FOODBORNE PATHOGENS AND DISEASE
Volume 8, Number 1, 2011

DOI: 10.1089/fpd.2010.0613

Prevalence and Antibiotic Resistance of Foodborne *Staphylococcus aureus* Isolates in Turkey

Ali Aydin,[1] Karlo Muratoglu,[1] Mert Sudagidan,[2] Kamil Bostan,[1] Burcu Okuklu,[3] and Sebnem Harsa[3]

Abstract

In this study, 154 *Staphylococcus aureus* isolates were detected from 1070 food samples (14.4%) collected from seven cities in Turkey. Antimicrobial susceptibility testing against 21 antibiotics was performed by agar disk diffusion method, and those isolates resistant to any antibiotic were further analyzed to determine minimum inhibitory concentration by *E*-test and polymerase chain reaction analysis of *vanA* and *mecA* genes. According to disk diffusion test results, a total of 139 strains were resistant to at least one tested antibiotic, with 39 (25.3%) strains being multidrug resistant (MDR) and the other 15 strains being susceptible to all antibiotics. Penicillin G, linezolid, erythromycin, and tetracycline took up 71.4%, 23.4%, 18.2%, and 15.6% of the tested strains, respectively. In addition, all of the strains were susceptible to vancomycin, oxacillin, cefoxitin, and imipenem. Only one strain (S158B) was resistant to both teicoplanin and cefazolin. On the other hand, the presence of *vanA* and *mecA* genes was not detected in the strains. Pulsed-field gel electrophoresis analysis was used to identify genetic-relatedness of the MDR strains. It is noteworthy that some strains from different sources showed 100% homology; however, some of MDR strains were found unrelated with 60% or less homology. The high diversity observed in pulsed-field gel electrophoresis results indicated the possible contamination of *S. aureus* from different sources and routes.

Introduction

STAPHYLOCOCCUS AUREUS is a common pathogen associated with community and nosocomial acquired diseases and has been considered as a major problem of public health (Pesavento *et al.*, 2007). Milk, dairy products, meat, meat products, and other foods are often contaminated with *S. aureus* (Moon *et al.*, 2007; Normanno *et al.*, 2007; Pereira *et al.*, 2009; Sudagidan and Aydin, 2009; Guven *et al.*, 2010), which was responsible for 59% of 177 outbreaks implicating milk and milk products in France over a 10-year period from 1988 to 1997 and appeared as the most frequent pathogen associated with cheeses from raw or unspecified milk in outbreaks (De Buyser *et al.*, 2001). In fact, foodstuff contamination may directly occur from infected food-producing animals or may result from poor hygiene during production processes or retail and storage of foods (Normanno *et al.*, 2007).

The use of antimicrobials in food animals creates an important source of resistant bacteria that can spread to humans through the food supply. Improved management of the use of antimicrobials in food animals, particularly reducing the usage of those defined as "critically important" for human medicine, is an important step toward preserving the benefits of antimicrobials for people (Collignon *et al.*, 2009). Moreover, food can be considered an excellent mechanism for introducing pathogenic bacteria into the general population and immune-compromised patients, and it serves as a vehicle for transfer of antibiotic-resistant bacteria to the intestinal tract, causing exchanging resistance genes between nonpathogenic bacteria and pathogenic bacteria in the intestine (Sørum and L'Abée-Lund, 2002).

The World Health Organization (WHO) classification, a core list of the most critical antimicrobial agents globally (WHO, 2007), was generated in an effort to provide a tool for developing risk management strategies and focusing resources to address antimicrobial use in agriculture and veterinary medicine (JETACAR, 1999; WHO, 2003). To categorize the relative importance of these drugs in human and veterinary medicine, three categories of antimicrobials were defined: critically important, highly important, and important (Collignon *et al.*, 2009). In the last decade, *S. aureus* strains from food have shown a considerable increase in resistance against most antibiotics in Turkey (Gundogan *et al.*, 2005; Sudagidan and Aydin, 2009) as well as in other countries (Chao *et al.*, 2007; Normanno *et al.*, 2007; Peles *et al.*, 2007; Pesavento *et al.*, 2007; Lin *et al.*, 2009), with some other reports concerning methicillin (Kitai *et al.*, 2005) and vancomycin (Manie *et al.*, 1998).

[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, Avcilar, Istanbul, Turkey.
[2]Biotechnology and Bioengineering Central Research Laboratory and [3]Department of Food Engineering, Izmir Institute of Technology, Urla, Izmir, Turkey.

The objectives of this study includes determination of the prevalence of *S. aureus* in Turkish food products, investigation of the antibiotic resistance patterns of *S. aureus* strains, and analysis of the genetic-relatedness of multidrug resistant (MDR) *S. aureus* strains by pulsed-field gel electrophoresis (PFGE).

