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Research Article

Molecular Detection and Antibiotic Resistance of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus* from Shrimp (*Penaeus monodon*) and Shrimp Environments in Bangladesh

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Some *Vibrio* species can cause food-borne diseases in humans, including cholera, vomiting, septicemia, and gastroenteritis, which are associated with the consumption of contaminated seafood products. The study was conducted to detect antimicrobial-resistant *Vibrio* species in shrimp and shrimp environments in Bangladesh. Samples of shrimp ($n = 50$), water ($n = 50$), and mud ($n = 50$) were collected aseptically from 50 different shrimp culture ponds in the Khulna region of Bangladesh. Identification of *Vibrio* species was based on cultural and staining characteristics, biochemical tests, and polymerase chain reaction (PCR). Antimicrobial resistance profiles were determined using a disk diffusion assay. By PCR, *Vibrio* isolates were found in 34% (95% CI: 26.9%–41.9%) of the samples, of which the detection rate was significantly higher in shrimp (54%), compared to mud (26%) and water (22%). Moreover, *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus* were detected in 24.7%, 15.3%, and 4% of the samples, respectively. Among them, the detection rate of *V. cholerae* and *V. alginolyticus* was significantly higher in shrimp samples than in other samples. *V. parahaemolyticus* was also higher in the shrimp samples, but the difference was not statistically significant. *Vibrio* isolates showed high to moderate resistance (92.2%–15.7%) to ampicillin, amikacin, cefotaxime, tetracycline, ceftazidime, gentamicin, nalidixic acid, levofloxacin, and ciprofloxacin, and low resistance (3.9%) to imipenem, meropenem, chloramphenicol, and trimethoprim-sulfamethoxazole. Interestingly, 52.9% of the isolates were multidrug resistant, and the multiple antibiotic resistance index was up to 1.0. To our knowledge, this is the first study in Bangladesh detecting these three *Vibrio* species (*V. parahaemolyticus*, *V. alginolyticus*, and *V. cholerae*) from shrimp and shrimp environments by molecular approach in the same study. These findings reveal the alarmingly high occurrence of antimicrobial-resistant *Vibrio* species in shrimp and shrimp environments, which should be of concern to both the shrimp industry and public health management.

1. Introduction

The aquaculture sector of Bangladesh has become a major food production sector, contributing to national economies and supporting rural and farming families. Total shrimp and prawn production in 2017-18 was 2.54×10^5 metric tons, as reported by the Deputy Director (Shrimp), Department of Fisheries, Dhaka, and District Fisheries Offices [1]. Aquaculture, fisheries, and related processing are important for food security in Bangladesh, contributing nearly 60% of animal proteins. In addition, they make a substantial contribution to gross domestic product (GDP) and export

earnings. This sector accounts for 3.57% of the national GDP and about a quarter of the agricultural GDP of Bangladesh (25.30%), making it vitally important to the rural economy and employment [1]. Globally, Bangladesh ranks 3rd in inland open water capture production and 5th in aquaculture production [2]. In terms of the value of exports, shrimp like the black tiger (*Penaeus monodon*) and freshwater scampi (*Macrobrachium rosenbergii*) bring in the most foreign currency, indicating the economic importance of shrimp in our country.

Consumption of contaminated fresh-raw shellfish, shrimp, and other sea foods has been linked to numerous cases of food-borne infection in humans [3, 4]. Shrimp contamination can occur due to improper and unhygienic conditions during cultivation, processing, preservation, and storage [5, 6]. Consequently, shrimp may be tainted with different *Vibrio* species that not only contribute to food spoilage but also to the spread of cholera and other food-borne diseases [7, 8]. *Vibrio* spp. are Gram-negative halophilic bacteria with a curved rod shape and a single flagellum that are typically found in aquatic environments. Moreover, *Vibrio* spp. are widespread in marine environments, including sea fish, mollusks, and crustaceans [9–11]. Twelve of the 30 *Vibrio* spp. are considered human pathogens; among these, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus* are the most frequently reported pathogens of zoonotic significance [10, 12, 13]. They can develop a wide range of human diseases, such as cholera, wound infection, bloody diarrhea, vomiting, abdominal pain, fever, and nausea [10, 14]. *V. cholerae* is the causal agent of cholera and has the potential to be transmitted to humans through contact with fish, seafood, and water [15]. *V. parahaemolyticus* causes gastroenteritis and food poisoning associated with the consumption of sea fish and foods [16, 17]. *V. vulnificus*, also known as the flesh-eating bacteria, is the leading cause of primary septicemia in people who consume raw or undercooked seafood [18]. *V. alginolyticus* is associated with wound infection through the exposure of cuts or abrasions of the skin to contaminated seawater [5].

