Journal of Applied Biology & Biotechnology Vol. 11(2), pp. 123-128, Mar-Apr, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110211

The occurrence of antibiotic resistance *Vibrio* isolates from brackish water shrimp ponds in the coastal area in Thua Thien Hue, Vietnam

Le Cong Tuan[1], Nguyen Van Khanh[2], Huyen Ton Nu Bao Tien[1], Phung Thao Phuong[1], Duong Van Hieu[1], Le Thi Ha Thanh[3], Nguyen Hoang Loc[3]*

[1]Department of Environmental Science, University of Science, Hue University, Hue 530000, Vietnam.
[2]Institute of Biotechnology, Hue University, Thua Thien Hue 530000, Vietnam.
[3]Department of Biotechnology, University of Science, Hue University, Hue 530000, Vietnam.

ARTICLE INFO

Article history:
Received on: June 06, 2022
Accepted on: October 08, 2022
Available online: January 22, 2023

Key words:
Vibrio,
Antibiotic resistance,
Shrimp,
Ciprofloxacin.

ABSTRACT

The indiscriminate and improper use of antibiotics in shrimp farming can have a number of negative consequences, including the development of drug-resistant bacteria that render disease prevention and treatment ineffective, negatively impact the user's health, and alter the natural microflora. In addition, exporting and consuming farmed shrimp items that have antibiotic residues will be also prohibited. The aim of this study was to detect antibiotic-resistant *Vibrio* species in extensive white leg shrimp farming systems in Thua Thien Hue, Vietnam. Microbial culture techniques were utilized to isolate *Vibrio*, determine antibiotic resistance, and estimate the minimal bacterial inhibitory concentration. The species of *Vibrio* were identified using 16S rRNA sequencing, and a phylogenetic tree was constructed using the maximum likelihood estimation with a bootstrap of 1000 replications. The results showed the supply brackish water consisted of $10^{2.5}$–$10^{3.8}$ CFU/mL of *Vibrio*. After 3 months of cultivation, the *Vibrio* concentration in white leg shrimp ponds increased rapidly, reaching 10^{4}–$10^{6.6}$ CFU/mL. Antibiotic susceptibility testing demonstrated the antibiotic resistance of *Vibrio* present in supply brackish water. *Vibrio* bacteria in shrimp ponds were resistant to gentamicin, kanamycin, streptomycin, oxytetracycline hydrochloride, and doxycycline, up to 98.87% of the antibiotics tested, ciprofloxacin was the most potent inhibitor of *Vibrio*. MIC evaluation of ciprofloxacin-resistant *Vibrio* isolates showed that they were resistant to many other antibiotics. This discovery demonstrates the need for using the optimum antibiotics in white leg shrimp farming to prevent the dissemination of antibiotic-resistant *Vibrio* bacteria.

1. INTRODUCTION

Shrimp culture has become an important industry which is accounting for approximately 18% of the total world's aquaculture economy. Asian and Latin America are major production areas whose economic value is estimated at USD 28 billion per year [1]. However, this high-value industry has faced serious disease outbreaks, particularly caused by *Vibrio* bacteria, which have caused widespread losses to farmers. A certain *Vibrio* species can cause high mortality to shrimp such as *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. mimicus*, or *V. vulnificus*. *Vibrio* bacteria are the major agent responsible for acute hepatopancreatic necrosis disease, shell disease, white gut disease, luminescent disease, red disease, and loose shell [2,3].

Antibiotics were widely applied in aquaculture to control pathogenic bacterial infections including *Vibrio* species. The overuse of antibiotics is recently being a serious problem to the water ecosystem by spreading antibiotic-resistant bacteria and antimicrobial resistance genes to the environment. Moreover, opportunistic *Vibrio* bacteria have been reported carrying out multiple virulence factors and multiple resistomes. The diversity of resistomes found higher in shrimp farms than in coastal waters and depended on the farming stage [4-6]. The overuse of antibiotics in shrimp farming in Vietnam has recently escalated, according to the National Agro-Forestry-Fisheries Quality Assurance Department (Nafiqad). Antibiotics including oxytetracycline, chloramphenicol, ciprofloxacin, ormetoprim, and enrofloxacin are typically to blame for violations of shrimp batches [7]. As a result, many antibiotics are now listed as banned substances including ciprofloxacin, chloramphenicol, erythromycin, oxytetracycline, and sulfonamides [2].

