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Original article

Molecular characterization and detection of enterotoxins, methicillin resistance genes and antimicrobial resistance of *Staphylococcus aureus* from fish and ground beef

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Abstract

A total of 120 samples including 40 freshwaterfish(*Oncorhynchus mykiss*), 40 seawater fish (*Sparus aurata*) and 40 ground beef samples were examined for the presence of *Staphylococcus aureus*. The isolates were identified using biochemical tests and a PCR for the species-specific fragment (Sa442) and thermonuclease gene (*nucA*). The presence of staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*), toxin genes (*eta*, *etb*, *tsst*), methicillin resistance gene (*mecA*) and some phenotypic virulence factors was also tested. Genotypic characterization of the isolates was analyzed by PCR-RFLP of the *coa* gene. Overall, 36 (30%) meat samples were contaminated with *S. aureus*. Of the 36 isolates, 3 (8.3%) were found to be positive for enterotoxin genes. Only 1 isolate (5.9%) from ground beef had the *sea* gene. In addition, 1 (12.5%) of the freshwater fish and 1 (9.1%) of the seawater fish carried both the *sea* and *sed* genes. The presence of *seb*, *sec*, *see*, *eta*, *etb* and *tsst* was not detected among the isolates of *S. aureus*. The amplified *coa* gene revealed five different clusters. Seven and six distinct RFLP patterns were obtained with *Alu*I and *Hae*III digestion, respectively. All isolates were found to be positive for slime, hemolytic and DNase activity while 41.7% of them were beta-lactamase positive. The presence of methicillin resistance was neither detected by PCR nor the disk diffusion method. A total of 94.4% of the isolates were resistant to at least one antimicrobial while 44.4% of them were resistant to at least two or more antimicrobials.

Key words: *Staphylococcus aureus*, enterotoxin, *nucA*, *mecA*, RFLP, meat, antimicrobial resistance

Introduction

Staphylococcus aureus is considered to be the most important pathogen among staphylococci responsible for a variety of severe infections including foodborne poisoning, skin and soft tissue infections, nosocomial pneumonia, mastitis and bacteremia in humans and animals (Le Loir et al. 2003, Becker and von Eiff 2011).

S. aureus is frequently present in the environment. The major habitats of *S. aureus* are the skin and mucous membranes of humans and animals. Nasal car-

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riers of *S. aureus* play a crucial role as a source of infection in dissemination of this pathogen among humans and to food through direct and indirect contact (Livermore 2000, Becker and von Eiff 2011). Therefore, *S. aureus* can be found commonly in various foods including meat (de Boer et al. 2009, Jackson et al. 2013) and fish (Da Silva et al. 2010, Saito et al. 2011). It is clear that contamination of food with this pathogen is often related to improper handling and storage conditions, as well as inadequate hygienic conditions and post-production microbial contamination (Ray 2004).

The presence of *S. aureus* in foods is of major public health concern due to its ability to produce a wide range of virulence factors that are involved in pathogenesis. These factors are enterotoxins, adhesion proteins, toxic shock syndrome toxin (TSST), exfoliative toxin (ETA, ETB), pore-forming hemolysins, ADP-ribosylating toxin and proteases (Bhunia 2008). In addition, staphylococcal enterotoxins (SEs) are involved in staphylococcal food poisoning (SFP) which occurs because of the consumption of food contaminated with one or more SEs preformed by enterotoxigenic *S. aureus* strains (Le Loir et al. 2003). SFP is associated with vomiting, nausea, abdominal cramps and diarrhea which occur 2 to 6 hours after food ingestion (Becker and von Eiff 2011). Since SEs are resistant to proteolytic enzymes, such as trypsin and pepsin, they keep their activity in the digestive tract after digestion (Le Loir et al. 2003). SEs encoding genes can be located on plasmids (*sed*), phages (*sea* and *see*) and pathogenicity islands (*seb* and *sec*) (Le Loir et al. 2003).

Molecular characterization can be accomplished by subtyping *S. aureus* using the restriction fragment length polymorphism (RFLP) method. Different sizes after PCR amplification of coagulase gene (*coa*) and DNA restriction endonuclease site polymorphisms at the 3' coding region of the *coa* gene have been exploited in the *coa* gene-based PCR-RFLP of *S. aureus* isolates (Hookey et al. 1998).

