Volume 7, Issue 1: KUS: MariNEX 1505: xx-xx: 15102025: 2026

KHULNA UNIVERSITY
STUDIES

journal homepage: www.kus.ku.ac.bd

Research article

Antibiotic Sensitivity of *Vibrio* spp. Isolated from the Hatchery Water of Tiger Shrimp *Penaeus monodon*

Anup Sardar*, Nurunnahar Nadira, Shikder Saiful Islam, Alokesh Kumar Ghosh and Abul Farah Md. Hasanuzzaman

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh

ABSTRACT

Penaeus monodon, one of the most traded aquatic species, fetch a premium price in national and international markets for its significant taste and nutritional value. But, vibriosis caused by *Vibrio* spp. is a threat to the sustainability of shrimp aquaculture. This study aimed at identifying and profiling antibiotic resistance of *Vibrio* spp. detected in the water samples, collected from June to September of 2023 from four shrimp hatcheries located in Cox's Bazar, Batiaghata and Dacope Upazillas of Khulna District and Debhata Upazilla of Satkhira Ditrict. The colonies of *Vibrio* spp. from TCBS isolation, included *Vibrio alginolyticus* ($9.55\pm0.14\times10^5$ CFU /ml) from Cox's Bazar samples, *Vibrio fluvialis* ($9.55\pm0.21\times10^6$ CFU /ml) from Dacope samples, and *Vibrio furnissii* ($3.66\pm0.41\times10^6$ CFU/ml) and *Vibrio parahaemolyticus* ($3.55\pm0.23\times10^5$ CFU/ml) from Debhata samples in accordance with biochemical tests. Considering two replications of all the experiments, the antibiotic sensitivity test (Kirby-Bauer method) determined that all *Vibrio* isolates were resistant to penicillin, 75% to erythromycin, 50% to ampicillin, 25% to oxytetracycline, nalidixic acid and azithromycin. The *V. alginolyticus* and *V. furnissii* isolates had multidrug resistance (MDR) with Multiple Antibiotic Resistance (MAR) values of 0.41 and 0.17, respectively. In contrast, all the tested isolates were susceptible to cefotaxime, chloramphenicol, sulphamethoxazole & trimethoprim, nitrofurantoin, ciprofloxacin, and tobramycin. Therefore, it has been evident that the shrimp hatchery's water is very prone to antibiotic resistant *Vibrio* spp. contamination with 5-41% MDR. This study recommends an effective implementation of biosecurity measures to reduce the transmission of disease from other sources and the driving factors associated with vibriosis in *P. monodon* hatchery must be identified.

ARTICLE INFO

Article timeline:
Date of Submission:
15 October, 2025
Date of Acceptance:
24 May, 2026
Article available online:
14 June, 2026

***Keywords*:**
Shrimp Hatchery
Bacterial diseases
Susceptibility
AMR

Introduction

Aquaculture becomes an important production system due to its contribution to supplying dietary protein for human consumption. The aquaculture production of the globe has been growing up to meet the public demand; a record production of 223.2 million tonnes in 2022, comprising 185.4 million tonnes of aquatic animals and 37.8 million tonnes of algae, where aquaculture production accounts for 130.9 million tonnes and capture fisheries 92.3 million tonnes (FAO, 2024). This growth is largely centered in Asia, which contributes 70% of global output, led by China, India, Indonesia, and Vietnam, Thailand and Bangladesh.

Bangladesh possesses a wealth of fisheries resources and currently ranks as the second-largest producer of freshwater fish worldwide, recently overtaking China in this category (FAO, 2024).The nation produced more than 4.92 million tons of fish during the 2022–2023 fiscal year, which contributed more than 22% to the agricultural GDP and 2.53% to the national GDP (DoF, 2024). Over the past ten years, the export of prawns and shrimp has accounted for around 80% of all fisheries export earnings, making it a significant economic driver. The income from the export of fish, shrimp, and other fisheries products contributes significantly to Bangladesh's foreign exchange profits, which make up about 0.91% of total export revenue (EPB 2024). Recent figures show that Bangladesh produced roughly 261,833 metric tons of shrimp and prawns in FY 2022-23, with exports valued at USD 347.54 million. (Hosain et al., 2021; DoF, 2024).

The southern part of Bangladesh, especially Cox's Bazar, Chattogram, Khulna, Bagerhat, Satkhira, and

*Corresponding author: <anupsardar141@gmail.com>

DOI: https://doi.org/10.53808/KUS.2026.SI.7.01.MariNEX1505-ls

surrounding districts is considered as the heart for the cultivation of gigantic tiger shrimp in Bangladesh (Matin et al., 2016). To sustain this industry, an annual supply of 8 to 9 billion post-larvae (PL) is required. Currently, only 52 of the 80 existing hatcheries are active, producing between 1.6 and 2.6 billion PL per year. Of this total, only a small fraction (2–9%) originates from specific pathogen-free (SPF) brood stock (DoF, 2023; Sultana and Biswas, 2022).

Specific pathogen free (SPF) PLs have recently been used in shrimp farms as a control measure of pathogenic infection, but the shrimp hatcheries have thousands of records of disease outbreaks and are still at risk of outbreaks. One of the common pathogenic causative agents of shrimp infections recorded are *Vibrio* species, such as *V. anguillarum, V. alginolyticus, V. splendidus, V. parahaemolyticus, V. harveyi, V. vulnificus, V. tubiashi, V. nigripulchritudo, V. fluvialis and V. damsel* (Rungrassameeet al., 2013). Vibriosis has been reported as the most devastating diseases in shrimp hatcheries in Bangladesh; primarily caused by *V. harveyi, V. metschnikovii, V. nereis, and V. alginolyticus* (Rahman et al., 2010). *Vibrio* spp. were commonly found throughout freshwater, estuaries, and marine environments. Additionally, there are more than twenty species known to exist; some of them are infections that affect humans (*V. vulnificus, V. cholerae*and *V. parahaemolyticus*), while others affect aquatic creatures, such as shrimp (*V. harveyi*, *V. penaecida, V. parahaemolyticus, V. anguillarum, V. splendidus* and *V. vulnificus*). Additionally early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND), is a bacterial prawn disease brought on by *Vibrio parahaemolyticus's* production of the PirABvp toxin. The AHPND pandemic has caused large financial losses to the aquaculture industry since 2009 (Lai et al. 2015; Kumar et al. 2018, 2019, 2020, 2021; Tran et al., 2020).*Vibrio harveyi*, *Vibrio owensii*, *Vibrio punensis*, *Vibrio alginolyticus* , and *Vibriocampbellii* are among the strains of *Vibrios* that can also cause AHPND as they carry the pVA1-type plasmid tha contains the toxin's genes (Dong et al. 2019).

Extensive use of antibiotics in aquaculture had become very common to treat pathogenic bacteria and improve fish health (Dawood et al., 2018; Chen et al., 2020; Chowdhury et al., 2022). Most commonly used antibiotics in fish and shellfish farms are ampicillin, streptomycin, chloramphenicol, oxytetracycline, malachite green, and other chemicals (Chelossi et al., 2003). However, because of the continued use of antibiotics in aquaculture, microbial populations are under more natural selection pressure, which can develop antibiotic-resistant strains that can spread widely and cause dangerous infections (Gao et al., 2012). Aquatic habitats, fish consumers, and aquaculture production may all suffer as a result of these resistant bacteria (Watts et al., 2017).

