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Research article

The antibiotic resistance of *Vibrio parahaemolyticus* originated from intensively farmed white shrimp (*Litopenaeus vannamei*) in Ben Tre and Soc Trang provinces of the Mekong Delta, Vietnam

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Abstract

The antibiotic resistance of bacteria in aquaculture is a concern for many scientists around the world. Both aquatic animals and humans were able to infect *Vibrio parahaemolyticus*, leading to economic losses for the aquaculture sector worldwide. Therefore, the goal of this research is to evaluate the antibiotic resistance of *V. parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND), in white-leg shrimp (*Litopenaeus vannamei*) intensively farmed in Ben Tre and Soc Trang provinces of the Mekong Delta, Vietnam. The antibiotic susceptibility of 32 strains of *V. parahaemolyticus* to 12 antibiotics was determined by the disk diffusion method. The findings revealed that *V. parahaemolyticus* isolates are highly sensitive to flumequine (94%), florfenicol (88%), ceftazidime (82%), rifampicin (76%), ciprofloxacin (74%), tetracycline (74%), and doxycycline (68%). Meanwhile, these isolates are highly resistant to amoxicillin (88%), colistin (72%), and cephalexin (66%). In addition, most bacterial strains in the study showed multidrug resistance (resistant to at least 3 antibiotics), of which *V. parahaemolyticus* bacterial strains resistant to 3 antibiotics account for the highest percentage (34%), followed by 4 antibiotics (19%), resistance to 5 antibiotics accounted for 16%, and the lowest rate was resistance to 7 antibiotics (3%).

Keywords: Antibiotic resistance, Mekong Delta, *Vibrio parahaemolyticus*, Vietnam, White-leg shrimp

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INTRODUCTION

With more than 700,000 hectares of black tiger and white-leg shrimp farming regions, particularly in the Mekong Delta, which accounts for 95% of shrimp production, Vietnam is one of the world's top producers and exporters of shrimp. The shrimp industry in this region contributes about 40-45% of the total seafood export value, equivalent to 3.5-4 billion USD per year. According to the expected development momentum, by 2025, shrimp export turnover can reach 5.6 billion USD. However, one of the issues influencing Mekong Delta shrimp farming's production and product quality is the outbreak of disease due to intensive shrimp farming with high stock densities, ranging from 30-80 shrimp/m^2 (Long and Chinh, 2017; Tri, 2020). According to Oanh and Phuong (2012), one of the most serious illnesses affecting white-leg shrimp in the Mekong Delta is known as AHPND disease or EMS (Early Mortality Syndrome).

V. parahaemolyticus is a Gram-negative, facultative aerobic, is the causative agent of AHPND disease (Tran et al., 2013). According to De Schryver et al. (2014), there were reports of this disease in China in 2009, Thailand in 2010, Vietnam in 2011, Malaysia in 2012, and Mexico in 2013. *V. parahaemolyticus* not only causes epidemics in humans but also causes great harm to aquatic animals such as crabs, mollusks, fish, and shrimp at all stages of development (Praja and Safnurbaiti, 2018; To et al., 2020). *V. parahaemolyticus* can infect the intestinal tract of shrimp, and toxins are secreted that cause the liver to swell or shrink with a mortality rate of up to 100% (Lightner et al., 2012; Dao et al., 2014), leading to significant economic damage for shrimp farmers (Hien et al., 2016). The bacteria are transmitted orally, then enter the digestive tract of shrimp, creating toxins that destroy tissue and cause dysfunction of the liver and pancreatic digestive organs.

Up to now, to control AHPND disease, most shrimp farmers have often used antibiotics to treat the disease in the Mekong Delta. However, overuse or improper use of antibiotics in farming households has led to antibiotic resistance in bacteria (Akinbowale et al., 2006; Hanh et al., 2016). This makes treatment ineffective, expensive, and more difficult. Therefore, the study was carried out to provide information about the antibiotic sensitivity of *V. parahaemolyticus*, thereby contributing to the choice of the appropriate antibiotic to treat the AHPND disease for the sustainable development of shrimp farming in the Mekong Delta.

MATERIALS AND METHODS

Shrimp source of bacterial isolation

V. parahaemolyticus was isolated from white-leg shrimp samples infected AHPND disease. Samples of AHPND infected shrimp were collected from 15 intensive shrimp ponds in Ben Tre and Soc Trang provinces of the Mekong Delta, Vietnam (Figure 1). Each pond collected 3-5 diseased shrimp with a weight ranging from 4-6 g/shrimp. Sick shrimp have pathological signs such as swimming slowly with wide eyes, atrophied hepatopancreas, toughness, pale color, and an empty intestine (Figure 2A&B).

