



Occurrence and antimicrobial resistance of *Staphylococcus aureus* and *Salmonella* spp. in retail fish samples in Turkey



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ARTICLE INFO

Article history:

Available online 18 November 2014

Keywords:

ELISA
Fish
PCR
Salmonella spp.
S. aureus

ABSTRACT

The aims of this study were to investigate the presence of *Staphylococcus aureus* and staphylococcal enterotoxins, as well as *Salmonella* spp. and to determine the antimicrobial susceptibilities of the isolates from fish samples. A total of 100 fish samples were analysed consisting of 30 anchovy, 35 trout and 35 sea bream. The presence of SEs was detected using ELISA and its genes confirmed by mPCR. Also, *S. aureus* and *Salmonella* spp. were detected in 9 (9%) and 5 (5%) samples, respectively. None of the *S. aureus* isolates had SEs and SEs genes. The resistance rates of the *S. aureus* isolates to erythromycin, tetracycline, and penicillin G were found to be 33% while *Salmonella* spp. isolates were resistant to trimethoprim-sulfamethoxazole, gentamicin and neomycin in 20%, 20% and 80%, respectively of the samples. It is of utmost important for public health that retail fish markets need to use hygienic practices in handling and processing operations.

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1. Introduction

Seafood products are appreciated worldwide for their high nutritional value and are increasingly popular among consumers (Amagliani et al., 2012). Seafood is responsible for a significant amount of foodborne diseases and represents a great concern from a public health perspective. Bacterial load of raw fish depends on the environmental condition and microbial quality of the water where fish is hunted, temperature of the water, salt content of the water, distance of hunting area from areas contaminated with human and animal feces, fishing method and cooling conditions (Feldhusen, 2000; Saito et al., 2011). *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Listeria monocytogenes*, *Clostridium botulinum* and *Aeromonas hydrophila* are pathogenic bacteria that found naturally in the sea and rivers and they may infect to humans carried by fisheries (Calki and Kislak, 2003; Da Silva et al., 2010; Eklund et al., 2004). Bacteria like *Salmonella* spp., *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., and *Yersinia enterocolitica* can be found in fisheries due to fecal contamination of water (Herrera et al., 2006; Vieira et al., 2001; Vural and Erkan, 2006). Also toxigenic strains of *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium perfringens* may transmit through the

process of handling and processing that can be dangerous in terms of consumption of fishery products (Da Silva et al., 2010; Grigoryan et al., 2010; Huss et al., 2003; Normanno et al., 2005; Simon and Sanjeev, 2007). Aquatic environments are the major reservoirs of *Salmonella* and aid its transmission among the hosts (Shabarinath et al., 2007). *Salmonella* may contaminate seafood during the processing, and may cross-contaminate products during the various stages of preparation (Amagliani et al., 2012). *Salmonella* is not a component of the normal flora of sea animals, thus contamination of seafood is the consequence of fecal contamination through polluted water, infected food handlers or cross-contamination during production or transport. High prevalence is frequently attributed to poor hygienic practices during handling and transportation from landing centers to fish markets (Carrasco et al., 2012). In addition, this organism finds its way into the river water, coastal and estuarine sediments through fecal contamination (Shabarinath et al., 2007). *S. aureus* is a type of bacteria commonly found on the skin and in the noses and throats of human that can produce superantigen exotoxin with different characteristics (Ezzeldeen et al., 2011; Jorgensen et al., 2005). Toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxins (SEA, SEB, SEC 1, 2, 3, SED, SEE, SEG, SEH, SEI, SEJ and SEK) are produced by this bacterium and they can cause toxin-mediated diseases, such as toxic shock syndrome and food poisoning (Balaban and Rasooly, 2000; Orwin et al., 2001). *Staphylococcal* food poisoning is an illness that results from eating food contaminated with heat stable toxins,

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resulting in vomiting, retching and abdominal cramps, often accompanied by diarrhoea and sometimes fever (Dinges et al., 2000).

Using of antibiotics for therapeutic and growth promoters in food animals suggested that the main factor of the emergence of resistant isolates (Barber et al., 2003). Several studies have reported that food animals, meat, dairy and fishery products are contaminated by multi-resistant *S. aureus* strains that have been one of the common causes of severe nosocomial infections for a long time (Beleneva, 2011; Enright, 2003; Lee, 2003; Normanno et al., 2007; Pereira et al., 2009). Otherwise, a high number of studies have been reported the increasing in the occurrence of resistance among *Salmonella* spp. isolated from poultry, beef and fishery products (Cailhol et al., 2006; Newaj-Fyzul et al., 2006; Van Duijkeren et al., 2003).