Antimicrobial resistance has dramatically increased worldwide due to the widespread use or abuse of antimicrobial agents (Livermore 2000). Beta-lactam antibiotics are frequently used in treatment of staphylococcal infections. However, an increasing resistance to beta-lactam antibiotics due to the production of beta-lactamase in *S. aureus* strains has been reported (Livermore 2000, Becker and von Eiff 2011). In the early 1960s, a new type of penicillin antibiotic called methicillin was developed and used to treat infections stemming from beta-lactamase producing strains of *S. aureus*. Nevertheless, most strains of *S. aureus* now show methicillin resistance which is mediated by the *mecA* gene located on a foreign, mobile DNA element called staphylococcal cassette chromosome *mec* (SCC*mec*). The *mecA* gene encodes a penicillin-binding protein (PBP), PBP2a which has an unusually low affinity for all beta-lactam antimicrobials (Livermore 2000, Becker and von Eiff 2011). Today, methicillin resistant *S. aureus* (MRSA) strains have become resistant to most common antibiotics. Therefore, treatment of infections in humans and animals caused by MRSA is quite difficult (Livermore 2000). In recent studies, antibiotic resistant *S. aureus* and MRSA strains have been reported in meat (de Boer et al. 2009, Guven et al. 2010) and fish (Hammad et al. 2012, Vazquez-Sanchez et al. 2012).

S. aureus causes serious diseases by producing various virulence factors and presents difficult therapeutic problems due to rapid development of resistance to many antimicrobial agents. The results of many studies on genetic diversity of *S. aureus* from different regions have been documented (Soltan et al. 2010, Jackson et al. 2013, Alibayov et al. 2014). In Turkey, limited data are available on genotypic characterization of *S. aureus* from fish and meat. Therefore, this study aimed to investigate the prevalence of *S. aureus* in fish and ground beef, to type the isolates based on the PCR-RFLP pattern analysis of the *coa* gene, to determine the presence of classical staphylococcal enterotoxin (*sea*, *seb*, *sec*, *sed* and *see*), exfoliative toxin genes (*eta*, *etb*), toxic shock syndrome toxin gene (*tsst*), methicillin resistance gene (*mecA*), and some phenotypic virulence factors as well as antimicrobial resistance profiles in *S. aureus* isolates.

Materials and Methods

Sample collection

A total of 120 meat samples, 40 freshwater fish (*Oncorhynchus mykiss*), 40 seawater fish (*Sparus aurata*) and 40 ground beef samples were purchased from various public bazaars, supermarkets and butchers in Bolu (Northwest Turkey) from October 2011 to December 2012. All samples purchased were placed in sterile bags and kept on ice during transportation and until they were analyzed in the laboratory.

Isolation and identification of *S. aureus*

10 g of ground beef and fish samples consisting of skin and flesh were taken aseptically and homogenized in 90 ml of sterile Tryptic Soy Broth (Merck, Darmstadt, Germany) supplemented with 7.5% NaCl (Merck) by stomacher (BagMixer 400, Interscience,

Paris, France) for 2 min. After incubation at 37°C for 24 h, all homogenates were serially diluted in sterile peptone saline solution (0.1% peptone and 0.85% NaCl) until 1:1000. The dilutions (0.1 ml) were streaked onto Baird-Parker Agar (Merck) supplemented with egg yolk tellurite emulsion (Merck). The plates were incubated for 48 h at 37°C. After incubation, presumptive *S. aureus* colonies were seen as circular, black, shiny, convex colonies with or without a white edge surrounded by a clear zone, with or without opaque zones from each plate which were transferred to Tryptic Soy Agar plates (Merck) (37°C/24 h). Typical *S. aureus* colonies were identified using the following tests: Gram staining, the production of coagulase, catalase, deoxyribonuclease (DNase), thermonuclease (TNase), hemolysis on 5% sheep blood agar, Voges-Proskauer test and fermentation of mannitol, trehalose and maltose (Götz et al. 2006, Becker and von Eiff 2011).

Identification of *S. aureus* by PCR

In addition to culturing and biochemical tests, identification of all *S. aureus* isolates was conducted using a PCR for the species-specific fragment (Sa442) and thermonuclease gene (*nuc*A) (Brakstad et al. 1992, Martineau et al. 1998). Genomic DNA was extracted using a GF-1 bacterial DNA extraction kit (Vivantis Technologies, Selangor DE, Malaysia) following the manufacturer's instructions for Gram positive bacteria.

The PCR reaction mixtures were prepared to make a final volume of 50 µl containing 1 µl of DNA template (50 ng/ µl), 5 µl of 10X PCR buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, 0.8% (v/v) nonidet P40) (Fermentas, Life Sciences, Burlington, Ontario, Canada), 1 mM of $MgCl_2$ (Fermentas), 0.2 mM of dNTP mix (Fermentas), 0.15 µM of each of the primers (Biomers, Ulm, Germany) and 1.25 U of Taq DNA polymerase (Fermentas). A total of 39.25 µl of molecular grade water (AppliChem, Darmstadt, Germany) was added to a final volume of 50 µl. All DNA amplifications were performed with the XP Thermal Cycler (Bioer Technology Co., Ltd., Tokyo, Japan). The amplification conditions proposed by Bartolomeoli et al. (2009) were used to target the Sa442 fragment. The amplification conditions of the *nucA* gene were as follows: an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, with a final extension at 72°C for 10 min. The PCR was performed three times with each isolate for all genes. The PCR products were subjected to electrophoresis on 2% (w/v) agarose gel containing ethidium bromide for 90 min at 75V. A DNA ladder of 100-3000 bp (Fermentas) was also included in all gels as a molecular size marker. Gels were visualized with a UV transillumination (DNR Minilumi Bio-imaging Systems, Jerusalem, Israel). During the experiments, *S. aureus* ATCC 25923 was used as a positive control for identification of *S. aureus* and detection of the Sa442 fragment as well as the *nuc*A gene.

