

Multi-drug resistant mesophilic aeromonads isolated from marketed scallops (*Patinopecten yessoensis*) harboring resistance genes

Benthotage Chamara Jayasankha De Silva, Sabrina Hossain, Pasan Sepala Dahanayake, Dae-Won Lee, Masimbule Vidanalage Kasun Sameera Wickramanayake, Gang-Joon Heo

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Abstract. Antimicrobial resistance properties of 32 *Aeromonas* strains isolated from fresh scallops (*Patinopecten yessoensis* (Jay)) marketed in Korea were assessed. All the *Aeromonas* spp., including *A. salmonicida*, were mesophilic and grew very well at 37°C. The isolates were tested for susceptibility to 19 antimicrobials belonging to eight antimicrobial classes. All isolates were multi-drug resistant, which means they were resistant to five or more antimicrobials. Higher resistance rates ($\geq 50\%$) were observed for ampicillin, piperacillin, cephalothin, imipenem, meropenem, trimethoprim-sulfamethoxazole, tetracycline, oxytetracycline, and nalidixic acid while intermediate resistance was also determined. PCR assays revealed the presence of many antimicrobial resistance genes among the isolates in varying combinations. Among them, some isolates harbored higher numbers of resistant genes, e.g., *A. veronii*-V1 (*aac(6')-Ib*, *tetE*, *qnrS*, *IntI1*), *A. salmonicida*-SL10 (*IntI1*, *blaCTX*, *aac(3')-Ib*, *aac(6')-Ib*, *qnrS*), *A. hydrophila*-H13 (*IntI1*, *blaTEM*, *qnrS*, *aac(6')-Ib*, *strA-strB*). However, neither the *blaSHV*, *blaIMP*, *tetB*, *qnrA*, *qnrB*, and *aphAI-IAB* genes nor class1 integrons were detected in any of the isolates. Discrepancies between

phenotypic and genetic resistance traits were observed in some isolates. With respect to outcomes, scallops are proposed as a source of multi-drug resistant *Aeromonas* spp. that harbor antimicrobial resistant genes.

Keywords: *Aeromonas* spp., antimicrobials, multi-drug resistance, resistance genes, scallop

Introduction

The genus *Aeromonas* comprises Gram-negative, rod-shaped, facultatively anaerobic, non-spore-forming bacteria that can be divided into two distinct groups. The non-motile psychrophilic group, growing best at temperatures between 22-28°C, is exemplified by the fish pathogenic *A. salmonicida*. The motile mesophilic group grows optimally between 35-37°C, and the motility of these species is facilitated by single polar flagella. This second, large group is responsible for human clinical infections of many categories like gastroenteritis, wound and soft tissue infections, blood-borne dyscrasias, and some less common infections of muscles, bones, and

B.C.J. De Silva, S. Hossain, P.S. Dahanayake, D.W. Lee, M.V.K.S. Wickramanayake, G.J. Heo 
Veterinary Medical Center and College of Veterinary Medicine,
Chungbuk National University, Cheongju, 28644, Korea
E-mail: gjheo@cbu.ac.kr

respiratory and urogenital tracts (Janda and Abbott 2010, Parker and Shaw 2011, Percival and Williams 2014). Of the approximately 30 recognized *Aeromonas* spp., *A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*, and *A. dhakensis* are most frequently detected in human infections (Janda and Abbott 2010, Teunis and Figueras 2016).

Aeromonas spp. are well-known to develop and disseminate antibiotic resistance. They show high resistance rates against penicillins and first-generation cephalosporins thanks to chromosome-encoded, inducible β -lactamases (Janda and Abbott 2010). Chromosomal mutations and plasmid-mediated genes cause quinolone resistance in *Aeromonas* (Shakir et al. 2012, Chenia 2016). However, aminoglycoside resistance in *Aeromonas* remains rare, and genetic elements are seldom reported (Aravena-Román et al. 2012, Sedaros 2015). Additionally, aeromonads can contain integrons bearing tetracycline, aminoglycoside, chloramphenicol, and trimethoprim resistance genes (Chang et al. 2007, Kadlec et al. 2011).