In the present study was conducted to determine the incidence of *S. aureus*, SEs and *Salmonella* spp. in fish samples marketed in Kayseri, Turkey. Furthermore, this study aimed to detect antibiotic resistance of the isolates for investigate their potential threat for public health.

2. Material and method

2.1. Samples

In this study, a total of 100 fish samples [30 anchovy (*Engraulis encrasicolus*), 35 trout (*Oncorhynchus mykiss*) and 35 sea bream (*Sparus aurata*)] were examined between February and April 2013 in Kayseri in Turkey. The fish samples were purchased from different retail market and were immediately transported to the laboratory in a cool box and examined within 1–2 h.

2.2. Reference strain

Salmonella Typhimurium (ATCC 13311) reference strain was used as positive control for the *Salmonella* spp. Also, reference strains of *S. aureus* ATCC 29213 (SEA), *S. aureus* NCTC 10652 (SEA, SED), *S. aureus* NCTC 10654 (SEB), *S. aureus* NCTC 10655 (SEC) were used as positive controls in this study.

2.3. Primers

Eight primers, SA-U, SA-A, SA-B, SA-C, ENT-C, SA-D for *S. aureus* enterotoxin genes (Sharma et al., 2000), ST11 and ST15 for *Salmonella* spp. (Aabo et al., 1993) were used for mPCR assay (Table 1).

2.4. Microbiological analysis

The method proposed by ISO 6579 (ISO, 2002) was used for the isolation and the definition of *Salmonella* spp. from samples. Furthermore, isolation and identification of *S. aureus* were done according to the standards ISO 6888-3 (ISO, 2003).

2.5. Enzyme-linked immunosorbent assay (ELISA) for *S. aureus* enterotoxins

SEs were determined by using ELISA technique (Thermo, Finland) with commercially available kits (Ridascreen® SET A,B,C,D,E, r-biopharm, Germany, Art.no:R1101).

2.6. DNA extraction and PCR amplification

Total genomic DNA was extracted by using a commercial DNA extraction kit (Axygen, Bioscience, USA) according to the manufacturer's instructions. PCR was performed in a reaction mixture of 50 µL final volume containing 5 µL template DNA, 5 µL 10 × PCR buffer (Vivantis), 1.5 U Taq polymerase (Vivantis), 0.2 mM dNTP Mix (Vivantis), 3 mM MgCl₂ (Vivantis) and 25 pmol of each primer for *Salmonella* spp. and SEs genes of *S. aureus*.

PCR amplification of *Salmonella* spp. was performed with an initial denaturation of 95 °C for 1 min followed by 30 cycles, each consisting of 94 °C for 15 s, 57 °C for 15 s and 72 °C for 30 s. The final extension cycle was performed at 72 °C for 8 min (Techne TC-512). For the SEs genes, thermal cycling consisted of one cycle at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s followed by one final extension cycle at 72 °C for 2 min (Techne TC-512).

All of the amplified DNA were separated by electrophoresis at 100 V for 50 min in 1.5% (w/v) agarose gel and stained with ethidium bromide. Gels were visualized under an ultraviolet transilluminator Vilber Lourmat, Marne La Vallee, France).

2.7. Antibiotic susceptibility testing

The susceptibilities of antibiotics were determined by the standardized agar diffusion test (Bauer et al., 1996) on Muller-Hinton agar (Oxoid, CM0337) using the following antibiotic impregnated disks: ampicillin (AMP, 10 µg), cephalosporin (KZ, 30 µg), danofloxacin (DFX, 5 µg), enrofloxacin (ENR, 5 µg), gentamicin (CN, 10 µg), nalidixic acid (NA, 30 µg), neomycine (N, 10 µg), oxytetracycline (OT, 30 µg) and trimethoprim-sulphamethoxazol (SXT, 25 µg). Amoxicillin-clavulanic acid (AMC, 30 µg), clindamycin (DA, 2 µg), erythromycin (E, 15 µg), penicillin G (P 10U), tetracycline (TE, 30 µg) and ciprofloxacin (CIP, 5 µg). Zones of growth inhibition were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) standard (CLSI, 2009).

