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Research article

Vibrio parahaemolyticus isolated from live shellfish marketed in Korea: antibiotic and heavy metal resistance

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Abstract

This study examined the antibiotic and heavy metal resistance properties in *Vibrio parahaemolyticus* isolated from Korea's five most popular shellfish. Thirty-four *V. parahaemolyticus* isolates were isolated by conducting biochemical tests and PCR. Isolation frequencies were 4 (cockles), 2 (scallops), 2 (mussels), 1 (Pacific abalone), and 25 (white-leg shrimp). The disc diffusion method was employed to detect antibiotic resistance. All isolates were resistant to ampicillin. Piperacillin, colistin, and vancomycin resistance were detected in 82%, 68%, and 50% of the isolates, respectively. Thirty-two isolates were multidrug resistant [Multiple Antibiotic Resistance (MAR) index ≥0.2]. β-lactam resistant *bla*$_{SHV}$ gene was the most prevalent gene detected in 68% of the isolates in PCR assays. In addition, *bla*$_{CTX}$, *aac(6')-Ib*, and *bls*$_{TEM}$ were detected in 21%, 44%, and 6% of the isolates, respectively. Phenotypic resistance to Pb, Cd, and Cu was detected in 25, 4, and 3 isolates, respectively in broth dilution test. *CzcA* gene was the prevalent detected in 16 isolates, followed by *CopA* and *merA* genes detected in 11 and 7 isolates, respectively. The findings of this study suggest that live shellfish marketed in Korea are a potential source of antibiotic-resistant *V. parahaemolyticus*.

Keywords: Multiple drug resistance, Resistance genes, Seafood, Public health, Shellfish.

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INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative bacterium that inhabits estuarine and marine habitats. It is considered a major food-borne pathogen that causes severe illnesses in humans. Especially after the consumption of undercooked or raw food (Su and Liu, 2007; Fasulkova and Stratev, 2024). *V. parahaemolyticus* food poisoning is predominantly associated with different kinds of seafood, including shrimp, crab, fish, lobster, and oysters (Wang et al., 2015; Farag et al., 2023). As filter feeders, shellfish are highly prone to contamination with pathogenic Vibrios in water (Drake et al., 2007; Campbell et al., 2022). Food-borne *Vibrio* infections usually occur during summer and late autumn when the environmental temperature increases. Because They grow well in warm (>15°C) conditions (Jung, 2018).

V. parahaemolyticus can cause acute gastroenteritis in humans, and it can also cause infections in open wounds exposed to contaminated seawater (Santos et al., 2015). Although *V. parahaemolyticus* infections can be treated with rehydration therapy, some cases require antibiotics to recover (Lopatek et al., 2015). Severe *Vibrio* infections are treated with tetracycline; however, a combination of 3rd generation cephalosporins and doxycycline or fluoroquinolone alone is also used occasionally (Han et al., 2007; Dutta et al., 2021). Despite, resistance to a structurally and functionally diverse range of antibiotics, including β-lactams, tetracyclines, macrolides, cephems, aminoglycosides, glycopeptides, ansamycins, and carbapenems have been reported in *V. parahaemolyticus* (Baker-Austin et al., 2008). Inappropriate and frequent use of antibiotics can cause bacteria to change so antibiotics do not work against them. This is called antibiotic resistance. The emergence of antibiotic resistance in food-borne bacteria is a growing concern worldwide. Also, the emergence of antibiotic resistance in food-borne human pathogens is an indirect health hazard (Banerjee and Farber, 2018; Almansour et al., 2023).

The origin of antibiotic resistance is believed to have started due to the interaction of many microbes with their environment to overcome the action of natural antibiotic compounds to survive. This is intrinsic resistance (Munita et al., 2016). In contrast, bacteria can acquire resistance by horizontal gene transfer (Džidić et al., 2008; Wang et al., 2023a). However, acquired resistance in a bacterial community that was initially susceptible to the antibiotic compound is more important. Conjugation, transformation, and transduction are the well-known gene transfer mechanisms that bacteria use to acquire antibiotic resistance (Blakely, 2024).

