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Biofilm, protease and lipase properties and antibiotic resistance profiles of staphylococci isolated from various foods

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In this study, a total of 209 isolates were isolated from raw calf meat (minced), chicken drumsticks, raw milk, ice cream and white cheese samples. One hundred and seventy-five (83.7 %) coagulase-negative Staphylococci (CNS) and 34 (16.3%) coagulase-positive staphylococci (CPS) were isolated from these samples. The majority of *Staphylococcus* isolates showed biofilm formation (75.1%) and slime formation (68.4%). The frequency of positive protease and lipase production for *Staphylococcus aureus* isolates were 23.5 and 11.8%, respectively. Proteolytic and lipolytic activitiy were not found in the other *Staphylococcus* species. The overall antimicrobial susceptibility profiles revealed that the highest percentage of resistance was detected for ampicillin (33.8%), tetracycline (26.3%), erythromycin (20.6%), methicillin (17.2%) and gentamicin (12.4%). The susceptibility to vancomycin, chloramphenicol, amikacin and clindamycin were 100% for all isolates.

Key words: Coagulase positive, coagulase negative *Staphylococcus*, biofilm, slime, lipase, protease, antibiotic resistance, foods.

INTRODUCTION

The coagulase-positive *Staphylococcus aureus* can cause a number of animal diseases such as mastitis, suppurative disease, and urinary tract infections, which may lead to pneumonia, wound infections and septicemia. Despite its pathogenicity, *S. aureus* is present on the skin and mucous membranes of humans and animals, and also as environmental contaminants (Abulreesh, 2011). As a consequence, food products may orginally become contaminated during or after processing (Acco et al., 2003). The contamination of foodstuffs by *S. aureus* is an important cause of food poisoning (Normanno et al., 2007). The Coagulase-Negative Staphylococci (CNS) are a major component of the normal microbial communities of the human body, colonizing preferably in the upper airways and skin. For long time, *S. aureus* has been believed to be the only pathogen in this genus, while the CNS have been expected to be saprophytic or rarely pathogenic. However, there are some CNS species such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* that play a role in nosocomial and bloodstream infections. Being essentially opportunistic microorganisms, CNS can cause severe infections, especially among immunocompromised people that are often difficult to treat due to its relatively high prevalence of multi resistant strains. Some CNS species (*S. carnosus*, *S. condimenti*, *S. equorum, S. piscifermentans*, *S. succinus* and *S. xylosus*) are either associated with foods or play a major role in the food processing industry (Irlinger, 2008).

Staphylococci have been described as bacteria which may attach to the contact surfaces in both milk and meat processing industries, form biofilms and survive on them.

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Their attachment to food contact surfaces in food processing plants and subsequent biofilm formation pose a risk of contamination in milk and meat products. Bac-terial contamination of foodstuffs can lead to their decay or transmission of diseases (Schlegelova et al., 2008). Extracellular lipase and protease are produced by most microorganisms under suitable conditions. Storage of milk for long periods at refrigertaion temperatures has resulted in quality problems for dairy industry. The psychrotrophs are able to grow at refrigeration temperatures and alter the milk by producing heat resistant proteolytic enzymes which include degredation of casein (Parkash et al., 2007). Many psychrotrophic bacteria are also lipolytic. Lipolytic bacteria catalyse the hydrolysis of fats to fatty acids and glycerol. The main source for isolation of lipolytic microorganisms is milk, butter and other dairy products (Cempirkova and Mikulova, 2009). The spoilage of dairy products thus result in the production of many off-flavours, which are characterized as fruity, musty, bitter, rancid and even putrid (Parakasah et al., 2007; Cempirkova and Mikulova, 2009).

Antimicrobial agents used in therapy and as feed supplements to promote growth in food animals may increase the spread of drug-resistant bacteria. Such bac-teria may contaminate milk or meat and are subsequently found in fermented food made of such raw material (Normanno et al., 2007). Staphylococci were isolated from different milk and meat products and screened for their antibiotic resistance pattern (Normanno et al., 2007; Abulreesh, 2011). The objective of the present study is to investigate the biofilm, slime, lipolytic and proteolytic properties and antibiotic resistance profiles of staphy-lococci isolates from meat and milk products processed for human consumption in Ankara, Turkey.

