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EDITED BY
Songzhe Fu,
Dalian Ocean University, China

REVIEWED BY
Xiaojun Zhang,
Yangzhou University, China
Duochun Wang,
National Institute for Communicable Disease Control and Prevention (China CDC), China

*CORRESPONDENCE
Jingfeng Fan
jffan@nmemc.org.cn

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Prevalence, antibiotic and heavy metal resistance of *Vibrio* spp. isolated from the clam *Meretrix meretrix* at different ages in Geligang, Liaohe estuary in China

Jie Su[1], Yingxue Zhang[1], Tian Hu[2], Hongxia Ming[1], Yuyang Xie[1,3], Yuan Jin[1], Tingting Shi[1] and Jingfeng Fan[1]*

[1]State Environmental Protection Key Laboratory of Coastal Ecosystem, National Marine Environmental Monitoring Center, Dalian, China, [2]College of Marine Technology and Environment, Dalian Ocean University, Dalian, China, [3]College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai, China

Vibrio as one of the main pathogens of shellfish diseases can cause serious human seafoodborne gastroenteritis and even death. In this study, we analyzed the bacterial communities from the clam, and compared the resistance phenotypes and genotypes of *Vibrio* spp. from *Meretrix meretrix* at different growth stages. High-throughput sequencing analysis revealed the predominance of *Proteobacteria* (50%) in the bacterial community and *Vibrio* was one of the dominant genera in the clam hepatopancreas in the summer. *Vibrio* abundance in *Meretrix meretrix* positively correlated with the water temperature ($p<0.05$). A total of 73 *Vibrio* isolates from *Meretrix meretrix* were classified into 19 species and the dominant strains included *V. mediterranei* (19%) and *V. harveyi* (11%), *V. algolyticus* (10%), and *V. parahaemolyticus* (8%). The species and abundance of *Vibrio* spp. were the highest in the 3-year-old of *Meretrix meretrix* compared with clams of other ages in the summer. Among the 73 isolates, 68 *Vibrio* strains were resistant to other 15 antibiotics except for sulfamethoxazole-trimethoprim with 57 resistant phenotypes. The most prevalent resistance was toward clindamycin (76%), followed by amikacin (63%), ampicillin (62%), rifampicin (62%), vancomycin (57%), and amoxicillin (50%). The ARI values of *Vibrio* spp. in different ages ranged from 0.13 to 0.18, and ARI values of 3-year-old (ARI=0.18) clams are higher than that of other ages clam. Approximately 72% of the resistant isolates showed multidrug-resistant phenotypes with maximum resistance to 15 antibiotics. Tolerance to heavy metals including Cd, Zn, and Cu was detected in the majority of antibiotic resistant isolates. In addition to the co-resistance to the same class of antibiotics, resistance to cephalosporin (CFP, CEP, CZ) were significantly correlated with penicillins (AMP, AMC) ($p< 0.01$), tetracycline ($p < 0.001$), sulfanilamide (SXT) ($p< 0.01$) and quinolone (CIP) ($p< 0.01$). The heavy metal resistance genes *copB* and *nccA* were significantly correlated with the clindamycin resistance phenotype ($p<0.01$). This study revealed that the habitat of *Meretrix meretrix* is in low exposure to antibiotics, and a link between heavy metal resistance genes and antibiotic resistance.

KEYWORDS

prevalence, antibiotic resistance, heavy metal resistance, *Vibrio* spp., *Meretrix meretrix*

1 Introduction

Shellfish mainly inhabit coastal and estuarine environments. Due to the nature of their habitats, shellfish contain a variety of bacterial microbiota, including the *Vibrio* spp. (Romalde et al., 2014). *Vibrio* is a Gram-negative bacterium with genetic and metabolic diversity and is an integral part of the global marine ecosystem (Thompson et al., 2004). *Vibrio* seriously affects shellfish farming. Moreover, it poses a potential danger to humans due to its high detection rate in shellfish farming. Twenty different pathogenic *Vibrio* species can cause large-scale death of shellfish. *Vibrio* infection-related diseases usually include gastrointestinal disorders with symptoms like diarrhea, abdominal cramps, nausea, vomiting, and fever (CDC, 2019b). These symptoms may occur within 24 h of ingestion and last for three days. Patients with low immunity or underlying diseases are at a higher risk of death (CDC, 2019a).