The rise of antimicrobial resistance (AMR) poses a serious risk to human and animal health, food production, and economic growth around the globe [19]. It is predicted that AMR will result in hundreds of millions of deaths, massive economic shortfalls, and a sharp decline in livestock production if it is not encapsulated by the year 2050 [20, 21]. Overuse of antibiotics in both animals and humans creates a selective pressure that leads to the evolution of AMR [22, 23]. Antibiotic-resistant bacteria can be spread throughout the environment due to the careless use of antibiotics and a general lack of knowledge among the public [24]. Consequently, antibiotic-resistant *Vibrio* spp. could be transferred from marine products (e.g., shrimp) to their environments (e.g., water and mud) and vice versa.

The shrimp export market in Bangladesh is at risk because of the possible deterioration in the quality of processed shrimp products brought on by improper handling and the subsequent spread of food-borne illnesses [25]. Shrimp contaminated with *Vibrio* spp. are of lower quality, leading to their rejection at export. So, if these pathogenic microorganisms can be detected early in the shrimp industry, it will increase the likelihood that high-quality shrimp will be produced, which in turn will increase the number of export markets, bring in more foreign currency, and ensure the safety of shrimp for human consumption. Several studies on shrimp and shrimp products have been conducted so far in Bangladesh, but most of them focused solely on identifying *Vibrio* spp. at the genus level and quantifying their numbers [26–35]. However, as far as we know, very limited studies on detecting *Vibrio* spp. from shrimp and shrimp environments have been conducted in Bangladesh regarding species identification based on molecular approaches. Considering the current situation, the present study was undertaken with the following objectives: (1) to detect *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* from shrimp and shrimp environments (water and mud) of the Khulna region of Bangladesh by molecular approach and (2) to determine antibiotic resistance patterns of isolated *Vibrio* species.

2. Materials and Methods

2.1. Ethical Statement. No ethical statement was required during the sample collection. Two expert veterinarians and one microbiologist were involved in the sample collection. Moreover, consent to collect samples was obtained verbally from the farm owners or authorities.

2.2. Sampling Site and Sampling. Samples were collected from 50 different shrimp culture ponds in three selected upazilas of Satkhira district (Debhata: 22.5809°N, 88.9892°E; Satkhira Sadar: 22.7185°N, 89.0705°E; and Tala: 22.7508°N, 89.2574°E) in the Khulna region of Bangladesh (Figure 1). These areas were selected based on the high number of shrimp farms and the high demand for shrimp and shrimp farming.

A total of 150 samples containing shrimp, water, and sediments were collected randomly from each pond. Casting nets were used to collect the shrimp, and in order to avoid cross-contamination, each shrimp sample was placed in an individual sterile plastic zipper bag. Falcon tubes were used to collect water and sediment samples from the ponds where shrimp were harvested. All the samples were thereafter transferred to the Cary-Blair transport medium and transported to the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, as soon as possible for further processing and analysis. If one of three samples taken from a certain pond tested positive for *Vibrio* spp., that pond was labeled as contaminated with the bacteria.

2.3. Sample Processing. A loop of mud or sediment samples was directly inoculated into BHI broth containing 2% NaCl (2% BHI broth). Water samples were centrifuged at 3000 rpm for 1 min, and then the sediments were inoculated into 2% BHI broth. The brain, leg, muscle, and intestine from each shrimp sample were blended and taken for enrichment in 2% BHI broth. After that, the broths were incubated overnight at 37°C for bacterial enrichment.