The incidences of antimicrobial resistance has become pressing issue around the word. For instance, AMR *Vibrio parahaemolyticus* strains have been detected in shellfishes from major fish markets in Cochin, South India (Silvesteret al., 2022), and shrimp ponds (Tendencia and de la Pena, 2001), and aquaculture habitats (Chelossi et al., 2003). The eggs from a mud crab hatchery of Bangladesh were contaminated with multidrug resistant *Vibrio* spp. V. *algiloniticus* and V. *parahaemolyticus* (Hasanuzzaman et al., 2025). The luminous strains of *V. splendidus*that and *Vibrio harveyi,* identified from shrimp larvae were resistant to pencicillin G, kanamycin, erythromycin, and streptomycin (Baticados et al., 1990). Abraham et al., (1997) have detected AMR in *V. harveyi* strains isolated from diseased shrimp to Novobiocin, ofloxacin, oxytetracycline, penicillin G, polymyxin B, rifampicin, streptomycin, sulphasomodine, sulphamethezole, ampicillin, chlorotetracycline, ceprofloxacin, erythromycin, furazolidone, gentamycin, nalidixic acid, neomycin, nitrofurantoin, and nitrofurazole. There were five multidrug-resistant *Enterobacterales*in the shrimp farms in Bagherhat of Bangaldesh; *Proteus penneri, Morganellamorganii, Proteus alimentorum, Enterobacterhormaechei*subsp. *xiangfangensis,* and *Plesiomonasshigelloides* (Khan et al., 2022).

In the shrimp industry, biosecurity refers to the technique of excluding certain pathogens from broodstock, hatcheries, grow-out farms, as well as the entire area in order to prevent disease (Lightner, 2003). In Bangladesh, only three hatcheries in the districts of Cox's Bazar and Khulna have received approval for production of SPF PLs production (DoF, 2020), while most of the hatcheries continue their production. These shrimp hatcheries are experiencing production crush, and using a variety of antibiotics and other antimicrobial agents. But, antimicrobial resistance (AMR) has likely emerged as a potential threat to aquaculture as well as public health. One of the main public health issues related to the usage of antibiotics is the fast growing number of antibiotic resistance genes (ARGs) detected in aquaculture environments (Huang et al., 2017;Chen et al.,2022; Sun et al., 2020). Significant infections brought on by bacteria resistant to antibiotics were thought to be the direct cause of about 1.27 million fatalities globally in 2019 and to have contributed to an additional 4.95 million deaths (Murray et al., 2022). By horizontal gene transfer, ARGs can also quickly spread to different bacterial species (Ben et al., 2019). In the aquatic environment, approximately 90% of tested bacteria showed at least one antibiotic resistance, and 20% of them may be multi-antibiotic resistant, which could affect human and fish health as well as the fish farm environment (Lin, 2015; Preena et al., 2020; Zaman et al., 2017).

But, the occurrence, susceptibility pattern and multidrug resistance profile of *Vibrio* are not well understood and reported, whereas the hatcheries are at risk of vibriosis disease. Therefore, a study of antibiotic resistance of pathogenic bacteria in shrimp hatcheries in Bangladesh is one of the prerequisites for sustainable shrimp aquaculture. Since the Cox's Bazar is a significant source of brackish and seawater, the majority of shrimp hatcheries of Bangladesh are located there. Additionally, there are a number of shrimp hatcheries in the Khulna and Satkhira regions, which provide PLs to the shrimp farms in these areas. This study aimed at isolating *Vibrio* spp. from the water of four tiger shrimp hatcheries, one from each location, Cox's Bazar, Dacope, Bothiaghata, and Debhata, and exploring their antibiotic resistance profile.

Materials and Methods

Sample Collection

Four shrimp hatcheries, one from each location, Cox's Bazar (June, 2023), Dacope (August, 2023), Bothiaghata (August, 2023), and Debhata (September, 2023), were chosen for sample collection (Fig. 1). As hatchery water is a significant source of bacterial infections, 100 ml of water sample from each rearing tank from these four hatcheries were collected. All samples were collected in sterile bottles; immediately placed in an ice box, and transported to the Fish and Shellfish Quality Control and Pathology Laboratory of the Fisheries and Marine Resources Technology (FMRT) Discipline at Khulna University. The samples were used for isolating *Vibrio* species and subsequently carrying out antibiotic sensitivity tests; all laboratory works were done in accordance with the code of practice (CoP) to care and use of animals for scientific purposes at Life Science School, Khulna Universityunder the ethical approval (Ref No: KUAEC-2023-04-08; Date: 30 04.2023) by Animal Ethics Committee of Khulna University, Bangladesh.

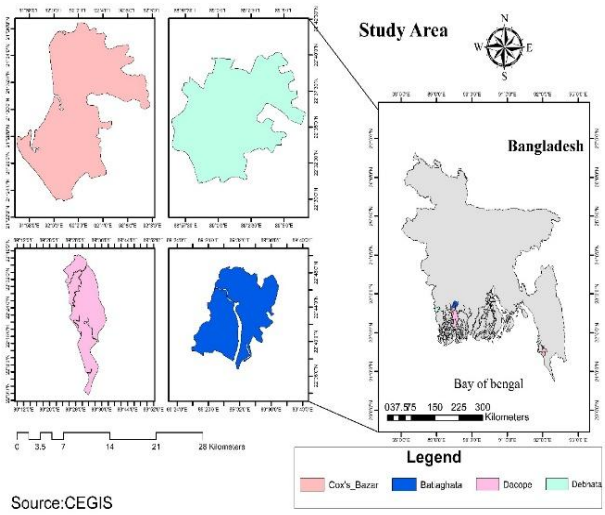

Figure 1: Map of Bangladesh indicating the study area. Salmon colour, Cox's Bazar; Aquamarine, Debhata; Orange, Dacope; and Blue, Batiaghata.

Sample Preparation and Bacterial Culture

At first, water from different rearing tank of a hatchery were mixed homogenously and 100 ml of the combined water was used for stock sample preparation. Then, each water sample was thoroughly mixed with physiological saline using a vortex machine, and the resulting mixture was treated as the stock solution. Sterile falcon tubes were used to make 10-fold serial dilutions of each stock solution. The serial dilution samples were enriched by adding sterile alkaline peptone water (APW) and incubated at 37±1°C for 8-12 hours. Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates were prepared and 0.1 ml of each diluted solution was added to each plate and spread homogenously using a sterile glass spreader. The inoculation plates were incubated at 37±1°C in an incubator for 18 to 24 hours. Post-incubation, distinct colonies were quantified to calculate the Total Viable Count (TVC) in colony-forming units per milliliter (CFU/ml). Each bacterial test had two replications to avoid manual error.

Biochemical Test of Bacteria

Morphologically isolated isolates were selected for pure culture and 2 pure culture plates of identical isolates were selected for biochemical tests. Biochemical test was carried out for confirming the isolates as *Vibrio* spp. following Bacteriological Analytical Manual (BAM) (Kaysner et al., 2004).

A sterile glass rod was used to move the pure-cultured bacterial colonies on filter paper, soaked with oxidase reagent for the oxidase test (FDA, 1992); a positive reaction was considered when a dark purple color appeared quickly, within a few seconds.

The Voges-Proskauer (VP) test involved inoculating a colony from the pure culture plate with MR-VP media and incubating it at 37ºC for 48 hours. Within 30 minutes, watch for the medium's surface to turn reddish-pink. During the thirty-minute waiting time, the tube was vigorously shaken continuously. (FDA, 1992).

To conduct additional experiments, a pure culture inoculum was aseptically transferred to a sterile tube containing broth for each test: to phenol red sucrose broth for the sucrose test, phenol red lactose broth for the lactose test, and phenol red mannitol broth for mannitol fermentation test The tubes were then incubated at 35–37 °C for 24 hours, and the color change was used to assess the results.

The NaCl growth test involved adding 3%, 6%, 8%, and 10% of NaCl to a sterile test tube of nutritional broth, and then each pure culture inoculum was added in the broth tube and the tube was thoroughly mixed. The bacterial growth was ascertained by monitoring the turbidity after 24 hours of incubation at 35–37 °C. (Kaysner et al., 2004).

Autoclaved sterile distilled water was used as a control group for each biochemical test and all the tests had two replications.