Molluscan shellfish are considered delicacies around the world. In Korea, mollusks are the second most cultured seafood, and the scallop *Patinopecten yessoensis* (Jay) is one of the species that is consumed throughout the country (FAO Fisheries & Aquaculture 2018). Koreans enjoy scallops in hot stews and in smoked, boiled, grilled, stir-fried, and raw preparations. The antimicrobial resistance of bacteria associated with food animals is an important public health concern (Marshall and Levy 2011). Although the direct application of antibiotics is less common in bivalve molluscan culture, studies report bacteria such as *Aeromonas* and *Vibrio* isolated from bivalves with multi-drug resistance (Evangelista-Barreto et al. 2006, Lopatek et al. 2015, Kang et al. 2017, Odeyemi and Ahmad 2017). However, there are no reports of genetic characterizations of antimicrobial-resistant aeromonads isolated from scallops. Therefore, this study sought to investigate the phenotypic antimicrobial resistance and the prevalence of antimicrobial resistance genes among *Aeromonas* spp. isolated from marketed fresh scallops in Korea.

Materials and Methods

Sampling scallops, isolation, and biochemical identification of mesophilic *Aeromonas* spp.

A total of 105 marketed fresh scallops were purchased randomly from several retail markets in Korea, shipped to the laboratory in sterile plastic bags, and processed immediately within an hour of receipt. Each scallop was shucked and the meat was scraped out aseptically. A homogenate composite of each scallop meat sample was prepared by blending it in a sterile blender jar and 1 g of the composite was enriched in alkaline peptone water (APW) at 37°C. Plates of *Aeromonas* agar (MB Cell, LA, CA) with ampicillin supplement (5 mg L⁻¹) were prepared and the enrichment in APW was streaked on the medium. Inoculated plates were incubated at 37°C for 24-48 h. The colonies that produced a typical green color were then biochemically identified following a set of biochemical tests (Abbott et al. 2003).

Genetic identification of *Aeromonas* spp.

All biochemically identified isolates were enriched again overnight in APW at 37°C, cells were separated by centrifugation (7500 rpm for 10 min), and genomic DNA was extracted using Exgene Cell SV kits (GeneAll, Seoul, Korea). PCR was performed for each DNA sample to amplify approximately 1100 bp fragment of the *gyrB* gene using the primers *gyrB*3F and *gyrB*14R in accordance with a previous report (Yáñez et al. 2003). Purified PCR products were submitted for sequencing at Cosmogenetech Co. Ltd, Daejeon, Korea. The isolates were identified to the species level by the BLAST compatibility of the *gyrB* sequences in the NCBI public database. A total of 32 *Aeromonas* spp. isolates including *A. hydrophila* (n = 13), *A. salmonicida* (n = 11), *A. media* (n = 3), *A. caviae* (n = 2), *A. veronii* (n = 2) and *A. enteropelogenes* (n = 1) were identified (see

supplementary Table S1 for NCBI accession numbers), and further experiments were performed on them.

Antimicrobial disc-diffusion test

The strains were cultured overnight in tryptic soy agar at 37°C prior to testing. Each bacterial inoculum was prepared in concentrations equivalent to 0.5 McFarland units (1.5×10^8 CFU ml⁻¹) in sterile saline. Bacterial inocula were spread on Mueller-Hinton agar (MHA) (MBCcell, LA, CA) using sterile cotton swabs in order to obtain evenly inoculated cultures. Under aseptic conditions, antimicrobial discs (Oxoid, Hampshire, UK) of a total of 19 antimicrobials belonging to eight antimicrobial classes were placed on the MHA plates and incubated for 18 h at 37°C. The inhibition zone diameters were measured to determine susceptibility patterns. *Escherichia coli* ATCC 25922 was employed as the quality control strain. Susceptibility testing was conducted in triplicate according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2014).

