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Characterisation of pathogenic *Vibrio* spp. isolated from live Pacific abalone (*Haliotis discus hannai* Ino, 1953) marketed in South Korea

M. V. K. S. WICKRAMANAYAKE, P. S. DAHANAYAKE, S. HOSSAIN, B. C. J. DE SILVA AND G. J. HEO*

Laboratory of Aquatic Animal Medicine, Veterinary Medical Center and College of Veterinary Medicine Chungbuk National University, Chungdae-ro 1, Seowon-gu, Cheongju 28644, Republic of Korea
e-mail: gjheo@cbu.ac.kr

ABSTRACT

This study aimed to evaluate the profile of virulence factors and antimicrobial resistance in *Vibrio* spp. isolated from live Pacific abalone (*Haliotis discus hannai* Ino, 1953) marketed in South Korea. A total of 32 *Vibrio* isolates comprising *V. alginolyticus* (n=15), *V. diabolicus* (n=14), *V. antiquarius* (n=2) and *V. parahaemolyticus* (n=1) were analysed. All isolates demonstrated DNase, lipase, phospholipase and amylase activities. Additionally, production of slime (97%), gelatinase (94%), α-haemolysin (22%), β-haemolysin (78%) and protease (53%) were detected. Virulence genes *viz*., *toxR*, *tlh*, *tdh*, *VAC*, *VPI*, *ctxAB* and *hupO* were recorded in 56, 59, 13, 100, 41, 9 and 9% of the isolates, respectively. All isolates were resistant to ampicillin and 88% of the isolates were resistant to cephalothin and colistin sulphate. Twentyone isolates (66%) showed multiple antimicrobial resistance (MAR) index ≥0.2. Antimicrobial resistance genes *blaCTX* (85%), *blaTEM* (10%), *blaSHV* (10%), *strAB* (13%) and *aphA-IAB* (22%) and class 1 integrons (19%) were detected. *V. diabolicus* and *V. antiquarius* were identified and characterised for the first time in the Pacific abalone. Our findings imply the significance of integrated monitoring and surveillance programmes for the occurrence, virulence and antimicrobial resistance patterns of vibrios in Pacific abalone.

Keywords: *Haliotis discus hannai*, Pacific abalone, Pathogenicity, South Korea, *Vibrio* spp., virulence

Introduction

Abalone (*Haliotis* spp.) is recognised as one of the most valuable seafood in the world. South Korea is the world's second largest abalone producer with over 10,000 t of annual production after China, the leading producer of farmed abalone in the world. Also, South Korea is considered one of the world's top seafood consuming countries where a variety of species including abalones are in abundance (FAO, 2017; Yonhap, 2017).

The statistics and studies related to food borne diseases reveal bacteria as the major causative agents. Among the pathogenic bacteria, *Vibrio* spp. have been identified as the major group of microbes which can cause severe food borne illnesses (Lee *et al.,* 2001). In South Korea, ten cases of *Vibrio* gastritis have been reported in 2017, which occurred due to the consumption of seafood in a restaurant (KCDC, 2018). *Vibrio* spp. are Gram negative bacteria that are ubiquitous in marine environment (Thompson *et al.,* 2004). Among the large number of *Vibrio* spp. identified, *V. parahaemolyticus, V. alginolyticus, V. cholerae, V. vulnificus* and *V. fluvialis* have been found to be frequently associated with seafood related disease outbreaks (Elhadi *et al.,* 2004).

Thermostable direct haemolysin (TDH) encoded *tdh* gene, TDH related haemolysin (TRH) encoded *trh* gene and thermolabile haemolysin (TLH) encoded *tlh* gene are considered as important virulence factors in food borne *Vibrio* infections (Zhang and Austin, 2005; Wang *et al.,* 2015). Moreover, production of transmembrane regulatory protein (*toxR*), cholera toxin (CTX) and secretions in *V. cholerae* pathogenicity island (VPI) also have a significant contribution to the pathogenicity of *Vibrio* spp. (Sarkar *et al.,* 2002). Besides the toxin production and haemolysins, there are many other virulence related extracellular enzymes such as protease, amylase, DNase, lipase and gelatinase (Bunpa *et al.,* 2016). Also, *Vibrio* spp. have been noted for their emerging antimicrobial resistance patterns (Harbottle *et al.,* 2006).