MATERIALS AND METHODS

Sample collection and preparation

In this study, 56 samples of raw calf meat (minced), 56 samples of chicken drumsticks, 56 samples of raw milk, 56 samples of ice cream and 56 samples of Turkish white cheese were collected from various supermarkets, dairy plants and pastry shops in Ankara, Turkey, between June 2009 and August 2010. White cheese and meat samples were collected in sterile polyethylene packs and milk samples were collected in disposable plastic bottles, transported on ice to the laboratory, and analyzed within 2 h. Ice cream samples were collected in sterile jars and transported to the laboratory in a deep freezer and stored at -18°C. The samples were kept at 4°C for 10 min before microbiological analyses.

Isolation and identification of isolates

Food samples of 25 g or 25 ml were diluted with 225 ml of 1% buffered peptone water (BPW; Oxoid, Basingstoke, Hampshire, UK) and homogenized in a stomacher (Lab. Lemco 400, Seward, Worthington, UK) for about 10 min. From each prepared sample, 0.1 ml was streaked on to 5% sheep blood agar and incubated aerobically at 37°C for 48 h. After incubation, suspect colonies were examined by Gram staining. The colonies with morphologies compatible with *Staphylococcus* spp. were transferred to tryptic soy broth (TSB; Oxoid, Basingstoke, Hampshire, UK) and tryptic soy agar (TSA; Oxoid, Basingstoke, Hampshire, UK). After growth, *Staphylococcus* spp. were identified on the basis of colony characteristics, Gram staining, pigment production, hemolysis and the following biochemical reactions: catalase activity, coagulase test (rabbit plasma), oxidase test, O/F test with glucose, resistance to bacitracin (0.04 U), mannitol fermentation on Chapman Agar, urease, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose, maltose, fructose, lactose, mannose) fermentation tests (Murray et al., 2003). The API ID Staph (Bio Merieux SA, Marcy-l'Etoile, France) was used to determine the species more precisely. The isolates were kept frozen at -20°C in skim milk containing 15% (v/v) glycerol, until assayed. The *S. aureus* ATCC 25923 was used as a control strain.

Coagulase test

Coagulase activity was determined by the method described by Quinn et al. (1994). This test was performed as a tube coagulase (TC) test. Several colonies of each organism were mixed with 0.5 ml of citrated rabbit plasma in a sterile test tube. The tube was incubated at 37°C and examined after 4 and 24 h. Clot formation at either reading was recorded as positive.

Detection of slime formation by the Congo Red Agar method (CRA)

Production of slime from all isolates was studied by cultivation of the isolates on Congo Red Agar (CRA). CRA plates [sucrose 50 g (Sigma, St. Louis, MO), brain heart infusion broth 37 g (Oxoid, Basingstoke, Hampshire, UK), agar 10 g, congo red 0.8 g (Sigma, St. Louis, MO), distilled water 1000 ml] were incubated at 37°C for 24 h. After incubation, bright black colonies were established as slime positive (Gundogan et al., 2006).

Detection of quantitative biofilm formation by the microplate method (MP)

Biofilm-forming ability was measured by determination of adhesion to polystyrene microtiter plates according to the protocol of Christensen et al. (1985). Briefly, isolates were inoculated in TSB (tryptic soya broth, Oxoid) and incubated for 18 h at 37°C. After a 1:40 dilution in TSB supplemented with 0.25% glucose, 200 µl of each dilution were distributed in flat-bottom 96-well polystyrene plates (Oxyvital, Hong Kong, China). The plates were incubated for 18 h at 37°C, washed 3x with phosphate buffer saline (PBS), pH 7.0, air-dried for 1 h at 60°C and stained with 0.25% crystal violet for 1 min. After washing, optical density (OD) of each well content was measured at 570 nm using an automated Multiskan reader (GIO. De Vitae, Rome, Italy). We defined the cut-off OD (OD_c) for the microtiter-plate test as three standard deviations above the mean OD of the negative control. The adherence ability of the tested strains was classified into four categories based on the OD: "$OD \leq OD_c$: non–adherent, $OD_c < OD \leq 2XOD_c$: weakly adherent, $2XOD_c < OD \leq 4XOD_c$: moderately adherent, $4XODc < OD$: strongly adherent". All tests were carried out three times and the results were averaged.

