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# Microbial contamination including *Vibrio cholerae* in fishery auction markets in West Sea, South Korea

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## Abstract

**Background:** The monitoring of pathogens of fishery auction markets is important to obtain safe fishery products regarding hygiene and sanitation. In this study, aerobic, coliform, *Escherichia coli*, and *Vibrio cholerae* were monitored in the fishery products and environmental samples obtained from fishery auction markets.

**Methods:** The fishery products (flounder, octopus, skate, rock cod, sea bass, snail, monkfish, flatfish, comb pen shell, corb shell, conger eel, hairtail, croaker, and pilchard) were placed in filter bags, and the environmental samples (samples from the water tanks at the fishery auction markets, seawater from the fishery distribution vehicles, ice from wooden or plastic boxes, and surface samples from wooden and plastic boxes used for fish storage) were collected. Aerobic bacteria, *E. coli*, and coliform in the samples were enumerated on aerobic count plates and *E. coli*/coliform count plates, respectively. For *V. cholerae* O1 and *V. cholerae* non-O1 quantification, most probable number (MPN)-PCR analysis was performed.

**Results:** Aerobic and coliform bacteria were detected in most samples, but *E. coli* was not detected. Wooden boxes were contaminated with high levels of aerobic and coliform bacteria in all seasons (spring, summer, and fall). During fall, *V. cholerae* non-O1 were detected in snails, hairtails, croakers, flatfishes, pilchards, plastic boxes, and water samples.

**Conclusions:** These results indicate an increased prevalence of *V. cholerae* contamination in fishery products in fall, including food contact samples, which can be vehicles for cross-contamination.

**Keywords:** Fish, Food safety, Microbial contamination, Environmental, Detection

## Background

Global fish production increased to 171 million tons in 2016, and the amount of fish consumed has been growing continually (20.5 kg/person/year in 2017) (FAO 2018). A considerably dynamic import and export of fishery products has been evidenced between countries (FAO 2019). More fish and fishery products were consumed in S. Korea in 2016 (59.9 kg/person/year) than meat (56.0 kg/person/year). The degree of self-sufficiency in S. Korea was 67.3% in 2016 (KREI 2017). Fishery products arrive at the auction market directly after harvesting. Sanitation from

collection to distribution is essential for obtaining safe fishery products that are protected from cross-contamination (Ahmed 1991). S. Korea is surrounded by the East, West, and South Sea (Chough et al. 2000). Especially, the West Sea is comprised of mudflats and has high tides and estuary waters (Cho et al. 1999; Koh and Shin 1988). Therefore, fishery products from the West Sea may become cross-contaminated from these environments. In particular, Di et al. (2017) detected *V. cholerae* (0.1%) in the tidal water collected from the southern coast in June and *V. cholerae* (0.5%) in the tidal water in September 2013. Therefore, the microbial contamination of products from the West Sea should be monitored.

Foodborne illness occurring through the consumption of fish (17%) is common, followed by dairy (11%) and chicken (10%) in the USA from 2009 to 2015 (Dewey-Mattia et al.

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2018). *Vibrio* spp. are gram-negative bacilli and major pathogens which present in coastal or estuarine environments (Horseman and Surani 2011; Reidl and Klose 2002). *V. cholerae* is a causative agent for cholera in humans which grows in 0–3% NaCl and relatively low salinity. There was a foodborne outbreak, caused by *V. cholerae* in 2016 through domestic sea water (KCDC 2017). For the case of 2016, raw seafoods (sea bass, sea squirt, abalone, crab, mackerel, flatfish, rockfish, shrimp, sea cucumber, octopus, and squid) were assumed as causative foods for three patients in the outbreak (Kim et al. 2018). The *V. cholerae* O1 isolated from the South Sea seawater and the fecal samples collected from three patients were Ogawa serotype, El Tor biotype, and contained cholera toxin (*ctx*) (KCDC 2017). The O1 serotype of *V. cholerae* is known as exhibiting explosive growth (Maheshwari et al. 2011; Labbé and García 2013). Since 2016, monitoring of *V. cholerae* has been ongoing, and the importance of tracking *V. cholerae* has been emphasized in S. Korea.