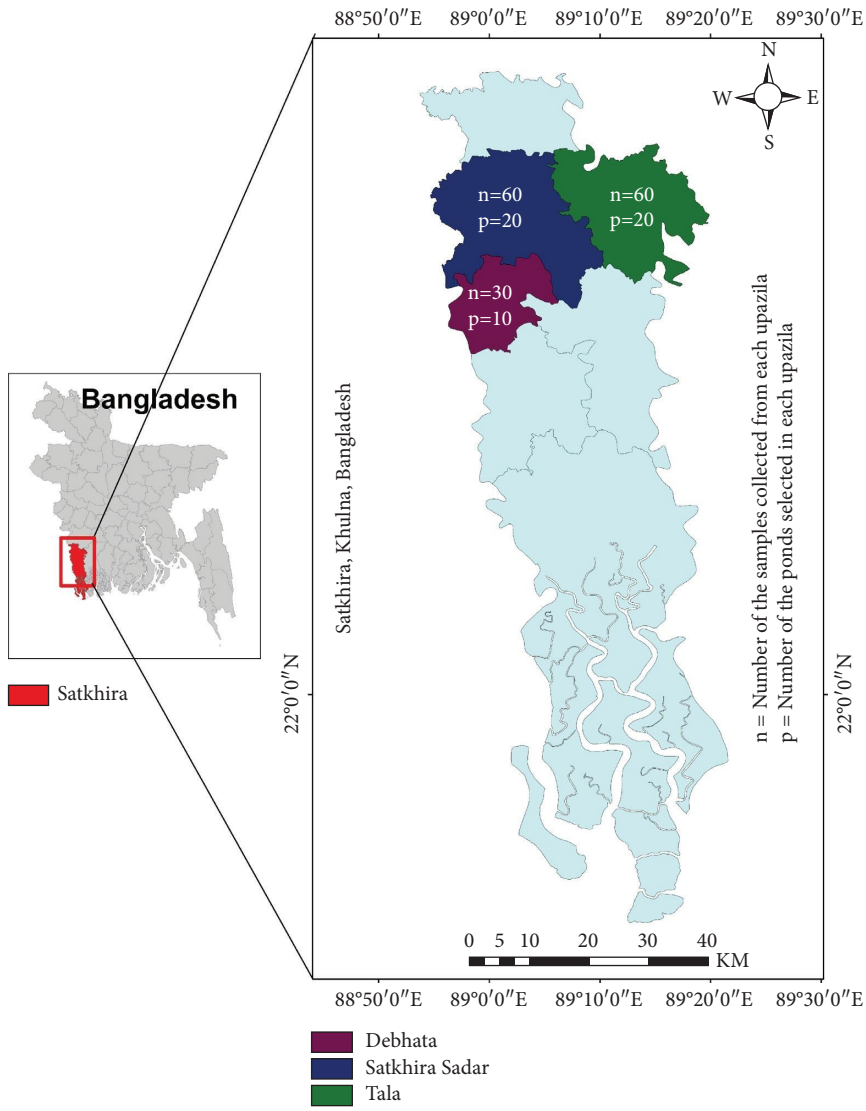

Figure 1: Sample location map of study areas, prepared by ArcMap 10.7 (ArcGIS Enterprise, ESRI, Redlands, CA, USA).

2.4. Isolation of Vibrio spp. A loop full of surface growth was streaked on a preset thiosulfate-citrate-bile salt-sucrose (TCBS) agar plate and incubated at 37°C for 18–24 h, as previously described [36]. After incubation, bacterial colonies were tentatively identified as *Vibrio* spp. based on their morphology (shape, size, and color). A single, clearly defined colony (yellow or green in color) was then streaked onto freshly prepared TCBS and blood agar plates for further purification. Isolates thought to be colonies of *Vibrio* spp. were Gram-stained to determine their morphology. As previously mentioned by Bergey et al. [37], other biochemical assays, including catalase and oxidase tests, were also conducted.

2.5. Molecular Detection of Vibrio spp. Final confirmation of *Vibrio* spp. was done by a polymerase chain reaction (PCR) test targeting the genus-specific *groEL* gene (Table 1).

Genomic DNA was extracted by the rapid boiling method following the procedures described by Talukder et al. [41]. In brief, 1 mL of overnight bacterial broth culture was centrifuged at 5,000 rpm for 3 min at 4°C. After centrifugation, the supernatant was discarded, and the pellet was resuspended in 500 μL of sterile distilled water. Again, the sample was centrifuged at 5,000 rpm for 3 min, and the supernatant was discarded. The pellet was resuspended in 200 μL of sterile distilled water. The mixture was then boiled for 10 min and immediately cooled at −20°C for 10 min. Subsequently, the sample was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant containing the genomic DNA was transferred into a sterile Eppendorf tube and stored at −20°C until used.

A 25 μL of PCR mixture comprising 12.5 μL of 2X master mix (Promega, Thermo Fisher Scientific, MA, USA), 5 μL of genomic DNA, one μL of each of the designated forward and reverse primers, and 5.5 μL of nuclease-free water were used for amplification. Thereafter, 5 μL of the PCR product was loaded onto a 1.5% agarose gel and run in 1X TAE buffer for 25 min at 100 V. Ethidium bromide was used for staining, and PCR products were visualized under an ultraviolet transilluminator (Biometra,

Table 1: List of primers used for specific detection of target *Vibrio* species.

Name of target gene	Name of *Vibrio* spp.	Primers	Sequence (5′-3′)	Annealing Tm	Amplicon size (bp)	Reference
groEL	*Vibrio* genus	gro-up gro-rp	TCCARAACATGGGCGCACAA ACGTTTTGYTCTTCGTTGTCRC	69	1117	[38]
	V. cholerae	groVc1 groVc2	GATCTTGACTGGCGGTGTTGTG GTCACCCACCAGAGAAGAGAGT	69	418	[39]
	V. parahaemolyticus	groVp1 groVp2	GTCAGGCTAAGCGCGTAAGCA GCATGCCTGCGCTTTCTTTTTG	69	644	
	V. vulnificus	gro Vv1 groVv2	GTTCGCGCTGGTGAAGGTTCA TGGCATACCAGAGTCTTTCTGTG	69	192	
	V. alginolyticus	groVa1 groVa2	GATTCGGTGAAGAAGAGATGATCTC TCTTCGTTGTCACCCGTTAGGTGA	66	301	[40]

Germany). For molecular weight DNA markers, 1 kb and 100 bp DNA ladders (Promega, Thermo Fisher Scientific, MA, USA) were used.