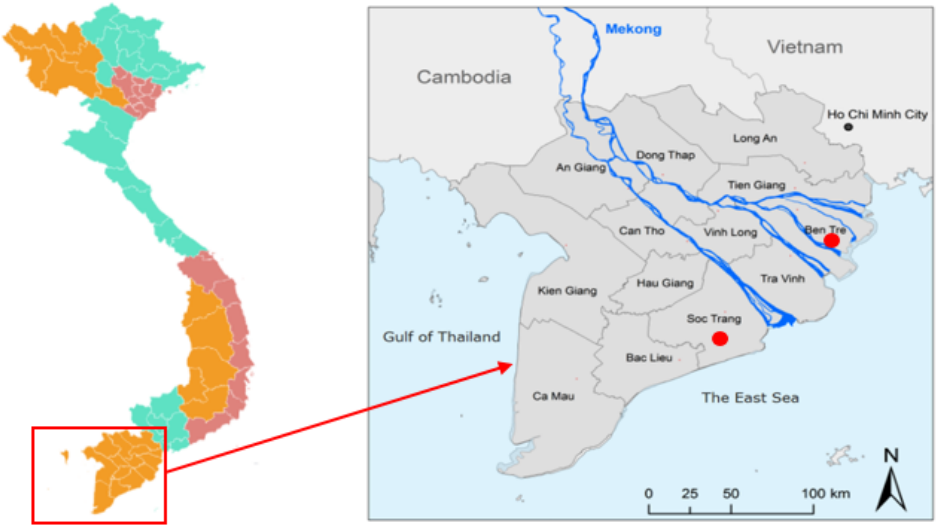

Figure 1 Sample collection locations (red circle) for isolation of *V. parahaemolyticus*.

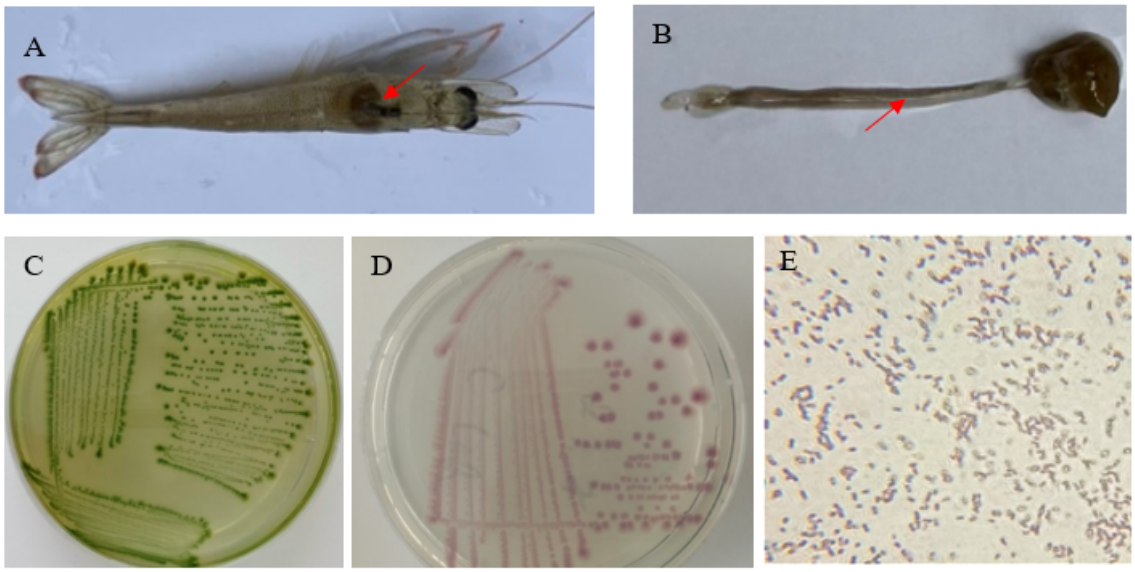

Figure 2 *V. parahaemolyticus* isolated from AHPND infected shrimp.
A. Diseased shrimp samples with signs of AHPND: atrophied hepatopancreas (arrow) and B. Empty intestine (arrow); C. V. parahaemolyticus strain on TCBS medium; D. V. parahaemolyticus strain on CHROMagar TM Vibrio medium; E. Gram staining (100X).