Presently, more than 120 species of Vibrio have been found; at least 12 species are known to cause human diseases. The list includes *Vibrio alginolyticus*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnisii*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, etc. (Oliver, 2015; Economopoulou et al., 2017; Huang et al., 2017; Zago et al., 2017). Antibiotics are often used to prevent and cure aquaculture diseases in recent years. However, the aquaculture industry lacks relevant drug regulations leading to the overuse of antibiotics. Consequently, the *Vibrio* develop antibiotic resistance, increasing the difficulty of treating human infections (Alsalem et al., 2018; Miranda et al., 2018). *Vibrio* spp. also showed resistant to the most clinically used antibiotics (Mala et al., 2014; Letchumanan et al., 2015). The incidence of human *Vibrio* infection and the drug resistance rate of drug-resistant bacteria are also increasing (Kitaoka et al., 2011). Therefore, it is imperative to detect pathogenic *Vibrio* in aquatic products and prevent food poisoning. The sudden outbreak of *Vibrio* will seriously affect marine biomass and cause severe economic loss to aquaculture (Moffitt and Cajas-Cano, 2014).

China remains the first major producer of fisheries and aquaculture in the world, with a 35 percent share of the total (FAO (Food and Agriculture Organization, 2022). In 2020, China's mariculture area covered 1996 thousand hectares. The shellfish occupied 1197 thousand hectares, accounting for 59.99% of the mariculture area. *Meretrix meretrix* is a beach-buried shellfish widely distributed in both the north and south coastal regions of China, especially in the estuary and tidal flat, such as Liaoning, Shandong, Jiangsu, and Guangxi. *Meretrix meretrix* grows in a wide range of temperatures and salinity and mainly feeds on planktonic and benthic diatoms. In 2020, the output of clam mariculture from the Liaoning Province was 1.353 million tons, and the area encompassed was 1,53,000 hectares, ranking first in China and far exceeding that of the other provinces (Ministry of Agriculture and Rural Affairs Fisheries Bureau et al., 2021). *Meretrix meretrix* is currently an important economic shellfish in the coastal mudflat culture in China. However, due to intensified aquaculture and the increasing eutrophication of coastal mudflats, *Vibrio* spp. as one of the primary pathogen have caused serious harm to the clam aquaculture industry and food safety (Li et al., 2018).

Geligang, as one of the important producing areas of clams *Meretrix meretrix* in northern China, is located in the east of Liaohe estuary, with an area of about 10000 ha. The annual output of *Meretrix meretrix* in Geligang area is more than 1000 t. In this study, we investigate the differences in the diversity and abundance of *Vibrio* isolated from *Meretrix meretrix*, and analyze antibiotic resistance and heavy metal resistance of *Vibrio* spp. in *Meretrix meretrix* at different ages in Geligang, Liaohe estuary in China. This study will support the need for food safety risk assessment of aquatic products.

2 Materials and methods

2.1 Sample collection

Meretrix meretrix samples of different ages were collected from the Geligang aquaculture area in Liaohe estuary in the spring (April), summer (July, August), and autumn (November) of 2019. The 1-year-old and 2-year-old clams were collected in spring. The 1-year-old, 2-year-old, 3-year-old, and 5-year-old of clams were collected in the summer. The 1-year-old, 2-year-old, 3-year-old, and 5-year-old clams were collected in autumn. The collected samples were stored in sterile plastic bags at 4°C and transported to the laboratory for analysis within 24 h. Water temperature and salinity were measured *in situ* by the YSI ProQuatro Handheld Multiparameter Instrument (YSI, Xylem Inc., NY, USA).

2.2 Isolation and identification of the *Vibrio* strains

The samples of *Meretrix meretrix* were divided according to different growth cycles. The hepatopancreas samples of the same age were mixed by three independent samples, and 2–5 groups of parallel samples were set aside for preservation. The clam samples were washed with sterile saline. The clam hepatopancreas (200 g) samples were homogenized in phosphate-buffered saline (PBS; 2.5 mM KH_2PO_4; pH-7.2) with a blender and subsequently serially diluted with PBS. The homogenate diluents were further plated on Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS Agar; Oxoid, Thermo Fischer Scientific, UK) and incubated at 37°C for 24 h. The presumptive colonies on the TCBS agar plates were re-streaked on Tryptone Soy Agar (Oxoid, Thermo Fischer Scientific, UK) supplemented with 3% Sodium Chloride (TSA + 3% NaCl) and incubated at 37°C for 24 h to achieve a pure isolate (Sujeewa et al., 2009). *Vibrio* isolates were identified by PCR and sequencing of16S rDNA. Chromosomal DNA from the *Vibrio* cells were extracted using a QIAGEN DNA extraction kit following the manufacturer's instructions. 16S rRNA gene amplification and DNA purification were determined as described previously (Park et al., 2018) and sequenced by Sangon Biotech (Shanghai) Co., Ltd. (China). The obtained consensus sequences were subjected to BLAST search at NCBI (http://www.ncbi.nlm.nih.gov/pubmed) for sequence alignment analysis.