2.6. Molecular Detection of V. cholerae, V. parahaemolyticus, V. Vulnificus, and V. alginolyticus. All the *Vibrio* isolates were subjected to a simplex PCR for the detection of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* molecularly. The primer sequence, annealing temperature, and their amplicon size are documented in Table 1.

2.7. Antibiotic Susceptibility Test. The antibiotic susceptibility test (AST) was performed on each *Vibrio* isolate by employing the disk diffusion method [42]. A total of eight antimicrobial categories, including 13 antibiotics, were utilized in this study, such as penicillins (ampicillin-10 μg), cephalosporins (cefotaxime-30 μg; ceftazidime-30 μg), carbapenems (imipenem-10 μg and meropenem-10 μg), aminoglycosides (amikacin-30 μg and gentamicin-10 μg), tetracyclines (tetracycline-30 μg), fluoroquinolones (ciprofloxacin-5 μg; levofloxacin-5 μg; and nalidixic acid-5 μg), folate pathway inhibitors (trimethoprim-sulfamethoxazole-25 μg), and phenicols (chloramphenicol-30 μg). At the end of 18–24 hours of cultivation on TCBS agar plates, 2-3 bacterial colonies were suspended in 0.85% sterile normal saline solution to bring the final concentration to 0.5 McFarland standard units for AST. Following a 24-hour incubation period at 37°C, the inoculum was distributed using sterile cotton swabs on Mueller–Hinton agar plates, and preselected antibiotics were dispensed on the plates. The Clinical and Laboratory Standard Institute [43] guidelines were used to depict the results (sensitive, intermediate, and resistant). Isolates showing resistance to at least three antimicrobial categories were deemed multidrug resistant (MDR) [44]. Multiple antibiotic resistance (MAR) indices were calculated by the following formula: MAR = μ/v [45], where "μ" is the number of antibiotics that were resistant to an isolate and "v" is the total number of antibiotics used in this study.

2.8. Statistical Analysis. All the data were recorded and incorporated in the Excel 365 (Microsoft/Office 365, Redmond, DC, USA) spreadsheet and checked for inconsistencies and errors before being sorted, coded, and tested to guarantee that they retained their authenticity. Finally, the data were transferred from Excel 365 to the statistical package for social sciences (IBM SPSS 25.0, Chicago, IL, USA) and GraphPad Prism 8.4.2 (GraphPad Software, Inc.) for further statistical analysis. Calculations of the binomial 95% confidence intervals (CI) were performed using GraphPad Prism in accordance with the Wilson/Brown hybrid approach [46]. A chi-square test for relatedness (with a Z-test for proportion) was performed to evaluate the variations in the occurrence of *Vibrio* species among different types of collected samples. The significant p value was fixed at ≤0.05. The GraphPad Prism was used to create a heatmap displaying the antibiotic resistance profiles of *Vibrio* isolates.

3. Results

3.1. Occurrence of Vibrio spp. In PCR, 34% (51/150, 95% CI: 26.9%–41.9%) of the samples were found to be positive for *Vibrio* spp. Among them, shrimp samples (54%, 27/50) constituted a significantly higher ($p < 0.05$) prevalence of *Vibrio* spp. compared to mud (26%, 13/50) and water (22%, 11/50) samples (Table 2). Moreover, 21 of 50 shrimp culture ponds (42%, 95% CI: 29.4%–55.8%) were contaminated by *Vibrio* isolates.

3.2. Occurrence of V. cholerae, V. parahaemolyticus, V. Vulnificus, and V. alginolyticus. A total of 37 (24.7%, 95% CI: 18.5%–32.1%) samples harbored *V. cholerae*. Among them, significantly ($p < 0.05$) higher *V. cholerae* were detected in shrimp (38%) than in mud (20%) and water (16%) samples (Table 2). About 15.3% (23/150; 95% CI: 10.4%–21.9%) of the samples were positive for *V. parahaemolyticus*, but there was no significant variation among the samples. The highest prevalence rate was recorded in shrimp (24%), followed by water (10%) and mud (12%) (Table 2). *V. alginolyticus* isolates were found in 6 (4%, 95% CI: 1.9%–8.5%) samples, where a significant ($p < 0.05$) variation in the prevalence rate of *V. alginolyticus* was observed among samples, i.e., shrimp (10%), mud (2%), and water (0%) (Table 2). All the isolates were negative for *V. vulnificus* (Table 2).

Moreover, out of 50 ponds, 13 (26%) were contaminated with *V. cholera*, 21 (42%) with *V. parahaemolyticus*, and 5 (10%) with *V. alginolyticus*.

Table 2: Prevalence of *Vibrio* spp., *V. cholera*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* in shrimp and shrimp environments.