Antibiotic Sensitivity Test

For the antibiotic sensitivity test (AST) investigation, the following 17 antibiotics frequently used in aquaculture were used; (azithromycin, AZM (15 µg), amoxicillin, AMO (30 µg), ampicillin, AMP (10 µg), erythromycin, E (15 µg), cefotaxime, CTX (30 µg), chloramphenicol, C (30 µg), ciprofloxacin, CIP (5 µg), nalidixic Acid, NA (30 µg), nitrofurantoin, F (300 µg), oxytetracycline, OT (30 µg), penicillin G, P (10 µg), sulfamethoxazole& trimethoprim, SXT (25 µg), levofloxacin, LEV (5 µg), streptomycin, S (10 µg), gentamicin, CN (10 µg), tobramycin, TBR (30 µg) and tetracycline, TE (30 µg)); and blank disc were considered as a control group. The antibiotic susceptibility test was carried out by the Kirby-Bauer disc diffusion method ((Bauer et al., 1966) considering two replications. The representative isolates from the pure culture plates selected for biochemical test were considered for AST and inoculated in Muller Hinton Broth (MHB; Himedia, India) supplemented with 2% NaCl; after a 24-hour incubation period and the suspension's concentration comparing with the 0.5 Mcfarland standard; the isolates were tested. A sterile swab, after being dipped into the suspension tube, was used to swab the Mueller Hinton Agar (MHA) plate's surface. The commercially manufactured antibiotic discs and a blank disk were strategically positioned onto the inoculated MHA plates. To ensure optimal diffusion,

each disc was gently pressed to confirm complete contact with the agar surface. To ensure optimal diffusion, each disc was gently pressed to confirm complete contact with the agar surface. The plates were then inverted and incubated aerobically at 37°C for a precise duration of 18–24 hours. Following incubation, a digital vernier caliper was used to measure the clear zones of inhibition (ZOI) to the closest millimeter. To classify the isolates as Susceptible (S), Intermediate (I), or Resistant (R), these parameters were evaluated in accordance with the CLSI susceptibility breakpoints. The Multiple Antibiotic Resistance (MAR) index was calculated to measure the degree of multidrug resistance (MDR).

Data Processing, Analysis and Interpretation

Microsoft Word and Excel (Office 2019) were used to gather, compile, assess, and present the experimental data; mean, and standard error were used as statistics SPSS 29.0 was used to perform ANOVA for determining statistical significance in the variation of *Vibrio* spp. load among the waters samples of the hatcheries. The antibiotic susceptibility test findings were interpreted and reported as either sensitive, resistant, or intermediate using the zone diameter interpretative standard for veterinary infections (CLSI, 2013). The multidrug resistance profile and multiple antibiotic resistance index of resistant isolates were evaluated (Schwarz et al., 2010). The MAR index of a single isolate was calculated as a/b (Krumperman, 1983), where "a" indicates the number of antibiotics to which the isolate was resistant and "b" indicates the number of antibiotics to which the isolate was exposed. A higher MAR index close to 1 was interpreted as having higher level of antibiotic resistance, while a lower MAR value close to 0 is an indication of little or no resistance. MAR index values greater than 0.2 indicate that the isolate originated from a source where antibiotics were used to a large degree.

Result

Occurrence of Vibrio infection

The water samples from three hatcheries were found to be positive for *Vibrio* spp. infection (Table 1), except the samples from Batiaghata hatchery which just started production when Specific Pathogen Free hatchery management was implemented effectively, keeping the *Vibrio* spp. load in the water is either lower than the detection limit of free of *Vibrio*. The prevalence of *Vibrio* spp. was significantly ($P<0.05$) higher in the water from the hatchery of Dacope having the colonies $9.5\pm0.21\times10^6$ CFU/ml of isolate VYCL, compared to $3.6\pm0.41\times10^6$CFU/ml of isolate VYST and $3.5\pm0.23\times10^5$ CFU/ml of isolate VGST in Debhata and $9.5\pm0.14\times10^5$ CFU/ml of isolate VYCX in Cox's Bazar.

Identification of Vibrio Isolates

The biochemical tests identified the isolate VYCX from Cox's Bazar as *Vibrio alginolyticus* and VYCL from Dacope as *Vibrio fluvialis*. The samples VYST and VGST from Debhata showed the presence of *Vibrio furnissii* and *Vibrio parahaemolyticus*, respectively (Table 2).

Antibiotic susceptibility pattern of isolated bacteria

The antibiotic susceptibility profile of the isolated bacteria revealed significant variations in their responses to different antibiotics (Table 3). Cefotaxime (CTX), chloramphenicol (C), ciprofloxacin (CIP), nitrofurantoin (F), and tobramycin (TBR) were the most effective; all isolates were 100% sensitive. In contrast, all isolates (100%) were resistant to penicillin (P) while 75% to erythromycin, 50% to ampicillin (AMP) and amoxicillin (AMO). Other antibiotics like trimethoprim-sulfamethoxazole (SXT), oxytetracycline (OT), azithromycin (AZM), and nalidixic acid (NA) showed mixed effectiveness, with varying proportons of sensitivity, intermediate susceptibility, and resistance (Figure2).

Multiple Antibiotic Resistance (MAR) Index of the isolates

Vibrio alginolyticus isolated from the sample VYCX was found resistant to ampicillin, erythromycin, azithromycin, tetracycline, oxytetracycline, penicillin G, and nalidixic acid antibiotics. Penicillin G resistance was observed in *Vibrio fluvialis* (VYCL), while resistance to ampicillin, Penicillin G, and Erythromycin was observed in *Vibrio furnissii* (VYST). Erythromycin and Penicillin G were not effective against *Vibrio parahaemolyticus* (VGST) (Table 4). The isolates *Vibrio alginolyticus*and *Vibrio furnissii* showed multidrug resistance Pattern with MAR values of 0.41 and 0.17, respectively (Figure 3).

Table 1: Prevalence of bacteria in different samples

Sample Code	Sample Source	Sampling Area	Culture Media	Culture Method	CFU/ml	Colony Color	Colony Shape	Colony Size
VYCX	Water	Cox's Bazar	TCBS	Enriched	$^a9.5\pm0.14\times10^5$	Yellow	Round	Large
VYCL	Water	Dacope	TCBS	Enriched	$^b9.5\pm0.21\times10^6$	Yellow	Round	Medium
VYST	Water	Debhata	TCBS	Enriched	$^a3.6\pm0.41\times10^6$	Yellow	Round	Medium
VGST	Water	Debhata	TCBS	Enriched	$^a3.5\pm0.23\times10^5$	Deep Green	Round	Medium

Different superscripts in the same column shows significant ($P<0.05$) difference.

Table 2: Biochemical test of different isolates from different samples

Isolates	Color in TCBS	NaCl (0%)	NaCl (3%)	NaCl (6%)	NaCl (8%)	NaCl (10 %)	Oxidase	Sucrose	Lactose	D-Mannitol	Voges-Proskauer	Putative bacterial species
VYCX	Yellow	-	+	+	+	+	+	+	-	+	+	*Vibrio alginolyticus*
VYCL	Yellow	-	+	+	+	-	+	+	-	+	-	*Vibrio fluvialis*
VYST	Yellow	-	+	+	+	-	+	+	-	+	-	*Vibrio furnissii*
VGST	Deep Green	-	+	+	+	-	+	+	-	+	-	*Vibrio parahaemolyticus*
'+' = Positive and '–' = Negative												