2.3 High-throughput sequencing

The genomic DNA was extracted from the clam hepatopancreas using the DNeasy Power Water Total DNA Isolation Kit (QIAGEN, Germany). Bacterial communities were identified using the 16S rRNA gene sequencing technology from Shanghai Personalbio Technology Co., Ltd. (China). The V3-V4 regions of the 16S rRNA gene were amplified using barcodes. The 175 primer sets with the forward primer 341F (5'-CCTACGGGNGGCWGCAG-3') and the reverse primer 765R with 176 primer sets (5'-GACTACNVGGGTATCTAAT-3') were used for sequencing. The PCR amplification was performed using the Pfu high-fidelity DNA polymerase (TransGen Biotech), and purification and recovery were managed with magnetic beads. The fluorescence of the PCR amplification product was quantified on the Fluorescence Microplate reader (BioTek, FLx800). The fluorescent reagent was used from the Quant-iT PicoGreen dsDNA Assay Kit. The sequencing library was prepared using the TruSeq Nano DNA LT Library Prep Kit (Illumina, Inc. (USA)). The community DNA fragments were sequenced using the Paired-end Illumina MiSeq platform.

2.4 Antimicrobial susceptibility and heavy metal resistance tests of the *Vibrio* isolates

The antimicrobial susceptibility of the *Vibrio* isolates was determined from the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (Clinical Laboratory Standard Institute, 2016). The isolates were tested for susceptibility toward 16 antimicrobials: Cefazolin (CZ, 30 μg), Cephalotin (CEP, 30 μg), Cefoperazone (CFP, 75 μg), Cefuroxime (CXM, 30 μ g), Ampicillin (AMP, 10 μg), Amoxicillin (AMC, 10 μg), Streptomycin (STR, 10 μg), Gentamicin (GM, 10 μg), Amikacin (AN, 30 μ g), Sulfamethoxazole-Trimethoprim (SXT, 23.75–1.25 μg), Ciprofloxacin (CIP, 5 μg), Clindamycin (DA, 2 μg), Vancomycin (VA, 30 μg), Tetracycline (TCY, 30 μg), Erythromycin (ERY, 15 μg), and Rifampicin (RFP, 5 μg). The results were interpreted as susceptible (S), intermediate (I), and resistant (R) after 12 h of incubation at 30°C using the CLSI standards. Multiple Antibiotic Resistant (MAR) strain is defined as a bacterium resistant to three or more antibiotics (Manjusha et al., 2005). Antibacterial Resistance Index (ARI) was used to analyze the prevalence of resistant isolates from clam and calculated for the same age (Mohanta and Goel, 2014).

The heavy metal resistance of the *Vibrio* isolates was determined according to a previous method (Kang et al., 2016). The minimal inhibitory concentration (MIC) of the tested heavy metals against the isolates was measured using broth dilution testing (Clinical Laboratory Standard Institute, 2016). The heavy metals used in this study were $CdCl_2$, $ZnCl_2$, and $CuCl_2$.

2.5 Detection of antibiotic resistance genes and heavy metal resistance genes

From the results of the antibiotic and heavy metal resistance phenotypes of *Vibrio*, 13 resistant genes were selected. The six antibiotic resistance genes included the penicillins resistance gene(*ampR*), aminoglycosides resistance gene (*aadA*, *strA*, and *strB*) and glycopeptides resistance gene(*vanM*). The heavy metal resistance genes included *copA*, *copB*, and *copC* genes for Cu^{2+}, *nccA* and *cadD* genes for Cd^{2+}, and *zntA* and *zntB* resistance genes for Zn^{2+}. The primers and PCR conditions are presented in Table S1 (Sambrook, 2001; Kamika and Momba, 2013; Jiang et al., 2014; Liu et al., 2014; Liu, 2016; Wu et al., 2019; Yang et al., 2020).

2.6 Statistical analyses

The statistical analyses of alpha diversity, beta diversity and differentially abundant taxa were carried out using QIIME2 2019.4. Pearson's correlation analysis was used to evaluate the relationship between the antibiotic resistant phenotypes and antibiotic resistant genotypes with SPSS version 25 (IBM, Armonk, NY, USA). p value < 0.05 was considered statistically significant.