Isolation and identification of V. parahaemolyticus

V. parahaemolyticus bacterial strains were isolated from the hepatopancreas of diseased shrimp (Nghia et al., 2015). First, the shrimp are sterilized with 70° alcohol. A small sample of hepatopancreas was then streaked and cultured on a plate on TCBS (Thiosulfate Citrate Bile Salts Sucrose, Himedia, India) medium and CHROMagar TM Vibrio medium (Himedia, India). Finally, the culture plate is incubated for 24 hours at 28°C. Bacteria after isolation will be tested for basic characteristics such as colony morphology, cell shape, Gram stain, motility, oxidase, and catalase activity. These characteristics are implemented based on the handbook of Cowan and Steel (Barrow and Feltham, 1993) and the document of

Buller (2014). Bacteria were identified using the API 20E kit (BioMerieux, France) based on the manufacturer's instructions.

Identification and sequencing of the 16S rRNA gene fragment of *V. parahaemolyticus*

V. parahaemolyticus bacterial strains after isolation will be identified by the PCR technique. Bacteria used for DNA extraction were grown in TSB (Tryptic Soy Broth, Himedia, India) medium and incubated overnight at room temperature on a shaker at 110 rpm. Then, bacterial DNA was extracted using the iVAaDNA Extraction Kit P (Thermo Scientific, USA). After extraction, bacterial DNA will be tested for concentration and purity at wavelengths of 260 nm and 280 nm. The well-extracted sample was used for the PCR reaction to amplify the 16S rRNA gene segment with primer pairs 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' (Heuer et al., 1997).

PCR reactions were performed in a volume of 25 µL, including the following components: 12.5 µL iStandard iVAPCR (Thermo Scientific, USA); 9.5 µL distilled water; 0.5 µL primer 27F (20 pmol); 0.5 µL primer 1492R (20 pmol); 2 µL template DNA (40 ng). The PCR reaction cycle and conditions include the following steps: initial denaturation at 94°C for 5 minutes, then 30 cycles including denaturation at 94°C for one minute, primer annealing at 63°C for one minute, extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes (Heuer et al., 1997). PCR products (1.500 bp) were electrophoresed on a 1.5% agarose gel and photographed using the Analytikjena gel imaging system.

Antibiotic susceptibility

The Kirby-Bauer disk diffusion assay method was used to determine *V. parahaemolyticus*'s antibiotic susceptibility on Muller-Hinton agar (MHA, Merck, Germany) medium (Bauer et al., 1966). Twelve antibiotics are used to perform antibiograms, including colistin (COL/10µg), doxycycline (DOX/30µg), erythromycin (ERY/15µg), flumequine (FLU/30µg), rifampicin (RIF/30µg), amoxicillin (AMO/25µg), cephalexin (CEP/30µg), ceftazidime (CEF/30µg), novobiocin (NOV/5µg), tetracycline (TET/30µg), florfenicol (FLO/30µg) and ciprofloxacin (CIP/5µg) (Oxoid, UK). The bacterial colony was put into a test tube containing physiological saline (0.85%) to reach a density of about 9×10^8 CFU/mL (the turbidity of the bacterial suspension is equivalent to a 0.5 McFarland standard). Then, the bacterial solution was spread on the MHA agar surface. Finally, the antibiotic disk was placed on the agar medium. The diameter of the inhibition zone was measured after 24-48 hours of incubation at 30°C. The antibiotic sensitivity (susceptible, S), intermediate (intermediate, I), and resistance (resistant, R) of bacteria to antibiotics were determined by the standards of the Clinical and Laboratory Standards Institute (CLSI, 2020). For quality control, *Escherichia coli* ATCC 25922 was employed as the bacteria. The MAR (multiple antibiotic resistance) index of bacterial strains was calculated according to the formula as follows: MAR = a/b, in which, a: the quantity of antibiotics to which the bacterium is resistant; b: the total amount of antibiotics to which the bacterium has been exposed (Sandhu et al., 2016). Multiple resistance (MAR) index ≥ 0.2 indicates a high-risk source of contamination where antibiotics are often used (Elexson et al., 2023).

Data analysis

The data and graphs in the experiment were entered and processed using Microsoft Excel 2010 software. Descriptive statistical methods were used to calculate the average values and the average percentage of resistant, intermediate, and susceptible. The phylogenetic tree showing the genetic relationships between *V. parahaemolyticus* strains was built using MEGA X software, and the bootstrap value was 1,000 replications (Tamura et al., 2013).

RESULTS

Isolation, morphological, physiological, and biochemical characteristics of isolated *V. parahaemolyticus* bacterial strains

From 65 diseased shrimp samples collected in 15 intensive farming ponds in Ben Tre and Soc Trang provinces, 32 bacterial strains were isolated on TCBS medium and CHROMagar TM Vibrio medium (Table 1).