Thua Thien Hue province has the advantage of a long coastline, many dunes along the coast, which are favorable for shrimp farming. Besides, this place has the Tam Giang-Cau Hai coastal lagoon system which is the largest lagoon of Southeast Asia, which has many favorable conditions for the development of shrimp culture [8]. Over the past years, white leg shrimp (*Litopenaeus vannamei*) farming becomes more popular due to great economic benefits to farmers. However, enormous difficulties and challenges have been rising for the shrimp

*Corresponding Author:
Nguyen Hoang Loc,
Department of Biotechnology, University of Science, Hue University,
Hue 530000, Vietnam
E-mail: nhloc @ hueuni.edu.vn

farming markets such as environmental pollution and epidemics outbreaks caused by *Vibrio* species [8,9]. According to a preliminary investigation, for instance, a 2000 m^2 pond stocked with 50,000 shrimp in Phong Dien district (Thua Thien Hue province) would require spending 50–60 million VND on mineral salts, digestive enzymes, and antibiotics (tetracycline, berberine, etc.) over the course of 2.5 months [10]. Moreover, the data on antibiotic-resistant *Vibrio* related to the shrimp farming industry in this area are not well documented. This study alerts the occurrence of antibiotic resistance *Vibrio* species in intensive shrimp farming systems, contributing to the approaches to minimize the antibiotic resistance *Vibrio* in aquaculture.

2. METHODS AND MATERIALS

2.1. Medium and Antibiotics

Thiosulfate Citrate Bile Sucrose agar (TCBS) agar medium was used to isolate *Vibrio* [11]. Mueller-Hinton broth was used for the antimicrobial susceptibility test. Antibiotics including ciprofloxacin, gentamycin, kanamycin, oxytetracycline hydrochloride, doxycycline, and streptomycin from Nam Thai Veterinary Medicine Co. (Vietnam) were used to evaluate the antibiotic resistance ability of *Vibrio* isolates.

2.2. Determination of Antibiotic-resistant *Vibrio* Isolates

Five hundred mL of water from each shrimp pond in four districts of Thua Thien Hue province (Quang Dien, Phu Vang, Phu Loc, and Phong Dien) was collected monthly for the study, with three ponds in each district. Water was diluted between 10^{-1} and 10^{-4} in a sterile 0.9% saline solution. One hundred µL of the diluted solution was then spread on TCBS agar plate supplemented with various antibiotics including ciprofloxacin (50 µg/mL), gentamycin (10 µg/mL), kanamycin (30 µg/mL), oxytetracycline hydrochloride (30 µg/mL), doxycycline (30 µg/mL), and streptomycin (10 µg/mL). A TCBS agar plate without antibiotics was used as a control. The cultures were kept at 37°C for 24 h. Bacterial concentrations were determined based on the number of *Vibrio* colonies growing on TCBS agar with or without antibiotics. The prevalence of antibiotic-resistant *Vibrio* bacteria (P_{ARV}) was calculated as follows:

$$P_{ARV} = \frac{C_{ARV}}{C_{TV}} \times 100$$

Where: C_{ARV}: antibiotic-resistant *Vibrio* cells, C_{TV}: total *Vibrio* cells

2.3. Determination of Minimum Bacterial Inhibitory Concentration (MIC)

MIC50 and MIC90 were determined as described by Ataee *et al*. [12]. Antibiotic concentrations were prepared from 30 to 300 µg/mL (in increment of 30) for each, except for ciprofloxacin was from 5 to 50 µg/mL (in increment of 5).