3 Results

3.1 Microbial community analysis

3.1.1 Microbial alpha diversity

The alpha diversity was determined for each treatment and different seasons. The Good's Coverage index of 24 clam samples of different ages was >0.99, which means more than 99% of the species diversity was detected with a high coverage (Table S2). The Chao1, Shannon, and Simpson indices showed a trend of first increasing and then decreasing. The highest value was reached in the 3-year-old clams collected in August, and those collected in autumn contributed the lowest. The Chao1 index between the samples in spring and autumn (p <0.05) differed significantly; the Shannon and Simpson indices did not show a seasonal difference. The changing trend of the Good's Coverage index was autumn>spring>summer, indicating the difference in the sequencing coverage of samples in the four months. The samples in spring and summer were significantly different from those in autumn (p<0.05). Therefore, significant differences existed in the community richness but not in community diversity among the clam samples collected during different seasons (see Table 1; Figure 1).

3.1.2 Beta diversity

Beta diversity was used to analyze the similarities and differences in the structure of two or more communities. Only the 1-year-old and 2-year-old of *Meretrix meretrix* were detected in three seasons. Hence, the beta diversity analysis was conducted

TABLE 1 *Vibrio* spp. of clams in different ages.

Vibrio spp.	% of Vibrio spp.(No. of Isolates)			
	1-year-old (n=10)	2-year-old (n=10)	3-year-old (n=46)	5-year-old (n=7)
V. pacinii	10(1)	–	–	–
V. harveyi	20(2)	10(1)	8(4)	14(1)
V. mediterranei	40(4)	10(1)	15(7)	29(2)
V. tubiashii	–	20(2)	4(2)	–
V. campbellii	–	10(1)	4(2)	29(2)
V. parahaemolyticus	10(1)	20(2)	6(3)	–
V. brasiliensis	–	–	6(3)	14(1)
V. rotiferianus	10(1)	–	4(2)	14(1)
V. shilonii	10(1)	–	–	–
V. jasicida	–	10(1)	–	–
V. pelagius	–	20(2)	2(1)	–
V. hangzhouensis	–	–	8(4)	–
V. nereis	–	–	2(1)	–
V. alginolyticus	–	–	15(7)	–
V. diabolicus	–	–	8(4)	–
V. neocaledonicus	–	–	6(3)	–
V. natriegens	–	–	2(1)	–
V. azureus	–	–	2(1)	–
V. coralliilyticus	–	–	2(1)	–

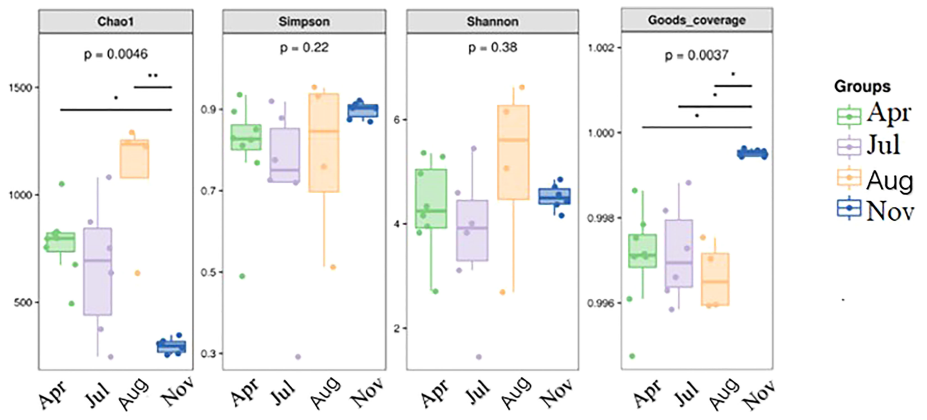

FIGURE 1
Differences in the Alpha Diversity Index of *Meretrix meretrix* Samples during different seasons.

only for the community structures of the 1-year-old and 2-year-old of *Meretrix meretrix*. Principal Coordinate Analysis (PCoA) revealed that the dissimilarities in community structure existed in different ages of *Meretrix meretrix*. Seasonal differences were prevalent in the bacterial community of the clam at the same age (see Figure 2).