The antimicrobials tested were as follows: Penicillins – ampicillin (10 µg), piperacillin (100 µg); Tetracyclines – tetracycline (30 µg), oxytetracycline (30 µg), doxycycline (30 µg); Phenicol – chloramphenicol (30 µg); Aminoglycosides – streptomycin (10 µg), gentamycin (10 µg), kanamycin (30 µg), amikacin (30 µg); Quinolones – nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg); Carbapenems – imipenem (10 µg), meropenem (10 µg); Folate pathway inhibitors – trimethoprim-sulfamethoxazole (25 µg); Cephalosporins – cefotaxime (30 µg), cephalothin (30 µg), ceftriaxone (30 µg).

PCR screening of antimicrobial resistance genes and integrons

A total of 16 antimicrobial resistance genes and class 1 integron variable regions were amplified by PCR using the primers and conditions presented in

Table 1. The PCR mixture was 20 µL of the total volume containing 2 µL of 10 × Taq reaction buffer, 2 µL of dNTP mixture, 0.2 µL of AmpOne Taq DNA polymerase (GeneAll, Seoul, Korea), 1 µL of each forward and reverse primer, 1 µL of template DNA and 12.8 µL of PCR water. PCR products were sent through 1.5% (w/v) agarose gel electrophoresis and visualized in a UV illuminator to capture the gel images.

Results and Discussion

The current study assessed the antimicrobial susceptibility patterns and the prevalence of antimicrobial resistance genes of 32 mesophilic *Aeromonas* strains isolated from marketed scallops. As shown in Table 2, all *Aeromonas* isolates were multi-drug resistant to five or more antimicrobials. Additionally, all the isolates exhibited intermediate resistance to one or more antimicrobials and positive bands for at least one resistance gene in PCR. The overall susceptibility patterns of antimicrobials are shown in Figure 1. Figure 2 illustrates the PCR gel images of antimicrobial resistance genotypes of three representative *Aeromonas* isolates that harbored the highest numbers of resistance genes.

All the isolates (100%) were resistant to ampicillin, and the piperacillin resistance rate was 94%. This has been observed in *Aeromonas* spp. isolated from seafood such as shrimp, fish, and bivalves (Odeyemi and Ahmad 2017, De Silva et al. 2018, Ramadan et al. 2018). In the cephalosporin group, 100% resistance was observed for cephalothin, but the resistance rates for ceftriaxone and cefotaxime were 16% and 9%, respectively. Among extended-spectrum β-lactamase (ESBL) genes, *blaCTX* was detected in one *A. caviae* isolate and one *A. salmonicida* isolate, whereas only one *A. hydrophila* isolate was positive for the *blaTEM* gene. These β-lactamases confer resistance to penicillins and some cephalosporins (Walsh et al. 1997); however, the prevalence of these genes in this study was less. ESBL genes have been detected previously in

Table 1Oligonucleotide primers used to amplify antimicrobial resistance genes and integrons of *Aeromonas* spp.