Environmental contaminants, such as antibiotics, heavy metals, and organic solvents, can provide selective pressure for microbes to develop and evolve mechanisms to tolerate such stressors (Ashbolt et al., 2013; Wang et al., 2023b). It is crucial to determine the causative factors that contribute to the development of antibiotic resistance and to introduce effective practices for fighting antibiotic resistance (Davies and Davies, 2010). Additionally, industrial pollutants, such as heavy metals, significantly threaten aquaculture. Marine bacteria can take up these metals, leading to bioaccumulation within the food chain (Çardak et al., 2023). The effects of heavy metals on microbes have been studied using diverse methods, including culturing bacteria in a medium enriched with known metal concentrations (Dickinson et al., 2019).

V. parahaemolyticus is one of the most frequently reported causes of seafood-borne illnesses in South Korea and also the most frequent seawater-isolated *Vibrio* species in South Korea (68.10 %) (Park et al., 2018; Lee et al., 2019). Nevertheless, food poisoning statistics from 2003 to 2016 published in 2017 by the Korea Ministry of Food and Drug Safety, reported that *V. parahaemolyticus* has caused infections in 4256 patients. Therefore, this study examined the antibiotic and heavy metal resistance properties of *V. parahaemolyticus* isolated from different shellfish marketed in South Korea.

MATERIALS AND METHODS

Sample collection

The five most popular shellfish items, including 120 Pacific abalone (*Haliotis discus hannai*), 257 Mussel (*Mytilus coruscus*), 120 Cockles (*Tegillarca granosa*), 115 scallops (*Patinopecten yessoensis*), and 65 Pacific white-leg shrimp (*Litopenaeus vannamei*), harvested in the southern and western coast of Korea, were purchased from five supermarkets and two retail markets in Korea from February to June 2020. Each sample type was brought to the laboratory in sterilized containers with ice packs and transported to the laboratory. Samples were processed immediately. Each sample was shuckled aseptically, and each flesh was separately blended in a sterile blender jar to make a homogenized composite. The sterile gloves were used and changed while handling each sample.

Isolation and biochemical identification of *V. parahaemolyticus*

One gram of each homogenized composite was placed in a conical tube with alkaline peptone water (APW) and incubated at 37°C for 24h. One loop full of each incubated enrichment was streaked onto thiosulphate bile salts sucrose (TCBS) agar (MB cell, LA, CA) plates and incubated for 24h at 37°C. Characteristic yellow or green colour colonies on TCBS agar were separated and stabbed onto triple sugar iron agar (TSI; MB cell, LA, CA) tubes and incubated for 24h at 37°C (Di Pintoet al., 2021). Isolates that showed alkaline or acidic slant and acidic butt were subjected to oxidase test. Oxidase-positive strains were examined for sensitivity with a vibriostatic disc diffusion test using DD150129 (150 µg) discs (Oxoid, Hamsphire, UK).

According to the manufacturer's protocol, the genomic DNA of the biochemically identified isolate was extracted using Exgene Cell SV extraction kit (Geneall, Seoul, Korea). The sequencing of the gyrB housekeeping gene was employed for species identification. *gyrB-F* and *gyrB-R* primers were used for PCR amplification (Luo and Hu, 2008). Amplified PCR products were purified using the Exgene PCR SV (Geneall, Seoul, Korea) kit and submitted for gene sequencing at Cosmogenetech Co. Ltd. (Daejon, Korea). BLAST compatibility with available gene sequences in the GenBank database (NCBI) was employed for species confirmation. This study used *V. parahaemolyticus* (DS267854) as a reference strain.