The monitoring of fishery auction markets for pathogens is essential for obtaining safe fishery products with regard to hygiene and sanitation. Therefore, the fishery auction markets in the West Sea, S. Korea were monitored in this study. Microbial contamination was evaluated by detecting *V. cholerae* and other hygiene indicator microorganisms in environmental samples from the fishery auction markets and the fishery products harvested in the West Sea.

## Methods

### Sample collection and preparation

Seventy-eight fishery products ( $N = 41$ ) and environmental samples ( $N = 37$ ) were collected at two fishery auction markets in the West Sea, S. Korea from March to September 2017. Of the 78 samples, 29, 24, and 25 were collected in spring (March–April), summer (July–August), and fall (September), respectively. Because of

season and daily circumstances in each market, types of fishery products samples were different between markets (Table 1). The fishery products that were harvested in each season were collected before, during, and after the auction. The environmental samples of the fishery auction markets were collected from water in tanks, seawater in fishery distribution vehicles, ice in wooden or plastic boxes, and from the wooden and plastic boxes used for fish storage. The surfaces ( $10 \times 10 \text{ cm}^2$ ) of the wooden and plastic boxes were swabbed using a swab-sampler (3 M, St. Paul, MN, USA). All samples were transported in an ice cooler to a laboratory. Twenty-five-gram samples were removed from the gills of the fish and the edible portion of the shellfish for microbial analysis.

### Quantification of aerobic, coliform, and *E. coli* bacteria

The fishery product samples were placed aseptically into filter bags (3 M) and 50 mL of 0.1% alkaline peptone water (APW; Becton, Dickinson and Company, Sparks, MD, USA) was added. After shaking 30 times, a 1-mL aliquot of the homogenate was serially diluted with 9 mL APW. The diluents were plated on an Aerobic Count Plate (Petrifilm™; 3 M) and an *E. coli*/Coliform Count Plate (Petrifilm™; 3 M). One milliliter of collected water, seawater, ice, and suspension was taken from swab-samples of wooden and plastic boxes were also diluted, and the diluents were plated on both plates of the environmental samples. All plates were incubated at 35 °C for 24 h. The red aerobic bacteria, blue with gas *E. coli*, and red and blue coliform colonies were manually counted.

### Quantification of *V. cholerae* by MPN-PCR analysis

The suspensions (10, 1, and 0.1 mL) from filter bags contained 25 g or 25 mL samples with 225 mL APW were inoculated in five test tubes containing 10 mL APW to target  $1 \times \text{APW}$  final concentration. All test

**Table 1** Information of the collected samples (fishery products and environmental samples)

Type of samples	Season	Samples	
		Market A	Market B
Fishery products ( $N = 41$ )	Spring (March–April)	Flounder (3), octopus (1), skate (1)	Monkfish (3), flatfish (3), comb pen shell (3)
	Summer (July–August)	Flounder (3), rock cod (3), sea bass (3)	Flounder (1), comb pen shell (1), snail (1), corb shell (1), flatfish (1), rock cod (1), conger eel (1)
	Fall (September)	Snail (3)	Hairtail (2), croaker (2), flatfish (2), pilchard (2)
Environmental samples ( $N = 37$ )	Spring (March–April)	Water (2), seawater (1), wooden box (2), plastic box (2)	Water (3), ice (1), wooden box (2), plastic box (2)
	Summer (July–August)	Water (1), seawater (1), wooden box (1), plastic box (1)	Water (1), ice (1), wooden box (1), plastic box (1)
	Fall (September)	Water (2), ice (1), wooden box (2), plastic box (2)	Water (2), ice (1), wooden box (2), plastic box (2)