3. Results

In this study, 5 (5%) of 100 fish samples were detected as positive for *Salmonella* spp., comprised of 3 (10%) anchovy and 2 (6%) trout samples (Table 2). All of *Salmonella* spp. isolates were confirmed by PCR and the expected band length for amplified products were as 429 bp (Fig 1). In addition, 9 (9%) of 100 fish samples were found to be positive for Coagulase positive Staphylococci (CPS),

Table 1
Primers used in the study.

Primer	Description	Nucleotide sequence	PCR product size (bp)
SA-U	Universal forward primer for SE	5'-TGATGTATGGAGGTGTAAC-3'	–
SA-A	Reverse primer for sea	5'-ATTAACCGAAGTTCTGT-3'	270
SA-B	Reverse primer for seb	5'-ATAGTGACGAGTTAGTA-3'	165
SA-C	Reverse primer for sec	5'-AAGTACATTTTGAAGTCC-3'	69
ENT-C	Reverse primer for sec	5'-AATTGTGTTCTTTTATTTTCATAA-3'	102
SA-D	Reverse primer for sed	5'-TTCGGGAAAATCACCCTTAA-3'	306
SA-E	Reverse primer for see	5'-GCCAAAGCTGTCTGAG-3'	213
ST11	Reverse primer for <i>Salmonella</i> spp.	5'AGCCAACCATGTCTAAATTGGCGCA3	429
ST15	Forward primer for <i>Salmonella</i> spp.	5'GGTAGAAATCCACGGGGTACTG 3'.	

Table 2
The distributions of *Salmonella* spp. and *S. aureus* in fish samples.

Fish samples	Number of samples	Number of <i>Salmonella</i> spp. positive samples	Total of CPS positive samples	Distributions of <i>S. aureus</i> count (CFU/g)		Number of CPS positive isolates/ numbers of <i>S. aureus</i> positive isolates (%)
				<10 ² –10 ²	10 ³ –10 ⁶	
Anchovy	30	3 (10%)	5 (17%)	2 (40%)	3 (60%)	25/8 (32%)
Trout	35	2 (6%)	1 (3%)	1 (100%)	–	5/2 (40%)
Sea bream	35	–	3 (9%)	1 (33%)	2 (67%)	15/5 (33%)
Total	100	5 (5%)	9 (9%)	4 (44%)	5 (56%)	45/15 (33%)

consisted of 5 (17%) from anchovy, 1 (3%) from trout and 3 (9%) from sea bream. A total of 45 isolates obtained from 9 positive samples in this study. Fifteen (33%) strains were identified as *S. aureus*, being 8 (32%) from 25 anchovy, 2 (40%) from 5 trout and 5 (33%) from 15 sea bream. Total *S. aureus* counts determined on BPM (Oxoid) were between 1×10^2 and 1×10^6 CFU/g in the fish samples (Table 2). None of the isolates were determined for the synthesis of enterotoxins with ELISA technique. No SEs genes were identified in the fish isolates by using mPCR technique.

In respect to antibiotic resistance, while all *Salmonella* spp. isolates were susceptible to enrofloxacin, nalidixic acid, oxytetracycline, cefazolin, danofloxacin and ampicillin. A small percentage of the isolates were resistance to trimethoprim-sulphamethoxazole (20%), gentamicin (20%) and neomycin (80%). Also, all *S. aureus* isolates were susceptible to ciprofloxacin, amoxicillin-clavulanic acid, gentamicin and cephazolin. Resistance rates to erythromycin, tetracycline, and penicillin G were found to be 33%.

4. Discussion

In Turkey, there is wide range of water sources for the production of seafood. According to Aydin et al. (2011), fish and fishery product consumption has been steady increasing in Turkey. The most consumed fish species are anchovy and trout (Erdal and Esengun, 2008; Sen et al., 2008).

In our study, *Salmonella* spp. was detected in fish samples (5%) collected from different retail market. Relatively higher results were reported by Hatha and Lakshmanaperumalsamy (1997), Kumar et al. (2003), Kusumaningrum et al. (2012) and Budiati et al. (2013) at rates of 14.25%, 30%, 10.3% and 43.8% in fish

samples, respectively. In contrast, *Salmonella* spp was not found in fish samples according to Adesiyun (1993). Also, the high *Salmonella* spp. contamination rate (90%) was described by Jegadeeshkumar et al. (2010).

