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Raw ready-to-eat seafood safety: microbiological quality of the various seafood species available in fishery, hyper and online markets

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Significance and Impact of the Study: Raw ready-to-eat seafood products like *sashimi* can be easily contaminated with various bacteria from aquatic environments and human reservoirs, which subsequently bring about a risk in food poisoning due to no heating process before consumption. The results of this study provide comprehensive microbiological data on various species of raw ready-to-eat seafood from various distribution channels. It may contribute to establish reasonable standard and effective strategies to ensure a good microbiological quality of raw ready-to-eat seafood for the safety of meals, like *sashimi* and *sushi*.

Keywords
foodborne pathogens, market survey, microbiological quality, ready-to-eat, seafood.

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Abstract

Microbiological quality of 206 raw ready-to-eat seafood samples was investigated according to species (gizzard shad, halibut, rockfish, tuna, oyster and squid) and distribution channels (fishery, hyper and online market). Enumeration of aerobic plate count and total coliforms (TC) and pathogenic bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*) was performed, and level of microbiological quality was classified into four groups: satisfactory, acceptable, unsatisfactory and unacceptable. Qualitative analysis was also performed for *Escherichia coli* and eight foodborne pathogens (*B. cereus*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *S. aureus*, *Vibrio cholerae*, *V. parahaemolyticus*, and *Vibrio vulnificus*). Raw ready-to-eat seafood products revealed 0·5% at an unsatisfactory level and 4·9% at an unacceptable level due to ≥4 log CFU g^{-1} of TC in squid and ≥3 log CFU g^{-1} of *V. parahaemolyticus* in gizzard shad respectively. Gizzard shad was shown to be potentially hazardous, as its *sashimi* is eaten with its skin attached. *Bacillus cereus*, *E. coli*, *S. aureus*, *V. parahaemolyticus* and *V. vulnificus* were qualitatively detected. Samples from the fishery market showed higher detection rate especially in *V. parahaemolyticus* (21·6%) and *V. vulnificus* (1·7%) which indicates the need to improve microbiological safety of raw ready-to-eat seafood products in fishery market.

Introduction

Seafood consumption has considerably increased globally due to its rising importance to food security and adequate nutrition for a global population (FAO 2016). In most cases, the fish undergo a thermal process before consumption to enhance its taste and ensure its food safety (Miguéis *et al.* 2015). However, Asian countries including Korea and Japan traditionally have a special diet involving a thinly sliced-up raw fish called *sashimi* or raw marine products such as oyster and squid, the popularity of which has been growing worldwide (Morgano *et al.* 2014). Such raw ready-to-eat seafood products can not only be found in Japanese-style restaurants but also in a variety of food premises that serve Chinese and Western dishes these days.

Raw ready-to-eat seafood products could be easily contaminated with various bacteria anywhere during the fishing, capture, distribution and sales stages (Huss *et al.* 2000). Specifically, the microbial status of seafood products is influenced by the aquatic environment (*Vibrio* spp.), general environment (*Bacillus* spp. and *Listeria monocytogenes*) and animal or human reservoir (*Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*) (Huss *et al.* 2000). As there is no chance to remove bacteria thoroughly from the contaminated raw seafood product before consumption, this could lead to an increased risk of food poisoning.

In the United States, more than 180 outbreaks were caused by seafood products from 1973 to 2006, causing 4020 illnesses and 11 deaths (Iwamoto *et al.* 2010). Numerous cases of *Vibrio parahaemolyticus* infection have been reported due to the consumption of contaminated shell fish including oyster (Velazquez-Roman *et al.* 2013), and more recently in 2015, a multistate outbreak of *Salmonella* infections linked to raw tuna resulted in 62 infections and 11 hospitalizations (USFDA 2015). It was reported that *sushi* and *sashimi* had contributed to 3% of foodborne outbreaks in Hong Kong between 1997 and 1999, including *Bacillus cereus, Salmonella, S. aureus* and *V. parahaemoyticus* (Centre for Food Safety 1999). In Japan and Taiwan, foodborne outbreaks related to *V. parahaemolyticus* have been reported due to contaminated *sashimi* consumption (Novotny *et al.* 2004).