After 24-48 hours of growth on TCBS medium and CHROMagar TM Vibrio medium, colonies of all bacterial strains were found to be small (from 2-3 mm), round, and smooth. The colonies are green on TCBS medium (Figure 2C), and on CHROMagar TM Vibrio medium, the colonies will be mauve (Figure 2D). The results also showed that the isolated bacterial strains were slightly curved, short rod-shaped, Gram-negative bacteria (Figure 2E). They are mobile and react positively with oxidase, catalase, and the O-F reaction.

Three isolates, VPST1, VPBT25, and VPBT23, were identified as *V. parahaemolyticus* by using the API 20E kit. These bacteria showed positive reactions to ortho-nitrophenyl galactosidase, lysine, ornithine, indole, gelatin, glucose, mannitol, amygdalin, and arabinose. Meanwhile, bacterial strains revealed negative reactions to arginine, sodium citrate, sodium thiosulphate, urea, tryptophane, voges-proskauer, inositol, sorbitol, rhamnose, sucrose, and melibiose. The morphological, physiological, and biochemical characteristics of *V. parahaemolyticus* are presented in detail in Table 2.

Table 1 Bacterial sources and distribution of *V. parahaemolyticus* bacterial strains in Ben Tre and Soc Trang province of the Mekong Delta

Geographical origin	Farm	Diseased shrimp samples*	No of isolates
Soc Trang	5	20	8
Ben Tre	10	30	24
Total	**15**	**50**	**32**

** Shrimp with signs of AHPND with a weight ranging from 4-6 g/shrimp.*

Identification of isolated *V. parahaemolyticus* bacterial strains by PCR technique

Identification results by PCR technique indicated that all tested bacterial strains amplified the 16S rRNA gene fragment, with DNA bands appearing at a size of 1,500 bp (Figure 3). In addition, the results of PCR product sequencing and comparison on the NCBI database showed that two representative bacterial strains, VPST1 and VPBT23, in the study had 98.67% similarity with *V. parahaemolyticus* strains on GenBank.

The results of phylogenetic tree analysis show that *V. parahaemolyticus* bacterial isolates are distributed into the same cluster (Figure 4). Hence, based on the results of the phylogenetic tree, PCR and gene sequencing combined with morphological, physiological, and biochemical characteristics have confirmed that two isolates, VPST1 and VPBT23, belong to *V. parahaemolyticus* species.

Table 2 Morphological, physiological, and biochemical characteristics of *V. parahaemolyticus*

Phenotypic characteristics	Isolate VPST1	Isolate VPBT9	Isolate VPBT23	*V. parahaemolyticus**
Gram stain	Positive	Positive	Positive	Positive
Shape	Curved rod	Curved rod	Curved rod	Short rod
Motility	Motile	Motile	Motile	Motile
Oxidase reaction	+	+	+	+
Catalase reaction	+	+	+	+
Oxidation/Fermentation test	+/+	+/+	+/+	+/+
Growth on NaCl**:				
0.5%	+	+	+	+
1%	+	+	+	+
1.5%	+	+	+	+
2%	+	+	+	+
ONPG	+	+	+	-
Arginine	-	-	-	-
Lysine	+	+	+	+
Ornithine	+	+	+	+
Citrate	-	-	-	-
H_2S	-	-	-	-
Urease	-	-	-	-
Tryptophane	-	-	-	-
Indole	+	+	+	+
Voges-Proskauer	-	-	-	+
Gelatin	+	+	+	+
Glucose	+	+	+	-
Manniton	+	+	+	-
Inositol	-	-	-	-
Sorbitol	-	-	-	-
Rhamnose	-	-	-	-
Saccharose	-	-	-	-
Melibiose	-	-	-	-
Amygdalin	+	+	+	-
Arabinose	+	+	+	+

*Note: ONPG: ortho-nitrophenyl galactosidase; oxidation/fermentation test; +: positive; -: negative; * Reference strain of Buller (2014); ** The ability of bacteria to grow on media with different salt concentrations (+: weak growth).*