In this study, the incidence of *S. aureus* was determined as 9% (Table 2). On the contrary, higher results were reported by some authors (Abraham et al., 1998; Papadopoulou et al., 2007; Simon and Sanjeev 2007; Vázquez-Sánchez et al., 2012). The incidence range described by Normanno et al. (2005) was lower than our results.

In addition, the count of *S. aureus* was above 10^3 CFU/g in 5 (5antibiotic resistance and RAPD analysis of food i) of fish samples (Table 2). However, *S. aureus* count was above the maximum tolerable microbiological limit (10^2 CFU/g) for fish according to the Turkish Food Codex (Anonymous, 2001). Similar results were reported by several authors (Grigoryan et al., 2010; Saito et al., 2011; Vázquez-Sánchez et al., 2012). However, Herrera et al. (2006), reported *S. aureus* count lower than 10^2 CFU/g fish samples.

In our study, none of the samples were positive for SEs and SEs genes. This might be due to the fact that staphylococcal counts should reach approximately 10^6 CFU/g to produce enterotoxin (Necidova et al., 2009; Pelisser et al., 2009). In contrast to our results, Normanno et al. (2005), Simon and Sanjeev (2007), Ayulo et al. (1994), found SEs including SEA, SEB, SEC, SED. Several authors (Saito et al., 2011; Vázquez-Sánchez et al., 2012) have also reported that enterotoxin genes *sea*, *seb*, *sec*, *seg*, *seh* and *sei* are the most common in staphylococci isolated from fish samples.

S. aureus has been stated as the third significant agent of food-borne illness by fish and fishery products (EFSA, 2009). Even if SEs and SEs genes could not be obtained from isolates in the present study, a high levels of this bacterium (above 10^3 CFU/g) might be indicate that the fish consumption is potential threat of staphylococcal food poisoning in Kayseri, Turkey.

The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern (Zhao et al., 2003). The mechanism of resistance to antimicrobial agents can be due to many factors, such as changes in the bacterial cell wall permeability or target sites, enzymatic drug modifications and energy-dependent removal of antimicrobials via membrane-bound efflux pumps (Chen et al., 2004).

In our study, a small percentage of *Salmonella* spp. were resistant to trimethoprim-sulphamethoxazole, gentamicin and neomycin. In contrast, Kakatkar et al. (2011), were found high percentages (97%) of the *Salmonella* isolates from marine fish were resistant to at least one antibiotic and 82% of the isolates were resistant to more than one antibiotic. Also, Broughton and Walker (2009), reported that all tested *Salmonella* isolates were susceptible to neomycin. In the study conducted by Kusumaningrum et al. (2012), 1 (33.3%) of 3 *Salmonella* spp. isolated from fish were resistant to erythromycin. Similar to our result (except from gentamicin), Ponce et al. (2008), found that the majority of isolates were sensitive to ampicillin, tetracycline, chloramphenicol, gentamicin, sulfisoxazole, streptomycin

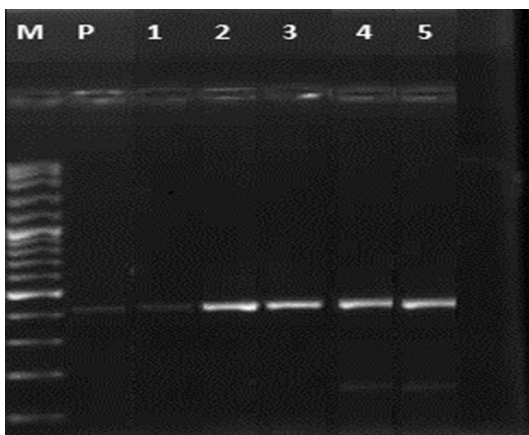


Fig. 1. Identification of *Salmonella* spp. genes from samples by PCR. Lane M, molecular weight marker (Gene Ruler™ 100 bp DNA Ladder Plus, Fermentas); lane P1: positive control for *Salmonella* spp. (ATCC 13311, 429 bp), Lane 1–5: fish isolates, for *Salmonella* spp.

and kanamycin. In contrast Broughton and Walker (2009), reported that all tested *Salmonella* isolates were susceptible to neomycin. Kakatkar et al. (2011), were found high percentages (97%) of the *Salmonella* isolates from marine fish were resistant to at least one antibiotic and 82 % of the isolates were observed multi-resistance. In the study conducted by Kusumaningrum et al. (2012), 1 (33.3%) of 3 *Salmonella* spp. isolated from fish were resistant to erythromycin.