Antibiotic susceptibility testing

Antibiotic susceptibility was examined against 20 antibiotics belonging to 11 antibiotic classes by disc diffusion test as follows: Aminoglycosides; streptomycin (10µg), gentamycin (10µg), kanamycin (30µg), amikacin (30 µg), Penicillins; ampicillin (10µg), piperacillin (100µg), Lipopeptides; colistin sulfate (10µg), Quinolones; nalidixic acid (30µg), ofloxacin (5µg), ciprofloxacin (5µg), Tetracyclines; doxycycline (30µg), Ansamycins; rifampicin (5µg), Carbapenems; imipenem (10µg), meropenem (10µg), Macrolides; erythromycin (15µg), Folate pathway inhibitors; trimethoprim-sulfamethoxazole (25µg), Cephalosporins; cephalothin (30µg), ceftriaxone (30µg), cefotaxime (30µg), Glycopeptides; vancomycin (30 µg). The testing procedure was carried out according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2021). The multiple antibiotic resistance (MAR) index was calculated according to the formula described by Krumperman (1983).

Heavy-metal tolerance testing

Heavy-metal resistance of the isolates was examined against five heavy-metal ions, including Cu, Pb, Cr, Cd, and Hg. The minimum inhibitory concentration

(MIC) of the tested heavy-metal ions was measured using the broth dilution testing method (microdilution) as described in He et al. (2016). $CuCl_2$, $PbCl_2$, $CrCl_3$, $CdCl_2$, and $HgCl_2$ (Samchun, Seoul, Korea) metal chlorides were used to obtain Cu, Pb, Cr, and Cd concentration series ranging between 3,200µg/mL and 6.25 mg/mL while 400 to 0.78 µg/mL concentration series was used for Hg. *E. coli* K-12 (MG 1655) strain was used as the control strain, and isolates that surpassed the MIC values of the control strain were considered resistant isolates.

Screening of heavy metal resistance genes and antibiotic resistance genes

The conventional PCR method examined the presence of 4 heavy metal resistance-related genes and 13 antibiotic resistance genes and class 1 integrons. The oligonucleotide primer pairs and conditions are given in Table 1. Each PCR reaction mixture was 30µL in final volume consisting 0.3µL of AmpOne Taq DNA polymerase (GeneAll, Seoul, Korea), 3µL of dNTP mix, 3µL of 10×Taq

Table 1 Oligonucleotide primers and PCR conditions used to amplify antibiotic and heavy metal resistance genes of *Vibrio parahaemolyticus*

Category	Gene		Sequence (5'-3')	Annealing temp (^{0}C)	Amplicon size (bp)	Reference
Extended-spectrum β-lactamase	*bla*$_{TEM}$	F R	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	58	1080	Dallenne et al. (2014)
	bla$_{SHV}$	F R	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	58	795	Dallenne et al. (2014)
	bla$_{CTX\text{-}M}$	F R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	52	550	Dallenne et al. (2014)
Tetracycline resistance	*tetA*	F R	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	62	1000	Henriques et al. (2006).
	tetB	F R	CTCAGTATTCCAAGCCTTTG CTAAGCACTTGTCTCCTGTT	58	400	Henriques et al. (2006).
	tetE	F R	GTGATGATGGCACTGGTCAT CTCTGCTGTACATCGCTCTT	62	1100	Henriques et al. (2006).
Plasmid-mediated quinolone resistance	*qnrA*	F R	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	56	580	He et al. (2016)
	qnrB	F R	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	53	496	He et al. (2016)
	qnrS	F R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	56	428	He et al. (2016)
Aminoglycoside resistance	*strA-strB*	F R	TATCTGCGATTGGACCCTCTG CATTGCTCATCATTTGATCGGCT	55	538	Sunde and Nostrom (2005)
	aphAI-IAB	F R	AAACGTCTTGCTCGA GGC CAAACCGTTATTCATTCGTGA	33	500	Frana et al. (2001)
	aac(3')-IIa	F R	ATGGGCATC ATTCGCACA TCTCGGCTTGAACGAATTGT	55	749	Samadi et al. (2015)
	aac(6')-Ib	F R	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	55	482	Park et al. (2006)
Integrons	*intI1* (Class 1 integron integrase)	F R	CTACCTCTCACTAGTGAGGGGCGG GGGCAGCAGCGAAGTCGAGGC	58	485	Diaz et al. (2006)
	Class 1 integron	5'-CS 3'-CS	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	56	variable	Lee et al. (2008)
Heavy metal resistance	*CopA* (copper translocating ATPase)	F R	CGGTCTCTACGAATACCGCTTCAA GAAATAGCTCATTGCCGAGGCGTT	55	1300	Bouskill et al. (2007)
	CzcA (cobalt/zinc/cadmium efflux protein)	F R	GTTCACCTTGCTCTTCGCCATGTT ACAGGTTGCGGATGAAGGAGATCA	55	320	Bouskill et al. (2007)
	ChrR (chromium resistance)	F R	ATGTCTGATACGTTGAAAGTTGTTA CAGGCCTTCACCCGCTTA	54	350	Rahman et al. (2007)
	merA (mercuric reductase)	F R	GTGCCGTCCAAGATCATGAT TAGCCYACRGTSGCSACYTG	57	933	Rahman et al. (2007)