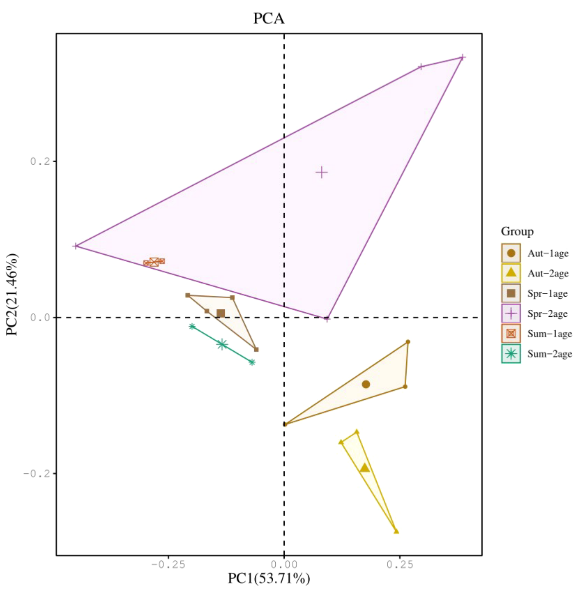

FIGURE 2
Comparison of the microbial community structures of *Meretrix meretrix* samples of the same age. Principal Coordinate Analysis (PCoA) of the 16S rRNA gene sequences using Bray-Curtis difference grouped by treatment.

3.1.3 Changes in the microbial community structure at different ages

Based on the high-throughput sequencing results, the dominant flora in the *Meretrix meretrix* included *Proteobacteria* (50%), *Firmicutes* (11%), *Bacteroides* (4%), *Spirochaetes* (1%), and *Cyanobacteria* (1%). *Proteobacteria* mainly included α-*Proteobacteria* (19%), γ- *proteobacteria* (19%), β- *Proteobacteria* (9%), and δ- *Proteobacteria* (0.5%).

The clam samples collected in the spring mainly comprised *Halomonas*, *Devosia*, *Cuprum*, *Lactobacillus*, *Paleobacterium* (1%), *Rhizobium* (1%), and *Sphingomonas* (1%). The dominant bacteria in the clam samples collected in the summer (July) included *Vibrio*, *Halomonas*, *Cuprum*, *Devosia*, *Palebacterium*, *Acinetobacter*, *Sphingomonas*, and *Seminibacterium*. The clam samples collected in mid-summer (August) mainly had *Vibrio* and *Palebacterium*. The dominant bacteria in the clam samples collected in autumn included *Ralstonia solanacearum*, *Devosia*, *Halomonas*, *Palebacterium*, *Pelomonas*, *Actinobacteria*, *Enterobacter*, *Staphylococcus*, sediment *Bacilli*, *Rhizobium*, and *Pseudomonas* (see Figure 3).

Vibrio accounted for 0.003%, 8%, 11%, and 0.1% of the bacterial community composition in the spring (April), summer (July), summer (August), and autumn (November), respectively. Thus, the *Vibrio* abundance in the clam samples increased first and then decreased with change in the season; it was the highest in summer. The relative abundance of *Vibrio* was the highest in the 3-year-old *Meretrix meretrix* in summer. *Ralstonia* caused the significant differences in the species abundance among the *Meretrix meretrix* during different seasons.

3.2 The abundance and species of *Vibrio*

The concentration of the culturable bacteria in the clam hepatopancreas ranged from 2.83×10^3 - 1.18×10^5 CFU/g in the

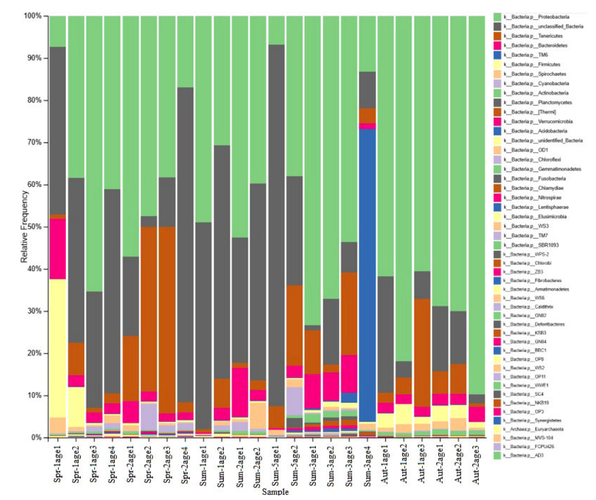

FIGURE 3
Bacterial community composition at the phylum level.