Targeted gene		Sequence (5'-3')	Annealing temperature (°C)	Amplicon size (bp)	Reference		
Extended-spectrum β -lactamase	<i>bla_{TEM}</i>	F	CATTTCCGTGTGCGCCCTTATTC	58	1080	(Dallenne et al. 2010)	
		R	CGTTCATCCATAGTTGCCTGAC				
	<i>bla_{SHV}</i>	F	AGCCGCTTGAGCAAATTAAC	58	795	(Dallenne et al. 2010)	
		R	ATCCCGCAGATAAATCACCAC				
	<i>bla_{CTX-M}</i>	F	CGCTTTGCGATGTGCAG	52	550	(Dallenne et al. 2010)	
		R	ACCGCGATATCGTTGGT				
	<i>bla_{IMP}</i>	F	GAATAGAGTGGATTAATTCTC	55	232	(Henriques et al. 2006)	
		R	GGTTAAYAAAACAACCACC				
	Tetracycline resistance	<i>tetA</i>	F	GTAATTCTGAGCACTGTGCG	62	1000	(Akinbowale et al. 2007)
			R	CTGCCTGGACAACATTGCTT			
		<i>tetB</i>	F	CTCAGTATCCAAGCCTTTG	58	400	(Akinbowale et al. 2007)
			R	CTAAGCACTTGTCTCCTGTT			
<i>tetE</i>		F	GTGATGATGGCACTGGTCAT	62	1100	(Akinbowale et al. 2007)	
		R	CTCTGCTGTACATCGCTCTT				
Plasmid-mediated quinolone resistance	<i>qnrA</i>	F	AGAGGATTTCTCACGCCAGG	56	580	(Cattoir et al. 2007)	
		R	TGCCAGGCACAGATCTTGAC				
	<i>qnrB</i>	F	GATCGTAAAAGCCAGAAAGG	53	496	(Cattoir et al. 2007)	
		R	ACGATGCCTGGTAGTTGTCC				
	<i>qnrS</i>	F	GCAAGTTCATTGAACAGGGT	56	428	(Cattoir et al. 2007)	
		R	TCTAAACCGTCGAGTTCGGCG				
Aminoglycoside resistance	<i>strA-strB</i>	F	TATCTGCGATTGGACCCTCTG	55	538	(Sunde and Norström 2005)	
		R	CATTGCTCATCATTGATCGGCT				
	<i>aphAI-IAB</i>	F	AAACGTCTTGCTCGA GGC	53	500	(Frana et al. 2001)	
		R	CAAACCGTTATTCATTCGTGA				
	<i>aac(3)-IIa</i>	F	ATGGGCATC ATTCGCACA	55	749	(Samadi et al. 2015)	
		R	TCTCGGCTTGAACGAATTGT				
	<i>aac(6)-Ib</i>	F	TTGCGATGCTCTATGAGTGGCTA	55	482	(Park et al. 2006)	
		R	CTCGAATGCCTGGCGTGTTT				
Integrons	<i>intI1</i> (Class 1 integron integrase)	F	CTACCTCTACTAGTGAGGGCGG	58	845	(Diaz et al. 2006)	
		R	GGGCAGCAGCGAAGTCGAGGC				
	Class 1 integron	5'-CS	GGCATCCAAGCAGCAAG	56	variable	(Lee et al. 2008)	
	3'-CS	AAGCAGACTTGACCTGA					

Aeromonas spp. isolated from marketed fish and other aquatic animals (Deng et al. 2016, Ramadan et al. 2018).

Half of the isolates (50%) were resistant to tetracycline and oxytetracycline, but only 13% of the isolates were resistant to doxycycline, the intermediate resistance rate to which was 28%. However, the *tetE* gene was detected in two isolates and *tetA* in only one

isolate. *Aeromonas* spp. from shrimp and farm-raised fish were positive for *tetA*, *tetE*, and other *tet* genes (Nawaz et al. 2006, De Silva et al. 2018). Six classes of these *tet* genes such as *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tet31* have been described in *Aeromonas* spp. encoding specific active efflux mechanisms conferring resistance to tetracyclines (Agersø et al. 2007).

Table 2
Antimicrobial resistance properties of *Aeromonas* spp. strains isolated from scallops