Previous studies have examined raw ready-to-eat seafood products for the prevalence of pathogenic bacteria such as *B. cereus*, *Listeria* species, *S. aureus* and *Vibrio* species (Johnston 2015; Miguéis *et al.* 2015, 2016; Wong *et al.* 2015). However, few studies considered the various distribution channels, although raw ready-to-eat seafood products are directly vended to consumers through fishery, hyper and online market these days. Therefore, it is important to evaluate and guaranty the microbiological safety of various raw ready-to-eat seafood products available at different distribution channels.

The standards to determine microbiological quality for raw ready-to-eat seafood products are not well established. Nevertheless, some information on ready-to-eat food has been indicated (Gilbert *et al.* 2000) and evaluation of ready to eat *sashimi* using this standard has been reported recently (Miguéis *et al.* 2015), which allows us to achieve some pathogenic indicators. The criteria for qualitative evaluation of micro-organisms, thus, were Aerobic Plate Counts (APC), total coliforms (TC), *B. cereus, S. aureus* and *V. parahaemolyticus* in quantitative analysis. We also tested raw ready-to-eat seafood products for the presence of *E. coli* and eight foodborne pathogenic bacteria to identify potential microbiological hazards of various raw ready-to-eat seafood products (gizzard shad (*Konosirus punctatus*), halibut (*Paralichthys olivaceus*), rock-fish (*Sebastes inermi*), tuna (*Thunnus albacares*), oyster (*Crassostrea gigas*) and squid (*Todarodes pacificus*)) from the different distribution channels (fishery, hyper and online market). The aim of this study was to assess the quality level of raw ready-to-eat seafood products directly vended to consumers and to determine if any improvements in distribution channels can be implemented.

Results and discussion

Microbiota that contribute to the microbiological quality

Microbiota that contributed to the low-quality level of raw ready-to-eat seafood products were APC, TC and *V. parahaemolyticus*. High APC is not likely to cause illness, but it indicates the level of micro-organisms in the product. TC is a hygiene indicator that is useful to understand if good hygiene practices are implemented as well as for showing some possible presence of enteropathogens (Kornacki and Johnson 2001). *Vibrio parahaemolyticus* is a cautious bacterium frequently reported in raw seafood products, which can lead to acute gastroenteritis characterized by diarrhoea, vomiting, nausea and abdominal cramps (Su and Liu 2007). In this study, a total of 2·4 and 0·5% of the 206 raw ready-to-eat seafood samples revealed unsatisfactory levels of APC ($\geq$6 log CFU g^{-1}) and TC ($\geq$4 log CFU g^{-1}) respectively (Fig. 1), while a total of 4·9% tested samples had unacceptable levels ($\geq$3 log CFU g^{-1}) of *V. parahaemolyticus* (Fig. 2).

In qualitative microbiological analysis, the presence of bacteria including *B. cereus*, *E. coli*, *S. aureus*, *V. parahaemolyticus* and *Vibrio vulnificus* in raw ready-to-eat seafood products was detected (Table 1). This suggests the possibility of bacterial contamination by cross-contamination or intrinsic of seafood (Busani *et al.* 2005). The presence of *B. cereus* in fish and *sushi* is frequently reported (Adams *et al.* 1994; Gram and Huss 1996). In this study, *B. cereus* presence in raw ready-to-eat seafood products (37·6%) could be explained by the contaminations during distribution and preparation in the markets. The occurrence of *E. coli* (5·3%) and *S. aureus* (5·3%) also can be an evidence of improper environment and human contact during the preparation of the products (Atanassova *et al.* 2008). This can denote a lack of good practices for handler hygiene, or the possibilities of food contamination during distribution. *Vibrio parahaemolyticus* and *V. vulnificus* are well observed in aquatic environment (Huss *et al.* 2007) and they are the most common pathogenic bacteria that contaminate ready-to-eat seafood products (Su and Liu 2007). Herein, *V. parahaemolyticus* and *V. vulnificus* were detected in 11·1% and 0·9% of tested samples respectively.