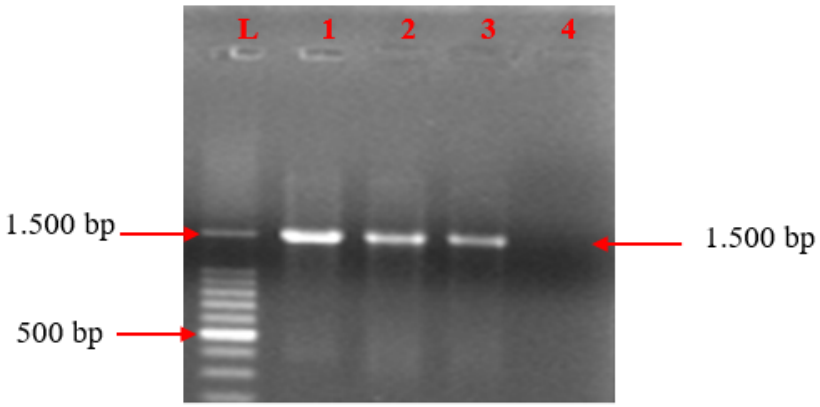

Figure 3 Amplification the 16S rRNA gene fragment of *V. parahaemolyticus* by PCR.
L. 100 bp DNA ladder; Lanes 1-3: isolates VPST1, VPBT9, and VPBT23, respectively; Lane 4: negative control.

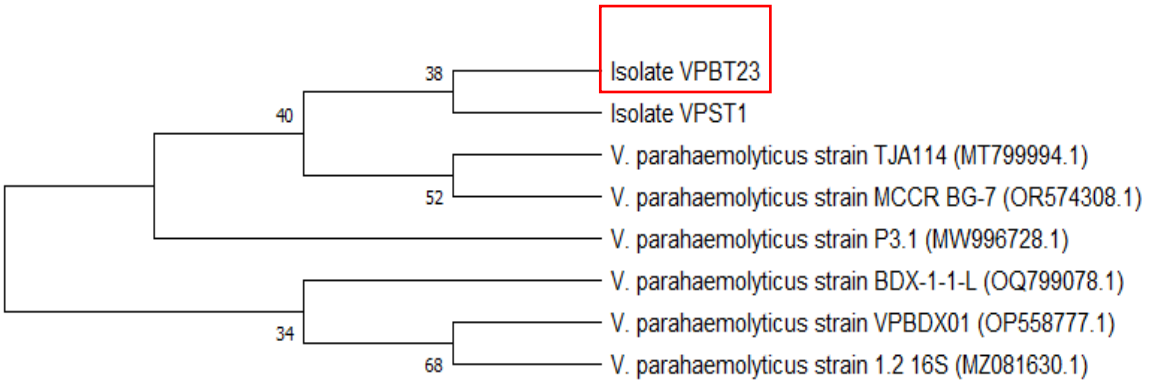

Figure 4 Phylogenetic tree showing genetic relationships between *V. parahaemolyticus* bacterial strains. The Neighbour-joining was used to build the phylogenetic tree from 16S rRNA fragments of two isolates and six reference strains. The bootstrap values on the nodes show the presence of two large clusters.

Antibiogram

The study performed antibiograms on 32 strains of isolated *V. parahaemolyticus* bacterial isolates with 12 antibiotics. Antibiogram results (Figure 5) illustrated that isolated *V. parahaemolyticus* bacterial isolates in the study were highly resistant to β-lactam antibiotics and polypeptides/polymyxins, specifically: the bacteria were highly resistant to amoxicillin (88%), cephalexin (66%), and colistin (72%). However, the findings revealed that *V. parahaemolyticus* isolates are highly sensitive to flumequine (94%), florfenicol (88%), ceftazidime (82%), rifampicin (76%), ciprofloxacin (74%), tetracycline (74%), and doxycycline (68%). The MAR index values of *V. parahaemolyticus* strains fluctuated from 0.08 to 0.58 (Table 3), with an average of 0.27, demonstrating the high-risk sources of contamination that originated from shrimp farms, where antimicrobials were commonly used in the Mekong Delta.