Materials and Methods

Sample collection and processing

Between July 2007 and December 2008, a total of 1070 food samples were collected from supermarkets, conventional markets, or bazaars in large cities in the Marmara Region of Turkey including Balikesir, Bursa, Canakkale, Edirne, Istanbul, Kirklareli, and Tekirdag. These samples included 115 meat (beef, mutton, chicken, and turkey meat), 15 meat products (Turkish type fermented sausage-sucuk, salami, and sausage), 303 raw milk, 452 dairy products (cheese, butter, yoghurt, and cream), 141 bakery products (pasta, thin sheet of dough, and cake), and 44 ready-to-eat foods.

Bacterial isolation and identification

The isolation of *S. aureus* strains from food samples was performed according to EN ISO 6881-1 standard procedure of the International Organization for Standardization (ISO, 1999). Typical colonies on Baird Parker Agar (Oxoid) were subcultured and identified by Gram staining, catalase test, coagulase test (Oxoid Dryspot Staphytect Plus), DNase activity (DNase agar; Oxoid), and mannitol fermentation (Mannitol Salt Agar; Oxoid). DNA isolation procedure was carried out accordingly (Sudagidan *et al.*, 2008). Presence of *S. aureus* specific genes (thermonuclease [*nuc*], coagulase [*coa*], and *S. aureus* protein A [*spa*]) was determined by polymerase chain reaction (PCR) analysis as previously described (Hookey *et al.*, 1999; Aires-De-Sousa *et al.*, 2006; Sudagidan and Aydin, 2009).

Antibiotic susceptibility

Antibiotic susceptibility testing was performed by agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2006); and antibiotics included gentamicin, kanamycin, tobramycin, rifampicin, imipenem, teicoplanin, vancomycin, erythromycin, linezolid, amoxicillin/clavulanic acid, penicillin G, levofloxacin, ofloxacin, cefazolin, cefoxitin, sulfamethoxazole/trimethoprim, tetracycline, chloramphenicol, fusidic acid, clindamycin, and oxacillin (Oxoid). The tested antibiotics represented the three main categories of antibiotic classification (Collignon *et al.*, 2009), with nine subcategories of the critically important category, four subcategories from the highly important category, and four subcategories from the important category being tested. The inhibition zone diameters were evaluated according to CLSI (2006) and Comité de L'antibiogramme de la Société Française de Microbiologie Communiqué (for fusidic acid) (1998). *S. aureus* ATCC 25293 was used as a quality control standard (Microbiologics). Further, those *S. aureus* strains found resistant to any tested antibiotics were then investigated and evaluated by *E*-test (AB Biodisk) or MICE test (Oxoid) according to CLSI (2006). Multidrug resistance was defined as showing resistance to at least three of the antimicrobials used.

Detection of vanA *and* mecA *genes*

The presence of vancomycin-resistance gene (*vanA*) (Clark *et al.*, 1993) and methicillin-resistance gene (*mecA*) (Lem *et al.*, 2001) was determined for all *S. aureus* strains by PCR analysis.

PFGE analysis

Genetic-relatedness of MDR *S. aureus* strains was determined by PFGE analysis. Agarose plugs were prepared as previously described (Sudagidan and Aydin, 2010). Bacterial DNA in plugs were digested with 30 U *Sma*I (Fermentas) overnight and were run in 1% (w/v) pulsed-field certified agarose (Bio-Rad) with 5–40 sec pulse time, 6 V/cm, 120° angle, at 14°C for 22 h using CHEF-Mapper PFGE system (Bio-Rad). After electrophoresis, the gel was stained with 20 μg/mL ethidium bromide and visualized with VersaDoc 4000MP image analyzer system (Bio-Rad). The band patterns were analyzed and compared with BIO-PROFIL Bio-1D++ software (Vilber Lourmat).

Results

Prevalence and antibiotic susceptibility of S. aureus *in food samples*

Out of 1070 food samples, 154 (14.4%) *S. aureus* strains were identified and characterized by biochemical and molecular tests. The prevalence of the strains on the basis of food samples and sampling cities were shown in Table 1. Most the strains were isolated from raw milk (41.6%) and dairy products (35.1%). In addition, Istanbul showed high prevalence (53.3%) in contaminated food and food products by *S. aureus*. In the critically important category, most of the strains (71.4%) were found to be resistant to penicillin G. In the highly important category and important category, 15.6% and 8.4% of *S. aureus* strains were resistant to tetracycline and clindamycin, respectively (Table 2). None of the strains were resistant to vancomycin, oxacillin, cefoxitin, and imipenem, with 15 strains (9.7%) being susceptible to all tested antibiotics. A total of 36 *S. aureus* strains (23.4%) were found resistant to only one antibiotic (penicillin G [21.5%] or teicoplanin [1.9%]), with 39 MDR strains, most of which were isolated