Isolate ^a	Antimicrobial resistance phenotype ^b		
	Intermediately resistant	Resistant	Antimicrobial resistance genes
H1	DO, STR, KAN, AMK, CIP, OFL	AMP, PRL, TET, OT, NAL, IMI, MRP, KF	<i>qnrS</i>
H2	STR, KAN, CIP	AMP, PRL, TET, OT, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H3	DO, STR, KAN, AMK, CIP	AMP, PRL, TET, OT, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H4	STR, KAN, CIP, OFL	AMP, PRL, TET, OT, GEN, NAL, IMI, MRP, SXT, KF	<i>qnrS</i>
H5	DO, CHL, GEN, KAN, AMK, MRP, CTX	AMP, PRL, TET, OT, STR, NAL, CIP, OFL, IMI, SXT, KF	<i>qnrS</i>
H6	STR, KAN, AMK, CIP, OFL	AMP, PRL, TET, OT, NAL, IMI, MRP, SXT, KF	<i>qnrS</i>
H7	STR, KAN, AMK, CIP	AMP, PRL, TET, OT, DO, CHL, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H8	DO, STR, KAN, AMK, CIP	AMP, PRL, TET, OT, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H9	STR, KAN, AMK, CIP	AMP, PRL, TET, OT, DO, CHL, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H10	DO, STR, KAN, AMK, CIP, OFL	AMP, PRL, TET, OT, CHL, NAL, IMI, MRP, SXT, KF	<i>qnrS</i>
H11	STR, KAN	AMP, PRL, IMI, MRP, KF	<i>qnrS</i>
H12	DO, STR, KAN, CIP	AMP, PRL, TET, OT, NAL, OFL, IMI, MRP, SXT, KF	<i>qnrS</i>
H13	AMK, CIP	AMP, PRL, STR, GEN, KAN, NAL, OFL, IMI, MRP, SXT, KF, CTX	<i>qnrS</i> , <i>aac(6')-Ib</i> , <i>strA-strB</i> , <i>blaTEM</i> , <i>Int11</i>
SL1	KAN, AMK, OFL	AMP, PRL, STR, IMI, MRP, SXT, KF	<i>qnrS</i>
SL2	STR, KAN, AMK, CIP, OFL	AMP, PRL, TET, OT, DO, IMI, SXT, KF	<i>tetA</i> , <i>qnrS</i>
SL3	STR, KAN, AMK, CIP, OFL	AMP, PRL, NAL, IMI, MRP, SXT, KF	<i>qnrS</i>
SL4	KAN	AMP, PRL, STR, AMK, IMI, MRP, SXT, KF	<i>qnrS</i>
SL5	DO, STR, KAN, AMK, CIP, OFL, SXT	AMP, PRL, TET, OT, NAL, IMI, MER, KF	<i>qnrS</i>
SL6	DO, STR, KAN, AMK, CIP, MRP	AMP, PRL, NAL, OFL, IMI, SXT, KF, CTX	<i>qnrS</i>
SL7	STR, KAN, AMK, CRO	AMP, PRL, TET, OT, IMI, MRP, SXT, KF	<i>tetE</i> , <i>qnrS</i>
SL8	OT, STR, KAN, AMK, CIP, OFL, CRO, CTX	AMP, PRL, NAL, IMI, MER, SXT, KF	<i>qnrS</i>
SL9	STR, GEN, KAN, AMK	AMP, PRL, IMI, MRP, SXT, KF	<i>qnrS</i>
SL10	OFL	AMP, PRL, TET, OT, STR, KAN, IMI, MER, SXT, KF	<i>qnrS</i> , <i>aac(6')-Ib</i> , <i>aac(3)-Ib</i> , <i>blaCTX</i> , <i>Int11</i>
SL11	AMK	AMP, PRL, DO, STR, KAN, NAL, IMI, MER, KF	<i>qnrS</i>
M1	KAN, AMK, CIP, OFL, CTX, CRO	AMP, PRL, STR, NAL, IMI, SXT, KF	
M2	KAN, AMK, CIP, OFL, MRP	AMP, PRL, STR, NAL, IMI, SXT, KF, CRO	
M3	GEN, KAN, CIP, MRP, CTX	AMP, PRL, STR, AMK, NAL, OFL, IMI, SXT, KF, CRO	
C1	KAN, AMK, IMI, MRP	AMP, PRL, STR, SXT, KF	<i>Int11</i>
C2	STR, KAN, AMK, CIP, OFL, CTX	AMP, PRL, IMI, MRP, SXT, KF, CRO	<i>blaCTX</i>
V1	PRL, GEN, CIP, OFL	AMP, TET, OT, STR, KAN, NAL, SXT, KF	<i>tetE</i> , <i>qnrS</i> , <i>aac(6')-Ib</i> , <i>Int11</i>
V2	KAN, AMK	AMP, PRL, STR, IMI, MRP, KF, CTX, CRO	
E1	PRL, DO, NAL, CIP, OFL, IMI	AMP, STR, KAN, AMK, SXT, KF	

^aIsolates; H - *Aeromonas hydrophila*, SL - *A. salmonicida*, M - *A. media*, C - *A. caviae*, V - *A. veronii*, E - *A. enteropelogenes*

^bAntimicrobials; AMP=ampicillin, PRL=piperacillin, TET=tetracycline, OT=oxytetracycline, DO=doxycycline, STR=streptomycin, GEN=gentamycin, KAN=kanamycin, AMK=amikacin, CHL=chloramphenicol, NAL=nalidixic acid, CIP=ciprofloxacin, OFL=ofloxacin, IMI=imipenem, MRP=meropenem, SXT=trimethoprim-sulfamethoxazole, KF=cephalothin, CRO=ceftriaxone and CTX=cefotaxime.