RESULTS

Identification of V. parahaemolyticus

A total of 34 *V. parahaemolyticus* strains were identified up to their species level. Of those, 1 strain from Pacific abalone, 4 strains from cockles, 2 strains from mussels, 2 strains from scallops, and 25 strains from white-leg shrimps were identified.

Antibiotic resistance profile

In this study, *V. parahaemolyticus* strains showed resistance against 17 antibiotics in different combinations. All the strains demonstrated ampicillin resistance. Piperacillin, colistin, and vancomycin resistance were also detected in 82%, 68%, and 50% of the isolates, respectively. Amikacin, cephalothin, and streptomycin resistance were detected in 35%, 32%, and 24% of the isolates, respectively. Antibiotic resistance patterns are presented in Table 2 and Figure 1. The occurrence of antibiotic resistance genes among *V. parahaemolyticus* spp. was detected by PCR, and the results are presented in Table 3. The highest prevalence was observed for the bla_{SHV} gene (68%). *aac(6")-Ib* and bla_{TEM} genes were detected in 44% and 26% of the isolated, respectively. Reported MAR index values ranged from 0.1 to 0.5. The highest MAR index value was reported in white-leg shrimp isolated strain LVP23. Thirty-two isolates scored MAR index ≥0.2.

Table 2 Antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from shellfish

Seafood source	Isolate	Resisted antibiotics[a]	MAR index
Pacific abalone	AVP01	AMP, COL, KF, PRL	0.2
Cockles	CVP01	AMP, PRL, COL, STR, ERY, RD, CRO, KF,	0.4
	CVP02	AMP, PRL, COL, STR, KF	0.3
	CVP03	AMP, PRL, COL, STR, KAN, AMK, CRO, KF	0.4
	CVP04	AMP, PRL, COL, KF	0.2
Scallop	SVP01	AMP, COL, VA, STR, AMK, KF	0.3
	SVP02	AMP, COL, VA, STR, RD, KF	0.3
Mussels	MVP01	AMP	0.05
	MVP02	AMP, COL, STR	0.2
White-leg shrimp	LVP01	AMP, PRL, COL, VA, AMK	0.3
	LVP02	AMP, PRL, COL, VA, KAN, AMK, KF	0.4
	LVP03	AMP, PRL, COL, VA, GEN, AMK, ERY, RD	0.4
	LVP04	AMP, PRL, COL, VA, KAN, AMK	0.3
	LVP05	AMP, PRL, COL, VA, RD, CTX	0.3
	LVP06	AMP, PRL, COL, VA, STR, RD, CTX	0.4
	LVP07	AMP, PRL, VA, DA, ERY, CTX, KF	0.4
	LVP08	AMP, PRL, COL	0.2
	LVP09	AMP, PRL, COL	0.2
	LVP10	AMP, PRL, COL, VA, DA, AMK	0.3
	LVP11	AMP, PRL, COL, VA, CTX	0.3
	LVP12	AMP, PRL, AMK, SXT	0.2
	LVP13	AMP, PRL, AMK	0.2
	LVP14	AMP, COL, VA, KF	0.2
	LVP15	AMP, PRL, COL, AMK, SXT	0.3
	LVP16	AMP, PRL, COL, KAN, AMK, CTX	0.3
	LVP17	AMP, PRL, COL, VA, KAN, CTX	0.3
	LVP18	AMP, PRL, ERY	0.2
	LVP19	AMP, PRL, VA	0.2
	LVP20	AMP, PRL, VA	0.2
	LVP21	AMP, PRL, STR	0.2
	LVP22	AMP, PRL, COL, VA, DA, KF	0.3
	LVP23	AMP, PRL, COL, DA, STR, GEN, KAN, AMK, NAL, RD	0.5
	LVP24	AMP, PRL, VA, DA	0.2
	LVP25	AMP, PRL	0.1