Vibrio isolates were cultured overnight in Mueller-Hinton broth at 37°C with 180 rpm of shaking, then diluted to an OD_{600} of 1 (approximately 1.5 × 10^8 CFU/mL). The diluted bacterial solution was mixed with 152.5 µL of Mueller-Hinton broth and antibiotic solution to a final concentration of 10^5 CFU/mL. The mixture was incubated at 37°C with 150 rpm of shaking for 18 h. The optical density of the mixture before and after incubation was measured at 600 nm using a UV-VIS spectrophotometer. The antibiotic susceptibility ratio (R_{AS}, %) was determined as follows [13]:

$$R_{AS}(\%) = \frac{A_b - A_a}{A_c - A_d}$$

Where, A_a and A_d are the initial optical density of sample and control (without antibiotics); A_b and A_c are the final optical density of sample and control (without antibiotics). MIC50 and MIC90 were defined as the antibiotic concentration that inhibits approximately 50% and 90% of *Vibrio* cells, respectively.

2.4. Molecular Identification of *Vibrio* Isolates

Total DNA from *Vibrio* isolates was extracted by the cetyltrimethylammonium bromide method as described by Sambrook [14]. 16S rRNA genes were amplified by PCR with universal primers (forward: 5'-AGAGTTTGATCMTGGCTCAG-3' and reverse: 5'-CTGCTGCSYCCCGTAG-3') and sequenced by the Sanger method. Their sequences were then searched homology against the GenBank database to identify species. The phylogenetic tree was generated using MEGA 11 software using the maximal likelihood method with a bootstrap of 1000 replicates [15].

2.5. Data Analysis

The data were processed with Minitab software version 16.2.0 and Microsoft Excel 2013 to calculate mean values. A one-way ANOVA with a significance level of 0.05 was used to compare the differences in means between treatments.

3. RESULTS

3.1. Determination of MIC

Based on inhibition rate data for the 10 antibiotics used, *Vibrio* isolates grown on ciprofloxacin-TCBS plates were selected to determine antibiotic resistance ability. The observation found five isolates exhibited a broad antibiotic resistance against gentamycin (10 µg/mL), kanamycin (30 µg/mL), oxytetracycline hydrochloride (30 µg/mL), doxycycline (30 µg/mL), and streptomycin (10 µg/mL). These isolates named as *Vibrio* KS01 (NCBI, accession number: OK560361), *Vibrio* KS03 (OK560362), *Vibrio* KS04 (OK560363), *Vibrio* KS06 (OK560364), and *Vibrio* KS07 (OK560365). The minimum inhibitory concentration of 10 antibiotics used to eliminate these five isolates is shown in Table 1.

The results showed that kanamycin, one of the four aminoglycoside antibiotics investigated, had the strongest inhibitory effect on *Vibrio*. *Vibrio* KS03 expressed high sensitivity to this antibiotic group than other isolates whose MIC50 was at 30 µg/mL. Meanwhile, *Vibrio* KS06 was strongly resistant to these antibiotics with MIC50 found to be above 120 µg/mL. MIC90 of all *Vibrio* isolates was ≥150 µg/mL. In contrast, streptomycin did not significantly affect bacterial growth.

Oxytetracycline hydrochloride did not show susceptibility to *Vibrio* strains KS03, KS06, and KS07, while the MIC50 of KS01 and KS04 was 180 µg/mL and 150 µg/mL, respectively. Although, the MIC50 of doxycycline ranges from 30 to 300 µg/mL. This antibiotic did not completely inhibit the growth of *Vibrio*.

Our study revealed that ciprofloxacin was not significantly effective against *Vibrio* isolates compared with other tested antibiotics. It only partially inhibited the growth of strains KS01 and KS03, but not strains KS04, KS06, and KS07 [Table 1]. This result indicated that ciprofloxacin-resistant ability of *Vibrio* was highly stable.