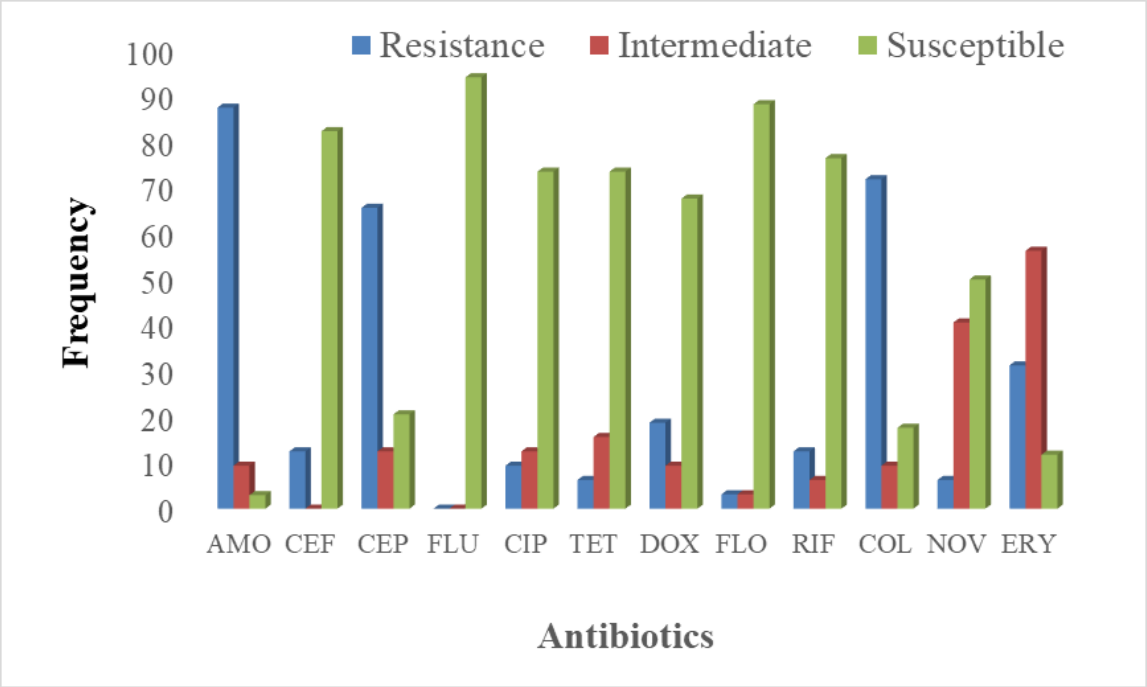

Figure 5 Antibiogram of isolated *V. parahaemolyticus* bacterial isolates.
Note: AMO: Amoxicillin, CEF: Ceftazidime, CEP: Cefalexin, FLU: Flumequine, CIP: Ciprofloxacin, TET: Tetracycline, DOX: Doxycycline, FLO: Florfenicol, RIF: Rifampicin, COL: Colistin, NOV: Novobiocin, and ERY: Erythromycin.

Multidrug resistance of *V. parahaemolyticus* strains

The investigation showed that the quantity of *V. parahaemolyticus* bacterial strains resistant to 3 antibiotics accounts for the highest percentage (34%), followed by 4 antibiotics (19%), resistance to 5 antibiotics accounted for 16%, and the lowest rate was resistance to 7 antibiotics (3%). No strains were resistant to 6 antibiotics or from 8 to 12 antibiotics tested (Table 3).

DISCUSSION

V. parahaemolyticus is not just a pathogen on aquatic animals but is a major food-borne pathogen, affecting public health and consumer safety in humans (Nelapati et al., 2012; Wang et al., 2015; Beshiru et al., 2023). Antibiotic resistance of *V. parahaemolyticus* has been commonly reported in humans and on aquatic animals (Saifedden et al., 2016; Huang et al., 2023). Most isolated *V. parahaemolyticus* bacterial strains in this study were highly resistant to amoxicillin (88%). This finding was in agreement with the study of Ha et al. (2023), which showed that 97.06% of *V. parahaemolyticus* isolated from AHPND shrimp extensively farmed in Bac Lieu province of the Mekong Delta was resistant to amoxicillin. The rate of resistance to amoxicillin in the study was higher than the finding of Saifedden et al. (2016). In Malaysia, otherwise, the study by Drais et al. (2016) showed that 98% of *V. parahaemolyticus* isolates were collected from sediments, and seawater was sensitive to amoxicillin. The high resistance rate of *V. parahaemolyticus* in these observations may be due to the fact that antibiotics are commonly used in shrimp farming (Rico et al., 2013; Thinh et al., 2020). However, the improper use and correct dosage of antibiotics by shrimp farmers is the cause of the antibiotic-resistant bacteria in this study.

Table 3 Antibiotic resistance phenotypes and MAR index of isolated *V. parahaemolyticus* strains