[a] Resisted antibiotics; AMP = ampicillin, PRL = piperacillin, COL = colistin, STR = streptomycin, KAN = kanamycin, ERY = erythromycin, RD = rifampicin, KF = cephalothin, NAL = nalidixic acid, SXT = trimethoprim-sulfamethoxazole, GEN = gentamycin, VA= vancomycin, DA=doxycycline, CTX= cefotaxime, AMK= amikacin, CRO= ciprofloxacin

Heavy-metal resistance profile

Lead (Pb) resistance, reported in 74% of the isolates, was the most prevalent. Resistance to other tested heavy metals was reported in very low percentages. Almost all isolates could grow at low metal concentrations. However, most of the isolated samples could not pass the threshold concentration that was used to categorize them as resistant strains. Cadmium and copper resistance was observed in four isolates, respectively. Heavy metal resistance genes were detected in contrast to the phenotypic heavy metal resistance. This study's most prevalent heavy metal resistance gene was *CzcA*, observed in 47% of the isolates. The genes, *copA,* and *merA* were detected in 33% and 21% of the isolates, respectively.

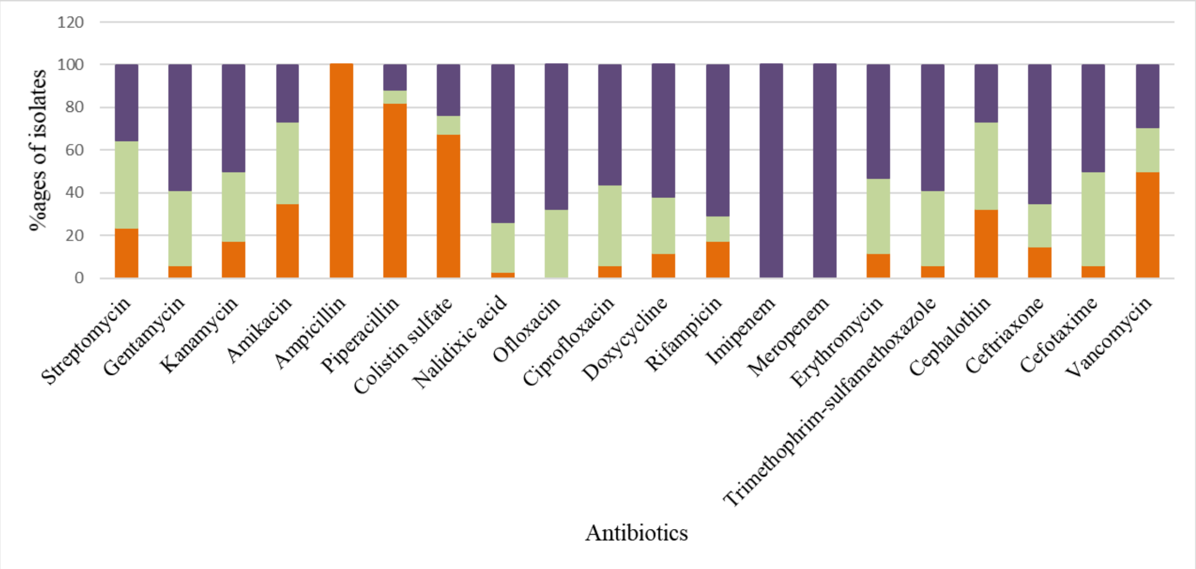

Figure 1 Antimicrobial susceptibility profile of *V. parahaemolyticus* isolated from shellfish.
🟧 Resistance 🟩 Intermediate resistance 🟪 Suseptible

Table 3 Antibiotic resistance genes and heavy metal resistance properties of *V. parahaemolyticus*

Seafood source	Isolate	Antibiotic resistance genes	Resisted heavy metals	Heavy metal resistance genes
Pacific abalone	AVP01	bla_{SHV}	Cu	*CzcA*
Cockles	CVP01	bla_{SHV}, bla_{TEM}, *aac(6')-Ib*		*CzcA*
	CVP02	bla_{SHV}, bla_{TEM}, *aac(6')-Ib*	Pb	*CopA*
	CVP03	*aac(6')-Ib*	Pb	
	CVP04	bla_{SHV}, bla_{TEM}, *aac(6')-Ib*	Pb	*CzcA, CopA*
Scallop	SVP01	bla_{SHV},*aac(6')-Ib*	Pb	
	SVP02	bla_{SHV}	Cd	
Mussels	MVP01	$bla_{CTX\text{-}M}$, bla_{TEM}		