In general, *Vibrio* KS01 isolate was completely resistant to streptomycin, while KS03 isolate resists streptomycin and oxytetracycline hydrochloride. Streptomycin and ciprofloxacin inhibited KS04 growth. Meanwhile, streptomycin, oxytetracycline

hydrochloride, and ciprofloxacin had no inhibitory effect against KS06. KS07 isolate still grew well in the presence of streptomycin, doxycycline, oxytetracycline hydrochloride, and ciprofloxacin.

3.2. Antibiotic Resistance Status of *Vibrio*

As shown in Table 2, the water contained *Vibrio* with concentrations ranging from $10^{2.5}$ to $10^{3.8}$ CFU/mL. *Vibrio* growth was slightly increased in the 1st month of culture but then more rapidly from the second to the 2nd month, reaching about 10^5 CFU/mL and 10^6 CFU/mL, respectively.

The percentage of antibiotic resistance varied and was quite different among the water sources and culture time [Table 3]. Water in Quang Dien was clean without any antibiotic resistance *Vibrio* except for doxycycline. On the contrary, antibiotic resistance *Vibrio* was found in water obtained from Phu Vang, Phu Loc, and Phong Dien. *Vibrio's* high resistance ratio with gentamycin and streptomycin was found in supply water at one shrimp pond in Phu Vang. Meanwhile, there was no ciprofloxacin resistance *Vibrio* in all investigated areas. The percentage of antibiotic resistance *Vibrio* dramatically increased over time during the cultivation of the shrimp.

Study results have shown that more than 90% of *Vibrio* present in water was resistant to antibiotics, especially to streptomycin and oxytetracycline hydrochloride [Table 3]. Among the investigated areas, the shrimp farm in Phong Dien had the highest rate of antibiotic-resistant *Vibrio* bacteria, 97.91% after 3 months of culture. In contrast, quinolone antibiotics such as ciprofloxacin inhibited most *Vibrio* isolates.

3.3. Identification Antibiotic Resistance *Vibrio*

Species identification of the five *Vibrio* isolates was carried out by sequencing their 16S rRNA gene. Comparison of 16S rRNA nucleotide sequences with the database on the GenBank showed that *Vibrio* KS03 has high similarity with *V. parahaemolyticus* (100%); *Vibrio* KS04, KS06, and KS07 like *V. alginolyticus* (100, 100, and 99%, respectively); while *Vibrio* KS01 was identified with *Vibrio* sp. (100%). Therefore, the three isolates of *Vibrio* KS04, KS06, and KS07 were named *V. alginolyticus* KS04, *V. alginolyticus* KS06, and *V. alginolyticus* KS07. The *Vibrio* KS03 isolate was named *V. parahaemolyticus* KS03. The phylogenetic tree of these isolates was constructed using the maximum likelihood method and the Tamura-Nei model, as shown in Figure 1. The nucleotide sequences of *Vibrio* isolates KS01, KS03, KS04, KS06, and KS07 have been deposited on NCBI with the accession numbers OK560361, OK560362, OK560363, OK560364, and OK560365, respectively.

4. DISCUSSION

A previous study by Chikwendu (2004) showed that the rate of *Vibrio* resistant to mezlocillin, doxycycline, carbenicillin, and ampicillin ranged from 81.3 to 97.8%, while the rate of resistance to kanamycin was only 40.9% [16]. The present study reported that *Vibrio* can resist other strong antibiotics including streptomycin, gentamycin, and ciprofloxacin which are often used to treat pathogenic bacteria for humans. Data in Table 3 indicated that ciprofloxacin is the last barrier to eliminating antibiotic resistance *Vibrio*. Ciprofloxacin has stronger antibacterial activity than antibiotics of other groups such as amino acids, β-lactams, tetracyclines, and penicillins [17,18]. Ciprofloxacin

Table 1: The minimum inhibitory concentration of antibiotics against *Vibrio* KS01, KS03, KS04, KS06, and KS07.