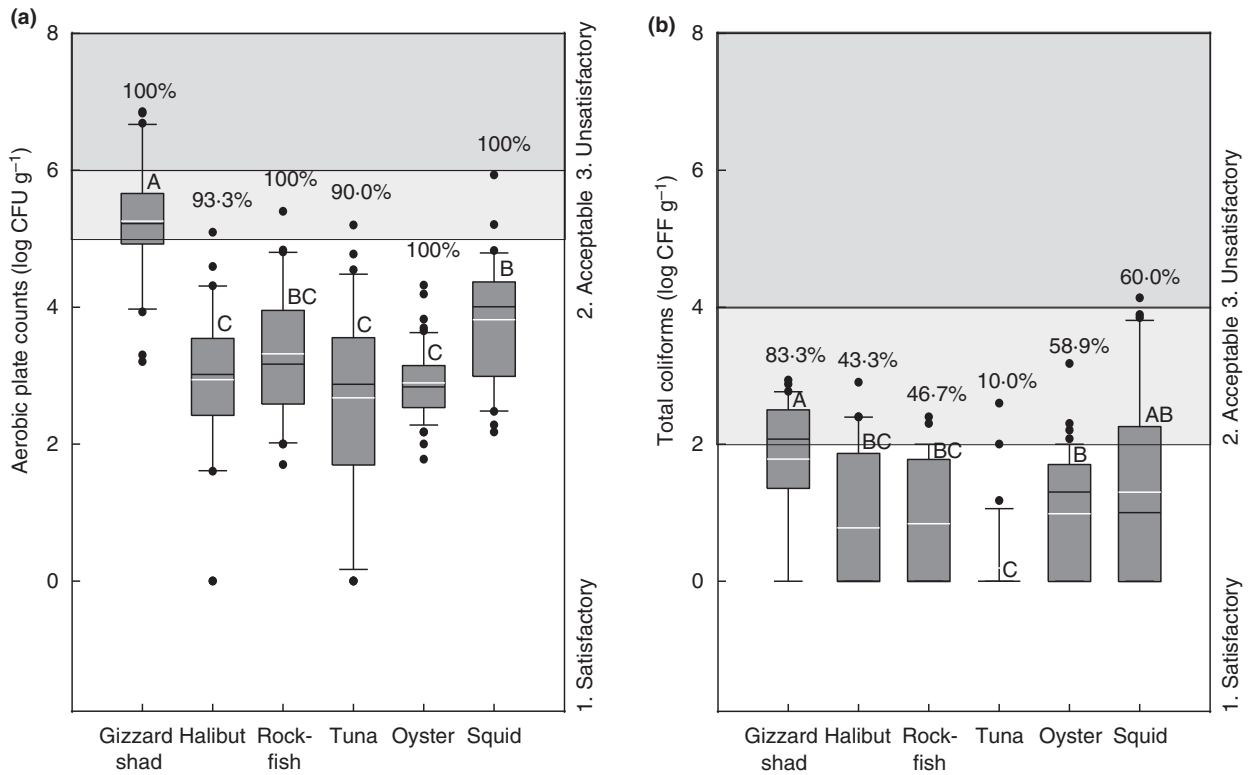

Figure 1 Box plots of (a) Aerobic plate counts and (b) total coliforms in gizzard shad, halibut, rockfish, tuna, oyster and squid: Square indicates the interquartile range for each data point, and blue values on the bars indicate the detection rate of each micro-organism in the samples. The black and blue lines denote the median and mean values respectively. The error bars above and below the square indicate the 90th and 10th percentiles, and the black circles represent outliers. A to C, values are significantly different ($P < 0{\cdot}05$).

Microbiological quality evaluation according to sample species

Among sample species, gizzard shad, the sardine-like shiny fish, was shown to be the most potentially hazardous. It contained the highest mean values of APC, TC and *V. parahaemolyticus* compared to other species ($P < 0{\cdot}05$): APC, 5·3 log CFU g^{-1} (detection rate = 100%); TC, 1·8 log CFU g^{-1} (83·3%); *V. parahemolyticus*, 1·1 log CFU g^{-1} (33·3%). Microbiological quality of gizzard shad samples revealed unacceptable levels due to *V. parahaemolyticus* of which populations of some samples (33·3%) were more than 3 log CFU g^{-1} (Table 2). As *V. parahaemolyticus* has a relatively high infective dose of 10^7–10^8 cells g^{-1} (Sanyal and Sen 1974), all collected unacceptable samples were below the limit. Nevertheless, as the ability of this bacterium to multiply rapidly at room temperature can result in the presence of sufficient bacteria to cause foodborne disease (Gooch *et al.* 2002), care must be taken to not consume *sashimi* contaminated with this bacterium. *Bacillus cereus* (13·3%), *S. aureus* (10·0%), *V. parahaemolyticus* (56·7%) and *V. vulnificus* (3·3%) were also qualitatively detected in this species. Gizzard shad *sushi* and *sashimi* is served with its skin still attached. Since fish are always in intimate contact with aquatic environments, their skin are permanently exposed to various bacteria (Benhamed *et al.* 2014), thus becoming a major source of these bacteria along with other raw materials.