	MVP02	$bla_{CTX\text{-}M}$, bla_{SHV}		*CzcA,merA*
White-leg shrimp	LVP01		Pb	*CopA*
	LVP02	bla_{SHV},*aac(6')-Ib*	Pb	*CopA*
	LVP03	bla_{SHV},*aac(6')-Ib*	Pb	*CzcA, CopA*
	LVP04	bla_{SHV},*aac(6')-Ib*	Pb	
	LVP05		Pb	*CzcA*
	LVP06	bla_{SHV},*aac(6')-Ib*	Cu, Pb	*CzcA*
	LVP07	bla_{SHV}, bla_{TEM},*aac(6')-Ib*	Pb	*CzcA,merA*
	LVP08		Pb	*CzcA,CopA*
	LVP09	bla_{SHV},*aac(6')-Ib*	Pb	*CzcA,merA*
	LVP10	bla_{SHV},*aac(6')-Ib*	Pb	*CzcA*
	LVP11	bla_{SHV}	Pb	*CzcA*
	LVP12	bla_{SHV},*aac(6')-Ib*	Pb	*CopA*
	LVP13	bla_{SHV},*aac(6')-Ib*	Pb	*merA*
	LVP14	bla_{SHV}, bla_{TEM}	Pb	*CopA,merA*
	LVP15	bla_{SHV}		*CopA*
	LVP16	bla_{SHV},*aac(6')-Ib*	Cu	*CzcA*
	LVP17	bla_{SHV}	Cd,Pb	*merA*
	LVP18	bla_{SHV}	Cd,Pb	*CzcA,CopA*
	LVP19		Cd,Pb	
	LVP20	bla_{SHV}	Pb	*CzcA,merA*
	LVP21	bla_{SHV}	Pb	
	LVP22	bla_{SHV}	Cu,Pb	
	LVP23		Pb	*CzcA, CopA*
	LVP24	bla_{SHV}, bla_{TEM}		
	LVP25			

DISCUSSION

Vibrio parahaemolyticus is a well-known food-borne pathogen with a global distribution. However, its densities in seafood vary depending on the sample type, location, season, and isolation methods (Letchumanan et al., 2015). A total of 34 *V. parahaemolyticus* strains were identified up to their species level. Of those, one strain from Pacific abalone, four strains from cockles, two strains from mussels, two strains from scallops, and 25 strains from white-leg shrimps were identified. *V. parahaemolyticus* has been isolated from various seafood items in different percentages in Korea. Incidence of *V. parahaemolyticus* was reported in fish and shrimps sampled from fish farms in Korea (Mok et al., 2021). Xie et al. (2020) reported *V. parahaemolyticus* isolates in fish and shrimps marketed in China. The presence of multidrug resistant *V. parahaemolyticus* in white-leg shrimp was significant.