Detection of toxin and the *mecA* genes

All *S. aureus* isolates were tested by PCR using primer pairs (Biomers) and cycling conditions for the presence of enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) and exfoliative toxin genes (*eta*, *etb*) as previously described by Johnson et al. (1991). The methicillin resistance gene (*mecA*) and toxic shock syndrome toxin gene (*tsst*) were analyzed according to Milheirico et al. (2007) and Lzvseth et al. (2004), respectively. PCR reaction mixture for the amplification of each toxin and the *mecA* gene was performed as mentioned above. The following strains were used as positive controls in this study: ATCC 25923 (*sea*), NCTC 10654 (*seb*), NCTC 10655 (*sec*), NCTC 10652 (*sed*), FRI913 (*see*, *tsst*) and a methicillin resistant *S. aureus* strain (*mecA*).

PCR-RFLP analysis of the *coa* gene

All identified *S. aureus* isolates were subjected to PCR for the *coa* gene determination using the primers and PCR conditions reported previously by Hookey et al. (1998). Amplified fragments of the *coa* gene were digested by both *Alu*I and *Hae*III restriction enzymes (Fermentas) according to the manufacturer's procedure. Phylogenetic analysis was carried out using the NTSYS-pc (version 2.10) software package. Each polymorphic RFLP band was scored 1 for presence and 0 for absence. Similarity among the isolates was determined using Dice's similarity and a dendogram was constructed with the unweighted pair group method using arithmetic average (UPGMA) clustering.

Determination of slime and beta-lactamase production

S. aureus isolates from fish and ground beef samples were examined for slime production using the Congo Red Agar method. In the test, the medium comprised of 37 g/l Brain Heart Infusion Broth, 50 g/l Sucrose, 10 g/l Agar, and 0.8 g/l Congo Red. Congo

Table 1. Phenotypic and genotypic characteristics of all *S. aureus* isolates from freshwater fish, seawater fish and ground beef.

Sample Type	Isolate	Phenotypic characteristics							Genotypic characteristics									
		DNase	TNase	Hemolysin	Slime	Beta-lactamase	Antimicrobial resistance profile[c]	Sa442 fragment	*nuc*A	*mecA*	*sea*	*seb*	*sec*	*sed*	*see*	*tsst*	*eta*	*etb*
Freshwater fish	A6/1	+[a]	+	+	+	–[b]	AMP	+[d]	+	–[e]	–	–	–	–	–	–	–	–
	A9/7	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	A11/2	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	A13/3	+	+	+	+	–	–[f]	+	+	–	–	–	–	–	–	–	–	–
	A27/7	+	+	+	+	–	AMP, E	+	+	–	–	–	–	–	–	–	–	–
	A32/1	+	+	+	+	+	AMP	+	+	–	+	–	–	+	–	–	–	–
	A34/6	+	+	+	+	–	AMP, FD	+	+	–	–	–	–	–	–	–	–	–
	A36/1	+	+	+	+	+	AMP, E, VA	+	+	–	–	–	–	–	–	–	–	–
Seawater fish	Ç 7/6	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç 8/2	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç11/3	+	+	+	+	–	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	Ç12/2	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç14/8	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç15/1	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç22/5	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	Ç29/1	+	+	+	+	+	AMP, VA	+	+	–	–	–	–	–	–	–	–	–
	Ç31/5	+	+	+	+	–	AMP, FD	+	+	–	–	–	–	–	–	–	–	–
	Ç32/3	+	+	+	+	+	AMP	+	+	–	+	–	–	+	–	–	–	–
	Ç33/4	+	+	+	+	–	E, VA	+	+	–	–	–	–	–	–	–	–	–
Ground beef	K4/7	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	K5/5	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	K6/2	+	+	+	+	+	AMP, VA	+	+	–	–	–	–	–	–	–	–	–
	K8/10	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	K11/3	+	+	+	+	+	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	K12/1	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	K13/1	+	+	+	+	+	AMP, VA	+	+	–	–	–	–	–	–	–	–	–
	K14/1	+	+	+	+	+	AMP, TE, VA	+	+	–	–	–	–	–	–	–	–	–
	K15/1	+	+	+	+	+	AMP	+	+	–	+	–	–	–	–	–	–	–
	K16/1	+	+	+	+	+	AMP	+	+	–	–	–	–	–	–	–	–	–
	K17/1	+	+	+	+	+	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	K19/2	+	+	+	+	+	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	K22/8	+	+	+	+	–	AMP	+	+	–	–	–	–	–	–	–	–	–
	K25/3	+	+	+	+	+	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	K28/3	+	+	+	+	+	AMP, TE	+	+	–	–	–	–	–	–	–	–	–
	K37/15	+	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–
	K40/10	+	+	+	+	–	AMP, TE	+	+	–	–	–	–	–	–	–	–	–