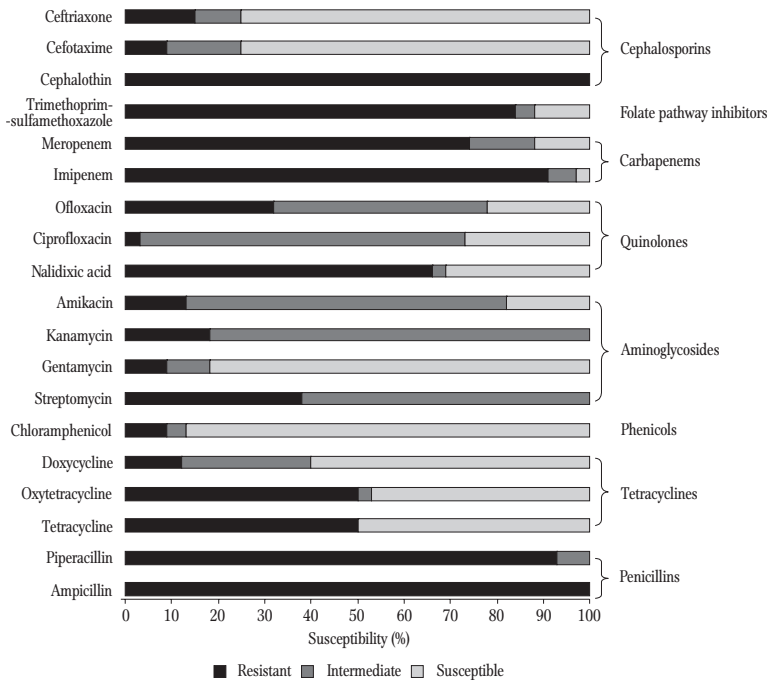


Figure 1. Susceptibility patterns of antimicrobials among *Aeromonas* spp. isolated from scallops.

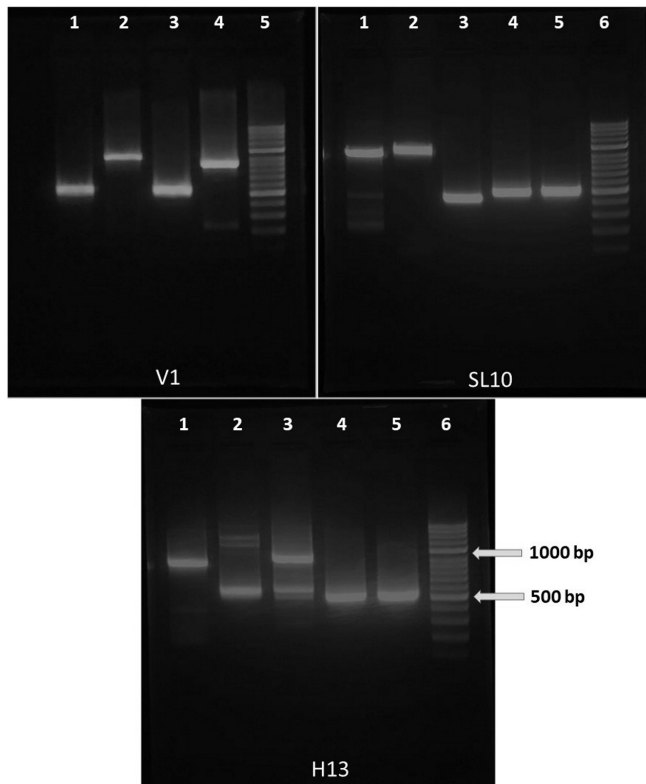


Figure 2. PCR gel images of three representative *Aeromonas* strains showing positive bands for resistance genes. V1 – Lanes 1-5; *aac(6')-Ib*, *tetE*, *qnrS*, *IntI1*, 100 bp DNA ladder. SL10 – Lanes 1-6; *IntI1*, *blaCTX*, *aac(3')-Ib*, *aac(6')-Ib*, *qnrS*, 100 bp DNA ladder. H13 – Lanes 1-6; *IntI1*, *blaTEM*, *qnrS*, *aac(6')-Ib*, *strA-strB*, 100 bp DNA ladder.