Screening for lipase activity

Nutient agar (NA, Oxoid, Basingstoke, Hampshire, UK) containing

1% tributyrin (Fluka, Buchs, Germany) was used to study lipolytic activity. The isolates were subcultured on TSA and incubated at 37°C for 24 h. They were inoculated on tributyrin agar plates and incubated at 37°C for 72 h. The presence of clear zones was taken as an indication of positive lipase activity (Saising et al., 2012).

Screening for protease activity

Nutrient agar supplemented with 2% casein (Sigma, St. Louis, MO) was used to screen for protease activity. The isolates were subcultured on TSA and incubated at 37°C for 72 h. They were inoculated on casein agar plates and incubated at 37°C for 72 h. The isolates producing opalescent zones around the colony were identified as protease positive (Saising et al., 2012).

Susceptibility to antimicrobial agents

Antibiotic susceptibilities of the staphylococci strains were determined by the disk diffusion method (CLSI, 2006) on Mueller-Hinton agar plates (Oxoid, Basingstoke, Hampshire, UK). The following antimicrobial susceptibility test disks (Oxoid, Basingstoke, Hampshire, UK) were used: oxacillin (1 µg), ampicillin (10 µg), gentamicin (10 µg), amikacin (30 µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30 µg), tetracyline (30 µg) and vancomycin (30 µg). The disks containing 1 µg oxacillin (zone diameter ≥ 13 mm sensitive, ≤ 10 resistant) were used for resistance to methicillin (CLSI, 2006). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 served as controls to ensure the accuracy of testing.

Statistical analysis

The Chi-square (χ^2) tests were used to determine statistically significant differences in the prevalence of CPS and CNS in food samples. Significant differences between CPS, CNS and slime and biofilm formation and, lipase and protease activity were also determined. P values of less than 0.05 were considered significant.

RESULTS

Origin of isolates and species distribution of the staphylococci are given in Table 1. Out of the total 209 staphylococci isolates, 34 (16.3%) isolates were identified as coagulase positive staphylococci (CPS), whereas 175 (83.7%) isolates were coagulase negative staphylococci (CNS) by the tube coagulase test. Present study showed that the prevalence of CNS species in food samples is significantly higher than those CPS species (P <0.05). The 46 CPS isolates were identified as *S. aureus* (8.1%), *S. intermedius* (6.2%) and *S. hyicus* (1.9%). The 175 CNS isolates were identified as 46 *S. cohnii* (22.0%), 36 *S. xylosus* (17.2%), 34 *S. hominis* (16.2%), 20 *S. simulans* (9.5%), 13 *S. capitis* (6.2%), 9 *S. haemolyticus* (4.3%), 7 *S. epidermidis* (3.3%), 6 *S. auricularis* (2.9%), 3 *S. warneri* (1.4%) and 1 *S. saprophyticus* (0.4%) (Table 1). The production rate of slime and biofilm of *Staphylococcus* species is shown in Table 2. The frequency of positive slime and biofilm formation for CPS strains were 50 and 52.9% and for CNS strains were 72 and 79.4%, respectively. The statistical analyses revealed that the prevalence of slime and biofilm production in CNS was significantly higher than those CPS (P <0.05). The slime production was detected in 86.1% *S. xylosus*, 85.7% *S. epidermidis*, 84.6% *S. capitis*, 77.8% *S. haemolyticus*, 73.6% *S. hominis*, 70.0% *S. simulans*, 69.6% *S. cohnii*, 64.7% *S. aureus* and 46.2% *S. intermedius* strains, but none of the *S. hyicus*, *S. auricularis*, *S. warneri* and *S. saprophyticus* strains.