[a] positive reaction for phenotypic characteristics; [b] negative reaction for phenotypic characteristics; [c] AMP, ampicillin; E, erythromycin; FD, fusidic acid; TE, tetracycline; VA, vancomycin; [d] presence of gene; [e] absence of gene; [f] no resistance to antimicrobials tested

Red stain prepared as a concentrated solution and the medium were autoclaved at 121°C for 15 min. The stain was then added when the agar cooled to 55°C. The isolates were inoculated on the plates and incubated at 37°C for 24 h. Slime positive and negative isolates appeared as black and red colonies, respectively (Freeman et al. 1989). Beta-lactamase activity of isolates was performed using the acidometric strip method. For the test, penicillin and bromocresol purple were dissolved in NaOH solution. A strip of filter paper (Whatman No 1) was placed in a Petri dish. A few drops of the solution were then added on to the strip until the paper was almost saturated. A test organism, taken with a sterile loop, was spread on the strip. The bacteria were accepted to be beta-lactamase positive if the purple color changed in 5 min (Oberhofer and Towle 1982).

Antimicrobial susceptibility

All *S. aureus* isolates were tested for susceptibility to a range of antibiotics by the disk diffusion method in line with the Clinical and Laboratory Standards Institute (CLSI 2006, CLSI 2009). The 18 antibiotics (Oxoid, Basingstoke, UK) were chosen: ampicillin, oxacillin, cefazolin, cefotaxime, cefepime, amoxicillin-clavulanic acid, gentamicin, erythromycin, tetracycline, ciprofloxacin, teicoplanin, vancomycin, linezolid, quinupristin-dalfopristin, chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole, fusidic acid. The inoculum density of each bacterial suspension in Mueller Hinton Broth (Merck) was adjusted to 0.5 McFarland standard turbidity. The suspensions were then spread on Mueller Hinton Agar (Merck). The antibiotic disks were placed on the agar surface in such a way that they were sufficiently separated from one another to avoid overlapping of the inhibition zones. All plates were incubated at 37°C for 18 to 24 h. After incubation, the diameter of the inhibition zones was measured and interpreted as susceptible, intermediate or resistant in accordance with the CLSI (2006), except for the susceptibility results of isolates to fusidic acid which were evaluated according to the CLSI (2009).

Results

Out of the 120 meat samples, 36 (30%) were found to be positive for *Staphylococcus aureus*. The identification of *S. aureus* isolates by conventional methods was consistent with the results of molecular methods (Sa442 and *nucA* gene). The prevalence of *S. aureus* was 20% in the freshwater fish, 27.5% in the seawater fish and 42.5% in the ground beef. In this study, DNase, TNase, hemolysin (β-hemolysin) and slime production were detected in all *S. aureus* isolates. However, 15 (41.7%) of the 36 isolates were found to be positive for beta-lactamase production. Table 1 shows the presence of DNase, TNase, hemolysin, slime and beta-lactamase production of *S. aureus* isolates.

As shown in Table 1, 3 (8.3%) of the 36 *S. aureus* isolates tested by PCR were positive for some of the classical staphylococcal enterotoxin genes. Of the ground beef isolates, 1 (5.9%) was found to be positive for the *sea* gene. One isolate (12.5%) from the freshwater fish and one isolate (9.1%) from the seawater fish carried both the *sea* and *sed* genes. The presence of *seb*, *sec*, *see*, *eta*, *etb* and *tsst* was not detected among the isolates of *S. aureus*. All *S. aureus* isolates tested were negative for the *mecA* gene.

In this study, 36 *S. aureus* isolates from fish and ground meat were also tested for typing using the *coa* gene based PCR-RFLP method (Table 2, Fig. 1, 2). A single amplicon of the *coa* gene was detected among all isolates tested. PCR amplification of the *coa* gene revealed five different PCR products (A-E) in size ranging from approximately 442 bp to 730 bp (Table 2). Among the five types, type B with an amplicon of 553 bp was predominant (33.3%) followed by type C (27.8%), type D (19.4%), type A (11.1%) and type E (8.3%). PCR- RFLP with *Alu*I and *Hae*III restriction enzymes of the *coa* gene in *S. aureus* isolates generated seven and six different profiles, respectively (Fig. 1, 2). Only five isolates (K11/3, K14/1, K19/2, K17/1 and K37/15) were not digested with *Alu*I. The results revealed that *Alu*I RFLP profile 2 predominated, with 30.6% isolates and *Hae*III profile 1 predominated with 41.7% isolates (Table 2). The 10 and 11 distinct *coa* gene genotypes were observed after digestion with *Alu*I and *Hae*III, respectively. Among the *Alu*I genotypes, B2 and C2 were the most prevalent while B1 and C2 were the most prevalent in *Hae*III genotypes (Table 2).