Antibiotics	KS01		KS03		KS04		KS06		KS07	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
1. Aminoglycoside										
Gentamycin	90 (51.5%)	180 (94.1%)	30 (56.4%)	150 (95.6%)	270 (54.9%)	-	240 (80.1%)	-	60 (51.6%)	-
Kanamycin	60 (55.3%)	150 (99.5%)	30 (85.8%)	150 (95.4%)	120 (69.3%)	150 (91.5%)	240 (57.9%)	-	120 (70.5%)	150 (96.6%)
Streptomycin	-	-	-	-	-	-	-	-	-	-
2. Tetracycline										
Oxytetracycline hydrochloride	180 (50.6%)	-	-	-	150 (54.7%)	-	-	-	-	-
Doxycycline	30 (84.2%)	-	300 (52.3%)	-	120 (62%)	-	30 (72.3%)	-	-	-
3. Quinolone										
Ciprofloxacin	50 (58.68%)	-	25 (50.47%)	-	-	-	-	-	-	-

No inhibition

Table 2: *Vibrio* in shrimp culture ponds in Thua Thien Hue.

Sample source	Culture time (month)	*Vibrio* concentration (CFU/mL)			
		Quang Dien	Phu Vang	Phu Loc	Phong Dien
Water	Supply water	$10^{2.9}$	$10^{2.5}$	$10^{3.2}$	$10^{3.8}$
	1	$10^{2.1}$	$10^{4.2}$	$10^{3.7}$	$10^{4.1}$
	2	$10^{5.3}$	$10^{4.4}$	$10^{3.8}$	$10^{5.5}$
	3	$10^{6.4}$	$10^{5.4}$	10^{4}	$10^{6.6}$
Vibrio isolates		KS03, KS01, KS04, KS07	KS03, KS01, KS06	KS03, KS01, KS06, KS07	KS03, KS01, KS07

Table 3: Percentage of antibiotic resistance *Vibrio* in shrimp pond.

Antibiotic group	Antibiotic	Collection time	Location			
			QD	PV	PL	PD
Aminoglycoside	Gentamycin	Supply water	0	21.21	43.00	8.29
		1st month	58.33	25.86	41.82	35.71
		2nd month	53.02	39.19	47.85	51.08
		3rd month	78.57	39.13	52.07	60
	Kanamycin	Supply water	0	13.13	30	2.86
		1st month	0	15.45	4.55	11.9
		2nd month	18.06	28.90	11.69	25.12
		3rd month	33.33	36.31	14.6	35.42
	Streptomycin	Supply water	8.75	16.16	42.67	7.14
		1st month	16.67	18.09	48.18	36.43
		2nd month	69.04	55.49	57.69	37.05
		3rd month	67.26	50.81	62.07	97.91
Tetracycline	Oxytetracycline hydrochloride	Supply water	0	0.00	23.67	5.57
		1st month	0	26.07	16.36	37.86
		2nd month	59.07	55.32	33.23	78.72
		3rd month	98.10	85.07	41.47	83.64
	Doxycycline	Supply water	7.5	24.24	0	3.71
		1st month	41.67	75.97	18.79	42.14
		2nd month	43.02	75.96	27.69	61.96
		3rd month	49.11	80.13	23.73	65.92
Quinolone	Ciprofloxacin	Supply water	0	0	0	0
		1st month	0	0.06	0	0.71
		2nd month	5.60	0.19	0	1.41
		3rd month	12.91	0.26	0	3.80