Biofilm producers were divided into three groups, namely strong, moderate and weak biofilm producers. Out of the 157 isolates, 87 (55.4%) were assessed as weak, 34 (21.7%) as moderate, 36 (22.9%) as strong biofilm producers (data not shown). *S. xylosus* was the highest biofilm-producing strain (100%), followed by *S. haemolyticus* (88.9%), *S. epidermidis* (85.7%), *S. capitis* (84.6%), *S. simulans* (80.0%), *S. hominis* (79.4%), *S. cohnii* (76.1%), *S. aureus* (70.6%) and *S. intermedius* (46.2%). None of the *S. hyicus*, *S. auricularis*, *S. warneri* and *S. saprophyticus* strains had biofilm formation. In the present study, the frequency of positive protease and lipase production for *S. aureus* isolates were 23.5 and 11.8%, respectively. Proteolytic and lipolytic activities were not found in the other *Staphylococcus* species (data not shown). Table 3 indicates the antimicrobial resistance rates of staphylococci in food samples investigated. Antimicrobial resistance tests of the isolates revealed that all isolates were susceptible to chloramphenicol, clindamycin, amikacin and vancomycin. Furthermore, all of the *S. auricularis*, *S. hyicus*, *S. warneri* and *S. saprophyticus* isolates were susceptible to all antibiotics which were tested.

The rest of the *Staphylococcus* species were resistant to ampicillin (31.6%), tetracycline (26.3%), erythromycin (20.6%), methicillin (17.2%) and gentamicin (12.4%). A high prevalence of ampicillin resistance was found in *S. epidermidis* (71.4%), *S. aureus* (58.8%), *S. cohnii* (45.7%) and *S. haemolyticus* (44.4%) isolates. The highest percentages of methicillin-resistant isolates were found among *S. epidermidis* (57.1%) and *S. aureus* (29.4%) isolates. In this study, 65.0 and 45.0% of *S. simulans* isolates were resistant to erythromycin and tetracycline, respectively. *S. aureus* (11.8%), *S. cohnii* (32.6%) and *S. epidermidis* (42.9%) isolates were equally resistant to erythromycin and tetracycline. The observed resistance of *S. epidermidis* and *S. aureus* to gentamicin was 42.9 and 23.5%, respectively. In the present study, 87 (41.6%) of 209 *Staphylococcus* isolates were resistant to two or more antibiotics. Multiple resistances to antimicrobial agents was very common in *S. xylosus* (66.7%), *S. aureus* (58.8%) and *S. epidermidis* (57.1%) isolates.

DISCUSSION

The results obtained in this study showed a high incidence of staphylococci in calf meat, chicken meat, raw milk, ice cream and Turkish white cheese samples examined. The incidence of CNS isolates (83.7%) found in this study is

Table 1. Origin of isolates and species distribution of the staphylococci.

Specie	Calf meat	Chicken	Raw milk	Ice cream	Cheese	Total	Percent (%)
S. aureus	6	2	9	-	-	17	8.1
S. intermedius	-	3	8	-	2	13	6.2
S. hyicus	-	-	4	-	-	4	1.9
S. cohnii	2	10	4	20	10	46	22.1
S. xylosus	36	-	-	-	-	36	17.2
S. hominis	7	6	11	6	4	34	16.3
S. simulans	-	13	4	1	2	20	9.6
S. capitis	3	9	-	1	-	13	6.2
S. hemolyticus	-	1	1	2	5	9	4.3
S. epidermidis	2	-	2	3	-	7	3.3
S. auricularis	-	2	1	-	3	6	2.9
S. warneri	-	-	-	2	1	3	1.4
S. saprophyticus	-	-	-	1	-	1	0.5
Total	56	46	44	36	27	209	100

Table 2. The production of slime and biofilm among *Staphylococcus* species isolated from food samples.

Specie	Slime formation [n (%)]	Biofilm formation [n (%)]
S. aureus (17)	11 (64.7)	12 (70.6)
S. intermedius (13)	6 (46.2)	6 (46.2)
S. hyicus (4)	-	-
S. cohnii (46)	32 (69.6)	35 (76.1)
S. xylosus (36)	31 (86.1)	36 (100)
S. hominis (34)	25 (73.6)	27 (79.4)
S. simulans (20)	14 (70.0)	16 (80.0)
S. capitis (13)	11 (84.6)	11 (84.6)
S. haemolyticus (9)	7 (77.8)	8 (88.9)
S. epidermidis (7)	6 (85.7)	6 (85.7)
S. auricularis (6)	-	-
S. warneri (3)	-	-
S. saprophyticus (1)	-	-
Total (209)	143 (68.4)	157 (75.1)