Organisms	Mud ($n = 50$) $N(\%)^{P}$ $(CI)^{Q}$	Water ($n = 50$) $N(\%)^{P}$ $(CI)^{Q}$	Shrimp ($n = 50$) $N(\%)^{P}$ $(CI)^{Q}$	p value	Total ($n = 150$) $N(\%)^{P}$ $(CI)^{Q}$
Vibrio spp.	13 (26^{a}) (15.9–39.6)	11 (22^{a}) (12.8–35.2)	27 (54^{b}) (40.4–67.0)	0.001	51 (34) (26.9–41.9)
V. cholerae	10 (20^{a}) (11.2–33.0)	8 (16^{a}) (8.3–28.5)	19 (38^{b}) (25.9–51.9)	0.025	37 (24.7) (18.5–32.1)
V. parahaemolyticus	5 (10^{a}) (4.4–21.4)	6 (12^{a}) (5.6–23.8)	12 (24^{a}) (14.3–37.4)	0.110	23 (15.3) (10.4–21.9)
V. alginolyticus	1 ($2^{a,b}$) (0.1–10.5)	0 (0^{b}) (0.0–7.1)	5 (10^{a}) (4.4–21.4)	0.026	6 (4) (1.9–8.5)
V. vulnificus	0 (0) (0.0–7.1)	0 (0) (0.0–7.1)	0 (0) (0.0–7.1)	NC	0 (0) (0.0–2.5)

Values with different superscripts differ significantly ($p < 0.05$) within the variable under assessment, CI = confidence interval, ^{P}number and percentage of positive isolates, ^{Q}95% confidence interval, and NC = not computed.

3.3. Antibiogram Profiles of Vibrio spp. By antimicrobial susceptibility test, the highest percentage of the *Vibrio* isolates were phenotypically resistant to ampicillin (92.2%; 95% CI: 81.5%–96.9%), followed by amikacin (50.9%; 95% CI: 37.7%–64.1%), cefotaxime and tetracycline (29.4%; 95% CI: 18.7%–42.9%), ceftazidime and gentamicin (27.5%; 95% CI: 17.1%–40.9%), nalidixic acid (25.5%; 95% CI: 15.6%–38.9%), levofloxacin (17.7%; 95% CI: 9.6%–30.3%), ciprofloxacin (15.7%; 95% CI: 8.2%–28.0%), and imipenem, meropenem, chloramphenicol, and trimethoprim-sulfamethoxazole (3.9%; 95% CI: 0.7%–13.2%) (Figure 2).

By bivariate analysis, a very high significant positive correlation was observed between the resistance patterns of imipenem and meropenem (Spearman coefficient, $\rho = 1.000$), imipenem and chloramphenicol ($\rho = 1.000$), meropenem and chloramphenicol ($\rho = 1.000$), cefotaxime and ceftazidime ($\rho = 0.953$), ciprofloxacin and levofloxacin ($\rho = 0.932$), nalidixic acid and ciprofloxacin ($\rho = 0.614$), gentamicin and amikacin ($\rho = 0.603$), amikacin and ceftazidime ($\rho = 0.603$), ciprofloxacin and gentamicin ($\rho = 0.580$), nalidixic acid and levofloxacin ($\rho = 0.555$), amikacin and cefotaxime ($\rho = 0.547$), tetracycline and amikacin ($\rho = 0.547$), and levofloxacin and gentamicin ($\rho = 0.522$) (Table 3). Also, the higher, moderate, and lower positive significant correlations were audited between any of the two antibiotics that showed resistance to *Vibrio* isolates (Table 3).

3.4. MDR and MAR Profiles of Vibrio spp. Twenty-seven *Vibrio* isolates (52.9%, 95% CI: 39.5%–65.9%) were MDR in nature. Isolates have a MAR index that is greater than 0.2 in a substantial number of isolates (27/51). The MAR index had a range of 0.0 to 1.0. One isolate showed resistance to all 13 used antibiotics (under eight antimicrobial classes). Also, there were 14 different resistant patterns observed among *Vibrio* isolates (Table 4).

4. Discussion

Vibrio species are marine and estuarine bacteria that pose a serious hazard to human health due to their role as primary microbial agents in food-borne infections. Consumption of seafood contaminated by antimicrobial-resistant *Vibrio* species has the potential to have serious consequences for humans. This study reported the molecular detection of antimicrobial-resistant *Vibrio* spp. in shrimp and shrimp-rearing environments in Bangladesh by a molecular approach.

In this study, 34% (51/150) of the shrimp and shrimp environmental samples were contaminated with *Vibrio* spp., of which 24.7%, 15.3%, and 4% of the samples were positive for *V. cholera*, *V. parahaemolyticus*, and *V. alginolyticus*, respectively. All the samples were negative for *V. vulnificus*. The prevalence of shrimp contamination found in the present study is comparable with the previous study [26], in which the prevalence of *Vibrio* spp. was 44%. Another study conducted in the southwest coastal area of Bangladesh on 216 shrimp and shrimp environmental samples found a 60.2% prevalence of *Vibrio* spp. Conversely, a similar study carried out in southern Bangladesh found that only 27% of shrimp samples were contaminated with *V. parahaemolyticus* [35], whereas shrimp and water samples from Gher farms were less frequently contaminated with *Vibrio* spp. Shrimp and water samples from Gher were found to be less contaminated and even absent of *Vibrio* spp. in comparison to the market samples in a study of the Kaliganj area in Satkhira district, which hold most of the shrimp farms in Bangladesh [47]. To the best of our knowledge, this is the first study in Bangladesh that has used a molecular approach to detect all three species of *Vibrio*-*V. parahaemolyticus*, *V. alginolyticus*, and *V. cholerae* from shrimp and shrimp environments in the same study. International studies make interesting comparisons (similar, higher, and lower) with the present data [5, 48–52]. The differences between these findings and our findings might be due to variations in the geographical distributions, environments, sample types and sizes, test methodologies, and others.