QD: Quang Dien, PV: Phu Vang, PL: Phu Loc, PD: Phong Dien

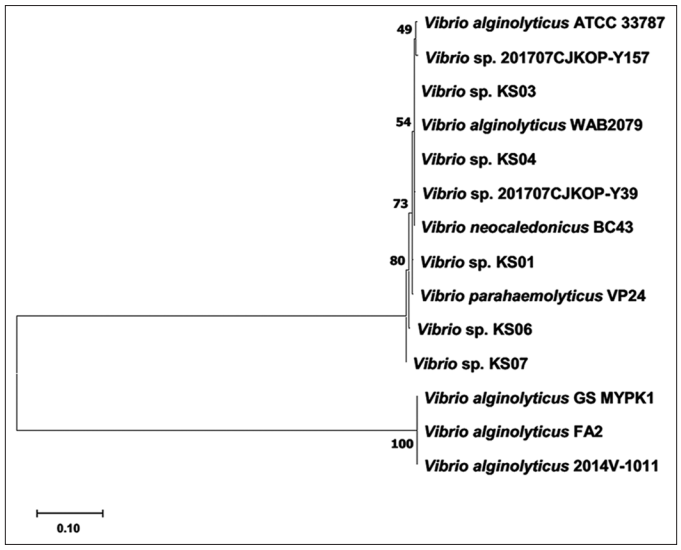

Figure 1: Phylogenetic tree among *Vibrio* sp. KS01, *Vibrio* sp. KS03, *Vibrio* sp. KS04, *Vibrio* sp. KS06, *Vibrio* sp. KS07, and other *Vibrio* sp. from GenBank. The tree was built using the maximum likelihood method. The tree was generated with branch lengths measured in the number of substitutions per site.

inhibited bacteria growth through inactivation of the DNA gyrase, which further prevents chromosome replication [17]. The supply water did not contain any *Vibrio* which can resist ciprofloxacin. However, it was found in pond-reared water after 2 months of cultivation. Similar studies also recorded ciprofloxacin resistance *Vibrio* from fish [19] and shrimp [20]. Gentamycin resistance of *Vibrio* has been reported as dominant as in their community in Nigeria and Iran, accounting for 93% and 83.3%, respectively [21,22]. A high prevalence of streptomycin-resistant *Vibrio* has also been noted here [21]. A study on antibiotic resistance of *V. parahaemolyticus* appeared in Korea and found that 27.7% of isolates were resistant to tetracycline [23]. Meanwhile, more than 90% of *V. parahaemolyticus* isolates from seafood samples in Malaysia were sensitive to tetracycline [24,25].

Kang *et al*. [26] identified that 15/16 *Vibrio* isolates from oysters had resistance profiles against 16 antibiotics in which five *V. alginolyticus* isolates showed multiple resistance to at least three antimicrobials. *V. parahaemolyticus* isolates also alerted high resistance to ampicillin, cefazolin, streptomycin, trimethoprim, kanamycin, and trimethoprim [27]. A report by Manjusha *et al*. [21] showed that 16.8% of *Vibrio* isolates collected from brackish water and coastal areas in India resisted all types of tested antibiotics. *Vibrio* isolated from aquaculture water in Nigeria resisted gentamycin, nitrofurantoin, tetracycline, augmentin, chloramphenicol, amoxicillin, ofloxacin, cotrimoxazole, ceftriaxone, and ciprofloxacin [28]. These results combine with our similar findings that demonstrated antibiotic resistance *Vibrio* present in brackish water, especially in aquaculture. This shows that strict

adherence to safe farming guidelines as well as regular assessment of bacterial susceptibility to antibiotics is essential.

5. CONCLUSION

Vibrio presents in brackish water with the number from $10^{2.5}$ to $10^{3.8}$ CFU/mL and dramatically increasing during shrimp culture, reaching 10^4 to $10^{6.6}$ CFU/mL after 3 months. Antibiotic-resistant *Vibrio* also occurred in brackish water and depends on farming location. Gentamycin, kanamycin, streptomycin, oxytetracycline hydrochloride, and doxycycline did not completely control the *Vibrio* growth. After 3 months of culture, pond water consists of up to 98.87% antibiotic resistance *Vibrio*. Ciprofloxacin exhibited the strongest inhibitory against *Vibrio*. MIC evaluation results in high resistance ability to other antibiotics of ciprofloxacin-resistant *Vibrio*. According to the present study, antibiotic-resistant *Vibrio* bacteria can be controlled by routinely using ciprofloxacin in shrimp farming.