Squid contained the second highest levels of APC (mean value = 3·8 log CFU g^{-1}, detection rate = 100%) ($P < 0{\cdot}05$); however, APC of all squid samples were <6 log CFU g^{-1} at a satisfactory (93·3%) and an acceptable level (6·7%). TC in squid also tended to be the second highest (1·3 log CFU g^{-1}, 60·0%), but there was no significant difference compared to that in other species ($P > 0{\cdot}05$). Nevertheless, one sample of squid showed more than 4 log CFU g^{-1} of TC, which revealed an unsatisfactory level (3·3%). *Bacillus cereus* (13·3%), *E. coli* (3·3%), *V. parahaemolyticus* (20·0%) and *V. vulnificus* (3·3%) were also qualitatively detected.

Oyster particularly contained the second highest populations of TC (1·0 log CFU g^{-1}, 85·9%) and the highest populations of *B. cereus* (1·1 log CFU g^{-1}, 83·9%) among sample species ($P < 0{\cdot}05$). However, TC detected in the samples was below 4 log CFU g^{-1} resulting in an

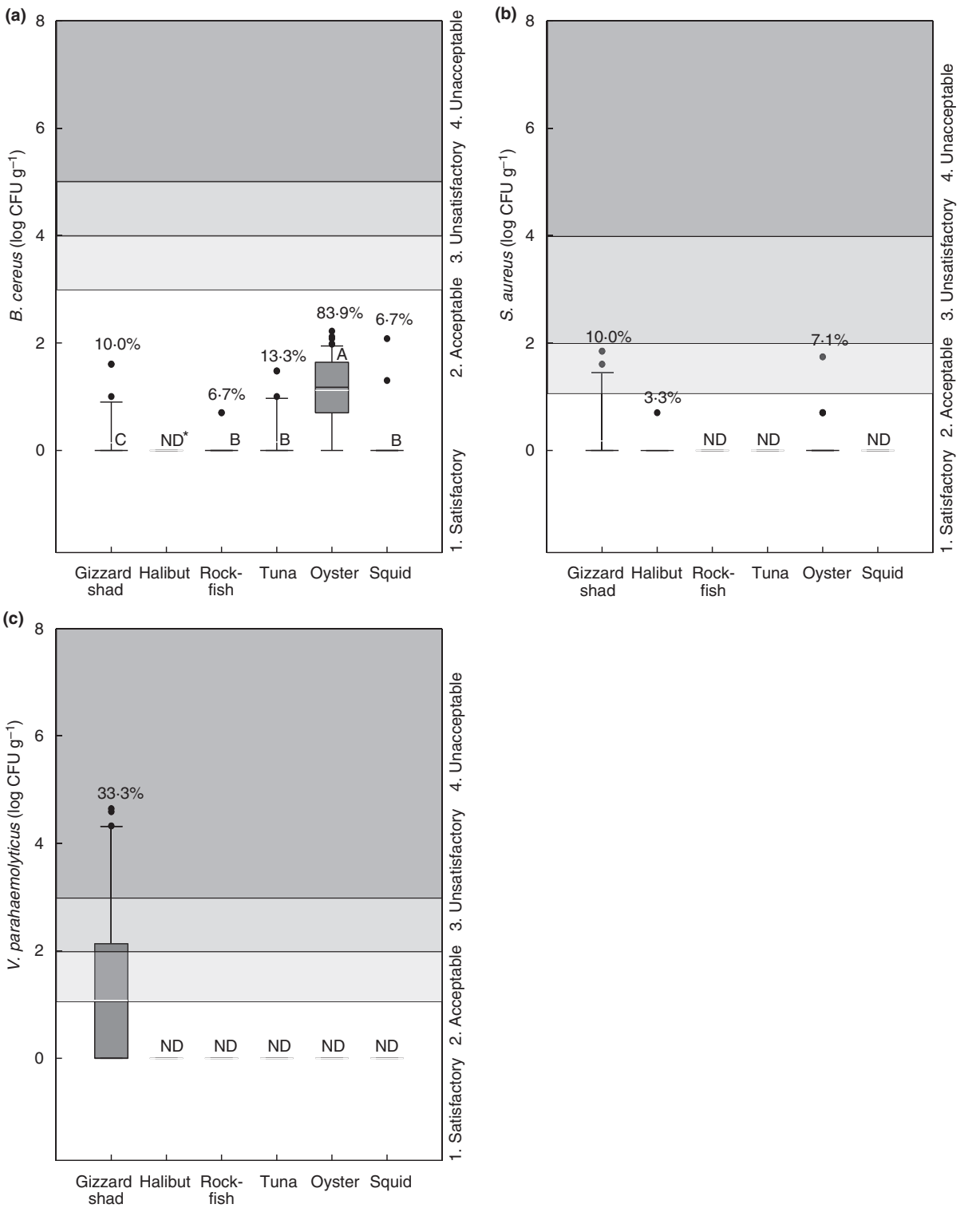

Figure 2 Box plots of (a) *Bacillus cereus*, (b) *Staphylococcus aureus* and (c) *Vibrio parahaemolyticus* population in gizzard shad, halibut, rockfish, tuna, oyster and squid: Square indicates the interquartile range for each data point, and blue values on the bars indicate the detection rate of each micro-organism in the samples. The black and blue lines denote the median and mean values respectively. The error bars above and below the square indicate the 90th and 10th percentiles, and the black circles represent outliers. A to C, values are significantly different ($P < 0{\cdot}05$). *ND, Not detected.

