Occurrence of *Vibrio*, *Salmonella* and *Staphylococcus aureus* in retail fresh fish, mussel and shrimp

Tulay Elal Mus, Figen Cetinkaya, Umut Celik

University of Uludag, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bursa, Turkey

Received June 12, 2013
Accepted March 26, 2014

Abstract

This study aimed to determine the presence of pathogenic *Vibrio* spp., *S. aureus* and *Salmonella* in 100 seafood samples purchased from retail outlets in Bursa city (Turkey). Of the samples examined including fish, mussel and shrimp, 67% were found to be contaminated with *Vibrio*. Presumed *Vibrio* spp. were identified by standard biochemical tests, and further confirmed by API 20E system. Identified *Vibrio* spp. were *V. parahaemolyticus* (28%), *V. vulnificus* (1%) and *V. cholerae* (1%), with the most prevalent being *V. alginolyticus* (37%). Six (6%) of the samples analysed were positive for *S. aureus*. However, no contamination of the samples with *Salmonella* was observed. Our results showed that seafood from retail outlets can be a likely vehicle for infections with *Vibrio* spp. and *S. aureus*.

Seafood, pathogen bacteria, incidence, microbiological quality

In addition to being a healthy food with nutritional value, seafood can also act as a source of foodborne pathogens (Hudecová et al. 2010; Bakr et al. 2011). Various outbreaks of bacterial disease associated with the consumption of seafood have been reported (Guerin et al. 2004; Friesema et al. 2012). From these seafood-borne bacteria, *Vibrio* spp. are Gram-negative rod-shaped and halophilic bacteria that generally widespread in the coastal and estuarine environments (Austin 2010). Among more than 20 *Vibrio* species known to be associated with human disease, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are the pathogenic species of *Vibrio* that pose the greatest threat to human health (Gopal et al. 2005; Cariani et al. 2012).

Salmonella and *S. aureus* are the leading foodborne pathogens, causative agents of the most common enteric infections to humans (Lei et al. 2008; Kumar et al. 2009). These bacteria can enter the aquatic environment through wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal wastes (Amagliani et al. 2012). Various authors have reported the incidence of *Salmonella* (Woodring et al. 2012) and enterotoxigenic *S. aureus* (Popovic et al. 2010) in seafood.

In this study, we investigated the prevalence of pathogenic *Vibrio* spp., *Salmonella* and *S. aureus* contamination in fish, mussel and shrimp from the retail level in order to assess health risks for consumers, and also determined the identification to species level of the *Vibrio* strains isolated from samples.

Materials and Methods

Sample collection

During 2012, a total of 100 fresh seafood samples including 78 fish, 12 mussel and 10 shrimp were collected from several supermarkets, fish markets and neighbourhood bazaars in Bursa province, Turkey. The samples were placed into sterile bags and transported to the laboratory under refrigerated conditions for analysis.

Isolation and identification

The detection of pathogenic *Vibrio* species was achieved according to the standard methods of the U.S. Food and Drug Administration (FDA) (DePaola and Kaysner 2004). Approximately 25 g of each sample were

Address for correspondence:
Figen Cetinkaya
Department of Food Hygiene and Technology
Faculty of Veterinary Medicine, University of Uludag
16059 Gorukle Campus, Bursa, Turkey

Phone: +90 224 294 1212
Fax: +90 224 294 1202
E-mail: figcetinkaya@yahoo.com
http://actavet.vfu.cz/

homogenized in 225 ml Alkaline Peptone Water (APW) (Merck 1.01800) with 2% NaCl and incubated overnight at 35 ± 2 ºC. A loop of the pre-enriched culture was streaked onto Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar (Merck 1.10263). After incubation at 35 ± 2 ºC for 18–24 h, presumptive colonies were firstly characterized by biochemical tests which included Gram staining, motility, oxidase production, 0129 vibriostat susceptibility (Oxoid DD0015), and then identified at the genus and species level using API 20E (BioMerieux, France) test kits.