6. ACKNOWLEDGMENTS

The authors would like to thank Prof Loc's Core Research Group from Hue University for supporting the study.

7. AUTHORS' CONTRIBUTION

Investigation: Tuan LC, Khanh NV, Tien HTNB, Phuong PT, and Thanh LTH; data analysis: Loc NH; writing-original draft preparation: Tuan LC; and writing-review and editing: Loc NH.

8. FUNDING SOURCES

This study was supported in part by grant from Hue University (Code: HH-2020-01-176) and VLIR - IUC Programme - Hue University (Project 3 - Coastal Ecosystem and Natural Resources Management).

9. CONFLICTS OF INTEREST STATEMENT

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Food and Agriculture Organization. Towards Sustainability in the Shrimp Industry 2020. Rome: Food and Agriculture Organization. Available from: https://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1261310 [Last accessed on 2022 Aug 3].
2. Valente CS, Wan AH. *Vibrio* and major commercially important vibriosis diseases in decapod crustaceans. J Invertebr Pathol 2021;181:107527.
3. Thao TT, Thoa LT, Ngoc LM, Lan TT, Phuong TV, Truong HT, *et al*. Characterization halotolerant lactic acid bacteria *Pediococcus pentosaceus* HN10 and *in vivo* evaluation for bacterial pathogens inhibition. Chem Eng Process 2021;168:108576.
4. Je Seong H, Kim JJ, Kim T, Ahn SJ, Rho M, Sul WJ. A case study on the distribution of the environmental resistome in Korean shrimp farms. Ecotoxicol Environ Saf 2021;20:112858.
5. Mok JS, Cho SR, Park YJ, Jo MR, Ha KS, Kim PH, *et al*. Distribution and antimicrobial resistance of *Vibrio parahaemolyticus* isolated from fish and shrimp aquaculture farms along the Korean coast. Mar Pollut Bull 2021;171:112785.
6. Ibrahim WN, Leong LK, Razzak LA, Musa N, Danish-Daniel M, Zainathan SC, *et al*. Virulence properties and pathogenicity of multidrug-resistant *Vibrio harveyi* associated with luminescent vibriosis in pacific white shrimp, *Penaeus vannamei*. J Invertebr Pathol 2021;186:107594.
7. The Saigon Times. Abuse of Banned Chemicals and Antibiotics in Shrimp Farming is Increasing. Vietnam: The Saigon Times. Available from: https://www.thesaigontimes.vn/lam-dung-hoa-chat-khang-sinh-cam-trong-nuoi-tom-dang-tang [Last accessed on 2022 Aug 3].
8. Truong DQ, Ariyawardana A. Small-scale shrimp grower-collector relationships: The case of Thua Thien Hue province, central Vietnam. Aquac Econ Manag 2015;19:404-22.
9. Quang HT, Lan TT, Hai TT, Yen PT, Van TQ, Tung HT, *et al*. Genetic diversity and toxic genes analysis of *Vibrio spp*. Isolated from white leg shrimp and marine fishes cultured in Tam Giang lagoon in Thua Thien Hue province, Vietnam. Indian J Sci Technol 2020;13:1412-22.
10. Thuysanvietnam.com.vn. Alarm of Overuse of Shrimp Culture Antibiotics. Available from: https://www.thuysanvietnam.com.vn/bao-dong-lam-dung-khang-sinh-nuoi-tom [Last accessed on 2022 Aug 3].
11. Pfeffer C, Oliver JD. A comparison of thiosulphate-citrate-bile salts-sucrose (TCBS) agar and thiosulphate-chloride-iodide (TCI) agar for the isolation of *Vibrio* species from estuarine environments. Lett Appl Microbiol 2003;36:150-1.
12. Ataee RA, Mehrabi-Tavana A, Hosseini SM, Moridi K, Ghorbananlizadegan M. A method for antibiotic susceptibility testing: Applicable and accurate. Jundishapur J Microbiol 2012;5:341-5.
13. Yen PT, Tram ND, Han HT, Phuong TV. Antibacterial activity of *Phyllanthus amarus* and effective microorganism (EM5) toward acute hepatopancreatic necrosis disease caused by *Vibrio* parahaemolyticus in white leg shrimps (*Litopenaeus vannamei*). Nat Sci 2019;128:107-14.
14. Sambrook S, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor; 2003.
15. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 2021;38:3022-7.
16. Chikwendu CI, Ibe SN, Okpokwasili GC. Multiple antimicrobial resistance in *Vibrio spp* isolated from river and aquaculture water sources in Imo State, Nigeria. Br Microbiol Res J Int 2014;4:560-9.
17. King DE, Malone R, Lilley SH. New classification and update on the quinolone antibiotics. Am Fam Physician 2000;61:2741-8.
18. Azargun R, Gholizadeh P, Sadeghi V, Hosainzadegan H, Tarhriz V, Memar MY, *et al*. Molecular mechanisms associated with quinolone resistance in *Enterobacteriaceae*: Review and update. Tran R Soc Trop Med Hyg 2020;114:770-81.
19. Chowdhury G, Pazhani GP, Dutta D, Guin S, Dutta S, Ghosh S, *et al*. *Vibrio fluvialis* in patients with diarrhea, Kolkata, India. Emerg Infect Dis 2012;18:1868-71.
20. De Melo LM, Almeida D, Hofer E, Dos Reis CM, Theophilo GN, Santos AF, *et al*. Antibiotic resistance of *Vibrio parahaemolyticus* isolated from pond-reared *Litopenaeus vannamei* marketed in Natal, Brazil. Braz J Microbiol 2011;42:1463-9.
21. Manjusha S, Sarita GB, Elyas KK, Chandrasekaran M. Multiple antibiotic resistances of *Vibrio* isolates from coastal and brackish water areas. Am J Biochem Biotechnol 2005;1:201-6.