Though abalone is considered as valuable seafood all over the world, only a few studies have been conducted to assess the potential health risk of abalone consumption. Lee *et al.* (2016) reported the prevalence of food poisoning microbiota in abalone. However, to the best of our knowledge, there have been no studies conducted to characterise virulence factors and antimicrobial resistance properties of *Vibrio* spp. isolated from Pacific abalone marketed live in south Korea. Therefore, the current

study intended to characterise the virulence factors, antimicrobial resistance patterns, antimicrobial resistance gene profiles and class 1 integrons of *Vibrio* spp. isolated from marketed Pacific abalone (*Haliotis discus hannai* Ino, 1953) in South Korea.

Materials and methods

Abalone sampling

A total of 120 live Pacific abalone samples were procured from retail markets from February to June 2018. Samples were transported to the laboratory and processed immediately. Each abalone was shucked and a homogenised composite was prepared by blending each sample in a sterile blender. The entire sampling procedure was conducted under aseptic conditions.

Isolation and biochemical identification of Vibrio spp.

One gram each of homogenised tissue sample was incubated in alkaline peptone water (APW) at 37ºC for 24 h. One loopful of each enrichment was streaked onto thiosulphate citrate bile salts sucrose (TCBS) agar (MB cell, California, USA) plates and incubated for 24 h at 37ºC. All colonies that appeared yellow or green in colour on TCBS agar were subcultured on tryptic soy agar (TSA) supplemented with 1% (w/v) NaCl to obtain pure colonies. Triple sugar iron agar (TSI) test, oxidase test and sensitivity to vibriostatic discs were employed for phenotypic confirmation of *Vibrio* spp. status.

DNA extraction, polymerase chain reaction and phylogenetic analysis

Each *Vibrio* sp. identified based on biochemical tests was incubated in APW at 37ºC for 24 h and genomic DNA was extracted using Exgene Cell SV extraction kit (Geneall, Seoul, Korea) according to the manufacturer's protocol. Polymerase chain reaction (PCR) and sequencing of *gyrB* housekeeping gene amplified with gyrB-F and gyrB-R primers were employed for species identification. Amplified PCR products were purified using Exgene PCR SV (Geneall, Seoul, Korea) kit and submitted for gene sequencing at Cosmogenetech Co. Ltd. (Daejeon, Korea). Sequencing data were checked for BLAST compatibility with available gene sequences in the GenBank database (NCBI). Neighbor joining phylogenetic tree was constructed according to Kumar *et al.* (2016). The following sequences were downloaded from GenBank database (NCBI) for analysis: *V. diabolicus* strain LMG 23867, *V. alginolyticus* strain DX 0406, *V. antiquarius* strain EX 25, *V. parahaemolyticus* strain GQ426112 and *Escherichia coli* strain KCTC 2441 (EU014649) as the outgroup taxa.

Phenotypic pathogenicity tests

A total of 32 *Vibrio* strains isolated were subjected to eight phenotypic pathogenicity tests. Tryptic soy agar (TSA) supplemented with 1% (w/v) NaCl was used to maintain test strains. Additionally, 1% (w/v) NaCl was supplemented in each test medium. Protease production was examined by incubating the isolates on TSA added with 0.5% (w/v) skim milk (MB Cell, Seoul, Korea) for 48 h at 37ºC (Zhang and Austin, 2000). To detect DNase activity, 1N HCl was added to the well grown colonies on DNase agar (MB Cell, California, USA) plates and the presence of halo effect around colonies was noted as positive (Twedt *et al.,* 1970). Slime production was detected by Congo red uptake method according Freeman and Falkine (1989). Production of lipase and phospholipase were examined employing TSA added with 1% (v/v) Tween 80 (Samchun, Pyeongtaek, Korea) and 5% (v/v) egg yolk emulsion (MB Cell, Seoul, Korea), respectively. The presence of opaque halos around the colonies was considered as positive (Liuxy *et al.,* 1996). Gelatinase activity was tested according to Elavarashi *et al.* (2017). Amylase production was examined by adding Gram's iodine onto well grown colonies in TSA supplemented with 0.2% (w/v) soluble starch. The presence of clear halos around the colony after overnight incubation at 37ºC was considered as positive. Haemolysin activity was assessed by streaking the strains on blood agar base (MB Cell, California, USA) supplemented with 5% (v/v) sheep blood. Greenish discolouration and clear zone around the colonies were considered as (α) and (β) haemolysis, respectively.