Table 3: Zone of inhibition (mm) with susceptibility criteria

Isolate ID	Zone of inhibition (mm) with susceptibility criteria															
	CTX	CN	C	LEV	SXT	OT	P	F	E	CIP	AZM	NA	S	AMO	TBR	B
VYCX	27±0.7(S)	25±0.7(S)	26±0.3(S)	29±0(S)	23±0.4(S)	0±0(R)	34±0.1(S)	21±0.7(S)	0±0.4 (R)	27±0.3(S)	0±0.7 (R)	7±0.1 (R)	21±0(S)	20±0.4(S)	21±0.3(S)	-
VYCL	34±0.4(S)	18±0.7(S)	36±0.1(S)	24±0.4(S)	19±0.3(S)	27±0.7(S)	8±0.1 (R)	19±0 (S)	16±0.4(I)	21±0.1(S)	22±0.7(S)	14±0.1(I)	18±0.7(S)	20±0.3(S)	22±0.4(S)	-
VYST	30±0.7(S)	16±0.4(S)	27±0.7(S)	14±0.3(I)	21±0.4(S)	18±0.1(I)	0±0.1 (R)	20±0.7(S)	8±0.4 (R)	26±0 (S)	16±0.1(I)	19±0 (S)	14±0.1(I)	16±0.4(I)	17±0.7(S)	-
VGST	31±0.3(S)	16±0.1(S)	20±0.4(S)	29±0.4(S)	25±0.3(S)	26±0 (S)	0±0.4 (R)	20±0.1(S)	12±0.1(R)	30±0.7(S)	24±0.7(S)	17±0.4(I)	15±0 (S)	16±0.7(I)	21±0.3(S)	-
CTX = Cefotaxime, CN = Gentamicin, AMP = Ampicillin, TE = Tetracycline, C = Chloramphenicol,, LEV = Levofloxacin, SXT = Sulfamethoxazole& Trimethoprim, OT = Oxytetracycline, P = Penicillin G, F = Nitrofurantoin, E = Erythromycin, CIP = Ciprofloxacin, AZM = Azithromycin, NA = Nalidixic Acid, S = Streptomycin, AMO = Amoxicillin, TBR = Tobramycin and B = Blank																

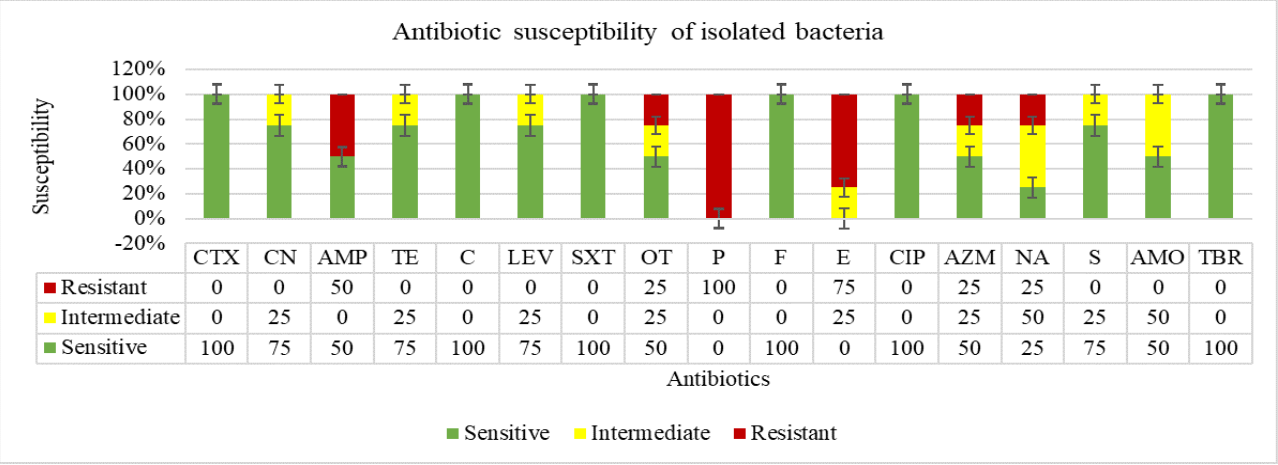

Figure 2: Antibiotic susceptibility pattern of isolated *Vibrio* spp. from the study samples; red: resistant percentage, yellow: intermediate percentage, and green: susceptible percentage. CTX = Cefotaxime, CN = Gentamicin, AMP = Ampicillin, TE = Tetracycline, C = Chloramphenicol, LEV = Levofloxacin, SXT = Sulfamethoxazole& Trimethoprim, OT = Oxytetracycline, P = Penicillin G, F = Nitrofurantoin, E = Erythromycin, CIP = Ciprofloxacin, AZM = Azithromycin, NA = Nalidixic Acid, S = Streptomycin, AMO = Amoxicillin, TBR = Tobramycin

Table 4: Antibiotic susceptibility profile of bacteria

Isolates	Antibiotic susceptibility profiles with %		
	Sensitive	**Intermediate**	**Resistant**
Vibrio alginolyticus	CTX-C-LEV-F-CIP-SXT-S-AMO-TBR (52.95%)	CN (5.88%)	AMP-OT-TE-AZM-NA-E-P (41.17%)
Vibrio fluvialis	AMP-CTX-C-CN-LEV-F-CIP-SXT-S-AMO-TBR-OT-TE-AZM (82.36%)	NA-E (11.76%)	P (5.88%)
Vibrio furnissii	CTX-C-CN -F-CIP-SXT-TBR-TE-NA (52.95%)	OT-LEV-AZM-S-AMO (29.41%)	AMP-P-E (17.64%)
Vibrio parahaemolyticus	AMP-CTX-C-CN-LEV-F-CIP-SXT-S -TBR-OT-TE-AZM (76.48%)	NA-AMO (11.76%)	P-E (11.76%)

CTX = Cefotaxime,, CN = Gentamicin, AMP = Ampicillin, TE = Tetracycline, C = Chloramphenicol,, LEV = Levofloxacin, SXT = Sulfamethoxazole& Trimethoprim, OT = Oxytetracycline, P = Penicillin G, F = Nitrofurantoin, E = Erythromycin, CIP = Ciprofloxacin, AZM = Azithromycin, NA = Nalidixic Acid, S = Streptomycin, AMO = Amoxicillin, TBR = Tobramycin

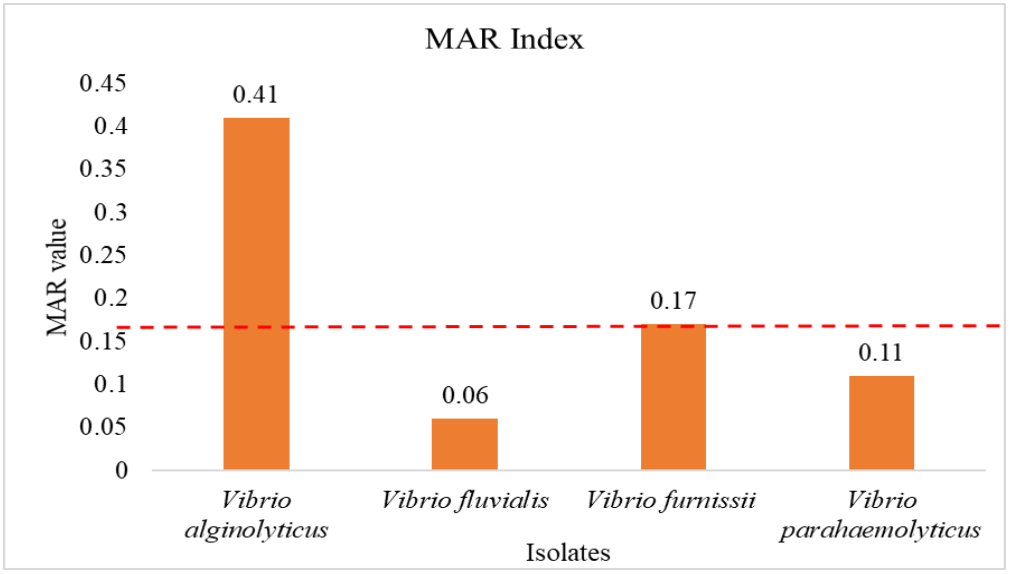

Figure 3: MAR index of isolated *Vibrio* species from shrimp hatcheries at Cox's Bazar, Dacope, Batiaghata and Debhata. Red disconnected line with value (0.17) indicates minimum value of the MAR index for a bacterial isolate to be multiple antibiotic resistant.