*Water* water in water tanks of fishery auction markets, *seawater* seawater in fishery distribution vehicles, *wooden box* surfaces of wooden boxes for fish storage, *plastic box* surfaces of plastic boxes for fish storage, *ice* ice in wooden or plastic boxes

tubes were incubated at 35 °C for 14 h. For PCR analysis, 1 mL aliquots of the cultures were centrifuged at 13,475×g for 2 min, and the supernatants were removed. The pellets were suspended with 0.1 mL distilled water then heated at 100 °C for 10 min. After centrifuging at 13,475×g for 2 min, the supernatants were used as a DNA template. The primers for *V. cholerae* (F: 5'-CAC-CAAGAAGGTGACTTTATTGTG-3', R: 5'-GAACTTATAACCACCCGCG-3'; 586 bp) and *V. cholerae* O1 (F: 5'-CTCAGACGGGATTGTAGGCACG-3', R: 5'-TC TATCTCTGTAGCCCTATTACG-3'; 302 bp) were used (Kim et al. 2015; Rajpara et al. 2013; Nandi et al. 2000). PCR amplification was performed using a FastMix kit (Intron Bio, Gyeonggi, Korea) composed of dNTP, DNA polymerase, reaction buffer, and MgCl<sub>2</sub>. For the amplification of *V. cholerae* and *V. cholerae* O1, the following steps were performed: initial denaturation at 94 °C for 4 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 90 s, extension at 72 °C for 90 s, and final extension at 72 °C for 10 min. The results of amplification were electrophoresed on 1.5% agarose gel for 20 min and visualized using UV light. The number of positive test tube samples per five test tubes that were analyzed by PCR analysis was counted for each dilution, and the most probable number (MPN) of *V. cholerae* and *V. cholerae* O1 was determined using an MPN table (FDA 2010).

## Results and discussions

From March to September 2017, 41 fishery products (March–April, 14; June–July, 16; and September, 11) and 37 environmental samples (March–April, 15; June–July, 8; and September, 14), which were collected from two fishery auction markets located in the West Sea of S. Korea, were analyzed for microbial contamination.

At fishery auction market A, aerobic bacteria were detected in the fishery products ( $1.5 \times 10^2$ – $2.2 \times 10^4$  CFU/g) and the environmental samples ( $2.7 \times 10$ – $2.2 \times 10^6$  CFU/mL or /100 cm<sup>2</sup>), and coliform were detected in the fishery products ( $7.2 \times 10$ – $1.9 \times 10^2$  CFU/g) and the environmental samples ( $6.0 \times 10$ – $1.6 \times 10^2$  CFU/mL or /100 cm<sup>2</sup>) in spring (Table 2). *E. coli* and *V. cholerae* were below the limit of detection in all samples. Aerobic bacteria and coliform were detected in flounder irrespective of the period of the fishery auction (before, during, and after the auction). Of the environmental samples, the wooden boxes for fish storage were the most contaminated with aerobic bacteria ( $1.7 \times 10^4$ – $2.2 \times 10^6$  CFU/100 cm<sup>2</sup>), followed by the plastic fish boxes ( $5.8 \times 10^3$ – $8.0 \times 10^3$  CFU/100 cm<sup>2</sup>), and even seawater in the fishery distribution vehicle ( $4.3 \times 10^3$  CFU/mL) and water in the tanks of the fishery auction market ( $2.7 \times 10$ – $3.0 \times 10$  CFU/mL). In particular, coliform was detected in the wooden ( $6.0 \times 10$ – $1.6 \times 10^2$  CFU/100