Antibacterial susceptibility testing

Antimicrobial susceptibility was examined against 20 antimicrobials belonging to 11 antimicrobial classes by the disc diffusion test. The antimicrobials used included Penicillins: ampicillin (10 µg) and piperacillin (100 µg); Lipopeptide: colistin sulfate (10 µg); Tetracyclines: tetracycline (30 µg) and oxytetracycline (30 µg); Phenicol: chloramphenicol (30 µg); Aminoglycosides: streptomycin (10 µg), gentamycin (10 µg) and kanamycin (30 µg); Quinolones: nalidixic acid (30 µg), ofloxacin (5 µg) and ciprofloxacin (5 µg); Macrolide: erythromycin (15 µg); Ansamycin: rifampicin (5 µg); Carbapenems: imipenem (10 µg) and meropenem (10 µg); Cephalosporins: cephalothin (30 µg), ceftriaxone (30 µg) and cefotaxime (30 µg) and Folate pathway inhibitor: trimethoprim-sulfamethoxazole (25 µg). The testing procedure was carried out according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014). The multiple antimicrobial resistance (MAR) index was calculated according to the formula described by Krumperman (1983).

Screening of virulence related and antimicrobial resistance genes

The presence of eleven virulence related genes, 6 antimicrobial resistance genes and integrons as detailed in Table 1 were examined by conventional PCR method. Details of primer pairs and conditions are also given in Table 1. Each PCR reaction mixture was 30 µl in final volume comprising 0.3 µl AmpOne Taq DNA polymerase (GeneAll, Seoul, Korea), 3 µl dNTP mix, 3 µl 10×Taq polymerase buffer, 1 µl of each forward and reverse primers, 1 µl of template DNA and 21.7 µl of PCR water. PCR cycling conditions for each reaction included: Initial denaturation at 94ºC for 2 min followed by a total of 35 cycles, each cycle comprising denaturation at 94ºC for 30s, annealing at respective temperature for 50s and extension at 72ºC for 1 min. Amplified PCR products were visualised by electrophoresis on 1.5% (w/v) agarose gels.

Results and discussion

Abalone samples were collected during the period of 7 months from February to September, when the food borne *Vibrio* outbreaks was frequently reported in South Korea as reported in Park *et al.* (2018). *Vibrio* spp were isolated from 28 samples (23%) out of the 120 abalone samples tested. From the 28 samples, a total of 32 *Vibrio* spp. comprising *V. alginolyticus* (*n*=15), *V. diabolicus* (*n*=14), *V. antiquarius* (*n*=2) and *V. parahaemolyticus* (*n*=1) species were identified by sequencing the *gyrB* housekeeping gene. *V. alginolyticus* and *V. diabolicus* were the dominant species. Pang (2006) reported *V. alginolyticus* as one of the dominant bacteria species in abalone aquaculture systems.

Neighbor-joining phylogenetic tree was constructed using the *gyrB* gene sequence data and the isolates were sorted into two major clades (Fig. 1). *V. parahaemolyticus* strain belonged to the first major clade with *V. parahaemolyticus* reference strain that has been deposited in NCBI database under reference number GQ426112 as food and waterborne pathogen. In the second major clade, two *V. antiquarius* isolates grouped in the same subclade along with *V. alginolyticus* isolates and the reference *V. antiquarius* strain. Also, *V. diabolicus* and *V. alginolyticus* isolates exhibited a scattered distribution pattern in the second major clade. *V. alginolyticus*, *V. diabolicus* and *V .antiquarius* isolated from Pacific oysters (*Crassostrea gigas*) have demonstrated similar phylogenetic distribution in the study by Turner *et al.* (2018).