Discussion

In fish and shellfish hatcheries, diseases caused by a range of pathogenic bacteria, most frequently *Vibrio* spp., pose significant threats to the production (Islam et al., 2023; Hasan et al., 2017; Rahman et al., 2012). This present study reports the presence of 4 *Vibrio* spp. (*V. alginolyticus*, *V. fluvialis*, *V. furnissii*, *V. parahaemolyticus*) in water samples from different shrimp hatcheries of Bangladesh, indicating that *Vibrio* is a prevalent pathogenic bacterial group in shrimp hatcheries. The previous investigation of shrimp PL nurseries which is directly linked to hatchery production of shrimp larvae, supported the present finding and reported *Vibrio* as the dominant group of bacteria (Yasinet al., 2022). Generally, *penaeid* shrimp naturally harbour bacterial species from the genus *Vibrio* (Gomez-Gil et al., 1998); of which some *Vibrio* species have been reported to have the potential to become pathogenic, and cause mortality of shrimp in a production system (Nash et al., 1992).

Among all the *Vibrio* species isolated in this investigation, the presence of *V. alginolyticus* in the samples of Cox's Bazar indicates an occurrence of vibriosis in juvenile penaeid shrimp caused by *V. alginolyticus* (Selvin& Lipton, 2003). *Vibrio alginolyticus* had also been reported to cause vibriosis from several shrimp farms in the southwestern coastal shrimp farms of Bangladesh, caused signs of illness and mortality in juvenile shrimps (Hannan et al., 2019). Therefore, *Vibrio* spp. could be transferred from hatchery to shrimp farm as most of the farm rear hatchery produced post larvae.

V. parahaemolyticus was identified as one of the strains in the present investigation, which is known as one of the causative agents of early mortality syndrome or acute hepatopancreatic necrosis disease (EMS/AHPND) in cultured shrimp. *V. parahaemolyticus* and other *Vibrio* spp. that carry pirA and pirB toxin genes in their plasmid caused AHPND is shrimps (Dong et al., 2017; Liu et al., 2018; Restrepoet al., 2018; Muthukrishnanet al., 2019). This disease has resulted in significant losses in shrimp production in China, Vietnam, Thailand and Malaysia

(FAO, 2013). AHPND carrying *V. parahaemolyticus* strains had been evident in Bangladesh shrimp farms in recent years (Eshik et al., 2017; Eshik et al., 2018).

The presence of *V. furnissii* and *V. fluvialis* from the shrimp hatchery water samples; indicates uncleaned water sources of hatcheries or unhygienic handling practices by the hatchery workers of technicians, as *V. furnissii* and *V. fluvialis*have been detected in the stool samples from diarrhea patients (Zhou et al., 2024).

On the other hand, *Vibrio* infections in human beings were frequently linked to consuming tainted seafood (Janda*et al*., 1988), which indicates that shrimp can be a carrier of human pathogens. Outbreaks and isolated cases of human gastroenteritis had been linked to *V. furnissii* (Schirmeister et al., 2014, Jandaet al., 1988, Magalhãeset al., 1993, Hickman et al., 1994, Lam et al., 1985). Additionally, the intestines of healthy brown shrimp and aquatic marine habitats are reported to harbor *Vibrio furnissii* (Janda et al., 1988; Hernandez et al., 1997).

Antibiotics are intensively employed to control bacterial infections in aquaculture systems, which could be a significant cause behind the antibiotic resistance formation of bacteria (Schar et al., 2020). The sensitivity of a bacterium to a particular antibiotic is the only factor that determines how effective an antibiotic is. This study demonstrated that *V. fluvialis* showed the highest sensitivity (82.36%) towards 14 out of 17 antibiotics, which is in line with a study where this species was also sensitive to sulfamethoxazole& trimethoprim, chloramphenicol, gentamicin and so on (Okoh et al., 2015). *V. parahaemolyticus* showed 76.48% sensitivity towards 13 out of 17 antibiotics, supporting earlier research demonstrating the sensitivity of this shellfish-isolated *Vibrio* species to ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, and gentamicin (Yasin et al., 2022; Tan et al., 2020; Letchumanan et al., 2019). *V. parahaemolyticus* isolated from seafood, including shrimp, grouper, shellfish, and small mackerel, showed extreme resistance to ampicillin (82.09–88%) and penicillin (92.54–100%) (Srinivasan and Ramasamy, 2009; Letchumananet al., 2015; Tan et al., 2017; Amalina et al., 2019). In addition to these antibiotics, some *V. parahaemolyticus* isolates from different sources have been reported to resistant to erythromycin, cefotaxime, chloramphenicol, gentamicin and nalidixic acid (Hasanuzzaman et al., 2025; Yasinet al., 2022; Tan et al., 2020; Letchumanan et al., 2019);

Both *V. alginolyticus* and *V. furnissii* were found sensitive (52.95%) to antibiotics; *V. alginolyticus*to cefotaxime, chloramphenicol, levofloxacin, nitrofurantoin, ciprofloxacin, sulfamethoxazole& trimethoprim, streptomycin, amoxicillin, and torbomycin; *V. furnissii*to sulfamethoxazole& trimethoprim, turbomycin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin, and tetracycline. Similarly, *V. alginolyticusisolated from fishes* and shellfishes *were found sensitive to* cefotaxime, chloramphenicol, ampicillin, erythromycin, gentamycin, tetracycline, penicillin and gentamicin (Genovese et al., 2012;Kang et al, 2016; Yasinet al., 2022).*V. fluvialis*have been indicatedto be resistant to erythromycin, sulfamethoxazole, cefuroxime, penicillin G, chloramphenicol, tetracycline, trimethoprim, polymyxin b (Okoh et al., 2015); and *V. furnissii* to cefazolin, tetracycline, streptomycin (Zhou et al., 2024). *V. alginolyticus*strains isolated from mud crab hatchery were resistant to erythromycin, nalidixic acid, penicillin G, and vancomycin antibiotics (Hasanuzzaman et al., 2025). This study has opined that the susceptibility of these *Vibrio* species varied with strains, host species and environment, resulting in contradictory resistance pattern of different *Vibrio* spp., and multi-drug resistance was evolved in bacterial strains.

This study reports that *Vibrio* spp. from shrimp hatcheries in Bangladesh exhibited great resistance to penicillin G (100%), erythromycin (75%), and ampicillin (50%); and *V. alginolyticusand V. furnissii* displayed a pattern of multidrug resistance. The aquaculture business has suffered significant financial setbacks as a result of multiple antibiotic resistance (MAR) strains of *V. alginolyticus* (Mechriet al., 2015; Mohamad et al., 2019). According to a prior study, *Vibrio* spp. isolated from maritime whiteleg shrimp farms had a significant incidence of tetracycline-class (38.7%) and ampicillin (45.2%) resistance, and further revealed that 29% of the isolates exhibited multidrug resistance (Reboucaset al., 2011). *V. alginolyticus* isolated from Korean oysters were showed multidrug resistant to rifampin and erythromycin (Kang et al., 2016).

To combat the evolution of antibiotic resistance in shrimp hatcheries and the shrimp industry, the implementation of appropriate measures and regulatory initiatives is strongly advised. Robust biosecurity measures with standard water quality parameters in the hatchery setting are essential, along with the implementation of alternative therapeutic agents and meticulous monitoring of antibiotic usage in both hatchery and farming contexts. Consequently, the World Health Organization (WHO) developed a Global Action Plan (GAP) based on a "One Health" strategy, which emphasizes the interconnectedness of public health, animal health, and the environment, to combat the global threat posed by antibiotic resistance (WHO, 2015). Eventually, to investigate the emergence of AMR multidisciplinary approach is a must. This study addressed the imperative public health issue that can be a threat to aquaculture, the aquatic environment and the food safety. Nevertheless, this study further, given the sample size and molecular identification, suggests meticulous investigation of antibiotic resistance mechanisms, analysis of transmission patterns, and the expression of resistance genes.