much higher than CPS isolates (16.3%). The presence of *S. aureus* in foods commonly indicates contamination that may be directly introduced into the food by workers who have skin lesions containing *S. aureus*, or by sneezing or coughing. Other contamination sources of *S. aureus* are soil, dust and air (Gundogan et al., 2006). Previous studies showed that *S. aureus* is the most important *Staphylococcus* species causing food-borne illness (André et al., 2008). Our results showed that most of the CPS isolates isolated from food samples were *S. aureus*. However, the incidence of *S. aureus* (8.1 %) in the present study was much lower than the rates of 83% reported by Bartolomeoli et al. (2009), 66.7% by André et al. (2008), 61.1% by Gundogan et al. (2006) and 47.2% by Citak and Duman (2011). Compared to our results, lower contamination rates of different foods with *S. aureus* were reported as 6% by Can and Celik (2012) and 6.7% by Cepoglu et al. (2010). It is generally considered that the numbers of *S. aureus* need to be > 10^5 cfu/g of food for the production of sufficient toxin to cause illness (Can and Celik, 2012). However, neither the absence of *S. aureus* nor the presence of a small numbers of organism can provide complete assurence that the milk and meat products are safe. Because even in the case of complete alleviation or reduction of *S. aureus*, enough toxins could have been produced and still cause symptoms of staphylococcal food poisoning (Can and Celik, 2012). Thus, general hygienic practices aimed at preventing *S. aureus* contamination of

Table 3. Number and percentage of *Staphylococcus* isolates isolated from milk and meat products that resistant to antimicrobial agents.

Microorganism	*n*	AMP	TE	E	MET	GM	CHL	CLIN	AMC	VA	multiresistance* (%)
S. aureus	17	10 (58.8)	2 (11.8)	2 (11.8)	5 (29.4)	4 (23.5)	0 (0)	0 (0)	0 (0)	0 (0)	10(58.8)
S. intermedius	13	4 (30.8)	3 (23.0)	2 (15.3)	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3(23.0)
S. hyicus	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
S. cohnii	46	21 (45.7)	15 (32.6)	15 (32.6)	11 (24.0)	8 (17.3)	0 (0)	0 (0)	0 (0)	0 (0)	15 (32.6)
S. xylosus	36	5 (13.9)	10 (27.8)	3 (8.3)	0 (0)	5 (13.9)	0 (0)	0 (0)	0 (0)	0 (0)	24 (66.7)
S. hominis	34	12 (35.2)	5 (14.7)	6 (17.6)	8 (23.5)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	16 (47.0)
S. simulans	20	4 (20.0)	13 (65.0)	9 (45.0)	3 (15.0)	2 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (40.0)
S. capitis	13	1 (7.7)	3 (23.0)	2 (15.3)	2 (15.3)	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	5 (38.4)
S. haemolyticus	9	4 (44.4)	1 (11.1)	1 (11.1)	2 (22.2)	2 (22.2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (22.2)
S. epidermidis	7	5 (71.4)	3 (42.9)	3 (42.9)	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	0 (0)	4 (57.1)
S. auricularis	6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. warneri	3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. saprophyticus	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	209	66 (31.6)	55 (26.3)	43 (20.6)	36 (17.2)	26 (12.4)	0	0	0	0	87 (41.6)

AMP, ampicillin; TE, tetracycline; E, erythromycin; MET, methicillin; GM, gentamicin; CHL, chloramphenicol; CLIN: clindamycin; AMC: amikacin; VA: vancomycin; *n*: number of the isolates tested.
*Resistance to at least two different antibiotic.

foods should be emphasized.

Out of the total 209 isolates identified in this study, 175 (83.7%) isolates were CNS, and the majority of CNS were *S. cohnii*, *S. xylosus* and *S. hominis.* The remaining minority isolates were *S. simulans*, *S. capitis*, *S. haemolyticus*, *S. epidermidis*, *S. auricularis*, *S. warneri* and *S. saprophyticus.* Al-Tarazi et al. (2003) showed that the most common isolates isolated from dairy and meat samples were *S. warneri*, *S. simulans*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus* and *S. xylosus*. In a previous study, Udo et al. (1999) revealed that CNS (81.6%) were more prevalent than *S. aureus* (18.4%) on the hands of restaurant workers in Kuwait City, but 92.3 and 7.6% of *S. aureus* and CNS, respectively, were isolated from the nares of the workers. The species distribution of CNS on the hands of the food workers in their study were *S. hominis*, *S. warneri*, *S. saprophyticus*, *S. xylosus*, *S. schleiferi*, *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. capitis*, *S. intermedius* and *S. lentus*. In this study, similar strains were identified. Considering that *S. aureus* and CNS inhabit at the human skin and mucous membranes, it is obvious that these microorganisms can contaminate food if not handled properly. The biofilm formation is serious risk to the food industry because the removal of irreversibly adhered cells is difficult and requires the application of strong mechanical force or chemical interruption of the microbial adhesion using surfactants, sanitizers or heat. Thus, there is a high probability that the irreversibly adhered cells will remain even after pasteurization. This is one of the main reasons for biofilm formation on surfaces in contact with food (Schlegelova et al., 2008).