All *Vibrio* spp. isolated in this study demonstrated DNase, lipase, phospholipase and amylase production (Table 2). DNase involves pathogenicity by the degradation of neutrophil extracellular traps and innate immune structures composed of chromatin and granule proteins (Buchanan *et al.,* 2006). In line with the current study, Dahanayake *et al.* (2018) reported DNase production among 100% of *Vibrio* spp. isolated from oyster (*Crassostrea gigas*) in Korea. Lipolytic activity may be important for the dissemination and/or nutrition of bacteria in infections. *In vitro* studies have shown that lipases influenced the actions of various immune cells, such as the chemotaxis of neutrophils (Stehr *et al.,* 2003). In this study, protease and gelatinase were observed in 57 and 94% of the isolates, respectively. Gelatinase enzymes were reported to hydrolyse collagen, haemoglobin and some other peptides. Protease secretion ensures establishment of the pathogen in contact with the host cell (Frees *et al.,* 2013). These extracellular secretions of *Vibrio* spp. have been studied and reported as virulent related exoensymes due to their involvement in virulence activities.

Haemolysis (α and β) is considered as one of the major virulence activity which facilitate the intercellular growth of pathogenic bacteria (Jia *et al.,* 2010). α-haemolysis can reduce the host cell resistance by inhibition of phagocytosis (Cavalieri and Snyder, 1982). In our study, we observed a high rate of β-haemolysis activity and a low rate of α-haemolysis. β-haemolysis plays a role in bacterial infection by releasing iron from red blood cells and making it available for bacterial growth (Janda and Abbott, 1993). However, α-haemolysis causes only an incomplete degradation of haemoglobin (Zhang and Austin, 2005).

Slime production (97%) was observed among all strains except *V. parahaemolyticus* strain. Slime is an important factor required for biofilm production which can increase colonisation and contamination by pathogenic microflora (Abdallah *et al.,* 2009). Snoussi *et al.* (2008) observed strong adhesive properties of slime producing *V. alginolyticus* which can cause contamination easily. Thus, the *Vibrio* spp. isolated from the Pacific abalone can easily colonise and can cause contaminations by sticking to surfaces, which would also help preventing from being washed away.

Virulence related gene profiles of *Vibrio* spp. isolated are presented in Table 2. Among the tested virulence genes, *V. alginolyticus* specific collagenase gene (VAC) was recorded as the most prevalent virulence gene (100%). Furthermore, we detected positive isolates for *tdh* gene among *V. alginolyticus* (6%) and *V. diabolicus* (21%) strains. *tdh* gene is often associated with pathogenicity of *V. parahaemolyticus*. However, Gargouti *et al.* (2015) reported the presence of *tdh* gene in *V. alginolyticus* strains isolated from shrimp. *V. alginolyticus* species specific *tlh* gene was detected in 59% of the isolates. Haemolysin production stimulating *hupO* gene was detected in 9% of

Table 1. Oligo sequences and PCR conditions[α] employed in the study

Gene	Target action	Nucleotide sequence 5'-3'	Size (bp)	Annealing temperature (ºC)	Reference
toxR (*V. alginolyticus*)	Intestinal colonisation and toxin production	F: GATTAGGAAGCAACGAAAG R: GCAATCACTTCCACTGGTAAC	658	54	Xie *et al.* (2005)
toxR (*V. parahaemolyticus*)	Intestinal colonisation and toxin production	F: GTCTTCTGACGCAATCGTTG R: ATACGAGTGGTTGCTTCATG	368	56	Kim *et al.* (1999)
tdh	Thermostable direct haemolysin	F: CCACTACCACTCTCATATGC R: GGTACTAAATGGCTGACATC	251	55	Gargouti *et al.* (2015)
trh	TDH-related haemolysin	F: TTGGCTTCGATATTTTCAGTATCT R: CATAACAAACATATGCCCATTTCCG	500	58	Bej *et al.* (1999)
tlh (*V. alginolyticus*)	Thermolabile haemolysin	F: AGCGGATTATGCAGAAGCAC R: GCTACTTTCTAGCATTTTCTC TGC	448	54	Woodring *et al.* (2012)
Collagenase (*VAC*)	Collagenase activity	F: CGAGTACAGTCACTTGAAAGCC R: CACAACAGAACTCGCGTTACC	737	58	Di pinto *et al.* (2005)
ctxAB	Gene encoding cholera toxin	F: GCCGGGTTGTGGGAATGCTCCAAG R: GCCATACTAATTGCGGCAATCGCATG	536	59	Miller *et al.* (1987)
hupO	Hemin-binding outer membrane protein	F: ATTACGCACAACGAGTCGAAC R: ATTGAGATGGTAAACAGCGCC	600	56	Liang *et al.* (2013)
vfh	Extracellular haemolysin	F: GCGCGTCAGTGGTGGTGAAG R: TCGGTCGAACCGCTCTCGCTT	800	61	Liang *et al.* (2013)
Pathogenicity Island (*VPI*)	*V. cholerae* pathogenicity island (VPI)	F: GCAATTTAGGGGCGCGACGT R: CCGCTCTTTCTTGATCTGGTAG	680	52	Sechi *et al.* (2000)
intI1	Class 1 integron integrase	F: CTACCTCTCACTAGTGAGGGGCGG R: GGGCAGCAGCGAAGTCGAGGC	485	58	Diaz *et al*. (2006)
Gene cassette	-	5'-CS: GGCATCCAAGCAGCAAG 3'-CS: AAGCAGACTTGACCTGA	Variable	56	Lee *et al*. (2008)