Antimicrobial susceptibilities of all 36 *S. aureus* isolates from fish and ground beef are given in Table 3. The *S. aureus* isolates had the highest frequency of resistance to ampicillin (91.7%), followed by tetracycline (22.2%), vancomycin (16.7%) and erythromycin (8.3%). Isolates showed intermediate resistance to teicoplanin (36.1%) followed by erythromycin (8.3%) and cefepime (5.6%). A total of 94.4% of *S. aureus* isolates were resistant to at least one antimicrobial agent and 44.4% of them to at least two or more antimicrobials.

Table 2. RFLP patterns of the *coa* amplicons after digestion with endonuclease *Alu*I and *Hae*III.

coa-PCR type (Product size)	*Alu*I RFLP pattern	Genotypes/ Restriction fragments in bp	*Hae*III RFLP pattern	Genotypes/ Restriction fragments in bp
A (442 bp)	Profile 1 (16.7%)	A1 (11.1%)/ 310, 339	Profile 1 (41.7%)	A1 (11.1%)/ 310, 380
B (553 bp)	Profile 2 (30.6%)	B1 (8.3%)/ 310, 339	Profile 2 (19.4%)	B1 (25%)/ 310, 475
C (609 bp)	Profile 3 (19.4%)	B2 (25%)/ 339, 420	Profile 3 (5.6%)	B2 (8.3%)/ 320
D (677 bp)	Profile 4 (2.8%)	C1 (5.6%)/ 339, 420	Profile 4 (13.9%)	C1 (5.5%)/ 310, 380
E (730 bp)	Profile 5 (8.3%)	C2 (16.7%)/ 339	Profile 5 (8.3%)	C2 (16.7%)/ 310, 475
	Profile 6 (2.8%)	C3 (2.8%)/ 310, 339, 375	Profile 6 (11.1%)	C3 (2.8%)/ 310, 580
	Profile 7 (5.6%)	C4 (2.8%)/ 375, 453		C4 (2.8%)/ 320
		D1 (2.8%)/ 375, 453		D1 (2.8%)/ 310, 380
		D2 (2.8%)/ 310, 420		D2 (2.8%)/ 310, 580
		E1 (8.3%)/ 310, 375, 420		D3 (13.8%)/ 395, 475
				E1 (8.3%)/ 310, 340, 395

Table 3. Antimicrobial susceptibility patterns of *S. aureus* from fish and ground beef samples.

Antimicrobials (Disk content)	Class of Antimicrobials	Number of isolates (%)		
		resistant	intermediate	susceptible
Ampicillin (10 µg)	Penicillins	33 (91.7)	0 (0)	3 (8.3)
Oxacillin (1 µg)	Penicillins	0 (0)	0 (0)	36 (100)
Cefazolin (30 µg)	Cephalosporins	0 (0)	0 (0)	36 (100)
Cefotaxime (30 µg)	Cephalosporins	0 (0)	0 (0)	36 (100)
Cefepime (30 µg)	Cephalosporins	0 (0)	2 (5.6)	34 (94.4)
Amoxicillin-clavulanic acid (20/10 µg)	Beta-lactamase inhibitors	0 (0)	0 (0)	36 (100)
Gentamicin (10 µg)	Aminoglycosides	0 (0)	0 (0)	36 (100)
Erythromycin (15 µg)	Macrolides	3 (8.3)	3 (8.3)	30 (83.3)
Tetracycline (30 µg)	Tetracyclines	8 (22.2)	0 (0)	28 (77.8)
Ciprofloxacin (5 µg)	Fluoroquinolones	0 (0)	0 (0)	36 (100)
Teicoplanin (30 µg)	Glycopeptides	0 (0)	13 (36.1)	23 (63.9)
Vancomycin (30 µg)	Glycopeptides	6 (16.7)	0 (0)	30 (83.3)
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	Folate pathway inhibitors	0 (0)	0 (0)	36 (100)
Linezolid (30 µg)	Oxalidinones	0 (0)	0 (0)	36 (100)
Quinupristin-dalfopristin (15 µg)	Streptogramins	0 (0)	0 (0)	36 (100)
Chloramphenicol (30 µg)	Phenicols	0 (0)	0 (0)	36 (100)
Clindamycin (2 µg)	Lincosamides	0 (0)	0 (0)	36 (100)
Fusidic acid (10 µg)	Steroidal	2 (5.6)	0 (0)	34 (94.4)

