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

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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 neomycine 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 parahemolyticus*, *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 Kisla, 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 stabil 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* entrotoxin 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 $MgCl_2$ (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), cephazolin (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′-TGTATGTATGGAGGTGTAAC-3′	–
SA-A	Reverse primer for sea	5′-ATTAACCGAAGGTTCTGT-3′	270
SA-B	Reverse primer for seb	5′-ATAGTGACGAGTTAGGTA-3′	165
SA-C	Reverse primer for sec	5′-AAGTACATTTTGTAAGTTCC-3′	69
ENT-C	Reverse primer for sec	5′-AATTGTGTTTCTTTTATTTTCATAA-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 *Salmonella* spp.	5′AGCCAACCATTGCTAAATTGGCGCA3	429
ST15	Forward primer for *Salmonella* spp.	5′GGTAGAAATTCCCAGCGGGTACTG 3′.	

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