Conclusions

This study provides evidence of the occurrence of infectious *Vibrio* spp. in four shrimp hatcheries water in Bangladesh, with several isolates exhibiting resistance to commonly used antibiotics especially to penicillin (100%), erythromycin (75%), and ampicillin (50%) and amoxicillin (50%). Notably, *Vibrio alginolyticus* and *Vibrio furnissii* were shown to be multidrug resistant with MAR values of 0.41 and 0.17, respectively, which raises questions about the sustainability of shrimp hatchery production as well as possible health dangers to the general public. In order to reduce the introduction and spread of resistant bacteria, the results emphasize the critical necessity for stringent

biosecurity measures and the responsible use of antibiotics in aquaculture. Future studies should concentrate on addressing the molecular mechanisms that underlie antibiotic resistance in *Vibrio* spp., surveillance of the resistance genes dissemination within hatchery environments, and assessing the transmission possibility of resistant strains to humans through the food chain.

Conflict of Interest

The authors declares no conflict of interest.

References

Abraham, T. J. (1997). Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. *Journal of Aquaculture in the Tropics 12*, 1-8.

Amalina, N. Z., Santha, S., Zulperi, D., Amal, M. N. A., Yusof, M. T., Zamri-Saad, M., & Ina-Salwany, M. Y. (2019). Prevalence, antimicrobial susceptibility and plasmid profiling of *Vibrio* spp. isolated from cultured groupers in Peninsular Malaysia. *BMC Microbiology*, *19*, 1-15.https://doi.org/10.1186/s12866-019-1624-2

Baticados, M. C. L., Lavilla-Pitogo, C. R., Cruz-Lacierda, E. R., de la Peña, L. D., & Sunaz, N. A. (1990). Studies on the chemical control of luminous bacteria *Vibrio harveyi*and *V. splendidus* isolated from diseased *Penaeusmonodon* larvae and rearing water.*Diseases of Aquatic Organisms*, *9*(2), 133-139. https://doi.org/10.3354/dao009133

Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M. H., & Zheng, C. (2019). Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environmental Research*, *169*, 483-493.https://doi.org/10.1016/j.envres.2018.11.040

Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Riccardi, G., & Banat, I. M. (2003).Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean.*Aquaculture*, *219*(1-4), 83-97. https://doi.org/10.1016/S0044-8486(03)00016-4

Chen, J., Sun, R., Pan, C., Sun, Y., Mai, B., & Li, Q. X. (2020).Antibiotics and food safety in aquaculture. *Journal of Agricultural and Food Chemistry*, *68*(43), 11908-11919.https://doi.org/10.1021/acs.jafc.0c03996

Chen, X., He, Z., Zhao, J., Liao, M., Xue, Y., Zhou, J., Hoare, R., Monaghan, S.J., Wang, N., Pang, H. & Sun, C. (2022).Metagenomic analysis of bacterial communities and antibiotic resistance genes in *Penaeusmonodon*biofloc-based aquaculture environments. *Frontiers in Marine Science*, *8*, 762345.https://doi.org/10.3389/fmars.2021.762345

Chowdhury, S., Rheman, S., Debnath, N., Delamare-Debouteville, J., Akhtar, Z., Ghosh, S., Parveen, S., Islam, K., Islam, M.A., Rashid, M.M. & Khan, Z.H. (2022). Antibiotics usage practices in aquaculture in Bangladesh and their associated factors. *One Health*, *15*, 100445.https://doi.org/10.1016/j.onehlt.2022.100445

Clinical and Laboratory Standards Institute (CLSI).(2013) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplements. M100 S21, 31:1. Clinical and Laboratory Standards Institute, Wayne.

Dawood, M. A., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture*, *10*(4), 950-974. https://doi.org/10.1111/raq.12209

DoF, 2023.National Fish Week Compendium (*in Bangla*).Department of Fisheries.Ministry of Fisheries and Livestock, Bangladesh. 160p.

DoF, 2024.National Fish Week Compendium (*in Bangla*).Department of Fisheries.Ministry of Fisheries and Livestock, Bangladesh. 21p.

DoF. 2020. National Fish Week 2020 Compendium (in Bangla). Bangladesh: Department of Fisheries, Ministry of Fisheries and Livestock, 160.

Dong, X., Song, J., Chen, J., Bi, D., Wang, W., Ren, Y., Wang, H., Wang, G., Tang, K.F., Wang, X. & Huang, J. (2019). Conjugative transfer of the pVA1-type plasmid carrying the pirABvp genes results in the formation of new AHPND-causing *Vibrio*. *Frontiers in Cellular and Infection Microbiology*, *9*, 195.https://doi.org/10.3389/fcimb.2019.00195

Dong, X., Wang, H., Xie, G., Zou, P., Guo, C., Liang, Y., & Huang, J. (2017). An isolate of *Vibrio campbellii* carrying the pirVP gene causes acute hepatopancreatic necrosis disease. *Emerging Microbes & Infections*, *6*(1), 1-3.https://doi.org/10.1038/emi.2016.131

Eshik, M. M. E., Abedin, M. M., Punom, N. J., Begum, M. K., & Rahman, M. S. (2017). Molecular identification of AHPND positive *Vibrio parahaemolyticus* causing an outbreak in south-west shrimp farming regions of Bangladesh. *Journal of Bangladesh Academy of Sciences*, *41*(2), 127-135. https://doi.org/10.3329/jbas.v41i2.35492

Eshik, M. M. E., Punom, N. J., Begum, M. K., Khan, T., Saha, M. L., & Rahman, M. S. (2018). Molecular characterization of acute hepatopancreatic necrosis disease causing *Vibrio parahaemolyticus* strains in cultured shrimp *Penaeusmonodon* in south-west farming region of Bangladesh. *Dhaka University Journal of Biological Sciences*, *27*(1), 57-68. https://doi.org/10.3329/dujbs.v27i1.46411

FAO, F. (2013).MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp. *FAO Fisheries and Aquaculture Report*, 25-27.

FAO. 2024. *The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action*. Rome. https://doi.org/10.4060/cd0683en

Food and Drug Administration.(1992). Bacteriological analytical manual.In *Bacteriological Analytical Manual* (pp. 529-529).

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., & Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Research*, *46*(7), 2355-2364.https://doi.org/10.1016/j.watres.2012.02.004

Genovese, G., Faggio, C., Gugliandolo, C., Torre, A., Spano, A., Morabito, M., & Maugeri, T. L. (2012). In vitro evaluation of antibacterial activity of *Asparagopsistaxiformis* from the Straits of Messina against pathogens relevant in aquaculture. *Marine Environmental Research*, *73*, 1-6.https://doi.org/10.1016/j.marenvres.2011.10.002

Gomez-Gil, B., Tron-Mayén, L., Roque, A., Turnbull, J. F., Inglis, V., & Guerra-Flores, A. L. (1998). Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeusvannamei*. *Aquaculture*, *163*(1-2), 1-9. https://doi.org/10.1016/S0044-8486(98)00162-8

Hannan, M. A., Rahman, M. M., Mondal, M. N., Chandra, D. S., Chowdhury, G. A. Z. L. I. M. A., & Islam, M. T. (2019). Molecular identification of causing vibriosis in shrimp and its herbal remedy. *Polish Journal of Microbiology*, *68*(4), 429-438. https://doi.org/10.33073/pjm-2019-042

Hasan, M. A. R., Siddique, M. A., Hasan, M., Hossain, M. A., & Rahman, M. S. (2017). 16S rRNA gene sequence based identification of *Vibrio* spp. in shrimp and tilapia hatcheries of Bangladesh. *Dhaka University Journal of Biological Sciences*, *26*(1), 45-58.