Bacterial isolates	Multiple antibiotic resistance phenotypes	MAR index
VPST1	AMO-CEP-CEP-RIF-ERY	0.42
VPST2	AMO-DOX-RIF-COL	0.33
VPST3	AMO-CEF-CEP-COL	0.33
VPST4	AMO-CEP-COL	0.25
VPST5	AMO-COL	0.17
VPST6	CEP-RIF-COL	0.25
VPST7	AMO-CEP-COL	0.25
VPST8	AMO-CEP-COL-ERY	0.33
VPBL1	AMO-CEP-COL	0.25
VPBL2	AMO-CEP-FLO	0.25
VPBL3	AMO	0.08
VPBL4	AMO-CEP-CIP-COL-ERY	0.42
VPBL5	AMO-ERY	0.17
VPBL6	AMO	0.08
VPBL7	AMO	0.08
VPBL8	AMO-CEF-COL	0.25
VPBL9	AMO-DOX	0.17
VPBL10	CIP-DOX	0.17
VPBL11	AMO-CEP-COL-NOV	0.33
VPBL12	AMO-DOX	0.17
VPBL13	CEP-COL	0.17
VPBL14	AMO-CEP-COL-ERY	0.33
VPBL15	AMO-CEP-COL	0.25
VPBL16	AMO-CEP-RIF-NOV-ERY	0.42
VPBL17	TET-DOX-FLO-COL-ERY	0.42
VPBL18	AMO-CEP-CIP-TET-DOX-COL-ERY	0.58
VPBT19	AMO-CEP-COL-ERY	0.33
VPBL20	AMO-CEP-COL	0.25
VPBL21	AMO-CEP-COL	0.25
VPBL22	AMO-CEP-COL	0.25
VPBL23	AMO-CEF-CEP-COL-ERY	0.42
VPBL24	AMO-CEP-COL	0.25

Note: AMO: Amoxicillin, CEF: Ceftazidime, CEP: Cefalexin, FLU: Flumequine, CIP: Ciprofloxacin, TET: Tetracycline, DOX: Doxycycline, FLO: Florfenicol, RIF: Rifampicin, COL: Colistin, NOV: Novobiocin, and ERY: Erythromycin.

High resistance to cephalexin (66%) was also recorded in this investigation. In general, this rate was higher than the previous study by Al-Othrubi et al. (2014), who demonstrated that *V. parahaemolyticus*, which originated from cockles and shrimp marketed in Selangor, Malaysia, was resistant to cephalexin at a rate of 35.4%. However, this study was lower than the findings of Zaafrane et al. (2022), who demonstrated a complete resistance to colistin in *V. parahaemolyticus*

collected from Tunisian coastal seawater. In Malaysia, the results of the research by Tan et al. (2020) presented that *V. parahaemolyticus* derived from food sources were highly sensitive to antibiotics of the β-lactam group such as ampicillin-sulbactam (93.33%), amoxicillin-clavulanic acid (75.73%), cefotaxime (71.67%), and ceftazidime (70.83%), while bacteria are highly resistant to antibiotics of this group such as ampicillin (84.17%) and cefazolin (84.17%). A study by Letchumanan et al. (2015) also showed that *V. parahaemolyticus*, which originated from shellfish, also showed high resistance to β-lactam groups such as cefotaxime (73%), and ceftazidime (51.5%).

Colistin is a member of the cationic polypeptide antibiotic family. It is the final line of defense against deadly infections brought on by gram-negative bacteria that are resistant to many drugs (Sharma et al., 2022; Panigrahi et al., 2022). In this investigation, *V. parahaemolyticus* isolates were resistant to colistin at a rate of 72%. Resistance to colistin in *V. parahaemolyticus* was due to the presence of *the mcr-1* gene (Lei et al., 2019). Previous research has revealed that *V. parahaemolyticus* isolates collected from human and food samples exhibit elevated levels of colistin resistance (Obaidat et al., 2017). In the Mekong Delta, according to To et al. (2020), *V. parahaemolyticus* causes AHPND in molluscan shellfish, shrimp, and water samples, which showed 100% resistance to colistin.

In this study, *V. parahaemolyticus* bacterial isolates are highly susceptible to flumequine (94%), florfenicol (88%), ceftazidime (82%), rifampicin (76%), ciprofloxacin (74%), tetracycline (74%), and doxycycline (68%). In China, Zhang et al. (2024) reported that *V. parahaemolyticus* obtained from Pacific white shrimp (*L. vannamei*) in the Ningde regions had the highest sensitivity rate (97.1%) for florfenicol. In the Mekong Delta, research by Ha et al. (2023) revealed that 71% of isolated *V. parahaemolyticus* strains derived from AHPND shrimp extensively farmed in Bac Lieu province are sensitive to flumequine. In Nigeria, the report of Odjadjare and Igbinosa (2017) indicated that *V. parahaemolyticus* strains isolated from abattoir effluents in the Niger Delta region were found to be susceptible to rifampicin at a rate of 20%. The results of the finding by Tan et al. (2020) revealed that *V. parahaemolyticus* was sensitive to chloramphenicol (100%), doxycycline (98.33%), and tetracycline (94.17%). Letchumanan et al. (2015) illustrated that *V. parahaemolyticus* isolated from shrimp samples was found to be highly susceptible to chloramphenicol (95%), tetracycline (82%), and trimethoprim-sulfamethoxazole (93%). Lopatek et al. (2015) also reported that all *V. parahaemolyticus* strains isolated from shellfish were sensitive to tetracycline and chloramphenicol. In North China, a similar study by Xu et al. (2016) indicated that most of the isolates of *V. parahaemolyticus* from seafood retail were resistant to streptomycin (86.2%), while fewer were resistant to ampicillin (49.6%), cefazolin (43.5%), cephalothin (35.9%), and kanamycin (22.1%). The sensitive rate to ciprofloxacin in this finding was higher than the study by Al-Othrubi et al. (2014), who revealed that *V. parahaemolyticus*, which originated from cockles and shrimp marketed in Selangor, Malaysia, was susceptible to ciprofloxacin at a rate of 49.03%.