cm<sup>2</sup>) and plastic boxes ( $1.5 \times 10^2$  CFU/100 cm<sup>2</sup>) (Table 2). In summer (June–July), aerobic bacteria were detected in the fishery products ( $7.5 \times 10^2$ – $2.0 \times 10^4$  CFU/g) and the environmental samples ( $1.6 \times 10^3$ – $1.3 \times 10^7$  CFU/mL or /100 cm<sup>2</sup>). Coliform was detected in the fishery products ( $1.4 \times 10^2$ – $2.6 \times 10^3$  CFU/g) and the environmental samples ( $5.7 \times 10^2$ – $2.5 \times 10^4$  CFU/mL or /100 cm<sup>2</sup>). However, *E. coli* and *V. cholerae* were below the limit of detection in all samples. In addition, there was no difference between aerobic and coliform bacteria respective to the period of the auction (before, during, and after the auction) and in the fishery products (flounder, rock cod, and sea bass). Among the environmental samples, wooden boxes were the most contaminated with aerobic ( $1.3 \times 10^7$  CFU/100 cm<sup>2</sup>) and coliform bacteria ( $2.5 \times 10^4$  CFU/100 cm<sup>2</sup>), compared to other environmental samples (Table 3). In fall (September), *V. cholerae* non-O1 were detected only in snails (20–5,400 MPN/100 g). Aerobic bacteria were detected in the snails ( $2.6 \times 10$ – $8.4 \times 10^3$  CFU/g) and the environmental samples ( $1.3 \times 10^3$ – $5.8 \times 10^7$  CFU/g). Similar to the results of contamination in spring and summer, the wooden boxes were the most contaminated with aerobic ( $1.8 \times 10^7$ – $5.8 \times 10^7$  CFU/100 cm<sup>2</sup>) and coliform bacteria ( $3.6 \times 10^5$ – $5.4 \times 10^5$  CFU/100 cm<sup>2</sup>) (Table 4).

For fishery auction market B, aerobic bacteria were detected in the majority of fishery products ( $3.9 \times 10$ – $1.3 \times 10^3$  CFU/g) and environmental samples ( $1.5 \times 10^2$ – $5.2 \times 10^7$  CFU/mL or /100 cm<sup>2</sup>) in spring (March–April). Among the environmental samples, aerobic bacteria were at the highest levels in the wooden boxes ( $1.1 \times 10^6$ – $5.2 \times 10^7$  CFU/100 cm<sup>2</sup>), followed by the plastic boxes ( $5.8 \times 10^3$ – $1.1 \times 10^4$  CFU/100 cm<sup>2</sup>), ice in the boxes ( $1.4 \times 10^3$  CFU/mL), and water in the tanks ( $1.5 \times 10^2$ – $1.1 \times 10^3$  CFU/mL) at the fishery auction market. In addition, aerobic bacteria were detected in the monkfish ( $9.0 \times 10$ – $1.2 \times 10^3$  CFU/g) and flatfish ( $3.9 \times 10$ – $1.3 \times 10^3$  CFU/g). Coliform were detected only in the monkfish ( $1.1 \times 10^2$  CFU/g) and the wooden box for fish storage ( $2.5 \times 10^2$  CFU/100 cm<sup>2</sup>). However, *E. coli* and *V. cholerae* were below the limit of detection in all fishery products and environmental samples (Table 2). In summer (June–July), aerobic (fishery products:  $1.4 \times 10^2$ – $1.1 \times 10^6$  CFU/g, environmental samples:  $1.4 \times 10^2$ – $1.3 \times 10^6$  CFU/mL or /100 cm<sup>2</sup>) and coliform bacteria (fishery products:  $4.2 \times 10$ – $1.2 \times 10^5$  CFU/g, environmental samples:  $1.4 \times 10^2$ – $4.0 \times 10^5$  CFU/mL or /100 cm<sup>2</sup>) were detected in higher quantities, compared to the samples in spring. *E. coli* and *V. cholerae* were below the limit of detection (Table 3). In fall (September), aerobic (fishery products:  $2.3 \times 10^4$ – $2.7 \times 10^5$  CFU/g, environmental samples:  $9.8 \times 10^2$ – $1.3 \times 10^8$  CFU/mL or /100 cm<sup>2</sup>) and coliform bacteria (fishery products:  $3.7 \times 10^2$ – $5.2 \times 10^4$  CFU/g, environmental samples:  $3.3 \times 10^2$ – $3.4 \times 10^4$  CFU/mL or /100 cm<sup>2</sup>) were similar to the samples from summer. *E. coli* were below the limit of detection (Table 4). Meanwhile, *V. cholerae* non-