For the isolation of *Salmonella,* 25 g of seafood samples were homogenized in 225 ml of Buffered Peptone Water (Oxoid CM0509) and then incubated at 37 °C for 16–20 h. 0.1 ml of the pre-enrichment culture was inoculated into tubes containing 10 ml Rappaport Vassiliadis medium (Oxoid CM0669) and incubated 24 h at 42 °C. A loopful from each tube was streaked on Xylose Lactose Tergitol 4 agar (XLT4) (Merck 1.13919). After incubation at 37 ºC for 20–24 h, presumptive *Salmonella* colonies were subjected to initial screening tests using triple sugar iron agar (Oxoid CM0277), lysine iron agar (Oxoid CM0381), urea broth (Oxoid CM0071) and lysine decarboxylase broth (Oxoid CM308). Presumptive positive results were confirmed by Poly O (Denka Seiken, Tokyo, Japan) and Poly H slide agglutination tests (Andrews and Hammack 1998).

For the detection of *S. aureus*, 10 g seafood samples were homogenized with 90 ml of a sterile 0.1% peptone water solution. Serial dilutions of the homogenate were made with sterile peptone water and plated in duplicates on Baird Parker agar (Merck 1.05406) supplemented with egg yolk-tellurite emulsion (Merck 1.03785). After incubation at 35 ºC for 48 h, the typical black with clear halo colonies of *S. aureus* were tested for coagulase activity using Dry Spot Staphytect Plus (Oxoid DR0100) (Harrigan 1998).

Results

This study was conducted to determine the incidence of *Vibrio* spp., *S. aureus* and *Salmonella* spp. in seafood (78 fish, 12 mussel and 10 shrimp) for sale in retail outlets. The results related to the incidence and identification at the species level of *Vibrio* spp. are summarized in Table 1. Sixty-seven (67%) out of 100 samples were found to be contaminated with *Vibrio* spp. The isolates were identified as *V. vulnificus* (one isolate), *V. cholerae* (one isolate), *V. parahaemolyticus* (28 isolates) and *V. alginolyticus* (37 isolates) using API 20E system. *V. vulnificus* was isolated from one fish sample while *V. cholerae* was detected in one shrimp sample. Among 28 *V. parahaemolyticus* isolates, 25 were found in fish samples and three in mussels. As seen in Table 2, 6 (6%) from 100 seafood samples were contaminated with *S. aureus*. *Salmonella* was not detected in the samples analysed in this study.

Discussion

Vibrio spp. was isolated in 67% of total count of the samples. By API 20E system, the isolates were identified as *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus*. The total rate of isolation of *V. parahaemolyticus* in the samples analysed was 28% (Table 1). In comparison to the results of our study, previous studies with seafood from several

Table 1. Incidence and species distribution of *Vibrio* spp. from seafood.

Sample type	No. of samples	No. of positive samples (%)	*Vibrio* isolated	
			No.	Species
Fish	78	53 (67.9)	1	*V. vulnificus*
			25	*V. parahaemolyticus*
			27	*V. alginolyticus*
Mussel	12	9 (75)	3	*V. parahaemolyticus*
			6	*V. alginolyticus*
Shrimp	10	5 (50)	1	*V. cholerae*
			4	*V. alginolyticus*
Total	100	67 (67)		

Table 2. Incidence of *S. aureus* and *Salmonella* in seafood.

Sample type	No. of samples	No. of samples with *S. aureus*	% of incidence	No. of samples with *Salmonella*
Fish	78	3	3.8	0
Mussel	12	1	8.3	0
Shrimp	10	2	20	0
Total	100	6	6.0	0

countries demonstrated higher prevalence of *V. parahaemolyticus*. In a study on seafood products in China (Chao et al. 2009), the incidence of this bacterium was found to be 56.6%. The rates of *V. parahaemolyticus* contamination of seafood by Chakraborty et al. (2008) in India and by Costa Sobrinho et al. (2011) in Brazil were reported as 64% and 100%, respectively. However, the incidence (28%) of *V. parahaemolyticus* in retail seafood reported here was relatively higher than that reported by Baffone et al. (2000) (14.8%) and by Ottaviani et al. (2005) (24.3%).