Table 1 Qualitative microbiological analysis of the raw ready-to-eat seafood products according to sample species and distribution channels

Criterion	*N*	Number of detections (Detection rate, %)				
		Bacillus cereus	*Escherichia coli*	*Staphylococcus aureus*	*Vibrio parahaemolyticus*	*Vibrio vulnificus*
Sample species						
Gizzard shad	30	4 (13·3)	–*	3 (10·0)	17 (56·7)	1 (3·3)
Halibut	30	5 (16·7)	1 (3·3)	1 (3·3)	–	–
Rockfish	30	7 (23·3)	–	1 (3·3)	–	–
Tuna	30	13 (43·3)	–	1 (3·3)	–	–
Oyster	56	52 (92·9)	10 (17·9)	6 (10·7)	2 (3·6)	–
Squid	30	4 (13·3)	1 (3·3)	–	6 (20·0)	1 (3·3)
Distribution channels						
Fishery market	116	46 (39·7)	8 (6·9)	5 (4·3)	25 (21·6)	2 (1·7)
Hypermarket	50	17 (42·5)	3 (7·5)	5 (12·5)	–	–
Online market	40	22 (44·0)	1 (2·0)	2 (4·0)	–	–
Total	206	85 (37·6)	12 (5·3)	12 (5·3)	25 (11·1)	2 (0·9)

*–, Not detected.

Table 2 Microbiological quality levels according to sample species and distribution channels

Criterion	*N*	Frequencies of samples (rate, %)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
Sample species					
Gizzard shad	30	5 (16·7)	15 (50·0)	0 (0·0)	10 (33·3)
Halibut	30	22 (73·3)	8 (26·7)	0 (0·0)	0 (0·0)
Rockfish	30	24 (80·0)	6 (20·0)	0 (0·0)	0 (0·0)
Tuna	30	28 (93·3)	2 (6·7)	0 (0·0)	0 (0·0)
Oyster	56	46 (82·1)	10 (17·9)	0 (0·0)	0 (0·0)
Squid	30	19 (63·3)	10 (33·3)	1 (3·3)	0 (0·0)
Distribution channels					
Fishery market	116	72 (62·1)	33 (28·4)	1 (0·9)	10 (8·6)
Hypermarket	50	41 (82·0)	9 (18·0)	0 (0·0)	0 (0·0)
Online market	40	31 (77·5)	9 (22·5)	0 (0·0)	0 (0·0)
Total	206	144 (69·9)	51 (24·8)	1 (0·5)	10 (4·9)