S. aureus has advanced multidrug resistance throughout the world but wide variations in incidence exist regionally (Beleneva, 2011; Normanno et al., 2007; Pereira et al., 2009; Vázquez-Sánchez et al., 2012). Similar to our study, 125 *S. aureus* isolated from fish product were resistant to penicillin and tetracycline described by Vázquez-Sánchez et al. (2012) and Beleneva (2011). In our study, while all *S. aureus* isolates were susceptible to ciprofloxacin. Beleneva (2011), also found a high resistance to ciprofloxacin in fishery products.

In present study, 14 fish samples were contaminated with *Salmonella* spp. and *S. aureus*, did not comply with limits of Turkish Food Codex (Anonymous, 2001). In particular, *S. aureus* has been stated as the third significant agent of foodborne illness by fish and fishery products (EFSA, 2009). The isolates showed antimicrobial resistance to penicillin G, tetracycline, trimethoprim-sulfamethoxazole, gentamicin and neomycin. The antimicrobial resistant isolates might be hazardous for public health. Because of this reason it is important to continue surveillance and take some preventive control measures about resistant bacteria obtained from fish.

These results demonstrated that some hygienic conditions in the stages of fishery production were not suitable for public health. Therefore, enhanced hygienic practices in handling and processing from fishing to retail outlet is advised to supply the safety of fishery products.