The single isolate of *V. vulnificus* in our study originated from fish. Canigral et al. (2010) also reported that this bacterium was recovered from two oyster samples in Spain. Studies conducted by Ji et al. (2011) suggested that 140 out of 239 shrimp samples from Chinese seafood markets were contaminated with *V. vulnificus*. *Vibrio cholerae*, the causative agent of cholera in humans, was detected in only one shrimp sample (1%). Higher incidence rate (3.7%) of *V. cholerae* in seafood products was suggested in a study conducted by Baffone et al. (2000). On the other hand, *V. cholerae* was not isolated from any of the shrimp samples investigated by Hosseini et al. (2004).

Although *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are the main species associated with seafood-borne infections, others including *V. alginolyticus*, *V. mimicus* and *Grimontia hollisae* (formerly *V. hollisae*) have been sporadically found in outbreaks (Cariani et al. 2012). In this study, *V. alginolyticus* was detected in 27 fish, 6 mussel and 4 shrimp samples, with a total incidence rate of 37%. In a study on fish, mussels and clams in Italy (Baffone et al. 2000), 81.5% of the samples were positive for *V. alginolyticus*. In India, an investigation showed that *V. alginolyticus* was present in 19% of 30 west coast shrimp samples analysed (Gopal et al. 2005).

Our study revealed that of 100 seafood products, six (6%) samples harboured *S. aureus*. Three isolates of *S. aureus* were obtained from fish samples, one isolate from mussels and two isolates from shrimp (Table 2). Five of the isolates were found to be coagulase positive except for one isolate from mussels. Much higher incidence rates of *S. aureus* were reported for fresh (43%) and frozen (30%) fishery products in Spain (Vazquez-Sanchez et al. 2012), and for low salt sardine (86.7%), feseikh (93.3%) and molouha (90%) fish samples in Egypt (Ezzeldeen et al. 2011).

Salmonella was not detected in the samples analysed in this study, which was in agreement with previous studies (Popovic et al. 2010; Rodriguez et al. 2011) in seafood products. Conversely, different workers (Shabarinath et al. 2007; Kumar et al. 2009; Bakr et al. 2011) reported varying incidence rates of *Salmonella* in seafood.

In summary, this work revealed the presence of potentially pathogenic bacteria in seafood including fish, mussel and shrimp, and a probable health risk for consumers of raw seafood. The data presented here are useful for risk assessment and management of pathogenic *Vibrio* spp. and *S. aureus* in seafood. Improvement of the effective sanitary conditions in handling and processing operations from fishing to marketing is needed to minimize the risk of infections associated with consumption of these products.

References

Amagliani G, Brandi G, Schiavano GF 2012: Incidence and role of *Salmonella* in seafood safety. Food Res Int **45**: 780-788

Andrews WH, Hammack TS 1998: *Salmonella*. In: Food and Drug Administration, Bacteriological Analytical Manual. 8th Edition (Revision A), AOAC International, Gaithersburg, MD

Austin B 2010: Vibrios as causal agents of zoonoses. Vet Microbiol **140**: 310-317

Baffone W, Pianetti A, Bruscolini F, Barbieri E, Citterio B 2000: Occurence and expression of virulence-related properties of *Vibrio* species isolated from widely consumed seafood products. Int J Food Microbiol **54**: 9-18

Bakr WMK, Hazzah WA, Abaza AF 2011: Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. J Am Sci **7**: 663-668

Canigral I, Moreno Y, Alonso JL, Gonzales A, Ferrus MA 2010: Detection of *Vibrio vulnificus* in seafood, seawater and waste water samples from a Mediterranean coastal area. Microbiol Res **165**: 657-664.

Cariani A, Piano A, Consolandi C, Severgnini M, Castiglioni B, Caredda G, Candela M, Serratore P, De Bellis G, Tinti F 2012: Detection and characterization of pathogenic vibrios in shellfish by a ligation detection reaction-universal array approach. Int J Food Microbiol **153**: 474-482

Chakraborty RD, Surendran PK, Joseph TC 2008: Isolation and characterization of *Vibrio parahaemolyticus* from seafoods along the southwest coast of India. World J Microb Biot **24**: 2045-2054

Chao G, Jiao X, Zhou X, Yang Z, Huang J, Zhou L, Qian X 2009: Distribution, prevalence, molecular typing, and virulence of *Vibrio parahaemolyticus* isolated from different sources in coastal province Jiangsu, China. Food Control **20**: 907-912

Costa Sobrinho PS, Destro MT, Franco BDGM, Landgraf M 2011: Occurence and distribution of *Vibrio parahaemolyticus* in retail oysters in Sao Paulo State, Brazil. Food Microbiol **28**: 137-140

DePaola A, Kaysner CA 2004: *Vibrio*, Chapter 9. In: Food and Drug Administration, Bacteriological Analytical Manual. 8th Edition (Revision A), 1998, Washington, DC.