In the case of aminoglycosides, the resistance rate of streptomycin was 38% whereas the resistance rates of gentamycin, kanamycin, and amikacin were 9, 19, and 13%, respectively. All isolates were either resistant or intermediately resistant to streptomycin and kanamycin, and none of them were susceptible. Still, the prevalence of aminoglycoside-resistant genes was lower. One *A. hydrophila* isolate (H13) was positive for both *aac(6')-Ib* and *strA-strB* genes, and one *A. salmonicida* isolate (SL10) was positive for both *aac(6')-Ib* and *aac(3')-Ib* genes. Despite the fact that the *strA-strB* gene is responsible for streptomycin resistance, only one of the 12 streptomycin resistant isolates was one positive for this gene. Similarly, *Aeromonas* spp. isolated from fish and shrimp had no amplicons for the *strA-strB* gene, although some isolates were streptomycin resistant (Deng et al. 2014), and *aac(6')-Ib* gene coding for the aminoglycoside modifying enzyme was also detected in shrimp-borne aeromonads (De Silva et al. 2018).

With respect to quinolones, 66% of the isolates were resistant to nalidixic acid, while 31% and 3% of the isolates were resistant to ofloxacin and ciprofloxacin, but there were considerable intermediate resistance rates to ciprofloxacin and ofloxacin. Aeromonads are reported to be almost universally susceptible to fluoroquinolones, while lower resistance rates have been reported (Janda and Abbott 2010). However, the majority of the *Aeromonas* spp. isolated from mangrove oysters were resistant to ciprofloxacin and nalidixic acid (Evangelista-Barreto et al. 2006). Of the quinolone resistance genes, only *qnrS* was detected, and it was the most prevalent gene that was harbored by 25 (78%)

isolates. Interestingly, all *A. hydrophila* and *A. salmonicida* isolates were positive for the gene. Nevertheless, there were five isolates that were either not resistant or intermediately resistant to the tested quinolones, but they were positive for the *qnr* gene.

Moreover, carbapenem resistance was high in this study. Resistance to imipenem and meropenem was observed in 91% and 75% of the isolates, respectively, but, the *blaIMP* gene was not detected. This gene has been studied previously, and a clinical isolate was positive for the gene, but the environmental isolates were not (Neuwirth et al. 2007, Balsalobre et al. 2009). Among the tested antimicrobials, chloramphenicol had the lowest resistance rate and was the most effective antimicrobial. Only three (9%) and one (3%) isolates were resistant and intermediately resistant, respectively. In previous reports, the lowest resistance rates for chloramphenicol were observed among fish and seafood-borne *A. hydrophila* (Stratev and Odeyemi 2016). Trimethoprim-sulfamethoxazole resistance was 84% in the present results. However, *Aeromonas* spp. isolated from mussels were 19% resistant to trimethoprim-sulfamethoxazole while 100% of them were resistant to sulfamethoxazole (Ottaviani et al. 2006).

In this study, no PCR amplicon was obtained for class 1 integron gene cassettes. Generally, class 1 integrons can contain genes responsible for resistance to several antibiotics. *Aeromonas* from shrimp harbored *qacE2*, *dfrA1*, *orfC*, *orfD*, *aadB*, *catB3*, *oxa-10*, and *aadA1* genes causing resistance to aminoglycosides, chloramphenicol, and trimethoprim (De Silva et al. 2018). Moreover, class 1 integron gene cassettes have been reported in *Aeromonas* isolated from farmed fish, shrimp, turtles, and amphibians where the predominant gene cassette array was *drfA12-orfF-aadA2* (Deng et al. 2016). Integrons are site-specific recombination systems that can add open reading frames, thus they are considered as one of the most efficient mechanisms for recruiting antimicrobial resistance genes. They are the efficient but simple machinery of adding new genes into bacterial chromosomes, along with the necessary mechanism to ensure their expression (Munita and Arias 2016).