In this study, *V. parahaemolyticus* strains showed resistance against 17 antibiotics in different combinations. Thirty-two isolates (94%) were multidrug resistant by scoring MAR index ≥0.2. Multidrug resistance *V. parahaemolyticus* isolated from seafood has been reported in China and other East Asian countries (Yang et al., 2017). All strains demonstrated ampicillin resistance. Lee et al. (2019) reported that all of the *V. parahaemolyticus* strains isolated from seafood in Korea for his study as ampicillin-resistant isolates. Kim et al. (2014) also reported that ampicillin-resistant *V. parahaemolyticus* in seawater was collected near marine aquaculture sites in Korea. In recent years, increasing resistance to ampicillin has been detected among *V. parahaemolyticus* strains (Lopatek et al., 2015).

V. parahaemolyticus also shows resistance to colistin sulfate and vancomycin. Colistin and vancomycin resistance were detected in 68% and 50% of the isolates. *V. parahaemolyticus* isolated from the shrimps and fish samples exhibit resistance against colistin (Lei et al., 2019). Colistin is a critical "last-resort" antibiotic used when other treatments fail, particularly against multidrug-resistant bacteria (Mondal et al., 2024). Although it is permitted in livestock and aquaculture, its overuse can foster resistant bacterial strains that may transfer to humans through the food chain, limiting effective treatment options (Pepi and Focardi, 2021). Vancomycin resistance was observed in 100% of *V. parahaemolyticus* isolates from shellfish in Korea (Jo et al., 2020). Vancomycin is banned in animals as it is essential for treating serious human infections, such as MRSA (methicillin-resistant *Staphylococcus aureus*) (Aqib and Alsayeqh, 2022). Finding vancomycin-resistant *V. parahaemolyticus* indicates possible environmental contamination or cross-resistance that could potentially reach humans (Kavya et al., 2023).

Moreover. 82% of the isolates in this study showed resistance to piperacillin, a β-lactam antibiotic. *V. parahaemolyticus* strains isolated from different types of seafood in Malaysia were highly resistant to β-lactam class antibiotics, including ampicillin and piperacillin (Tan et al., 2020). Both environmental and clinical isolates of *V. parahaemolyticus* exhibit resistance to β-lactam antibiotics, narrowing treatment options (Li et al., 2016). Resistance to β-lactam antibiotics is primarily due to the secretion of β-lactamases that hydrolyze and inactivate β-lactam antibiotics (Jacoby and Munoz-price, 2005). The genes regulate the secretion of β-lactamases. β-lactamases encoding bla_{SHV}, bla_{TEM}, and bla_{CTX} gens were reported in 68%, 21%, and 6% of the isolates, respectively. These highly mobile genes, often inserted in plasmids and transposons, suggest that *V. parahaemolyticus* may act as a reservoir of β-lactamases encoding genes (Cantón et al., 2012). Plasmid-mediated bla_{TEM} and bla_{SHV} are the most frequently reported β-lactamases encoding genes (Chikwendu, 2014). However, CTX type β-lactamase producing genes are rarely found in Korea (Kim and Lim, 2005). Cephalothin resistance was reported as 32%. Seafood-isolated *V. parahaemolyticus* has shown cephalothin resistance (54%) in the study of Tan et al. (2020).