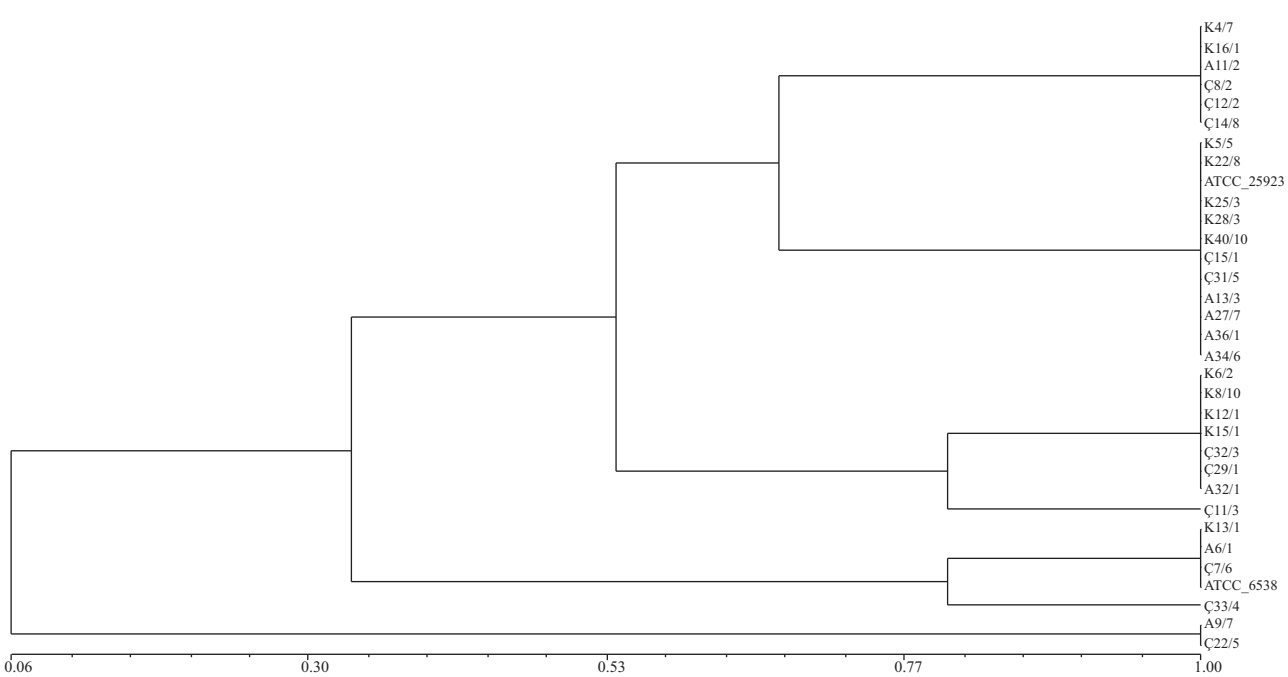

Fig. 1. Dendogram of *S. aureus* isolates after digestion with *Alu*I restriction enzyme.

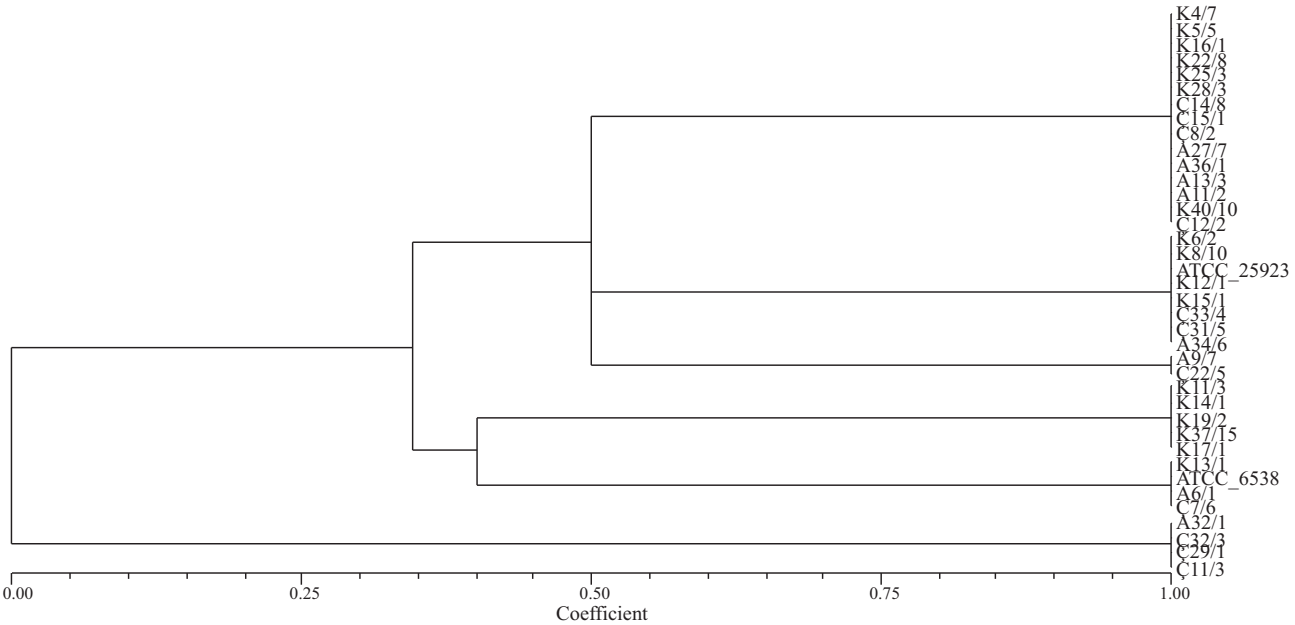

Fig. 2. Dendogram of *S. aureus* isolates after digestion with *Hae*III restriction enzyme.