In addition, the *IntI1* gene was observed in four isolates (12.5%), one from each of the species *A. veronii*, *A. caviae*, *A. hydrophila*, and *A. salmonicida*. The high prevalence of this gene is reported in *Aeromonas* and *Salmonella* isolated from seafood (Khan et al. 2009, De Silva et al. 2018). The *IntI1* gene is considered to be a proxy marker that is a response to diverse environmental pressures, and it is also linked to genes conferring resistance to antibiotics and heavy metals. In particular, it can transfer between bacteria by horizontal gene transfer (Gillings et al. 2015).

However, our results revealed an inconsistency between phenotypic resistance and the prevalence of resistance genes. Some isolates showed strong resistance to antimicrobials, but they were not positive for the corresponding resistance genes. This kind of resistance can be the outcome of complex bacterial machinery such as efflux pumps. These systems are capable of extruding toxic compounds out of the bacterial cell that can also result in eliminating antibiotics, thereby causing antimicrobial resistance (Munita and Arias 2016). On the contrary, some isolates possessed resistance genes, but they were not phenotypically resistant to the corresponding antimicrobials. Plasmid-mediated resistance genes can be transferred horizontally from one bacterium to another while they thrive in the same environments. However, these acquired genes could sometimes not be expressed because of internal suppression. There are many known methods of regulating gene expression that can sometimes cause genes to not express (Bervoets and Charlier 2019). In addition, bacterial strains can harbor resistance “pseudogenes,” which are DNA sequences that are homologous to known genes but that have undergone mutations that eliminate their ability to be expressed (Davis et al. 2011).

All the strains tested in this study were mesophilic, including *A. salmonicida*, which is of particular interest since it has been a well-known fish pathogen for decades. It was reported to grow only at temperatures below 25°C until a subspecies of *A. salmonicida* was detected growing efficiently at 37°C (Pavan et al. 2000, Austin and Austin 2016).

Subsequently, knowledge about the mesophilic *A. salmonicida* increased significantly. *A. salmonicida* was previously isolated from seafood such as clams (Rodriguez et al. 2011). More importantly, some cases of human infection by multi-drug resistant and mesophilic *A. salmonicida* have been reported (Tewari et al. 2014, Varshney et al. 2017, Ruppé et al. 2018). Moreover, a recent study investigated the pathogenicity of *A. salmonicida* isolated from two human clinical cases (an acute gastroenteritis patient and a patient with cellulitis of the foot) and from the environment in a mouse model; this study showed that these strains were highly virulent and some of them caused necrotizing fasciitis and tissue damage in mice livers (Vincent et al. 2019). Thus, the occurrence of these multi-drug resistant mesophilic *A. salmonicida* in marketed scallops might be of interest in terms of consumer health.

Conclusion

The outcomes of our study together with previous reports imply that the aeromonads from bivalves, including scallops, can exhibit multi-drug resistance even though they are not treated with antimicrobials during culture or processing. Furthermore, the prevalence of antimicrobial resistance genes, especially the plasmid-mediated genes, indicated the potential dissemination of these genes among different bacteria. Therefore, scallops marketed in Korea are a potential source of multi-drug resistant mesophilic *Aeromonas* spp. harboring antimicrobial resistance genes that could pose a risk of human infection.

Author contributions. B.C.J.D.S. and G.J.H. designed the research; G.J.H. supervised experiments; B.C.J.D.S. performed most of the experiments; S.H. helped in PCR amplification; P.S.D. and D.W.L. helped in sampling and bacterial isolation; M.V.K.S.W. carried out laboratory analysis; B.C.J.D.S. analyzed the data and wrote the manuscript with contributions from M.V.K.S.W. and G.J.H.

The online version of this article offers the following supplementary material: Supplementary file Table S1. NCBI accession numbers of identified *Aeromonas* spp. strains.

ORCID ID

Gang-Joon Heo.  <https://orcid.org/0000-0003-4602-4086>

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