Table 2. Virulence associated genes and phenotypic pathogenicity profile of *Vibrio* spp. isolated from live Pacific abalone

Species	Virulence genes[a]											No. of positive isolates for virulence test								
	VP specific		VA specific					VF specific												
	toxR	*tlh*	*toxR*	*tlh*	*tdh*	*trh*	*VAC*	*hupO*	*vfh*	*VPI*	*ctxAB*	DNase	Protease	Gelatinase	Lipase	Phospolipase	Amylase	Slime	α-Haemolysis	β-Haemolysis
V. parahaemolyticus (n=1)	1	1	1	1	-	-	1	-	-	1	-	1	1	1	1	1	1	0	0	1
V. alginolyticus (n= 15)	-	-	14	9	1	-	15	3	-	7	2	15	7	14	15	15	15	15	2	13
V. diabolicus (n= 14)	-	-	3	8	3	-	14	-	-	5	-	14	7	13	14	14	14	14	5	9
V. antiquarius (n=2)	-	-	-	1	-	-	2	-	-	-	1	2	2	2	2	2	2	2	0	2
Total (%) (n = 32)	1 (3%)	1 (3%)	18 (56%)	19 (59%)	4 (12%)	-	32 (100%)	3 (9%)	-	13 (41%)	3 (9%)	32 (100%)	17 (53%)	30 (94%)	32 (100%)	32 (100%)	32 (100%)	31 (97%)	7 (22%)	25 (78%)

a VP = *V. parahaemolyticus,* VA = *V. alginolyticus,* VF = *V. fluvialis*

the isolates. Moreover, 94% of *V. alginolyticus* isolates, 21% of *V. diabolicus* and *V. parahaemolyticus* isolates recorded the presence of *V. alginolyticus* species-specific *toxR* gene. Distribution of the virulence gene in this study was not found to follow the species specific pattern. Xie *et al.* (2005) reported the presence of homologous genes in different *Vibrio* spp. However, the presence of the most important virulence genes in vibrio pathogenicity

Fig. 1. Neighbor joining phylogenetic tree based on *gyrB* gene sequences showing the relationship between *Vibrio* spp. isolated from Pacific abalone and and reference sequences from the GenBank database. VA= *V. alginolyticus*, VD= *V. diabolicus*, VP= *V. parahaemolyticus*, VN= *V. antiquarius*

discloses the risk of infection of *Vibrio* spp. isolated from the Pacific abalone.

The gene *ctxAB*, encoding cholera toxin (CT) and the gene representing *V. cholerae* pathogenicity island (*VPI*) are associated with the epidemic strains of *V. cholerae* (Waldor and Mekalanos, 1996). Xie *et al.* (2005) reported the presence of *V. cholerae* originated virulence determinants among *V. parahaemolyticus* and their close genetic relatives. In agreement with this statement, we detected *VPI* (41%) and *ctxAB* (9%) positive isolates among all species. Though these genes are often linked with the virulence of *V. cholerae,* there could be a specific virulence mechanism in *V. alginolyticus* which is activated with the presence of these genes (Ren *et al.,* 2013).