In our study, the rate of CRA and MP methods positiveness was for CPS 50 and 52.9% and for CNS 72 and 79.4%, respectively. Recently, Krukowski et al. (2008) reported that out of the 59 *S. aureus* isolates isolated from the inflammatory secretion of mammary glands of cows, 47.45% produced slime used by the MT method and 42.37% by the CRA method. Fox et al. (2005) demonstrated that 41.4% of *S. aureus* recovered from bovine milk formed biofilm. Gundogan et al. (2006) found that 58 (52.7%) out of 110 *S. aureus* isolated from raw milk, pasteurized milk and ice cream samples were slime producers. Ciftci et al. (2009) found that only 22 of 59 (37.2%) of *S. aureus* isolated from mastitic milk samples were slime producing. The results that we have found are higher than these values. In the present study, the frequency of positive slime and biofilm formation for *S. aureus* isolates were 64.7 and 70.6%, respectively. Higher percentage of slime producers were reported by Vasudevan et al. (2003) who found that 32 (91.4%) of 35 *S. aureus* isolated from bovine mastitis were slime positive. Other investigators suggest that CNS (42.2%) isolates produce a slime more often than *S. aureus* (5.1%) (Citak et al., 2003). The results of our study do not confirm these findings. However, some of the previous studies have shown that the nutrient content of the growth medium influences slime/biofilm development (Vasudevan et al., 2003; Zell et al., 2008). Even considering that *Staphylococcus* strains played an important role in proteolysis and lipolysis (Sorensen et al., 1993; Aravindan et al., 2007), our results indicated that only 23.5 and 11.8% of *S. aureus* isolates obtained from food samples displayed proteolytic and lipolytic activities, respectively.

Parakash et al. (2007) reported that though *S. aureus*

recorded highest percentage in raw milk, it did not show any proteolytic activity, but showed lipolytic activity. However, in the present study, the *S. aureus* isolates isolated from meat, chicken and raw milk samples are proteolytic rather than lipolytic. Lipase and protease enzymes have also been demonstrated in staphylococci associated with infection and considered as the major virulence factors in staphylococci. Takeuchi et al. (1999) used skim milk agar to detect protease of the isolates and reported that protease positive strains of *S. aureus* are isolated frequently from diseased chicken. Other workers reported 100 to 65.9% of CNS and 65.6 to 26.6% of CPS isolated from acne lesions had lipase and protease activity, respectively (Saising et al., 2012). In the present study, none of the CNS species isolated from food samples were lipase and protease producers. Casaburi et al. (2006) showed that most of the *S. xylosus*, *S. saprophyticus*, *S. equorum*, *S. carnosus* and *S. simulans* strains isolated from different types of fermented sausages had lacked detectable levels of proteinase activity but most of them had esterase activity. In contrast, Fiorentini et al. (2010) did not observe any significant change on fatty acids content when evaluating the influence of a native strain of *S. xylosus* in Milano salami. Bacterial lipolytic and proteolytic activities are influenced by several factors such as physiological state of culture, substrate type, pH, temperature, etc. (Fiorentini et al., 2010).