Hasanuzzaman, A. F. M., Nadira, N., Sardar, A., & Islam, S. (2025). Antibiotic Sensitivity of *Vibrio* spp. and *Shewanellaalgae* Isolated From Brood and Egg of Mud Crab Hatchery. *Animal Research and One Health*. https://doi.org/10.1002/aro2.70008

Hernández-López, J., Gollas-Galván, T., Magallón-Barajas, F., & Vargas-Albores, F. (1997).Isolation of *Vibrio* and *Pseudomonas* from brown shrimp (*Penaeuscaliforniensis* Holmes) intestine. *Revista Latinoamericana de Microbiologia*, *39*(3-4), 109–115.

Hickman-Brenner, F. W., Brenner, D. J., Steigerwalt, A. G., Schreiber, M., Holmberg, S. D., Baldy, L. M., Lewis, C. S., Pickens, N. M., & Farmer, J. J., 3rd (1984). *Vibrio fluvialis* and *Vibrio furnissii* isolated from a stool sample of one patient. *Journal of Clinical Microbiology*, *20*(1), 125–127.https://doi.org/10.1128/jcm.20.1.125-127.1984

Hosain, M. A., Ullah, K., Al Sayam, M. A., Mohiuddin, K., & Rahman, E. (2021).Present Status and Future Direction of Bangladeshi Shrimp Resources.*Fisheries and Aquaculture Journal*, *12*(3), 1c-1c.

Huang, L., Xu, Y.B., Xu, J.X., Ling, J.Y., Chen, J.L., Zhou, J.L., Zheng, L. & Du, Q.P. (2017).Antibiotic resistance genes (ARGs) in duck and fish production ponds with integrated or non-integrated mode. *Chemosphere*, *168*, 1107-1114. https://doi.org/10.1016/j.chemosphere.2016.10.096

Islam, M.R., Hasanuzzaman, A.F.M, Islam, M.L., & Banu, G.R. (2023).Total bacterial load and enumeration of pathogenic bacteria *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas*spp.In the production line of mud crab (*Scylla olivacea*) hatchery in Bangladesh.*Advances in Animal and Veterinary Sciences*, *11*(6), 968-976. http://dx.doi.org/10.17582/journal.aavs/2023/11.6.968.976

Janda, J. M., Powers, C., Bryant, R. G., & Abbott, S. L. (1988).Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clinical Microbiology Reviews*, *1*(3), 245-267.https://doi.org/10.1128/cmr.1.3.245

Kang, C. H., Shin, Y., Jang, S., Jung, Y., & So, J. S. (2016). Antimicrobial susceptibility of *Vibrio alginolyticus* isolated from oyster in Korea. *Environmental Science and Pollution Research*, *23*, 21106-21112. https://doi.org/10.1007/s11356-016-7426-2

Khan, M., Paul, S. I., Rahman, M. M., & Lively, J. A. (2022).Antimicrobial Resistant Bacteria in Shrimp and Shrimp Farms of Bangladesh. *Water*, *14*(19), 3172.https://doi.org/10.3390/w14193172

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*, *46*(1), 165-170.https://doi.org/10.1128/aem.46.1.165-170.1983

Kumar, V., Baruah, K., Nguyen, D. V., Smagghe, G., Vossen, E., & Bossier, P. (2018).Phloroglucinol-mediated Hsp70 production in crustaceans: protection against *Vibrio parahaemolyticus* in *Artemiafranciscana* and *Macrobrachiumrosenbergii*. *Frontiers in Immunology*, *9*, 1091.https://doi.org/10.3389/fimmu.2018.01091

Kumar, V., Bels, L. D., Couck, L., Baruah, K., Bossier, P., &Broeck, W. V. D. (2019).PirABVP toxin binds to epithelial cells of the digestive tract and produce pathognomonic AHPND lesions in germ-free brine shrimp. *Toxins*, *11*(12), 717.https://doi.org/10.3390/toxins11120717

Kumar, V., Roy, S., Baruah, K., Van Haver, D., Impens, F., & Bossier, P. (2020). Environmental conditions steer phenotypic switching in acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus*, affecting PirAVP/PirBVP toxins production. *Environmental Microbiology*, *22*(10), 4212-4230.https://doi.org/10.1111/1462-2920.14903

Kumar, V., Roy, S., Behera, B. K., Bossier, P., & Das, B. K. (2021). Acute hepatopancreatic necrosis disease (AHPND): virulence, pathogenesis and mitigation strategies in shrimp aquaculture. *Toxins*, *13*(8), 524.https://doi.org/10.3390/toxins13080524

Lai, H.C., Ng, T.H., Ando, M., Lee, C.T., Chen, I.T., Chuang, J.C., Mavichak, R., Chang, S.H., Yeh, M.D., Chiang, Y.A. & Takeyama, H. (2015).Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. *Fish & Shellfish Immunology*, *47*(2), 1006-1014.https://doi.org/10.1016/j.fsi.2015.11.008

LajqiBerisha, N., PocevaPanovska, A., & Hajrulai-Musliu, Z. (2024). Antibiotic resistance and aquatic systems: Importance in public health. *Water*, *16*(17), 2362.https://doi.org/10.3390/w16172362

Lam, S. Y., &Goi, L. T. (1985). Isolations of "group F vibrios" from human stools. *Singapore Medical Journal*, *26*(3), 300-302. https://doi.org/10.1128/jcm.19.1.87-88.1984

Letchumanan, V., AbMutalib, N. S., Wong, S. H., Chan, K. G., & Lee, L. H. (2019). Determination of antibiotic resistance patterns of *Vibrioparahaemolyticus* from shrimp and shellfish in Selangor, Malaysia. *Progress in Microbes & Molecular Biology*, *2*(1).https://doi.org/10.36877/pmmb.a0000019

Letchumanan, V., Yin, W. F., Lee, L. H., & Chan, K. G. (2015). Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. *Frontiers in microbiology*, *6*, 33.https://doi.org/10.3389/fmicb.2015.00033

Lightner, D. V. (2003).Exclusion of specific pathogens for disease prevention in a *penaeid*shrimp biosecurity program. *Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables. The World Aquaculture Society, Baton Rouge, LA, USA*, 81-116.

Lin, J., Nishino, K., Roberts, M. C., Tolmasky, M., Aminov, R. I., & Zhang, L. (2015).Mechanisms of antibiotic resistance. *Frontiers in Microbiology*, *6*, 34.https://doi.org/10.3389/fmicb.2015.00034

Liu, L., Xiao, J., Zhang, M., Zhu, W., Xia, X., Dai, X., Pan, Y., Yan, S., & Wang, Y. (2018).A *Vibrio owensii* strain as the causative agent of AHPND in cultured shrimp, *Litopenaeusvannamei*. *Journal of Invertebrate Pathology*, *153*, 156–164.https://doi.org/10.1016/j.jip.2018.02.005

Magalhães, V., CastelloFilho, A., Magalhães, M., & Gomes, T. T. (1993).Laboratory evaluation on pathogenic potentialities of *Vibrio furnissii*. *Memórias do Instituto Oswaldo Cruz*, *88*, 593-597. https://doi.org/10.1590/S0074-02761993000400017

Matin, M. A., Chakraborty, S., Al Amin, M., & Ghosh, A. (2016). An assessment of shrimp aquaculture in selected coastal areas of Bangladesh. *Journal National Oceanographic and Maritime Institute*, *33*, 103-116.