**Table 2** Microbial contaminations (aerobic bacteria, coliform, *Escherichia coli*, and *Vibrio cholerae*) of the fishery products and the environmental samples collected from the fishery auction market A and B in spring (March–April)

Samples	Time	Bacteria									
		Aerobic bacteria (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		Coliform (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>E. coli</i> (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>V. cholerae</i> (MPN/100 g or MPN/100 mL or MPN/100 cm <sup>2</sup> )			
		Market A	Market B	Market A	Market B	Market A	Market B	<i>V. cholerae</i> O1		<i>V. cholerae</i> non-O1	
Fishery products											
Flounder	Before auction	1.0 × 10 <sup>3</sup>	–	7.2 × 10	–	< 15	–	< 20	–	< 20	–
	During auction	1.5 × 10 <sup>2</sup>	–	1.9 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	After auction	6.6 × 10 <sup>3</sup>	–	9.3 × 10	–	< 15	–	< 20	–	< 20	–
Octopus	During auction	2.2 × 10 <sup>4</sup>	–	1.2 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
Skate	During auction	2.2 × 10 <sup>2</sup>	–	< 15	–	< 15	–	< 20	–	< 20	–
	Before auction	–	9.0 × 10	–	< 15	–	< 15	–	< 20	–	< 20
Monkfish	During auction	–	7.5 × 10 <sup>2</sup>	–	1.1 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20
	After auction	–	1.2 × 10 <sup>3</sup>	–	< 15	–	< 15	–	< 20	–	< 20
Flatfish	Before auction	–	5.1 × 10	–	< 15	–	< 15	–	< 20	–	< 20
	During auction	–	3.9 × 10	–	< 15	–	< 15	–	< 20	–	< 20
Comb pen shell	After auction	–	1.3 × 10 <sup>3</sup>	–	< 15	–	< 15	–	< 20	–	< 20
	Before auction	–	< 15	–	< 15	–	< 15	–	< 20	–	< 20
Comb pen shell	During auction	–	< 15	–	< 15	–	< 15	–	< 20	–	< 20
	After auction	–	< 15	–	< 15	–	< 15	–	< 20	–	< 20
Environmental samples											
Water		2.7 × 10	1.5 × 10 <sup>2</sup>	< 15	< 15	< 15	< 15	< 20	< 20	< 20	< 20
		3.0 × 10	6.1 × 10 <sup>2</sup>	< 15	< 15	< 15	< 15	< 20	< 20	< 20	< 20
		–	1.1 × 10 <sup>3</sup>	–	< 15	–	< 15	–	< 20	–	< 20
Seawater		4.3 × 10 <sup>3</sup>	–	< 15	–	< 15	–	< 20	–	< 20	–
Ice		–	1.4 × 10 <sup>3</sup>	–	< 15	–	< 15	–	< 20	–	< 20
Wooden box		2.2 × 10 <sup>6</sup>	1.1 × 10 <sup>6</sup>	1.6 × 10 <sup>2</sup>	< 15	< 15	< 15	< 20	< 20	< 20	< 20
		1.7 × 10 <sup>4</sup>	5.2 × 10 <sup>7</sup>	6.0 × 10	2.5 × 10 <sup>2</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Plastic box		8.0 × 10 <sup>3</sup>	1.1 × 10 <sup>4</sup>	< 15	< 15	< 15	< 15	< 20	< 20	< 20	< 20
		5.8 × 10 <sup>3</sup>	5.8 × 10 <sup>3</sup>	1.5 × 10 <sup>2</sup>	< 15	< 15	< 15	< 20	< 20	< 20	< 20

*Water* water in water tanks of fishery auction markets, *seawater* seawater in fishery distribution vehicles, *ice* ice in wooden or plastic boxes, *wooden box* surfaces of wooden boxes for fish storage, *plastic box* surfaces of plastic boxes for fish storage, – not analyzed

O1 were detected in the hairtail (200 MPN/100 g), croaker (40–110 MPN/100 g), flatfish (20 MPN/100 g), large-eyed herring (45 MPN/100 g), water in the tanks at the fishery auction market (20 MPN/100 mL), and the plastic boxes (20 MPN/100 cm<sup>2</sup>) in fall, which was little bit higher than market A sample numbers for *V. cholerae* presence (Table 4).

