 92 degrees Celsius, annealed for 90 seconds at 50 degrees Celsius, and finally extended for 10 minutes at 68 degrees Celsius. The 5 L PCR amplicons were seen and associated to molecular mass values after electrophoresis on 1% agarose gels stained with ethidium bromide (100-basepair ladder).

Separate Clustering
Dielectrolysis data from rep-PCR was used to classify the isolates into subsets, and the Free Tree software was used to analyse band position on the gel. Then, we randomly selected an isolate from each cluster in the dendrogram tree.

Identifying Bacteria

We amplified the 16S rRNA gene with the GM3F and GM4R primers. The freeze-thaw technique was used in order to get genomic DNA from Vibrio strains. As was done before, we amplified, purified, and sequenced the 16S rRNA gene. After sequencing the PCR products, we used BLAST to look for similarities between them. The Mega 7 programme was used to create the phylogenetic tree.

3. RESULTS

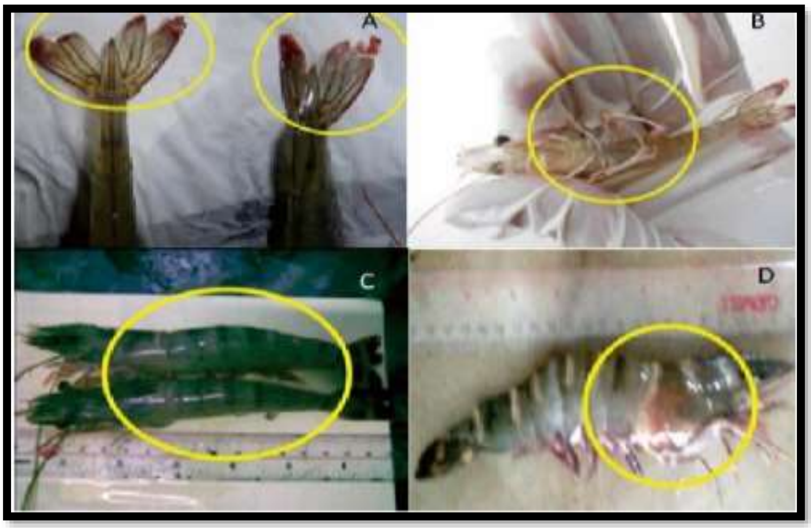

Fig 1. Shrimps with clinical signs of vibriosis

Table 2. Identification of five Vibrio species linked to shrimp vibriosis using 16S rDNA sequencing

No	Isolate	Strictly relation	Homology (%)
1	JKM06	Shewanella algae	99
2	JKM01	V. alginoluticus	97
3	JKP19	V.parahaemolyticus	94
4	JKP05	V.diabolocus	99
5	JKP03	Vibrio rotiferianus	100