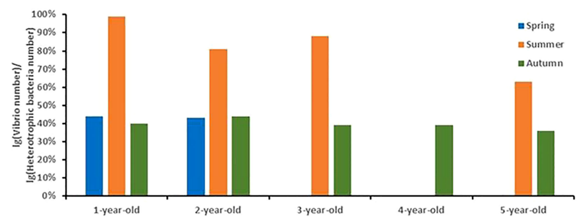

FIGURE 4
Seasonal variation of *Vibrio* spp. abundance in *Meretrix meretrix* at Different Ages.

spring, summer, and autumn. The abundance of the culturable bacteria increased first and then decreased with the season. The bacterial abundance was the highest in summer, 1–2 orders of magnitude higher than in spring. In the same season, the abundance of the culturable bacteria in the hepatopancreas of the clams of different ages was different. The quantity of the culturable bacteria in the 1-year-old and 2-year-old of *Meretrix meretrix* collected during the spring, summer, and autumn differed by 2 and 1 orders of magnitude, respectively. Thus, the abundance of the culturable bacteria in the younger *Meretrix meretrix* was more susceptible to seasonal changes. There was a significant positive correlation between the abundance of the culturable bacteria and temperature ($p<0.05$).

Vibrio abundance in the clam hepatopancreas ranged from 33 to 1.10×10^5 CFU/g in the spring, summer, and autumn. The abundance of the *Vibrio* and culturable bacteria in the clam hepatopancreas showed the same seasonal variation trend, reaching the highest in summer. The ratio of the logarithmic abundance of *Vibrio* to culturable bacteria also increased first and then decreased with the season, ranging from 0.36 – 0.99. Moreover, the ratio reached the maximum in summer, ranging from 0.63 – 0.99, indicating that *Vibrio* was the dominant bacteria in the clam hepatopancreas in summer (see Figure 4).

The 16S rRNA gene sequencing technology identified 170 bacterial isolates, and a total of 73 *Vibrio* isolates of 19 species were obtained. *Vibrio mediterranei* (19%), *V. harveyi* (11%), *V. algolyticus* (10%), and *V. parahaemolyticus* (8%) were the dominant species. Among them, the 1-year-old, 2-year-old, 3-year-old, and 5-year-old of *Meretrix meretrix* contained 6, 7, 16, and 5 species of *Vibrio*, respectively. The species and number of *Vibrio* isolates in the 3-year-old of *Meretrix meretrix* were the highest (63%).

3.3 Resistant phenotype analysis

All *Vibrio* isolates(n=73) were sensitive to sulfamethoxazole-trimethoprim. Among the 73 isolates, 68 *Vibrio* strains were resistant to other 15 antibiotics with 57 resistant phenotypes. *Vibrio* showed relatively high resistance to clindamycin (76%), amikacin (63%), ampicillin (62%), rifampicin (62%), vancomycin (57%) and amoxicillin (50%), respectively (Table 2). The ARI values of *Vibrio* spp. in different ages ranged from 0.13 to 0.18, and ARI values of 2-year-old (ARI=0.17) and 3-year-old (ARI=0.18) clams are higher than that of 1-year-old and 5-year-old clams. Forty-nine MAR *Vibrio* has been detected in the clams of different ages, and the proportion of MAR *Vibrio* in the clams at the 3-year-old is the highest (91%) compared with samples of other ages (Figure 5). Among the 49 multiple antibiotic-resistant isolates from the clam, 43 isolates were resistant to three to ten antibiotics and 1 isolate was resistant up to 15 antibiotics(Table S3). Sixteen isolates (24%) and fifteen isolates (22%) from clam samples showed resistance to four antibiotics and two antibiotics with high prevalence, respectively.

The MIC values of the 73 *Vibrio* strains for Cd^{2+}, Cu^{2+}, and Zn^{2+} metal ions were 25 – 125 mg/L, 25 - 300 mg/L, and 50 - 400 mg/L, respectively. The maximum MIC value of *Vibrio* showed the order of $Cd^{2+} < Cu^{2+} < Zn^{2+}$. The tolerance of *Vibrio* to Zn^{2+} increased with age (Table S4).

3.4 Resistant genotype analysis

Based on the results of the antibiotic-resistant phenotypes and heavy metal-resistant phenotypes of the 73 *Vibrio* strains, 13 associated resistance genes were selected for detection. The resistance genes with a higher detection rate were *nccA* (14%), *aadA* (14%), and *copB* (12%) (Table S5). The *copB* (20.00%) and *nccA* (20.00%) genes were the frequently detected resistant genes in the 1-year-old clam. The 2-year-old clam showed more resistance toward the *copA* (30.00%), *ampR* (20.00%), and *strA*

TABLE 2 Antibiotic resistance phenotypes of *Vibrio* isolates from *Meretrix meretrix* in different age.