The seasonal differences in microbial contamination for fishery products and environmental samples at two fishery auction markets were observed. Aerobic bacteria were detected in most fishery products and environmental samples in all seasons (spring, summer, and fall). Coliform was detected in most samples in fall and summer, followed by spring. *E. coli* and *V. cholerae* O1 were not detected in any sample collected in all seasons (spring, summer, and fall). Meanwhile, *V. cholerae* non-

O1 of the fishery products (20–5,400 MPN/100 g in the snail, hairtail, croaker, flatfish, and pilchard) and the environmental samples (20 MPN/100 mL or /100 cm<sup>2</sup> in water and plastic boxes) were detected only in fall (Tables 2, 3, and 4). *V. cholerae* detected in the fishery products may have been contaminated by seawater, as cross-contamination between these products and environmental samples in fishery auction markets can occur. Aerobic, coliform, and *E. coli* bacteria are hygiene indicator microorganisms for sanitary quality. *Vibrio* spp. are a cause of foodborne illness caused by the consumption of fishery products. *V. cholerae* is a pathogen in marine environments which causes cholera by producing the cholera toxin (CT), a vital virulence factor. *V. cholerae* O1 and O139 are representative serotypes (Halpern and

**Table 3** Microbial contaminations (aerobic bacteria, coliform, *Escherichia coli*, and *Vibrio cholerae*) of the fishery products and the environmental samples collected from the fishery auction market A and B in summer (June–July)

Samples	Time	Bacteria									
		Aerobic bacteria (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		Coliform (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>E. coli</i> (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>V. cholerae</i> (MPN/100 g or MPN/100 mL or MPN/100 cm <sup>2</sup> )			
		Market A	Market B	Market A	Market B	Market A	Market B	<i>V. cholerae</i> O1		<i>V. cholerae</i> non-O1	
						Market A	Market B	Market A	Market B		
Fishery products											
Flounder	Before auction	1.6 × 10 <sup>3</sup>	–	3.2 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	During auction	1.9 × 10 <sup>3</sup>	–	1.9 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	After auction	2.0 × 10 <sup>4</sup>	8.4 × 10 <sup>4</sup>	6.6 × 10 <sup>2</sup>	1.0 × 10 <sup>4</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Rock cod	Before auction	7.5 × 10 <sup>2</sup>	–	1.4 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	During auction	1.3 × 10 <sup>3</sup>	–	1.9 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	After auction	1.0 × 10 <sup>3</sup>	–	3.3 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
Sea bass	Before auction	2.2 × 10 <sup>3</sup>	–	2.6 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	During auction	1.9 × 10 <sup>3</sup>	–	2.0 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
	After auction	9.9 × 10 <sup>3</sup>	–	2.6 × 10 <sup>3</sup>	–	< 15	–	< 20	–	< 20	–
	Comb pen shell	After auction	–	3.5 × 10 <sup>2</sup>	–	4.5 × 10	–	< 15	–	< 20	< 20
Snail	After auction	–	1.4 × 10 <sup>2</sup>	–	4.2 × 10	–	< 15	–	< 20	< 20	
Corb shell	After auction	–	3.9 × 10 <sup>3</sup>	–	6.6 × 10 <sup>2</sup>	–	< 15	–	< 20	< 20	
Flatfish	After auction	–	1.1 × 10 <sup>6</sup>	–	1.2 × 10 <sup>5</sup>	–	< 15	–	< 20	< 20	
Rock cod	After auction	–	3.8 × 10 <sup>4</sup>	–	8.4 × 10 <sup>3</sup>	–	< 15	–	< 20	< 20	
Conger eel	After auction	–	3.6 × 10 <sup>4</sup>	–	3.6 × 10 <sup>3</sup>	–	< 15	–	< 20	< 20	
Environmental samples											
Water		8.1 × 10 <sup>3</sup>	6.6 × 10 <sup>2</sup>	3.2 × 10 <sup>3</sup>	1.4 × 10 <sup>2</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Seawater		1.6 × 10 <sup>3</sup>	–	5.7 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20	–
Ice		–	1.4 × 10 <sup>2</sup>	–	< 15	–	< 15	–	< 20	–	< 20
Wooden box		1.3 × 10 <sup>7</sup>	1.3 × 10 <sup>6</sup>	2.5 × 10 <sup>4</sup>	4.0 × 10 <sup>5</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Plastic box		3.3 × 10 <sup>6</sup>	1.5 × 10 <sup>5</sup>	1.0 × 10 <sup>3</sup>	2.4 × 10 <sup>4</sup>	< 15	< 15	< 20	< 20	< 20	< 20