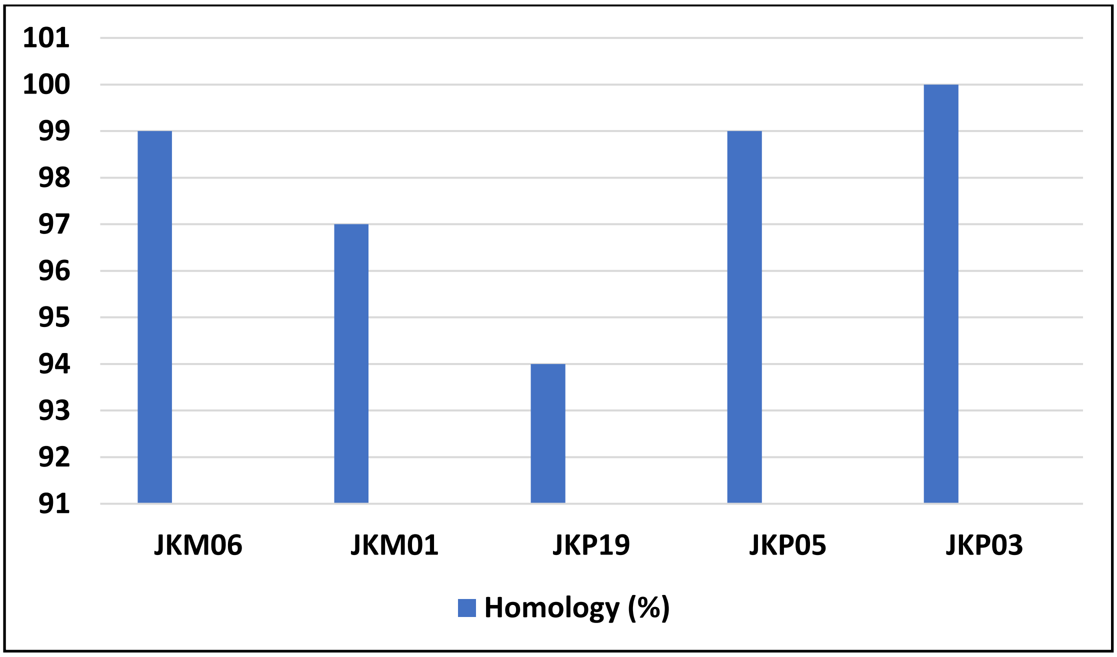

Fig 2. Graphical representation of isolate of shrimp vibriosis

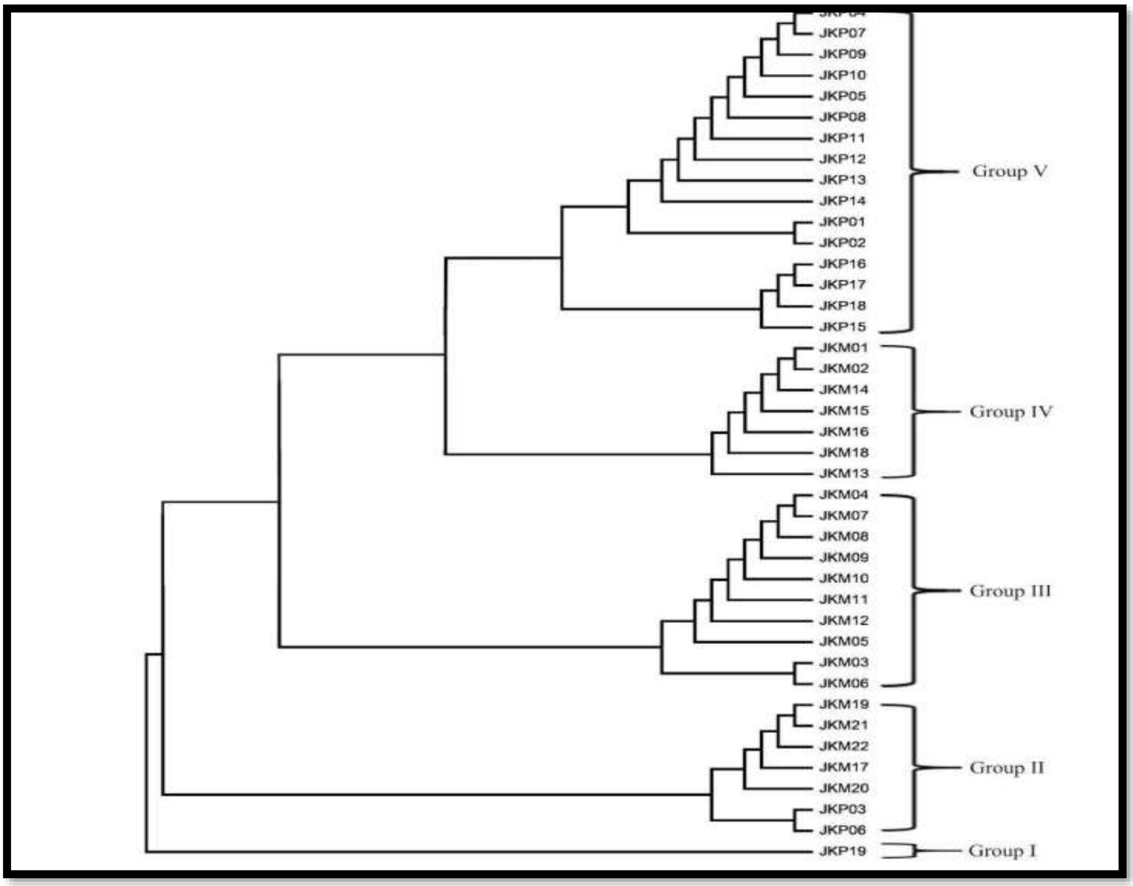

Fig 3. Repeated polymerase chain reaction of 41 shrimp Vibrio isolates

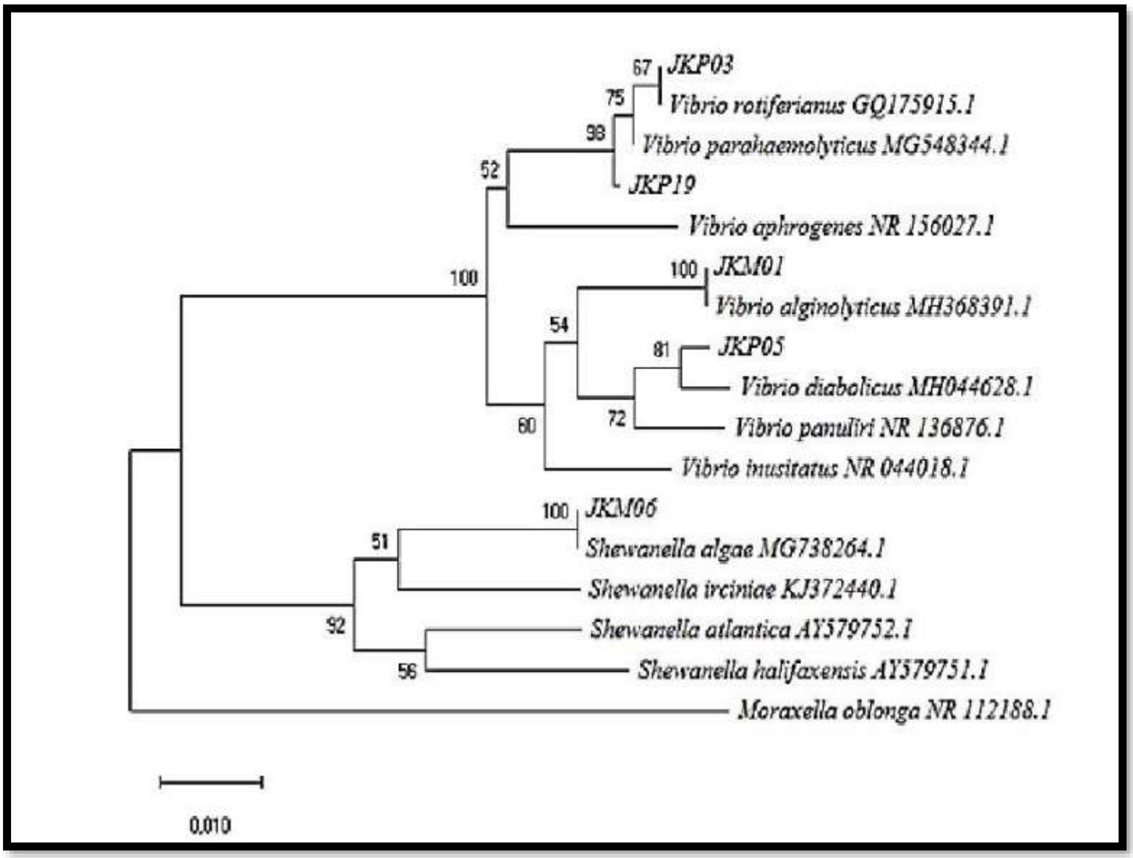

Fig 4. Vibrio phylogenetic tree in relation to shrimp vibriosis

4. DISCUSSIONS

Marine and estuarine habitats are typical habitats for vibrios species. There are a few species, however, that have been shown to have clinical relevance for aquatic animals and are thus considered potential pathogens. [16] Vibrio bacteria linked to shrimp vibriosis have been found. For this reason, vibriosis could be caused by any member of the genus vibrio. These vibrio strains are typically found in water, silt, or other aquatic environments. However, the current investigation did not uncover the previously recognised clinical symptoms of septicemia, including red to brown gills, lack of appetite, an empty gut, decreased feeding, or systemic infection. [19] The varying levels of pathogenicity may explain this outcome. Different Vibrio isolates can also vary in terms of their virulence depending on their origin and the conditions of the pond in where they were found. The virulence of various Vibrio species and isolates varies as well. DNA evidence suggests a tight genetic link between *V. diabolicus* and *V. vulnificus, V. harveyi, V. parahaemolyticus, V. fischeri* and *V. alginolyticus,*. S. algae is commonly originate in biofloc due to its potential to improve health through the usage of probiotics. Shrimp vibriosis was used to cultivate *S. algae* for this study.[15]

5. CONCLUSIONS

Old-style salty aquatic pools on the southern. shore of Tajpur,WestBengal are home to a rich biodiversity of Vibrio, the bacteria responsible for the disease vibriosis in shrimp.[19] Bacteria like *S. algae, V. diabolicus, V. parahaemolyticus, V. alginolyticus,* and *V. rotiferianus* were shown to be linked to these organisms. [21] Given the prevalence and diversity of vibriosis in aquaculture settings, novel vaccines, probiotics, and immunostimulant formulations are needed to combat the disease in shrimp raised in the conventional brackish water ponds.[22]

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