V. parahaemolyticus isolates from seafood are generally resistant to β-lactam antibiotics, and rising resistance to aminoglycosides has been observed worldwide (Lopatek et al., 2015). Thirty-five and thirty-two percent of the isolates showed resistance to amikacin and streptomycin in this study. Aminoglycoside 6'-Nacetyltransferase encoding *aac(6")-Ib* was detected in 44% of the isolates. Almost all isolates that could amplify *aac(6")-Ib* gene demonstrated either streptomycin or amikacin resistance. Similar to our study, fresh and marine fish isolated *V. parahaemolyticus* isolates have shown a high percentage of resistance to ampicillin and amikacin in the study by Lee et al. (2018). Streptomycin-resistant *V. parahaemolyticus* has been reported from seafood in previous studies conducted in Korea and Italy (Ottaviani et al., 2001; Oh et al., 2011).

Industrial pollutants, including heavy metals, were supposed to increase the selection for antibiotic resistance and vice versa (He et al., 2016). Lead (Pb) resistance, reported in 74% of the isolates, was the most prevalent in this study (Table 2). Resistance to other tested heavy metals was reported in very low percentages. Almost all isolates could grow at low metal concentrations. However, most of the isolates could not grow over the threshold concentration used to categorize a resistant strain to particular heavy metals. Cadmium and copper resistance was observed in four isolates. Antibiotic-resistant *V. parahaemolyticus* strains isolated from shellfish and crustaceans in China have demonstrated resistance to Cu, Pb, and Cd (Hu and Chen, 2016).

Heavy metal resistance genes were detected in contrast to the phenotypic heavy metal resistance. This study's most prevalent heavy metal resistance gene was cobalt-zinc-cadmium resistance protein-encoding *CzcA* gene, observed in 47% of the isolates. However, cadmium tolerance was weak in this study. *CzcA* is considered the actual efflux-transportation protein, making heavy metals tolerable. *CzcA*'s specificity is regulated by the presence of 2 other structural proteins (Hu and Chen, 2016). Lack of these associated factors may change the heavy metal tolerance of the bacteria that bears *CzcA* gene.

Moreover, *CopA* gene was detected in 33% of the isolates. The gene *CopA* is highly conserved among Vibrios and contributes to resisting the action from phagocytosis actions colonizing the host (Hernández-Montes et al., 2012). In contrast to the phenotypic Hg resistance, 21% of the isolates amplified *merA* gene. It has been reported that a relatively low level of Hg resistance occurs when the *merA* gene exists alone (Naguib et al., 2018). However, Hg-resistant genes are usually located on transposons and plasmids, which also carry antibiotic-resistance genes (Mirzaei et al., 2013). Thus, this study's *merA* present *V. parahaemolyticus* isolates may have transposons that transfer antibiotic-resistant traits. This study shows a complex link between heavy metal resistance genes and actual resistance in *Vibrio parahaemolyticus* isolates. Findings emphasize the need to monitor both phenotypic resistance and resistance genes in polluted environments, as they could influence antibiotic resistance spread.

CONCLUSIONS

This study provides information on the incidence of multidrug-resistant *V. parahaemolyticus* in marketed live shellfish in Korea, especially in white-leg shrimp. Resistance to β-lactam antibiotics and the presence of β-lactamases encoding genes were detected in different frequencies. These findings suggest that β-lactam antibiotics can be ineffective soon as a treatment option for infections caused by *V. parahaemolyticus.* Although the detected heavy metal resistance was not strong, reported heavy metal resistance genes recommend the further study of heavy metal resistance genes and their contribution to antibiotic resistance in *V. parahaemolyticus*; a specie that has not been widely studied for its heavy metal resistance properties.

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AUTHOR CONTRIBUTIONS

Masimbule Vidanalage Kasun Sameera Wickramanayake, Sana Majeed and Gang-Joon Heo contributed to the conception and design of the study Masimbule Vidanalage Kasun Sameera Wickramanayake, Sana Majeed, P.M. Kumarage, and Gang-Joon Heo performed material preparation, data collection, and analysis. Masimbule Vidanalage Kasun Sameera Wickramanayake and Sana Majeed wrote the first draft of the manuscript, and all authors contributed to reviewing and editing the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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