The MAR index in this investigation was found to be high, ranging from 0.08 to 0.58, with a value greater than 0.2 for 75% of the isolates. These observations are in line with many previous findings. In a study by Huang et al. (2023), it was presented that *V. parahaemolyticus* isolates from fecal samples of patients in Nantong, China, had a MAR index ranging from 0.07 to 0.36. On the other hand, Letchumanan et al. (2015) demonstrated that 85% of *V. parahaemolyticus* isolates derived from shellfish in Selangor, Malaysia, had a MAR index greater than 0.2. Similarlly, Elexson et al. (2023) showed that 31.38% of *V. parahaemolyticus* isolates in shrimp farms in Sarawak, Malaysia, had a MAR index > 0.2, with the MAR index of *V. parahaemolyticus* isolates ranging from 0.11 to 0.36. According to Saifedden et al. (1916), a MAR index ≥ 0.2 indicated that these isolates might

originate from high-risk sources, and aquatic animals frequently exposed to antibiotics.

The multidrug resistance of *V. parahaemolyticus* in aquaculture and food has been reported by numerous earlier investigations (Amalina et al., 2019; Elexson et al., 2023; Loo et al., 2023). All 32 bacterial strains in the current finding showed multidrug resistance (resistant to at least 3 antibiotics), of which multidrug resistance to 4 antibiotics accounted for the highest rate (66.67%). Similarlly, in a survey to evaluate the antibiotic resistance of *V. parahaemolyticus* obtained from shrimp with AHPND in the Quynh Luu shrimp farming area in Nghe An province, Hanh et al. (2016) revealed that *V. parahaemolyticus* strains had multidrug resistance (resistant to 2–6 antibiotics). The highest resistant rate of strains was 4 antibiotics (33.3%), followed by resistance to 6 and 3 antibiotics (22.2%), and resistance to 2 and 5 antibiotics (11.1%). In North China, the research by Xu et al. (2016) also illustrated that *V. parahaemolyticus* strain 58, which originated from a fish sample, was a multidrug-resistant strain that presented resistance to seven antibiotics, including cephalothin, ampicillin, cefazolin, tetracycline, kanamycin, streptomycin, and trimethoprim-sulfamethoxazole. The multidrug-resistance of *V. parahaemolyticus* in this investigation showed the potential risk of antibiotic resistance gene transmission from pathogenic bacteria in aquatic animals to bacterial pathogens in humans and other aquatic animals (Nishino et al., 2021; Pepi and Focardi, 2021). The study by Ha et al. (2023) also proved that isolated *V. parahaemolyticus* isolates from extensive shrimp ponds in Bac Lieu province were multidrug resistant. However, most multidrug resistant phenotypes of *V. parahaemolyticus* strains from the findings by Ha et al. were different from the current study. The difference in resistance patterns may be due to the different antibiotics used by shrimp farmers in the Mekong Delta provinces. The findings show that antibiotics need to be controlled and managed more strictly to control *V. parahaemolyticus*. In the future, it is necessary to reduce antibiotics and use alternative measures such as herbs, probiotics, immune stimulants, phage therapy, and especially developing vaccines to control these bacteria.

CONCLUSIONS

V. parahaemolyticus strains in this investigation are resistant to many antibiotics used in aquaculture, such as amoxicillin, cephalexin, and colistin. *V. parahaemolyticus* is also sensitive to florfenicol, flumequine, ceftazidime, rifampicin, ciprofloxacin, tetracycline, and doxycycline. Most bacterial strains in this observation showed multidrug resistance. The findings show that antibiotics need to be controlled and managed more strictly to control *V. parahaemolyticus*.

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