Antibiotics		% of Resistance (No. of Isolates)			
		1-year-old (n=10)	2-year-old (n=10)	3-year-old (n=46)	5-year-old (n=7)
Cephalosporin	CZ	0	20(2)	35(16)	0
	CEP	0	10(1)	33(15)	0
	CFP	0	0	17(8)	0
	CXM	0	10(1)	20(9)	0
Penicillins	AMP	50(5)	50(6)	61(28)	43(3)
	AMC	30(3)	40(3)	54(25)	43(3)
Aminoglycosides	STR	0	10(1)	26(12)	14(1)
	GM	0	10(1)	30(14)	0
	AN	40(4)	70(7)	67(31)	14(1)
Sulfanilamide	SXT	0	0	0	0
	CIP	0	0	20(9)	0
Lincomycin	DA	30(3)	50(5)	93(43)	14(1)
Glycopeptides	VA	60(6)	60(6)	56(26)	14(1)
Tetracyclines	TCY	0	10(1)	6(3)	0
Macrolides	ERY	0	0	11(5)	0
Ansamycins	RFP	40(4)	30(3)	72(33)	0
Total		100(10)	100(10)	96(44)	57(4)

(20.00%) genes. The 5-year-old clam showed more resistance toward the *copA* (14%), *nccA* (14%), and *zntB* (14%) genes.

In addition to the co-resistance to the same class of antibiotics, resistance to cephalosporin (CFP, CEP, CZ) were significantly correlated with penicillins (AMP, AMC) ($p< 0.01$), tetracycline ($p < 0.001$), sulfanilamide (SXT) ($p< 0.01$) and quinolone (CIP) ($p< 0.01$). Aminoglycoside (GM) resistance phenotype is closely correlated with quinolone (CIP) resistance phenotype ($p < 0.001$). The bla_{SHV} gene was positively correlated with *aadA*, *ampR* and *cadD* gene ($p<0.01$). The *strA* gene was positively correlated with *sul1*($p<0.01$), *qnrS* ($p<0.01$) and *tetB* ($p<0.05$), and negatively correlated with *qyrA* ($p<0.05$). The *copA* was significantly correlated with *strB*, *ermC* and qnr_{VC457} (p<0.01). The heavy metal resistance genes *copB* and *nccA* were significantly correlated with the clindamycin resistance phenotype ($p<0.01$) (Figure 6).

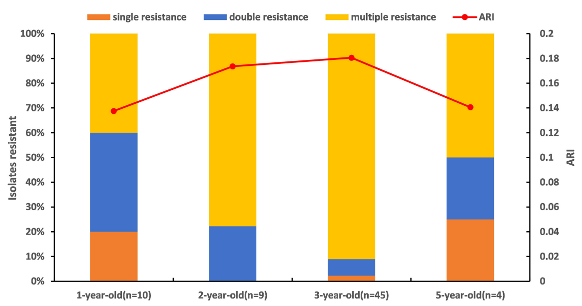

FIGURE 5
Prevalence of resistant *Vibrio* spp. and antibiotic resistance index (ARI) from clam *Meretrix meretrix* in different ages. Single resistance: resistance to one antibiotic; Double resistance: resistance to two antibiotics; Multiple resistance: resistance to three or more antibiotics.

Discussion

Temperature predicts *Vibrio* abundance quite well (Thompson et al., 2004). The prevalence of *V. parahaemolyticus*, *Vibrio vulnificus*, and *V. mimicus* in the environment positively correlated with temperature (León Robles et al., 2013). In this study, the temperature was higher in the summer (July and August), and lower in the spring (April) and autumn (November). The abundance of *Vibrio* in *Meretrix meretrix* in the summer was higher than in the spring and autumn. *Vibrio* was the dominant bacteria in the clam hepatopancreas in the summer, and there was a significant positive correlation between *Vibrio* abundance and temperature (p<0.05). Our results were consistent with a previous study demonstrating the increase in *Vibrio*

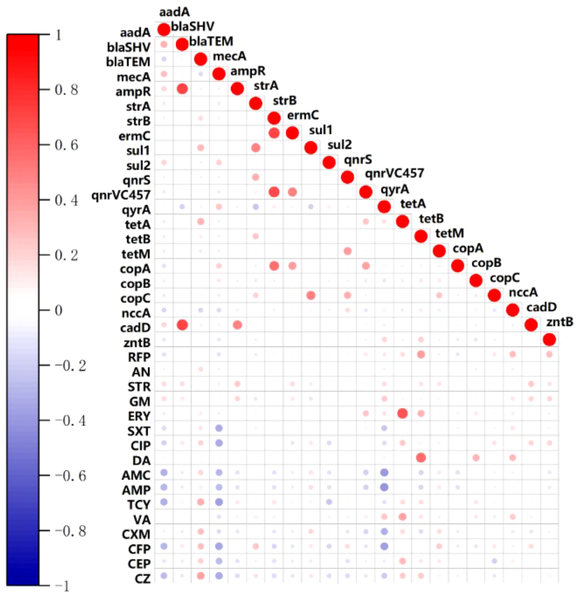

FIGURE 6
Correlation between the resistant phenotype and resistant genotype of *Vibrio* spp.

abundance upon an increase in the temperature within a certain temperature range (Cruz et al., 2016).