Temperature and salinity are the two most important factors affecting *Vibrio* distribution worldwide [53]. The concentration of marine *Vibrio* spp. is directly linked to water temperature. A study conducted in an island in Brazil revealed *Vibrio* spp. contamination in oysters was higher in warm water [54]. As water surface temperatures and salinity influence concentrations of most *Vibrio* spp., infections usually arise during the summer and fall when surface waters are comparatively warm [53]. Thus, the lower prevalence of *Vibrio* spp. found in the present study might be explained by the fact that the sampling was done in the winter. However, the variation among the studies might be due to the environmental conditions, nutrient concentration, the salinity of the water, and sample collection time.

Based on the present results, the prevalence of *Vibrio* spp. contamination in shrimp and the shrimp culture environment, together with the identification of zoonotic species (*V. parahaemolyticus*, *V. alginolyticus*, and *V. cholerae*), indicates a significant risk to public health. It is

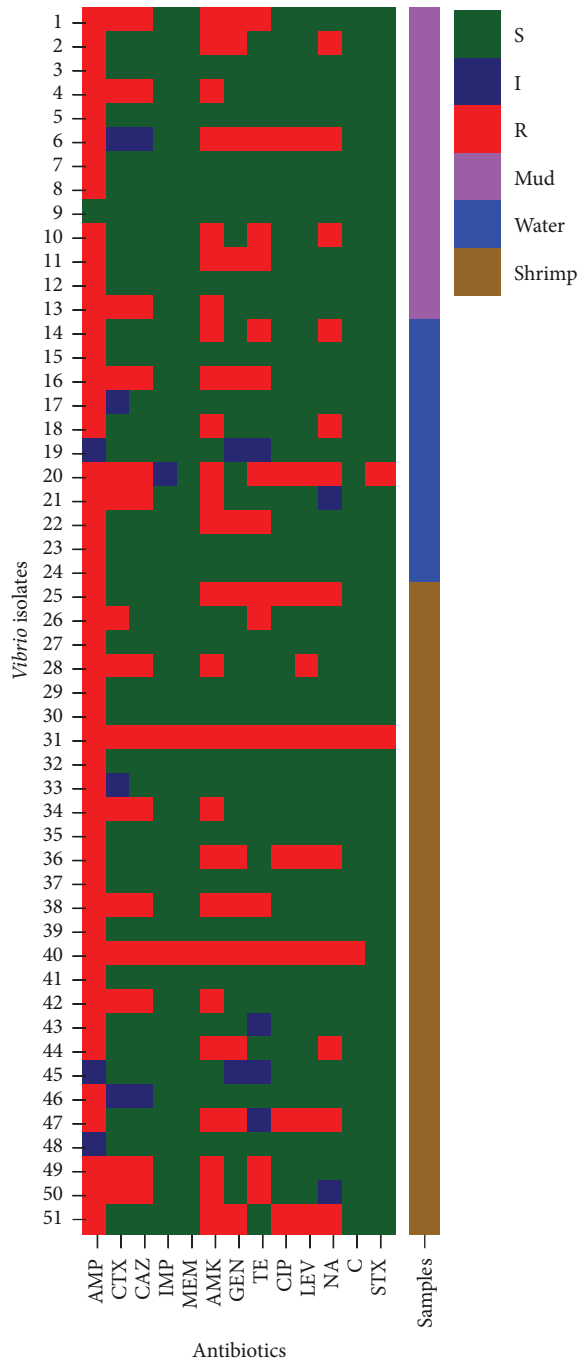

FIGURE 2: Heatmap showing the antimicrobial resistance patterns of each *Vibrio* isolate sourced from shrimp and shrimp environments, AMP = ampicillin, CTX = cefotaxime, CAZ = ceftazidime, IMP = imipenem, MEM = meropenem, AMK = amikacin, GEN = gentamicin, TE = tetracycline, CIP = ciprofloxacin, LEV = levofloxacin, NA = nalidixic acid, C = chloramphenicol, STX = trimethoprim-sulfamethoxazole, R = resistant, and I = intermediate, S = sensitive.

feasible that other toxicogenic strains could have been present, but identification of these was beyond the scope of the present study. Nonetheless, future studies should focus on attempting to identify additional pathogenic species, as well as considering the effects of season and water temperature and correlating the pH and salinity of the sampling source water for the best clarification of the factors that promote the multiplication of this organism in the shrimp industry. A routine surveillance program could be carried out to monitor the pathogenic bacteria, which would, in turn, allow for an effective management program to be implemented to control the risk of spreading diseases. In such programs, applications of conventional and molecular methods are strongly recommended to confirm and differentiate the pathogenic strains of *Vibrio* spp.