*Water* water in water tanks of fishery auction markets, *seawater* seawater in fishery distribution vehicles, *ice* ice in wooden or plastic boxes, *wooden box* surfaces of wooden boxes for fish storage, *plastic box* surfaces of plastic boxes for fish storage, – not analyzed

Izhaki 2017). Although the isolates in this study were identified as *V. cholerae* non-O1, and most *V. cholerae* non-O1 do not produce this toxin, it has been reported as the third most common group of *Vibrio* bacteria that causes diarrheal disease (CDC 2019). The prevalence of *Vibrio* in fishery products may be affected as the sea surface temperature of S. Korea continues to increase, having increased by 1.1 °C over the last 50 years (East Sea 1.7 °C, West Sea 0.3 °C, and South Sea 1.4 °C increase) (NIFS 2019). Chávez et al. (2005) and Singleton et al. (1982) suggest that warm temperatures may influence the occurrence of *V. cholerae* O1 and non-O1. Thus, a detection rate of *V. cholerae* in fishery products will be gradually increased.

Little increase was observed in the bacterial cell counts (aerobic and coliform bacteria) of the fishery products (flounder, monkfish, flatfish, rock cod, sea bass, snail, hairtail, croaker, and pilchard), as the time period of the fishery auction (before, during, and after auction)

progressed (Tables 2, 3, and 4). The bacterial cell counts in the fishery products may increase as temperature increases, and fishery products can be cross-contaminated by storage facilities (wooden or plastic boxes) that have not been decontaminated. Coliform in the wooden boxes were detected in spring ( $6.0 \times 10^{-2.5} \times 10^2$  CFU/100 cm<sup>2</sup>), summer ( $2.5 \times 10^4-4.0 \times 10^5$  CFU/100 cm<sup>2</sup>), and fall ( $9.6 \times 10^3-4.3 \times 10^5$  CFU/100 cm<sup>2</sup>) (Tables 2, 3, and 4). Therefore, the replacement or decontamination of storage facilities at fishery auction markets is required to prevent cross-contamination. In particular, the bacteria in wooden boxes could accumulate if the boxes are not decontaminated to be microbiologically safe.

## Conclusions

In conclusion, *V. cholerae* can be detected in fall and can cross-contaminate between the fishery products and environmental factors such as water and storage boxes in the fishery auction markets. Therefore, food safety

**Table 4** Microbial contaminations (aerobic bacteria, coliform, *Escherichia coli*, and *Vibrio cholerae*) of the fishery products and the environmental samples collected from the fishery auction market A and B in fall (September)