acceptable (17·9%) and satisfactory (82·1%) level, and *B. cereus* detected in the samples was below 3 log CFU g^{-1} resulting in a satisfactory level (100%). As the infective dose of *B. cereus* is 10^5–10^7 cells g^{-1} for intestinal symptoms, it was thought to be safe from *B. cereus* infections by oyster in this study. In the qualitative analysis, *B. cereus* (92·9%), *E. coli* (17·9%), *S. aureus* (10·7%) and *V. parahaemolyticus* (3·6%) was detected. Several studies have reported the presence and outbreaks of *Vibrio* spp. in oysters (DePaola *et al.* 1990; McLaughlin *et al.* 2005), but the detection rate of *V. parahaemolyticus* in oyster (3·6%) was relatively small compared to that in gizzard shad (56·7%) and squid (20·0%) in this study. *Vibrio vulnificus* was not detected in the oyster samples in this study.

The other fish *sashimi* including halibut, rockfish and tuna showed relatively low levels of micro-organisms compared to that in other species. The analysis showed not detected (ND) to 5·4 log CFU g^{-1} of APC and ND to 2·9 log CFU g^{-1} of TC, within a satisfactory (82·2%) and an acceptable (17·8%) level. Only one sample of halibut (3·3%) contained *S. aureus*, and two and four samples of rockfish (6·7%) and tuna (13·3%) contained *B. cereus* respectively. *Vibrio parahaemolyticus* were not quantitatively detected in any of these samples. Qualitatively, *B. cereus* and *S. aureus* were detected in these samples, while *E. coli* was detected in one sample of halibut (3·3%). *Vibrio* spp. was not qualitatively detected in these samples.

Microbiological quality evaluation according to distribution channels

Among distribution channels, samples only from the fishery market were observed in an unsatisfactory (0·9%) and an unacceptable level (8·6%), while all the other samples from hyper and online markets were satisfactory or

acceptable. This is due to *V. parahaemolyticus* contamination of gizzard shad and TC contamination of squid purchased from the fishery market. In the case of squid, the mean value of TC in fishery market samples was significantly higher than that in online market samples (1·5 and 0·9 log CFU g^{-1}, respectively; $P < 0{\cdot}05$; data not shown). *Vibrio parahaemolyticus* (21·6%) and *V. vulnificus* (1·7%) were also qualitatively detected in gizzard shad, oyster and squid samples from the fishery market. Since handlers in the fishery market usually wash raw seafood with marine water, *Vibrio* spp. was likely from the washing water. Consequentially, it was thought that various seafood species, use of marine water and hygienic status of food handlers may contribute to the bacterial contamination of samples from the fishery market.

Overall, the present results suggest that the microbiological quality of raw ready-to-eat seafood products was generally good, with 69·9% of satisfactory and 24·8% of acceptable samples. However, APC, TC and *V. parahaemolyticus* contributed to the unsatisfactory or unacceptable levels of raw ready-to-eat seafood products. Among sample species, gizzard shad *sashimi* was potentially hazardous since it is eaten with its skin. Thus, a notice for consumers and handlers for this kind of raw seafood eaten with skin is needed. *B. cereus, E. coli, S. aureus, V. parahaemolyticus* and *V. vulnificus* were qualitatively detected from samples, and they seemed to be derived from intrinsic of seafood products or cross-contamination during distribution and preparation. Among distribution channels, only the fishery market dealt in unsatisfactory and unacceptable seafood products; thus, it is needed to be properly managed. The results of this study provide comprehensive microbiological data on various species of raw ready-to-eat seafood products from various distribution channels. It can contribute to establish reasonable standard and draw effective strategies to ensure the microbiological safety of raw ready-to-eat seafood products.