Table 3: Spearman coefficient to assess the significant association between any of the two antibiotics resistant to *Vibrio* isolates sourced from shrimp environments.

		AMP	CTX	CAZ	IMP	MEM	AMK	GEN	TE	CIP	LEV	NA	C	STX
AMP	ρ	1												
CTX	ρ	0.188	1											
CAZ	ρ	0.179	0.953**	1										
IMP	ρ	0.059	0.313*	0.328*	1									
MEM	ρ	0.059	0.313*	0.328*	1.000**	1								
AMK	ρ	0.298*	0.547**	0.603**	0.198	0.198	1							
GEN	ρ	0.179	0.085	0.114	0.328*	0.328*	0.603**	1						
TE	ρ	0.188	0.433**	0.374**	0.313*	0.313*	0.547**	0.471**	1					
CIP	ρ	0.126	0.077	0.097	0.468*	0.468**	0.423**	0.580**	0.313*	1				
LEV	ρ	0.135	0.153	0.176	0.436**	0.436**	0.454**	0.522**	0.266	0.932**	1			
NA	ρ	0.171	−0.18	−0.158	0.114	0.114	0.484**	0.447**	0.215	0.614**	0.555**	1		
C	ρ	0.059	0.313*	0.328*	1.000**	1.000**	0.198	0.328*	0.313*	0.468**	0.436**	0.114	1	
STX	ρ	0.059	0.313*	0.328*	0.480**	0.480**	0.198	0.102	0.313*	0.468**	0.436**	0.345*	0.480**	1

*Correlation is significant at the 0.05 level (2-tailed), **correlation is significant at the 0.01 level (2-tailed), ρ = spearman correlation coefficient, AMP = ampicillin, CTX = cefotaxime, CAZ = ceftazidime, IMP = imipenem, MEM = meropenem, AMK = amikacin, GEN = gentamicin, TE = tetracycline, CIP = ciprofloxacin, LEV = levofloxacin, NA = nalidixic acid, C = chloramphenicol, and STX = trimethoprim-sulfamethoxazole.

Antimicrobial resistance is becoming an increasingly serious threat to public health [55, 56]. Antibiotic resistance among *Vibrio* species is a problem that affects not only human health on a worldwide scale but also poses a possible obstacle to the process of treating infectious diseases that affect aquatic animals. Although chromosomal mutations can be involved in AMR in *Vibrio* species, the greatest contributors to *Vibrio* drug resistance are the frequent acquisition of extrachromosomal mobile genetic elements (e.g., replicating plasmids and integrating conjugative elements) and/or insertion sequences from closely related or distantly related bacterial species [57]. In this study, a higher percentage of *Vibrio* isolates (92.2%) were resistant to ampicillin, which shows agreement with the previous studies in Bangladesh [34, 35]. Resistance to penicillin may have resulted from the widespread use of antibiotics in aquaculture and the impact of leftover antibiotics in aquaculture systems. As a result, infections caused by *Vibrio* spp. are difficult to treat using penicillin-based antibiotics. Also, *Vibrio* isolates showed a higher percentage of resistance to cefotaxime (29.4%) and ceftazidime (27.5%), which is analogous to other studies reported in different countries [58–60]. In the present study, *Vibrio* isolates were highly resistant to nalidixic acid, ciprofloxacin, levofloxacin, amikacin, gentamicin, and tetracyclines; however, a low level of resistance was exhibited to imipenem, meropenem, chloramphenicol, and trimethoprim-sulfamethoxazole. In line with our findings, Heenatigala and Fernando [61] reported that *Vibrio* isolates showed a very high level of resistance to antibiotics that are commonly used in aquaculture, including gentamycin, kanamycin, oxytetracycline, chloramphenicol, and trimethoprim. The presence of resistance to these antibiotics reveals a serious threat by limiting the treatment options because antibiotics under the classes of cephalosporins, aminoglycosides, fluoroquinolones, and folate pathway inhibitors are recommended to treat *Vibrio* spp. infections [60].

In bivariate analysis, we found a very high significant positive correlation between the resistance patterns of imipenem and meropenem, cefotaxime and ceftazidime, ciprofloxacin and levofloxacin, nalidixic acid and ciprofloxacin, nalidixic acid and levofloxacin, gentamicin and amikacin, imipenem and chloramphenicol, meropenem and chloramphenicol, amikacin and ceftazidime, ciprofloxacin and gentamicin, amikacin and cefotaxime, tetracycline and amikacin, and levofloxacin and gentamicin. Since they belong to the same class of antibiotics, it is not surprising to find a high degree of similarity between most of the antibiotics. The random administration of antibiotics to aquaculture systems is a possible cause of the other significant associations. The significance of these findings is tied to the fact that *Vibrio* isolates have shown signs of developing resistance against various types of antimicrobials [62].