Ezzeldeen NA, Mansour HA, Ahmed AA 2011: Phenotypic and molecular identification of *Staphylococcus aureus* isolated from some Egyptian salted fish. World Appl Sci J **15**: 1703-1712

Friesema IH, De Jong AE, Fitz James IA, Heck ME, van den Kerkhof JH, Notermans DW 2012. Outbreak of *Salmonella* Thompson in the Netherlands since July 2012. Euro Surveill **17**: 1-4

Gopal S, Otta SK, Kumar S, Karunasagar I, Nishibuchi M, Karunasagar I 2005: The occurence of *Vibrio* species in tropical shrimp culture environments, implications for food safety. Int J Food Microbiol **102**: 151-159

Guerin PJ, De Jong B, Heir E, Hasseltvedt V, Kapperud G, Styrmo K, Gondrosen B, Lassen J, Andersson Y, Aavitsland P 2004: Outbreak of *Salmonella* Livingstone infection in Norway and Sweden due to contaminated processed fish products. Epidemiol Infect **132**: 889-895

Harrigan WF 1998: Laboratory Methods in Food Microbiology. 3rd edn, Academic Press, London

Hosseini H, Cheraghali AM, Yalfani R, Razavilar V 2004: Incidence of *Vibrio* spp. in shrimp caught off the South coast of Iran. Food Control **15**: 187-190

Hudecová K, Buchtová H, Steinhauserová I 2010: The effects of modified atmosphere packaging on the microbiological properties of fresh common carp (*Cyprinus carpio* L.). Acta Vet Brno **79**: 93-100

Ji H, Chen Y, Guo Y, Liu X, Wen J, Liu H 2011: Occurence and characteristics of *Vibrio vulnificus* in retail marine shrimp in China. Food Control **22**: 1935-1940

Kumar R, Surendran PK, Thampuran N 2009: Distribution and genotypic characterization of *Salmonella* serovars isolated from tropical seafood of Cochin, India. J Appl Microbiol **106**: 515-524

Lei IF, Roffey P, Blanchard C, Gu K 2008: Development of a multiplex PCR method for the detection of six common foodborne pathogens. J Food Drug Anal **16**: 37-43

Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, Bacchiocchi I 2005: Presence of pathogenic *Vibrio parahaemolyticus* strains in mussels from the Adriatic Sea, Italy. Food Microbiol **22**: 585-590

Popovic NT, Skukan AB, Dzidara P, Coz-Rakovac R, Strunjak-Perovic I, Kozacinski L, Jadan M, Brlek-Gorski D 2010: Microbiological quality of marketed fresh and frozen seafood caught of the Adriatic coast of Croatia. Vet Med **55**: 233-241

Rodriguez AI, Hariharan H, Nimrod S 2011: Occurence and antimicrobial drug resistance of potential bacterial pathogens from shellfish, including queen conchs (*Strombus gigas*) and whelks (*Cittarium pica*) in Grenada. Webmedcentral Microbiol **2**: WMC001943

Shabarinath S, Kumar HS, Khushiramani R, Karunasagar I, Karunasagar I 2007: Detection and characterization of *Salmonella* associated with tropical seafood. Int J Food Microbiol **114**: 227-233

Vazquez-Sanchez D, Lopez-Cabo M, Saa-Ibusquiza P, Rodriguez-Herrera JJ 2012: Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). Int J Food Microbiol **157**: 286-296

Woodring J, Srijan A, Puripunyakom P, Oransathid W, Wongstitwilairoong B, Mason C 2012: Prevalence and antimicrobial susceptibilities of *Vibrio, Salmonella* and *Aeromonas* isolates from various uncooked seafoods in Thailand. J Food Protect **75**: 41-47