Materials and methods

Sample collection

A total of 206 raw ready-to-eat seafood products including fish (gizzard shad, $n = 30$; halibut, $n = 30$; rockfish, $n = 30$; tuna, $n = 30$), shellfish (oyster, $n = 56$) and mollusc (squid, $n = 30$) were directly collected from various distribution channels (fishery, hyper and online markets) in South Korea from January to October in 2013 considering they are seasonal products (Table 2). Since raw oyster is a well known vector of infectious pathogens (Rippey 1994), an additional 26 samples compared to others were tested (total of 56 oyster samples). Samples were maintained in their original packaging, transported to the laboratory with ice packs within 4 h and then subjected to microbiological analyses immediately.

Quantitative microbiological analysis (bacterial enumeration)

All microbiological analyses were performed according to the US Food and Drug Administration Bacteriological Analytical Manual (USFDA 2011) and the Korea Food Code (MFDS 2012), with some modifications as described previously (Choi *et al.* 2014; Jeon *et al.* 2015).

Twenty-five grams of each sample was cut into small pieces and aseptically transferred into the stomacher bags (Circulator 400 standard bags; Seward, Worthing, UK). Samples were homogenized with 225 ml of sterile 0·85% saline using a stomacher at 230 rev min^{-1} for 2 min (Circulator 400; Seward). Homogenized samples were serially diluted using 10-fold dilution methods with 9 ml of 0·85% sterile saline. One hundred microlitres of diluents were spread-plated on selective agar plates in duplicate, and 1 ml of sample was spread plated onto five plates to reduce the detection limit (10 CFU g^{-1}). The selective agar plates and incubation conditions were as follows: Plate Count Agar (PCA; Difco, Becton Dickinson, Sparks, MD) for APCs (35°C, 48 h), Violet Red Bile Agar (VRBA, Difco) for (TC 37°C, 24 h), Mannitol Egg Yolk Polymyxin agar (MYP, Difco) supplemented with 50% egg yolk and antimicrobial vial P (Difco) for *B. cereus* (30°C, 24 h), Baird–Parker medium (BP, Difco) supplemented with 5% egg yolk tellurite emulsion for *S. aureus* (35°C, 48 h) and Thiosulphate Citrate Bile Salts sucrose agar (TCBS, Difco) for *V. parahaemolyticus* (35°C, 24 h). In each selective agar, five colonies were selected and identified by VITEK2 (bioMerieux, MarchI'Etoile, France) with GN, GP or BCL card (bioMerieux) for Gram negative, Gram positive bacteria and *Bacillus* spp. respectively. The final confirmation of *V. parahaemolyticus* was provided by PCR. The identified colonies were calculated by multiplication of the ratio of counts.

Microbiological quality evaluation

Evaluation levels to determine the microbiological quality of ready-to-eat seafood products were adapted by Miguéis *et al.* (2015) and Gilbert *et al.* (2000). Criteria for and level of microbiological quality (CFU g^{-1}) were as follows: satisfactory (APC, $<10^5$; Enterobacteriaceae (including TC), $<10^2$; *B. cereus*, $<10^3$; *S. aureus* <20; *V. parahaemolyticus*, <20), acceptable (APC, $10^5 \leq 10^6$; Enterobacteriaceae, $10^2 \leq 10^4$; *B. cereus*, $10^3 \leq 10^4$; *S. aureus* $20 \leq 10^2$; *V. parahaemolyticus*, $20 \leq 10^2$), unsatisfactory (APC, $\geq 10^6$; Enterobacteriaceae, $\geq 10^4$; *B. cereus*, $10^4 \leq 10^5$; *S. aureus* $10^2 \leq 10^4$; *V. parahaemolyticus*,

$10^2 \leq 10^3$), and unacceptable/potentially hazardous (APC, not applicable (NA); Enterobacteriaceae, NA; *B. cereus*, $\geq 10^5$; *S. aureus* $\geq 10^4$; *V. parahaemolyticus*, $\geq 10^3$).