Samples	Time	Bacteria									
		Aerobic bacteria (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		Coliform (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>E. coli</i> (CFU/g or CFU/mL or CFU/100 cm <sup>2</sup> )		<i>V. cholerae</i> (MPN/100 g or MPN/100 mL or MPN/100 cm <sup>2</sup> )			
		Market A	Market B	Market A	Market B	Market A	Market B	Market A	Market B	Market A	Market B
Fishery products											
Snail	Before auction	2.6 × 10 <sup>2</sup>	–	3.2 × 10	–	< 15	–	< 20	–	5400	–
	During auction	8.4 × 10 <sup>3</sup>	–	5.9 × 10 <sup>2</sup>	–	< 15	–	< 20	–	130	–
	After auction	2.6 × 10	–	< 15	–	< 15	–	< 20	–	20	–
Hairtail	Before auction	–	6.3 × 10 <sup>4</sup>	–	1.1 × 10 <sup>3</sup>	–	< 15	–	< 20	–	< 20
	After auction	–	1.6 × 10 <sup>5</sup>	–	1.9 × 10 <sup>4</sup>	–	< 15	–	< 20	–	200
Croaker	Before auction	–	1.9 × 10 <sup>5</sup>	–	1.2 × 10 <sup>3</sup>	–	< 15	–	< 20	–	40
	After auction	–	2.7 × 10 <sup>5</sup>	–	5.9 × 10 <sup>2</sup>	–	< 15	–	< 20	–	110
Flatfish	Before auction	–	2.3 × 10 <sup>4</sup>	–	3.7 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20
	After auction	–	5.8 × 10 <sup>4</sup>	–	2.0 × 10 <sup>3</sup>	–	< 15	–	< 20	–	20
Pilchard	Before auction	–	2.9 × 10 <sup>4</sup>	–	5.2 × 10 <sup>4</sup>	–	< 15	–	< 20	–	45
	After auction	–	9.9 × 10 <sup>4</sup>	–	5.3 × 10 <sup>2</sup>	–	< 15	–	< 20	–	< 20
Environmental samples											
Water		1.3 × 10 <sup>3</sup>	9.8 × 10 <sup>2</sup>	1.1 × 10 <sup>3</sup>	6.0 × 10 <sup>2</sup>	< 15	< 15	< 20	< 20	< 20	20
		2.9 × 10 <sup>3</sup>	1.6 × 10 <sup>3</sup>	1.1 × 10	6.9 × 10 <sup>2</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Ice		5.5 × 10 <sup>4</sup>	1.1 × 10 <sup>6</sup>	3.9 × 10	3.3 × 10 <sup>2</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Wooden box		1.8 × 10 <sup>7</sup>	3.2 × 10 <sup>7</sup>	5.4 × 10 <sup>5</sup>	< 15	< 15	< 15	< 20	< 20	< 20	< 20
		5.8 × 10 <sup>7</sup>	5.5 × 10 <sup>6</sup>	3.6 × 10 <sup>5</sup>	9.6 × 10 <sup>3</sup>	< 15	< 15	< 20	< 20	< 20	< 20
Plastic box		5.2 × 10 <sup>6</sup>	1.3 × 10 <sup>8</sup>	2.0 × 10 <sup>4</sup>	3.4 × 10 <sup>4</sup>	< 15	< 15	< 20	< 20	< 20	20
		9.6 × 10 <sup>6</sup>	6.0 × 10 <sup>7</sup>	8.2 × 10 <sup>3</sup>	3.4 × 10 <sup>3</sup>	< 15	< 15	< 20	< 20	< 20	< 20

Water water in water tanks of fishery auction markets, seawater seawater in fishery distribution vehicles, ice ice in wooden or plastic boxes, wooden box surfaces of wooden boxes for fish storage, plastic box surfaces of plastic boxes for fish storage, – not analyzed

practices at fishery auction markets such as the frequent replacement and decontamination of storage facilities and tools should be performed to prevent foodborne disease outbreaks. Overall, the results of this study may be useful in establishing food safety practices for fishery auction markets in S. Korea.

#### Abbreviations

APW: Alkaline peptone water; MPN: Most probable number

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#### Authors' contributions

ISS and YY participated in the design of this study. YC and YL carried out the sample collection and data analysis. JL, JH, and HO helped to analyze the data, and SL and SK helped to draft the manuscript. All authors read and approved the final manuscript.

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#### Availability of data and materials

All datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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