Antimicrobial resistance patterns and the MAR indices recorded from disc diffusion assay results are shown in Table 3. All *Vibrio* spp. isolates in this study were identified as ampicillin resistant (Fig. 2). First generation antimicrobials including ampicillin have been extensively used in aquaculture leading to occurrence of ampicillin resistant *Vibrio* spp. in aquatic environment (Sudha *et al.,* 2014). Similar to our study, Dahanayake *et al.* (2018) detected antimicrobial resistant *Vibrio* spp.

from live oysters marketed in Korea and the majority of them were reported as ampicillin resistant. In this study, 66% of isolates showed multidrug resistance by demonstrating resistance against four or more antimicrobial agents. Highest MAR index value of our study was scored as 0.3 by a *V. alginolyticus* (VA13) isolate. Bacteria having MAR index ≥0.2 originate from a high-risk source of contamination where several antimicrobials are used (Paul *et al.,* 1997).

Majority of the *Vibrio* isolates in this study were resistant against β-lactam antimicrobials (ampicillin, piperacillin and cephalothin). The production of extended spectrum β-lactamases (ESBLs) is described as the major reason of being resistant to β-lactam antimicrobials (Shaikh *et al.,* 2015). In the present study, *blaCTX* (85%) gene was recorded as the most prevalent gene coding for β-lactamase and *blaSHV* (10%) as well as *blaTEM* (10%) genes were also recorded. These have been identified as emerging β-lactamase producing genes (Overdevest *et al.,* 2011).

strAB and *aphA-IAB* genes regulate streptomycin and kanamycin resistance mechanisms, respectively (Frana *et al.,* 2001; Bush and Fisher, 2011). Although we detected 22% of the isolates harbouring α-*IAB* gene, only one isolate (3%) showed kanamycin resistance in the disc diffusion test. Also, the number of isolates that

Table 3. Antimicrobial susceptibility, MAR index values and antimicrobial resistance genes of *Vibrio* spp. isolated from live Pacific abalone

Species	Isolate	Resisted antimicrobials [a]	Antimicrobial resistance gene	Class 1 integron and gene cassette	MAR index
V. parahaemolyticus	VP1	AMP, COL, CPL, PRL	*blaSHV*	-	0.2
V. alginolyticus	VA1	AMP, COL, PRL	*blaCTX*	-	0.15
	VA2	AMP, COL, CPL, PRL	*blaCTX*	-	0.2
	VA3	AMP, COL, CPL	*blaCTX*	-	0.15
	VA4	AMP, CPL, COL	-	*Intl 1*, *qacE2*	0.15
	VA5	AMP, STR, CPL, PRL, COL	*blaCTX, blaTEM, aphA-IAB*	-	0.25
	VA6	AMP, COL, CPL, PRL	*blaCTX*	-	0.2
	VA7	AMP, COL, CPL, PRL	*blaCTX,strAB*	-	0.2
	VA8	AMP, PRL, COL, CPL	-	*Intl 1*, *qacE2*	0.2
	VA9	AMP, CPL, COL	*blaCTX*	-	0.15
	VA10	AMP, STR, CPL, COL, PRL	*blaCTX, strAB*	-	0.25
	VA11	AMP, CPL, COL	*blaCTX*	*Intl 1*, *qacE2*	0.15
	VA12	AMP, COL, PRL	*aphA-IAB*	*Intl 1*, *qacE2*	0.15
	VA13	AMP, CPL, COL, PRL, STR, RD	*blaCTX*	-	0.3
	VA14	AMP, COL, PRL, CPL	*blaCTX*	*Intl 1*, *qacE2*	0.2
	VA15	AMP, COL, PRL, CPL	*blaCTX, blaSHV*	*Intl 1*, *qacE2*	0.2
V. diabolicus	VD1	AMP, COL	*blaCTX*	-	0.1
	VD2	AMP, CPL, COL, PRL	*blaCTX*	-	0.2
	VD3	AMP, STR, CPL, COL, KAN	*blaCTX, aphA-IAB*	-	0.25
	VD4	AMP, CPL, COL	*blaCTX*	-	0.15
	VD5	AMP, COL, PRL, STR	*blaCTX, strAB*	-	0.2
	VD6	AMP, CPL, COL, RD	*blaCTX, blaTEM*	-	0.2
	VD7	AMP, STR, COL, RD, CPL	-	-	0.25
	VD8	AMP, COL, CPL	*blaCTX*	-	0.15
	VD9	AMP, CPL, COL	*blaCTX, aphA-IAB*	-	0.15
	VD10	AMP, CPL, COL, STR	*blaCTX, blaTEM, strAB, aphA-IAB*	-	0.2
	VD11	AMP, CPL, COL, PRL	*blaCTX*	-	0.2
	VD12	AMP, CPL, COL, CRO	*blaCTX*	-	0.2
	VD13	AMP, STR, ERY, RD, CPL	*blaCTX, aphA-IAB*	-	0.25
	VD14	AMP, CPL, PRL, RD	*blaCTX, aphA-IAB*	-	0.2
V. antiquarius	VA1	AMP, STR, CPL, PRL	*blaCTX, blaSHV*	-	0.2
	VA2	AMP, CPL, COL, RD, PRL	*blaCTX*	-	0.25