In this study, 209 food-associated *Staphylococcus* isolates were investigated for their resistance to antibiotics (Table 3). Our results showed that all of the *S. auricularis*, *S. hyicus*, *S. warneri* and *S. saprophyticus* isolates were susceptible to all antibiotics which were tested. Besides, all of the *Staphylococcus* isolates were found to be sensitive to chloramphenicol, amikacin, clindamycin and vancomycin, which agrees to previously published data for staphylococci from food (Acco et al., 2003; Resch et al., 2008, Marino et al., 2010). On the other hand, *Staphylococcus* species showed the highest rates of resistance against ampicillin (31.6%), followed by tetracycline (26.3%), erythromycin (20.6%), methicillin (17.2%) and gentamicin (12.4%). Resistance to ampicillin was observed particularly for the *S. aureus* (58.8%) and *S. epidermidis* (71.4%). These results are expected since β-lactams are commonly used antimicrobials for the treatment of infections in humans and food-producing animals. A high level of CNS resistance to penicillin (41.7%), ampicillin (29.8%) and oxacillin (29.8%) were also reported by Kenar et al. (2012). Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MR-CNS) are the most commonly identified antibiotic-resistant pathogens in many countries including Turkey (Citak et al., 2003; Gundogan et al., 2006; Abulreesh, 2011). The highest percentages of methicillin-resistant isolates were found among *S. aureus* (29.4%) and *S. epidermidis* (57.1%).

Recently, Marino et al. (2010) did not find any sample among the ones they have examined to be harboring the MRSA and MR-CNS strains. This finding is in disagreement with the results in this study. Methicillin resistance found in our study is in agreement with recently reported MRSA (50%) and MR-CNS (20.4%) from chicken samples in our country (Citak and Duman, 2011) but higher than those reported in studies conducted by Normanno et al. (2007) (0.36%) and Kitai et al. (2005) (0.45%). Previously, we also reported higher levels of methicillin resistance for *S. aureus* isolated from meat and chicken samples (67.5%) and milk and ice cream samples (97.2%) in Ankara, Turkey (Gundogan et al., 2006). Resistance to tetracycline and erythromycin was observed especially for the CNS species which was similar to the findings of Marino et al. (2010). The *S. aureus* isolates were equally resistant to erythromycin and tetracyline (11.7%). In our country, erythromycin resistance was reported as 7.5% for meat, 7.2% for milk isolates (Gundogan et al., 2006; Ciftci et al., 2009). Yucel et al. (2011) showed that 9.5% of *S. aureus* isolates isolated from foods were resistant to tetracycline. Recently, Citak and Duman (2011) reported that 40.2 and 36.9% of *S. aureus* and 29.1 and 41.7% of CNS isolates were resistant to tetracycline and erythromycin, respectively. Compared with earlier reports that have examined the susceptibility to tetracycline and erythromycin of *Staphylococcus* isolates in Turkey, there is a clear tendency towards decreased susceptibility for aforementioned antibiotics. A high frequency of gentamicin resistance was found in *S. epidermidis* (42.9%), *S. aureus* (23.5%) and *S. haemolyticus* (22.2%). This finding is in contrast to published data showing that all food-associated *S. aureus* and CNS were sensitive to gentamicin (Resch et al., 2008; Pereira et al., 2009; Can and Celik, 2012).

In the present study, the frequency of antibiotic resistance varied between the species and was the highest in CNS accordingly to Marino et al. (2010) who showed that CNS were phenotypically less susceptible to antimicrobial agents than CPS. In contrast, Simeoni et al. (2008) reported that there was a similar pattern of antibiotic resistance between the CNS and *S. aureus* isolates. The differences of antibiotic susceptibility between individual species may arise from several reasons. For instance, the geographical variations in resistance profiles of *Staphylococcus* species have a considerable impact on antimicrobial prescription. Therefore, because of the differences occur in the efficacy of antibiotics against individual *Staphylococcus* species, the identification of bacterial agents has been recommended along with the antibiotic susceptibility test (Kenar et al., 2012). Our results demonstrate that *S. aureus* and CNS can often contaminate milk and meat products. Considering *S. aureus* and CNS are usual floral members of human skin and mucous membranes, the existence of these bacteria in nutriment indicates poor sanitary conditions during processing. Due to that reason, hygiene practices during food processing, distribution and con-

sumption of the final product need to be improved in the food processing plants.

Our results also indicate that most of the *S. aureus* and CNS isolates are resistant to one or more antibiotics. The increasing prevalence of resistance in the isolates from animal origin may have important therapeutic implications. More restrictive policies on the use of antibiotics on animals may improve the current situation.

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