References

- Aabo, S., Rasmussen, O.F., Rossen, L., Sorensen, P.D., Olsen, J.E., 1993. *Salmonella* identification by the polymerase chain reaction. *Mol. Cell. Probes* 7, 171–178.
- Abraham, A., Papa, A., Soultos, N., Ambrosiadis, I., Antoniadis, A., 1998. Antibiotic resistance of *Salmonella* spp. and *Listeria* spp. isolates from traditionally made fresh sausage in Greece. *J. Food Protect.* 61, 1378–1380.
- Adesiyun, A.A., 1993. Prevalence of *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp. and toxigenic *Escherichia coli* on meat and seafoods in Trinidad. *Food Microbiol.* 10, 395–403.
- Amagliani, G., Brandi, G., Schiavano, G.F., 2012. Incidence and role of *Salmonella* in seafood safety. *Food Res. Int.* 45, 780–788.
- Anonymous, 2001. Regulation which was published in official paper numbered as 24511. The Microbiological criteria regarding raw and milk products in Turkish Food Codex, Turkey.
- Aydin, H., Dilek, M.K., Aydin, K., 2011. Trends in fish and fishery products consumption in Turkey. *Turkish J. Fish Aquat. Sci.* 11, 499–506.
- Ayulo, A.M., Machado, R.A., Scussel, V.M., 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. *Int. J. Food Microbiol.* 24, 171–178.
- Balaban, N., Rasooly, A., 2000. Staphylococcal enterotoxins. *Int. J. Food Microbiol.* 61, 1–10.
- Barber, D.A., Miller, G.Y., McNamara, P.E., 2003. Models of antimicrobial resistance and food-borne illness: examining assumptions and practical application. *J. Food Protect.* 66, 700–709.
- Bauer, A.W., Kirby, M.M., Sherris, J.C., Truck, M., 1996. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45, 493–496.
- Beleneva, I.A., 2011. Incidence and characteristics of *Staphylococcus aureus* and *Listeria monocytogenes* from the Japan and South China seas. *Mar. Poll. Bull.* 62, 382–387.
- Broughton, E.L., Walker, D.G., 2009. Prevalence of antibiotic-resistant *Salmonella* in Fish in Guangdong, China. *Foodborne Pathog. Dis.* 6, 519–521.
- Budiati, T., Rusul, G., Wan-Abdullah, W.N., Arip, Y.M., Ahmad, R., Thong, K.L., 2013. Prevalence, antibiotic resistance and plasmid profiling of *Salmonella* in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture* 372, 127–132.
- Cailhol, J., Lailler, R., Bouvet, P., La Vieille, S., Gauchard, F., Sanders, P., Brisabois, A., 2006. Trends in antimicrobial resistance phenotypes in non-typhoid *Salmonellae* from human and poultry origins in France. *Epidemiol. Infect.* 134, 171–178.
- Calki, S., Kisla, D., 2003. Microbial spoilage of fishery products and prevention method. *EgeJFAS* 20, 239–245.
- Carrasco, E., Morales-Rueda, A., Garcia-Gimeno, R.M., 2012. Cross-contamination and recontamination by *Salmonella* in foods: a review. *Food Res. Int.* 45, 545–556.
- Chen, S., Zhao, S., White, D.G., Schroeder, C.M., Lu, R., Yang, H., McDermott, P.F., Ayers, S., Meng, J., 2004. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Appl. Environ. Microbiol.* 70, 1–7.
- Clinical and Laboratory Standards Institute (CLSI), 2009. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. CLSI document M100-S19. Wayne, PA.
- Da Silva, M.L., Matté, G.R., Germano, P.M.L., Matté, M.H., 2010. Occurrence of pathogenic microorganisms in fish sold in São Paulo, Brazil. *J. Food Safety* 30, 94–110.
- Dinges, M.M., Orwin, P.M., Schlievert, P.M., 2000. Enterotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev.* 13, 16–34.
- European Centre for Disease Prevention and Control (EFSA), 2009. The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA J.* 8 (1), 1496–1906.
- Eklund, M.W., Peterson, M.E., Poysky, F.T., Paranjpye, R.N., Pelroy, G.A., 2004. Control of bacterial pathogens during processing of cold-smoked and dried salmon strips. *J. Food Protect.* 67, 347–351.
- Enright, M.C., 2003. The evolution of resistant pathogen – the case of MRSA. *Curr. Opin. Pharmacol.* 3, 474–479.
- Erdal, G., Esengun, K., 2008. The analysis of the factors affecting fish consumption in Tokat province by logit model. *EgeJFAS* 25, 203–209.
- Ezzeldeen, N.A., Mansour, H.A., Ahmed, A.A., 2011. Phenotypic and molecular identification of *Staphylococcus* a isolated from some Egyptian salted fish. *World Appl. Sci. J.* 15, 1703–1712.
- Feldhusen, F., 2000. The role of seafood in bacterial foodborne diseases. *Microbes Infect.* 2, 1651–1660.
- Grigoryan, K., Badalyan, G., Andriasyan, D., 2010. Prevalence of *Staphylococcus aureus* in fish processing factory. *Potravinarstvo* 4, 25–28.
- Hatha, A.A.M., Lakshmanaperumalsamy, P., 1997. Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. *Food Microbiol.* 14, 111–116.
- Herrera, F.C., Santos, J.A., Otero, A., García-López, M.L., 2006. Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *J. Appl. Microbiol.* 100, 527–536.
- Huss, H.H., Ababouch, L., Gram, L., 2003. Assessment and management of seafood safety and quality. In: Huss, H.H., Ababouch, L., Gram, L. (Eds.), *FAO Fisheries Technical Paper*, 444. Food Agriculture Organization of the United Nations, Rome, Italy.
- International Organization for Standardization (ISO), 2002. Microbiology of food and animal feeding stuffs–horizontal method for the detection of *Salmonella* spp. International Standard (ISO 6579).
- International Organization for Standardization (ISO), 2003. Microbiology of food and animal feeding stuffs–horizontal method for the enumeration of coagulase-positive *Staphylococci* (*Staphylococcus aureus* and other species)–Part 3 Detection and MPN technique for low numbers. International Standard (ISO 6888-3).
- Jegadeeshkumar, D., Saritha, V., Moorthy, K., Sureshkumar, B.T., 2010. Prevalence, antibiotic resistance and RAPD analysis of food isolates of *Salmonella* species. *Int. J. Biol. Technol.* 1, 50–55.
- Jorgensen, H.J., Mork, T., Hogasen, H.R., Rorvik, L.M., 2005. Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *J. Appl. Microbiol.* 99 (1), 158–166.
- Kakatkar, A.S., Pansare, L.S., Gautam, R.K., Shashidhar, R., Karani, M., Bandekar, J.R., 2011. Molecular characterization of antibiotic resistant *Salmonella* isolates from Indian foods. *Food Res. Int.* 44, 3272–3275.
- Kumar, H.S., Sunil, R., Venugopal, M.N., Karunasagar, I., Karunasagar, I., 2003. Detection of *Salmonella* spp. in tropical seafood by polymerase chain reaction. *Int. J. Food Microbiol.* 88, 91–95.
- Kusumaningrum, H.D., Suliantari, Dewanti-Hariyadi, R., 2012. Multidrug resistance among different serotypes of *Salmonella* isolates from fresh products in Indonesia. *Int. Food Res. J.* 19, 57–63.
- Lee, J.H., 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microb.* 69, 6489–6494.
- Necidova, L., Stastkova, Z., Pos piová, M., Jans tova, B., Strejcek, J., Duskova, M., Karpiskova, R., 2009. Influence of soft cheese technology on the growth and enterotoxin production of *Staphylococcus aureus*. *Czech J. Food Sci.* 27, 127–133.
- Newaj-Fyzul, A., Adesiyun, A.A., Mutani, A., 2006. Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from apparently healthy ornamental fish and pond water in Trinidad. *J. Food Agric. Environ.* 4, 27–29.
- Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Decastelli, L., Mioni, R., Scutoa, S., Bolzoni, G., Di Giannatale, E., Salinetti, A.P., La Salandra, G., Bartoli, M., Zuccon, F., Pirino, T., Sias, S., Parisi, A., Quaglia, N.C., Celano, G.V., 2005. Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *Int. J. Food Microbiol.* 98, 73–79.
- Normanno, G., La-Salandra, G., Dambrosio, A., Quaglia, N.C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E., Celano, G.V., 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *Int. J. Food Microbiol.* 115, 290–296.
- Orwin, P.M., Leung, D.Y., Donahue, H.L., Novik, R.P., Schlievert, P.M., 2001. Biochemical and biological properties of staphylococcal enterotoxin K. *Infect. Immun.* 69, 360–366.
- Papadopoulou, C., Economou, E., Zakas, G., Salamoura, C., Dontorou, C., Apostolou, J., 2007. Microbiological and pathogenic contaminants of seafood in Greece. *J. Food Quality* 30, 28–42.