Mechri, B., Ben Salem, I., Medhioub, A., Medhioub, M. N., & Aouni, M. (2015). Isolation and genotyping of potentially pathogenic *Vibrio alginolyticus* associated with *Ruditapesdecussatus* larva and juvenile mass mortalities. *Aquaculture International*, *23*, 1033-1047.https://doi.org/10.1007/s10499-014-9862-7

Mohamad, N., Amal, M. N. A., Saad, M. Z., Yasin, I. S. M., Zulkiply, N. A., Mustafa, M., & Nasruddin, N. S. (2019). Virulence-associated genes and antibiotic resistance patterns of *Vibrio* spp. isolated from cultured marine fishes in Malaysia. *BMC Veterinary Research*, *15*, 1-13.https://doi.org/10.1186/s12917-019-1907-8

Murray, C.J., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E. & Johnson, S.C. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, *399*(10325), 629-655.https://doi.org/10.1016/S0140-6736(21)02724-0

Muthukrishnan, S., Defoirdt, T., Ina-Salwany, M. Y., Yusoff, F. M., Shariff, M., Ismail, S. I., & Natrah, I. (2019).*Vibrio parahaemolyticus* and *Vibrio harveyi* causing Acute Hepatopancreatic Necrosis Disease (AHPND) in *Penaeusvannamei* (Boone, 1931) isolated from Malaysian shrimp ponds. *Aquaculture*, *511*, 734227.https://doi.org/10.1016/j.aquaculture.2019.734227

Nash, G. (1992). Vibriosis and its control in pond-reared Penaeusmonodon in Thailand. *Diseases in Asian aquaculture*, 143-155.

Okoh, A. I., Sibanda, T., Nongogo, V., Adefisoye, M., Olayemi, O. O., &Nontongana, N. (2015). Prevalence and characterisation of non-cholerae*Vibrio* spp. in final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. *Environmental Science and Pollution Research*, *22*, 2008-2017. http://dx.doi.org/10.1007/s11356-014-3461-z

Preena, P. G., Swaminathan, T. R., Kumar, V. J. R., & Singh, I. S. B. (2020). Antimicrobial resistance in aquaculture: a crisis for concern. *Biologia*, *75*, 1497-1517. https://doi.org/10.2478/s11756-020-00456-4

Rahman, M. M., Rahman, F., Afroze, F., Yesmin, F., Fatema, K. K., Das, K. K., & Noor, R. (2012). Prevalence of pathogenic bacteria in shrimp samples collected from hatchery, local markets and the shrimp processing plant. *Bangladesh Journal of Microbiology*, *29*(1), 7-10. http://dx.doi.org/10.3329/bjm.v29i1.28422

Rahman, S., Khan, S. N., Naser, M. N., &Karim, M. M. (2010).Isolation of *Vibrio* spp.From *penaeid* shrimp hatcheries and coastal waters of Cox's Bazar, Bangladesh.*Asian Journal of Experimental Biological Sciences*, *1*(2), 288-293.

Rebouças, R. H., de Sousa, O. V., Lima, A. S., Vasconcelos, F. R., de Carvalho, P. B., & dos Fernandes Vieira, R. H. S. (2011). Antimicrobial resistance profile of *Vibrio* species isolated from marine shrimp farming environments (*Litopenaeusvannamei*) at Ceará, Brazil. *Environmental Research*, *111*(1), 21-24.https://doi.org/10.1016/j.envres.2010.09.012

Restrepo, L., Bayot, B., Arciniegas, S., Bajaña, L., Betancourt, I., Panchana, F., & Reyes Muñoz, A. (2018).PirVP genes causing AHPND identified in a new *Vibrio* species (*Vibrio punensis*) within the commensal Orientalis clade. *Scientific Reports*, *8*(1), 13080.https://doi.org/10.1038/s41598-018-30903-x

Rungrassamee, W., Klanchui, A., Chaiyapechara, S., Maibunkaew, S., Tangphatsornruang, S., Jiravanichpaisal, P., &Karoonuthaisiri, N. (2013).Bacterial population in intestines of the black tiger shrimp (*Penaeusmonodon*) under different growth stages.*PloS One*, 8(4), e60802.https://doi.org/10.1371/journal.pone.0060802

Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M., & Van Boeckel, T. P. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports*, *10*(1), 21878.https://doi.org/10.1038/s41598-020-78849-3

Schirmeister, F., Wieczorek, A., Dieckmann, R., Taureck, K., &Strauch, E. (2014).Evaluation of molecular methods to discriminate the closely related species *Vibrio fluvialis*and *Vibrio furnissii*. *International Journal of Medical Microbiology*, *304* (7), 851-857. https://doi.org/10.1016/j.ijmm.2014.09.001

Selvin J, Lipton AP. 2003. *Vibrio alginolyticus* associated with white spot disease of *Penaeusmonodon*. *Diseases of Aquatic Organisms* 57:147–150. https://doi.org/10.3354/dao057001

Silvester, R., Saji, A., Divakaran, A. R., Dilshana, P. M., Nair, R., Hatha, M., &Harikrishnan, M. (2022). Increased incidence and antimicrobial resistance among *Vibrio parahaemolyticus* in shellfishes from major fish markets in Cochin, South India: Seafood risk assessment. *Annals of Animal Science*, *22*(3), 1105-1114. https://doi.org/10.2478/aoas-2021-0077

Sultana & Biswas (2022, May). Shrimp and prawn prospect in Bangladesh. *The Financial Express.* https://icsf.net/newss/bangladesh-shrimp-and-prawn-prospect/.

Sun, S., Korheina, D. K., Fu, H., &Ge, X. (2020). Chronic exposure to dietary antibiotics affects intestinal health and antibiotic resistance gene abundance in oriental river prawn (*Macrobrachiumnipponense*), and provokes human health risk. *Science of the Total Environment*, *720*, 137478.https://doi.org/10.1016/j.scitotenv.2020.137478

Tan, C. W., Malcolm, T. T. H., Kuan, C. H., Thung, T. Y., Chang, W. S., Loo, Y. Y., Premarathne, J. M. K. J. K., Ramzi, O. B., Norshafawatie, M. F. S., Yusralimuna, N., Rukayadi, Y., Nakaguchi, Y., Nishibuchi, M., &Radu, S. (2017). Prevalence and Antimicrobial Susceptibility of *Vibrio parahaemolyticus* Isolated from Short Mackerels (*Rastrelligerbrachysoma*) in Malaysia. *Frontiers in Microbiology*, *8*, 1087.https://doi.org/10.3389/fmicb.2017.01087

Tan, C. W., Rukayadi, Y., Hasan, H., Thung, T. Y., Lee, E., Rollon, W. D., Hara, H., Kayali, A. Y., Nishibuchi, M., &Radu, S. (2020). Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia. *Saudi Journal of Biological Sciences*, *27*(6), 1602–1608.https://doi.org/10.1016/j.sjbs.2020.01.002

Tendencia, E. A., & de la Peña, L. D. (2001).Antibiotic resistance of bacteria from shrimp ponds.*Aquaculture*, 195(3-4), 193-204. https://doi.org/10.1016/S0044-8486(00)00570-6

Tran, P. T. N., Kumar, V., & Bossier, P. (2020). Do acute hepatopancreatic necrosis disease-causing PirABVP toxins aggravate vibriosis?. *Emerging Microbes & Infections*, *9*(1), 1919-1932.https://doi.org/10.1080/22221751.2020.1811778

Watts, J. E., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. *Marine drugs*, *15*(6), 158.https://doi.org/10.3390/md15060158

World Health Organization. (2015). *Global antimicrobial resistance surveillance system: manual for early implementation*. World Health Organization.https://www.who.int/initiatives/glass

Yasin, A., Begum, M. K., Eshik, M. M. E., Punom, N. J., Ahmmed, S., &Rahman, M. S. (2022). Molecular identification and antibiotic resistance patterns of diverse bacteria associated with shrimp PL nurseries of Bangladesh: suspecting *Acinetobactervenetianus* as future threat. *PeerJ*, *10*, e12808.https://doi.org/10.7717/peerj.12808

Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., &Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. *Cureus*, *9*(6).https://doi.org/10.7759/cureus.1403

Zhou, Y., Yu, L., Liu, M., Liang, W., Li, Z., Nan, Z., &Kan, B. (2024). Virulence, antibiotic resistance phenotypes and molecular characterisation of *Vibrio furnissii* isolates from patients with diarrhoea. *BMC Infectious Diseases* , *24*(1), 412.https://doi.org/10.1186/s12879-024-09273-5