Discussion

S. aureus has a ubiquitous nature; it is found in the environment, humans, animals and foods. Therefore, contamination of foods with *S. aureus* can occur frequently. The presence of *S. aureus* in foods may be considered a significant risk for foodborne diseases (Ray 2004, Bhunia 2008, Soltan Dallal et al. 2010).

In this study, the incidence of *S. aureus* in the ground beef was 42.5%, which was similar to the result (40%) reported by Gundo0an et al. (2013). A considerably high prevalence (69.2%) of *S. aureus* in ground beef was reported by Jackson et al. (2013). On the other hand, a quite low incidence (11.1%) of *S. aureus* from ground beef was observed by Guven et al. (2010). The prevalence of *S. aureus* in meat and meat products in other studies has ranged from 3.5% to 63% (Soltan Dallal et al. 2010, Guven et al. 2010, Jackson et al. 2013). The contamination rates of meat with *S. aureus* may be affected by several factors such as the environment, water quality, equipment, personnel, the health of the animal, slaughterhouse sanitation, slaughtering practices, and carcass contamination with intestinal contents during slaughtering, transport, handling and storage. It is quite clear that contamination of meat with *S. aureus* is common and often inevitable (Ray 2004, de Boer et al. 2009).

The present study also provides information on the incidence, enterotoxigenic potentials and antimicrobial resistance of *S. aureus* in fish as a vital source of nutrition to humans due to their proteinaceous nature. Although many researchers have reported the presence of *S. aureus* in various foods such as meat and dairy products, there are a limited number of reports on contamination of fish with *S. aureus* (Simon and Sanjeev 2007, Aydyn et al. 2011, Saito et al. 2011). In our study, the prevalence of *S. aureus* in all fish samples tested was 23.8% (19/80). The isolation rates of *S. aureus* were found to be 10% and 19.6% in fishes reported by Da Silva et al. (2010) and Saito et al. (2011), respectively. It is possible to say that the incidence rate of *S. aureus* in fish may be influenced by geographical location, the season, the method of harvest, post-harvest handling and storage (Ray 2004, Hammad et al. 2012).

S. aureus as a major pathogen produces a number of virulence factors which are responsible for pathogenicity such as adhesions, enzymes and toxins. This study showed that all *S. aureus* isolates were positive for production of DNAse which is responsible for the degradation of the host cell DNA (Bhunia 2008). Compared with our result, a lower incidence of production of DNase (53%) was reported by Gundo0an et al. (2013) in meat and milk products. Furthermore, in the present study, the percentage of hemolytic activity of *S. aureus* which involves hemolysins in the destruction of blood and tissue cells (Götz et al. 2006) was higher than those obtained by Pereira et al. (2009), Gündoğan et al. (2013) and Alibayov et al. (2014). Slime, another virulence factor, makes strains much more able to survive in the normally hostile environment of tissue and blood (Götz et al. 2006). Our slime production rate of *S. aureus* (100%) was found to be higher than that (80%) obtained by Gündoğan et al. (2013).

Many studies have implicated food-harbored staphylococcal enterotoxins as major factors associated with staphylococcal food poisoning (SFP). The presence of enterotoxigenic *S. aureus* in foods poses a potential hazard for the development of SFP (Le Loir et al. 2003, Ray 2004). Our study showed that only 1 (5.9%) of the 17 *S. aureus* isolates from the ground beef had the *sea* gene. Enterotoxigenic *S. aureus* strains, in previous studies, were reported to be present in 85% of retail meats (Pu et al. 2011), 87% of raw meat samples (Pereira et al. 2009), and 100% of meat samples (Aydyn et al. 2011). In contrast, a study by Sudagidan and Aydyn (2008) in Turkey failed to detect any enterotoxigenic *S. aureus* strains in meat and meat products. On the other hand, in our study, 2 (10.5%) *S. aureus* isolates from fish were found to be enterotoxigenic. Compared with our result, a much higher incidence (68.1%) of the enterotoxigenic strains was observed in the retail fish samples reported by Saito et al. (2011). Furthermore, prevalence rates of enterotoxigenic *S. aureus* strains in fishery products ranging from 17% to 91.2% were reported in previous studies (Simon and Sanjeev 2007, Vazquez-Sanchez et al. 2012, Alibayov et al. 2014). Among the SEs, SEA and SED are the two most often reported enterotoxins responsible for food poisoning (Bhunia 2008). In this study, the *sea* and *sed* were the most commonly detected genes. Also, Aydyn et al. (2011) reported that 8.6% of *S. aureus* strains from food were positive for the *sea* gene, similar to our result. Our results showed that none of the isolates contained the *seb, sec*, *see*, *eta*, *etb*, *tsst*, genes, which is consistent with the report by Pu et al. (2011).