Qualitative microbiological analysis

To examine whether the raw ready-to-eat seafood products contained potentially pathogenic micro-organisms, 25 g of each sample was transferred to a stomacher bag and mixed with 225 ml of the buffer solution or media. The mixture was then homogenized by the stomacher for 2 min at 230 rev min^{-1}. The following target micro-organisms were enriched in the appropriate buffer solution or media under certain conditions: *B. cereus* (in Tryptic soy polymyxin broth (Difco) at 30°C for 24 h), *E. coli* and *E. coli* O157:H7 (in modified EC broth (Difco) at 37°C for 24 h), *L. monocytogenes* (in UVM-modified Listeria enrichment broth (Difco) at 30°C for 24 h), *Salmonella* spp. (in buffered peptone water (Difco) at 37°C for 18 h), *S. aureus* (in Brain–Heart Infusion broth (Difco) at 35°C for 24 h) and pathogenic *Vibrio* spp. (in Alkaline peptone water (Difco) at 36°C for 24 h). The enriched UVM-modified Listeria enrichment broth (0·1 ml) was inoculated into 10 ml of Fraser Listeria broth (Difco) supplemented with Fraser Listeria broth supplement and incubated at 30°C for 24 h. The pre-enriched buffered peptone water (0·1 and 1 ml) was transferred to 10 ml of Rappaport-Vassiliadis broth (Oxoid) and selenite F broth (Difco), respectively, and then incubated at 42 and 37°C for 24 h to recover *Salmonella* spp.

Next, to check whether the target micro-organisms were present in the enriched samples, one loopful of enriched culture was streaked onto selective media and typical colony was isolated as follows: *B. cereus* (MYP at 30°C for 24 h, pink colony encircled with a precipitation zone), *E. coli* (eosin methylene blue agar at 37°C for 24 h, purple with green metallic sheen colony), *E. coli* O157:H7 (MacConkey sorbitol agar supplemented with cefixime and tellurite (Difco) at 37°C for 24 h, colourless colony), *L. monocytogenes* (modified Oxford agar (Oxoid) 30°C for 24 h, black and shiny colony with a black halo), *Salmonella* spp. (Xylose Lysine Desoxycholate agar (Difco) 37°C for 24 h, black colony), *S. aureus* (BP at 35°C for 48 h, grey-black to jet black circular colony), pathogenic *Vibrio* spp. (TCBS at 36°C for 24 h, large yellow colony for *V. cholerae* and green colony for *V. parahaemolyticus* and *V. vulnificus*).

To screen the suspected isolates of *L. monocytogenes* and *S. aureus*, CAMP test (positive for *S. aureus* ATCC 25923 and negative for *Rhodococcus equi* ATCC 6939) and coagulase test with EDTA (Becton Dickinson, BBL™, Franklin Lakes, NJ) were performed respectively. Isolated colonies were confirmed by VITEK 2 (bioMerieux) with BCL (*B. cereus*), GN (*E. coli, E. coli* O157:H7, *Salmonella* spp., and *Vibrio* spp.) and GP (*L. monocytogenes* and *S. aureus*) cards.

Multiplex PCR analysis for *Vibrio* spp.

Each isolated *V. parahaemolyticus* and *V. vulnificus* was analysed by multiplex PCR (MyCycler™ Thermal Cycler; Bio-Rad, Hercules, CA) with specific primers (Table S1, all primers were taken from Bioneer, Daejeon, Korea) (Kim *et al.* 2015).

Each PCR tube contained 25 μl of reaction mixture with 1× PCR buffer, 200 μmol l^{-1} of dNTP, the adjusted concentration of each primer (0·24 μmol l^{-1} for *V. parahaemolyticus*; 0·4 μmol l^{-1} for *V. vulnificus*), 25 ng of template DNA and 0·5 unit of taq polymerase (Bioneer). The cycle conditions used in the thermal cycler (Bio-Rad) were as follows: 94°C for 5 min, 94°C for 30 s, 60°C for 30 s, 72°C for 30 s for 25 cycles, with a final 10-min extraction at 72°C. The amplified PCR products (5 μl) were detected by electrophoresis (TaKaRa Bio, Otsu, Japan) with a 3·0% (wt/vol) agarose gel (Neogen, Lansing, MI) and a molecular marker (100 kb DNA ladder; Elpis biotech, Daejeon, Korea) was concurrently run. The DNA bands were visualized and photographed with UV light.

Statistical analysis

Significant differences in microbial populations among the different raw ready-to-eat seafood products were determined by general linear model. Means were compared by Tukey's studentized range test ($P < 0·05$) using SAS program (SAS ver. 9.3, SAS Institute Inc., Cary, NC).

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Conflict of Interest

There are no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Primer pairs for *Vibrio* PCR used in this study and their sources.