Humans are vulnerable to infections produced by MDR microorganisms [63]. Pathogens pose a significant threat to healthcare systems everywhere because MDR strains are spreading due to AMR [64]. In this study, 52.9% of the *Vibrio* isolates were phenotypically MDR in nature. It has become a common practice to use MAR analysis to discern bacteria isolated from settings where conventional antimicrobials are safe and effective for human treatment. Bacterial MAR is typically linked to the presence of plasmids that carry single or multiple resistance genes [65]. Bacterial strains with MAR indices above 0.2 were likely exposed to many antibiotics or isolated from tainted environments, while those with indices below 0.2 were less likely to have been treated with antibiotics [45, 66]. More than half (52.9%) of the *Vibrio* isolates in this investigation had a MAR index value larger than 0.2, suggesting that these samples came from a high-risk contamination source where several antibiotics were utilized. Previously, Lee et al. [67] reported that 70% of the *Vibrio* isolates showed a MAR index of more than 0.2, which is more than in our present study. There may be a correlation between the heavy use of antibiotics in the aquaculture industry to combat bacterial infections and the high prevalence of multiple antibiotic-resistant isolates observed in this study. Antimicrobial resistance in aquatic bacteria is a result of environmental contamination in coastal and estuarine

Table 4: Multidrug resistance and multiple antibiotic resistance index of *Vibrio* isolates sourced from shrimp environments.

Pattern no	Antibiotic resistance patterns	No. of antibiotics (classes)	No. of isolates	Overall no. of MDR isolates (%)	MAR index
1	AMP, CTX, CAZ, IMP, MEM, AMK, GEN, TE, CIP, LEV, NA, C, and STX	13 (8)	1		1.0
2	AMP, CTX, CAZ, IMP, MEM, AMK, GEN, TE, CIP, LEV, NA, and C	12 (7)	1		0.9
3	AMP, CTX, CAZ, AMK, TE, CIP, LEV, NA, and STX	9 (6)	1		0.7
4	AMP, AMK, GEN, TE, CIP, LEV, and NA	7 (4)	1		0.5
5	AMP, CTX, CAZ, AMK, GEN, and TE	6 (4)	3		0.5
6	AMP, AMK, GEN, CIP, LEV, and NA	6 (3)	4		0.5
7	AMP, CTX, CAZ, AMK, and TE	5 (4)	3	27/51 (52.9)	0.4
8	AMP, AMK, TE, and NA	4 (4)	2		0.3
9	AMP, AMK, GEN, and NA	4 (3)	2		0.3
10	AMP, AMK, GEN, and TE	4 (3)	2		0.3
11	AMP, CTX, CAZ, and AMK	4 (3)	5		0.3
12	AMP, CTX, and TE	3 (3)	1		0.2
13	AMP, AMK, and NA	3 (3)	1		0.2
14	AMP	1 (1)	20		0.1
15	—	—	4		0

MDR = multidrug-resistant, MAR = multiple antibiotic resistance, AMP = ampicillin, CTX = cefotaxime, CAZ = ceftazidime, IMP = imipenem, MEM = meropenem, AMK = amikacin, GEN = gentamicin, TE = tetracycline, CIP = ciprofloxacin, LEV = levofloxacin, NA = nalidixic acid, C = chloramphenicol, and STX = trimethoprim-sulfamethoxazole.

waters, most notably from wastewater treatment plants and agricultural runoff, which may carry a variety of antimicrobials and heavy metals [35].

5. Conclusion

The findings observed in this study embrace the comprehensive reports about the occurrence and antimicrobial resistance profiles of *Vibrio* species, especially *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus* isolates sourced from shrimp and shrimp environments. Our study suggests that shrimp and shrimp environments are contaminated with antimicrobial-resistant *Vibrio* species, indicating the requirement for extensive surveillance with a large number of farms and samples across the country. Hence, it is recommended that the shrimp culture industry should be aware of the risks of antimicrobial-resistant *Vibrio* contamination and take appropriate precautions during the culture, harvest, and processing of shrimp. Monitoring the antibiotic resistance of *Vibrio* species on a continuous basis is essential for determining the most effective treatment for patients with gastroenteritis and ensuring the safety of seafood. Moreover, it is necessary to do additional research in order to determine the risk factors that could result in disease outbreaks in shrimp farms with *Vibrio* species.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

ZFH, AAMS, and MSI performed the research. A.K.S. contributed to the sampling. MSI and ZFH analyzed the data. MSI, ZFH, and AAMS prepared the first draft of the manuscript. MSI, AP, MGH, and SS reviewed the manuscript. SS conceptualized, designed, and supervised the study, managed funds, and critically reviewed and rewrote the final draft of the manuscript. All authors have read and approved the final manuscript.

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