22. Raissy M, Momeni M, Ansari M, Rahimi E. Antibiotic resistance pattern of some *Vibrio* strains isolated from seafood. Iran J Fish Sci 2012;11:618-26.
23. Park K, Mok JS, Kwon JY, Ryu AR, Kim SH, Lee HJ. Food-borne outbreaks, distributions, virulence, and antibiotic resistance profiles of *Vibrio parahaemolyticus* in Korea from 2003 to 2016: A review. Fish Aquatic Sci 2018;21:21-3.
24. Tan CW, Malcolm TT, Kuan CH, Thung TY, Chang WS, Loo YY, *et al*. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from short mackerels (*Rastrelliger brachysoma*) in Malaysia. Front Microbiol 2017;8:1087.
25. Tan CW, Rukayadi Y, Hasan H, Thung TY, Lee E, Rollon WD, *et al*. Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia. Saudi J Biol Sci 2020;27:1602-8.
26. Kang CH, Shin Y, Jang S, Jung Y, So JS. Antimicrobial susceptibility of *Vibrio alginolyticus* isolated from an oyster in Korea. Environ Sci Pollut Res Int 2016;23:21106-12.
27. Mok JS, Ryu A, Kwon JY, Park K, Shim KB. Abundance, antimicrobial resistance, and virulence of pathogenic *Vibrio* strains from molluscan shellfish farms along the Korean coast. Mar Pollut Bull 2019;149:110559.
28. Adeyemi A, Enyinnia V, Nwanze R, Smith S, Omonigbehin E. Antimicrobial susceptibility of potentially pathogenic halophilic *Vibrio* species isolated from seafoods in Lagos, Nigeria. Afr J Biotechnol 2008;7:3791-4.

How to cite this article:
Tuan LC, Khanh NV, Tien HTNB, Phuong PT, Hieu DV, Thanh LTH, Loc NH. The occurrence of antibiotic resistance *Vibrio* isolates from brackish water shrimp ponds in the coastal area in Thua Thien Hue, Vietnam. J App Biol Biotech. 2023;11(2):123-128.
DOI: 10.7324/JABB.2023.110211