Some studies found the greater prevalence of *V. alginolyticus*, *V. cholerae*, *Vibrio communis*, and *V. parahemolyticus* among all the *Vibrio* spp. isolated from the clams (Yücel and Balci, 2010; Adebayo-Tayo et al., 2011; Amalina et al., 2019; Abdalla et al., 2022). However, our study indicated the predominance of *V. mediterranei* in the *Vibrio* spp. of the clams. The difference in the dominant *Vibrio* species may be due to different sites or locations of shellfish collection. *Vibrio* is an indigenous marine bacterium (Hsiao and Zhu, 2020), and not all *Vibrio* variants are pathogenic (Song et al., 2017). However, three other dominant pathogenic *Vibrio*, including *V. harveyi*, *V. algolyticus*, and *V. parahaemolyticus*, were also detected in this study. *V. parahaemolyticus* is the major *Vibrio* species causing human illness and has emerged as a severe global threat to human health through the consumption of raw or undercooked seafood (Yue et al., 2010; Yue et al., 2011; Silva et al., 2018; Park et al., 2019). *V. harveyi* is reported to be the primary pathogen of cultured prawns (Stalin and Srinivasan, 2016).

The antibiotic resistance of *Vibrio* in marine resources is a major global concern for human health (Yang et al., 2017; Silva et al., 2018). The current results showed that 73 *Vibrio* strains were resistant to 15 other antibiotics except for sulfamethoxazole-trimethoprim. This observation was consistent with a previous study (Abdalla et al., 2022). Moreover, clindamycin (76%), amikacin (63%), ampicillin (62%), rifampicin (62%), vancomycin (57%) and amoxicillin (50%) resistance were very prevalent among the *Vibrio* isolates in this study. High prevalence of resistances to ampicillin and rifampicin have also been reported in *Vibrio* isolated from many aquatic products in different regions of the world (Obaidat et al., 2017), which indicating the existence of intrinsic resistance to these antibiotics (Su and Chen, 2020).

ARI is usually used to analyze the prevalence of bacterial resistance in a given population at a specific sites (Mohanta and Goel, 2014). When ARI value >0.2,it means that the isolates are exposed to contamination where antibiotics are often used, and when ARI ≤0.2,it means that antibiotics are seldom or never used (Krumperman, 1983). The ARI value of *Vibrio* in *Meretrix meretrix* at all ages was less than 0.2, indicating that the antibiotic level in the meretrix was low. A higher ARI value is detected in the 2-year-old and 3-year-old clams compared to the other ages, indicating the 2-year-old and 3-year-old clams may be more susceptible to antibiotic contamination.

Due to the toxicity, non-biodegradability and bioaccumulation of heavy metals in the food chain, heavy metal pollution is considered as a serious threat to aquatic ecosystems and human health (Diagomanolin et al., 2004). Geligang is reported to have suffered from heavy metal pollution, including zinc, copper and cadmium (Zhang et al., 2016). In this study, our results showed that the heavy metals Cd^{2+}, Cu^{2+}, and Zn^{2+} were tolerated in the majority of the *Vibrio* isolates, which may be explained by the existence of these three heavy metal ions in Geligang. Moreover, the concentration of these three heavy metal ions in the clam may be higher due to the enrichment of the clam itself. About 70% of the *Vibrio* isolates with conjugative elements (ICEs) derived from Yangtze River Estuary displayed strong resistance to Hg (≥1 mM) and Cr (≥10 mM), and the heavy metal contamination is relatively serious in Yangtze River Estuary in China (Song et al., 2013). The tolerance to heavy metals was also found to be prevalent in the V. parahaemolyticus strains with more than two antibiotic resistance phenotypes (Kang et al., 2018), which is consistent with our results. Indeed, the co-resistance between antibiotic resistance and heavy metal resistance has been well confirmed in many studies (Zhao et al., 2012; Kang et al., 2018).

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA901517.

Author contributions

JF: conceptualization. JS and YZ: experimental operation. JS, YZ and TH: manuscript writing. JF and JS: review and acquisition of funding. HM and TS: field sampling. YX and YJ: data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1071371/full#supplementary-material

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