Coagulase gene-based PCR-RFLP has been considered a simple, relatively rapid and accurate method for differentiating *S. aureus* isolates (Hookey et al. 1998). In a previous study (Soltan Dallal et al. 2010), *S. aureus* strains from meat and dairy products were genotyped using PCR-RFLP based on the *spa* gene. Many studies are available on *coa* typing of *S. aureus* isolates from various clinical laboratories and mastitis cases (Saei et al. 2009, Lim et al. 2012). However, there are few data related PCR-RFLP analyses of the *coa* gene in *S. aureus* isolates from food. In this study, only five *S. aureus* isolates from ground meat samples were not digested with restriction enzyme *Alu*I. Similarly, a study by Saei et al. (2009) also showed that *Alu*I enzyme did not digest the *coa* gene PCR products of *S. aureus i*solates from bovine mastitis milk samples. Consequently, Saei et al. (2009) suggest that there is no restriction site for this enzyme in the variable region of *coa* gene in these isolates, and this finding can be explained by the presence of genetic alterations in a polymorphic repeated region of the *coa* gene because of point mutations. As mentioned earlier, isolates belonging to type B and C were the predominant *coa*- PCR types. Remarkably, all isolates included type A (442) and E (730) yielded the same RFLP pattern, indicating no heterogeneity in both *Alu*I and *Hae*III recognition sites. The other *coa*-PCR types such as B, C, and D generated different RFLP patterns. Among the *S. aureus* isolates, type C with an amplicon of 609 bp digested with *Alu*I and *Hae*III has a greater genetic diversity than other *coa*-PCR types. Hookey et al. (1998) indicated that the use of both *Alu*I and *Hae*III restriction endonucleases to digest the *coa* gene was beneficial in confirming the distinct RFLP patterns in *S. aureus* isolates, similar to our result.

An increasing resistance to beta-lactam antibiotics including penicillin derivatives, cephalosporins, monobactams and carbapenems in *S. aureus* strains has been established. Beta-lactam antibiotics are frequently used in the treatment of staphylococcal infections (Livermore 2000, Becker and von Eiff 2011). Our results indicated that most of the *S. aureus* isolates (91.7%) had resistance to ampicillin, a beta-lactam antibiotic. Similarly, high levels of ampicillin resistance in *S. aureus* have been reported by several researchers (Gündoğan et al. 2013, Alibayov et al. 2014). On the other hand, production of beta-lactamases is the most common mechanism of bacterial resistance to beta-lactam antibiotics. In our study, only 15 isolates (41.7%) were found to be positive for beta-lactamase production while a high level of ampicillin resistance was obtained. In the present study, resistance levels of *S. aureus* isolates to tetracycline, vancomycin, and erythromycin were found as 22.2%, 16.7%, and 8.3%, respectively. However, Alibayov et al. (2014) demonstrated slightly higher resistance percentages to tetracycline (37.6%) and erythromycin (12.9%) among the *S. aureus* strains. A similar result with respect to vancomycin (21.7%) was demonstrated by Guven et al. (2010).

Methicillin resistance mediated by the *mecA* gene has been detected firstly in hospitals and is now also increasingly recognized in the community (Becker and von Eiff 2011). In this study, none of the isolates was oxacillin (methicillin) – resistant and none of them carried the *mecA* gene. Similarly, the absence of the *mecA* gene in *S. aureus* from meat was reported in earlier studies (Sudagidan and Aydin 2008, Pereira et al. 2009). However, the incidence of MRSA strains containing the *mecA* gene in various foods, such as fish and meat, has been reported in several countries: 1.1% in retail ready-to-eat raw fish in Japan (Hammad et al. 2012), 11.1% in fish in the Czech Republic, 4% in beef in the USA (Jackson et al. 2013), 10.6% in raw beef in the Netherlands (de Boer et al. 2009).

In the present study, the prevalence of *S. aureus* as an important foodborne pathogen as well as its genotypic characterization, enterotoxin and methicillin resistance gene (*mecA*) have been determined. The antimicrobial resistance profiles and some phenotypic characteristics responsible for pathogenicity of *S. aureus* have also been revealed. As a result, a low incidence of enterotoxigenic *S. aureus* isolates has been detected in fish and ground beef. No matter how low the incidence is, the prevalence, enterotoxigenic potentials and antimicrobial resistance of *S. aureus* should regularly be monitored all together because this microorganism is considered as a potential hazard for consumers and food safety.

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