[a] Antibiotics: AMP = ampicillin, PRL = piperacillin, COL = colistin, STR = streptomycin, KAN = kanamycin, ERY = erythromycin, IMI = imipenem, CPL = cephalothin, CRO = ceftriaxone, OT = oxytetracycline, RD = rifampicin

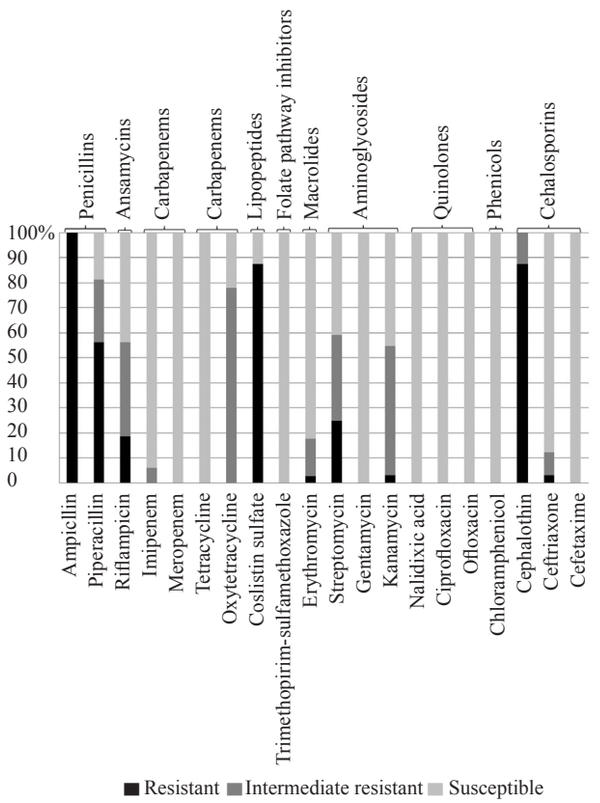

Fig. 2. Antimicrobial susceptibility profile of *Vibrio* spp. isolated from live Pacific abalone. (= Resistant, = Intermediate resistant, = Susceptible)

showed streptomycin resistance in the disc diffusion test was higher than the number of isolates that showed *strAB* gene in molecular analysis. Similarly, Randall *et al.* (2004) detected different ratios between phenotypic and genotypic expressions of antimicrobial resistance characteristics.

Integrons that contain a site specific recombination system called gene cassettes are considered as the elements that can facilitate the horizontal transfer of antimicrobial resistance genes among bacteria (Stokes and Gillings, 2011). Class 1 integron-related integrase gene, *intI1* has long evolution history initiated from the environmental contaminants and this gene aid to accumulate antimicrobial resistance determinants in the bacterial genome (Bahl *et al.,* 2006; Davies 2007). The *qacE2* gene cassette is an efflux pump for the cationic compounds which causes resistance to disinfectants (Ghaly *et al.,* 2017). Moreover, *qac* genes in class 1 integrons are known as the genetic marker derived from clinical ancestors (Gillings *et al.,* 2009). Hence, six *V. alginolyticus* isolates that were identified as harbouring *qacE2* gene cassette and *intI1* gene in this study could have been derived from the clinical ancestors.

Results of the present study indicate occurrence of pathogenic *Vibrio* spp. in marketed Pacific abalone in South Korea. *V. alginolyticus* and *V. diabolicus* were the most prevalent species among the *Vibrio* isolates and also, we detected virulence and antimicrobial resistance properties in higher percentages. *V. diabolicus* and *V. antiquarius* were identified and characterised for the first time in Pacific abalone. The presence of virulence related determinants as well as the presence of multidrug resistance properties among the abalone borne *Vibrio* isolates is a real concern and warrants ongoing surveillance.

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