- Pelisser, M.R., Klein, C.S., Ascoli, K.R., Zotti, T.R., Arisi, A.C.M., 2009. Occurrence of *Staphylococcus aureus* and multiplex PCR detection of classic enterotoxin genes in cheese and meat products. *Braz. J. Microbiol.* 40, 145–148.
- Pereira, V., Lopes, C., Castro, A., Silva, J., Gibbs, P., Teixeira, P., 2009. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiol.* 26, 278–282.
- Ponce, E., Khan, A.A., Cheng, C.M., Summige-West, C., Cerniglia, C.E., 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. *Food Microbiol.* 25, 29–35.
- Saito, E., Yoshida, N., Kawano, J., Shimizu, A., Igimi, S., 2011. Isolation of *Staphylococcus aureus* from raw fish in relation to culture methods. *J. Vet. Med. Sci.* 73, 287–292.
- Sen, B., Canpolat, O., Sevim, A.F., Sonmez, F., 2008. The Evaluation of fish consumption in Elazığ. *SEJFU* 20, 433–437.
- Shabarinath, S., Kumar, H.S., Khushiramani, R., Karunasagar, I., Karunasagar, I., 2007. Detection and characterization of *Salmonella* associated with tropical seafood. *Int. J. Food Microbiol.* 114, 227–233.
- Sharma, N.K., Rees, C.E.D., Dodd, C.E.R., 2000. Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. *Appl. Environ. Microbiol.* 66, 1347–1353.
- Simon, S.S., Sanjeev, S., 2007. Prevalence of enterotoxigenic *Staphylococcus aureus* in fishery products and fish processing factory workers. *Food Control* 18, 1565–1568.
- Van Duijkkeren, E., Wannet, W.J.B., Houwers, D.J., Van Pelt, W., 2003. Antimicrobial susceptibilities of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. *J. Clin. Microbiol.* 41, 3574–3578.
- Vázquez-Sánchez, D., López-Cabo, M., Saá-Ibusquiza, P., Rodríguez-Herrera, J.J., 2012. Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). *Int. J. Food Microbiol.* 157, 286–296.
- Vieira, R.H.S.F., Rodrigues, D., Gocalves, F.A., Menezes, F.G.R., Aragao, J.S., Sousa, O.V., 2001. Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Rev. Inst. Med. Trop. São Paulo* 43, 145–148.
- Vural, A., Erkan, E.M., 2006. Microbiological quality parameters in fish of Dicle (Tigris) river near Diyarbakır City. *Dicle Med. J.* 33, 153–156.
- Zhao, S., Datta, A.R., Ayers, S., Friedman, S., Walker, R.D., White, D.G., 2003. Antimicrobial-resistant *Salmonella* serovars isolated from imported foods. *Int. J. Food Microbiol.* 84, 87–92.