TABLE 1. DISTRIBUTION OF *STAPHYLOCOCCUS AUREUS* STRAINS IN FOOD PRODUCTS

Food products	n *(%)*	*Balikesir (%)*	*Bursa (%)*	*Canakkale (%)*	*Edirne (%)*	*Istanbul (%)*	*Kirklareli (%)*	*Tekirdag (%)*
Meat	16 (10.4)	—	5 (31.3)	—	5 (31.3)	6 (37.4)	—	—
Meat products	8 (5.1)	—	—	—	—	8 (100)	—	—
Raw milk	64 (41.6)	1 (1.6)	—	—	6 (9.4)	45 (70.3)	5 (7.8)	7 (10.9)
Dairy products	54 (35.1)	13 (24.1)	2 (3.7)	6 (11.1)	10 (18.5)	16 (29.6)	6 (11.1)	1 (1.9)
Bakery products	10 (6.5)	2 (20.0)	1 (10.0)	—	2 (20.0)	5 (50.0)	—	—
Ready-to-eat food	2 (1.3)	—	—	—	—	2 (100.0)	—	—
Total (%)	154 (100)	16 (10.4)	8 (5.1)	6 (3.9)	23 (15.0)	82 (53.3)	11 (7.2)	8 (5.1)

from raw milk samples in Istanbul and from dairy products in Istanbul, Balikesir and Kirklareli. The highest multidrug resistance was detected in 2 *S. aureus* strains (S174A and S175A, against 11 antibiotics) from raw milk samples in Istanbul (Fig. 1).

Determination of minimum inhibitory concentration of antibiotic-resistant S. aureus *strains*

Minimum inhibitory concentration (MIC) values of eight antibiotics were further determined for resistant *S. aureus* strains (Table 3). For penicillin G, tetracycline, linezolid, erythromycin, and sulfamethoxazole/trimethoprim, 99.1% (109/110), 58.3% (14/24), 33% (12/36), 21.4% (6/28), and 5% (1/20) of the tested strains were found in higher resistance than maximum detection limits. Three *S. aureus* strains from raw milk (S15D, S158B, and S264) were resistant to oxacillin by disk diffusion test. However, these strains were determined as susceptible with MIC values 1 μg/mL or lower.

PCR and PFGE analysis

None of the strains was positive for *vanA* or *mecA* genes in PCR analysis. The genetic-relatedness of 39 MDR *S. aureus* strains was investigated by PFGE, and the results exhibited wide variation in banding patterns (Fig. 1). Two major clusters with 60% homology were obtained, with six MDR *S. aureus* strains determined as distinct for showing less than 60% homology. It is noteworthy that strains with different isolation resources or resistance patterns reveal 100% homology, such as strains sampled from raw milk in Istanbul, five MDR strains (two from raw milk, two from dairy products, and one from a bakery product), the most resistant strains (S174A and S175A), and two other MDR strains (S137A and S143A).

Discussion

Investigation and determination of *S. aureus* contamination in food samples is significant with regard to food safety. Isolation rate of the current study (14.4%) (154/1070) (Table 1) was similar to a previous study, in which 209 (12.8%) *S. aureus* strains were identified from 1634 food samples (Normanno *et al.*, 2007). However, it was lower than that from another study in Turkey, in which 138 out of 413 (33.4%) food samples (48.7% [80/164] for meat products and 23.2% [58/249] for dairy products] were found contaminated with *S. aureus* (Guven *et al.*, 2010). Further, Andre *et al.* (2008) found that raw milk appears to be the most probable source of *S. aureus* contamination in cheese.

Foodstuffs were studied on the basis of specific food groups with regard to antibiotic susceptibility of *S. aureus* strains shown in Table 4. Similarly, in most previous studies, penicillin, tetracycline, and erythromycin resistance had been mainly reported in *S. aureus* strains from meat, meat products, raw milk, and dairy products (Gundogan *et al.*, 2005; Andre *et al.*, 2008; Guven *et al.*, 2010). The reason of this high resistance to penicillins (e.g., amoxicillin/clavulanic acid or bacitracin/tetracycline combinations) could be explained as to the extensive use of these drugs for treatment and prophylaxis in farm animals in Turkey (Budak, 2008). In addition, it is important to point out that *S. aureus* strains from ready-to-eat

TABLE 2. PREVALENCE OF ANTIBIOTIC RESISTANCE IN THE *STAPHYLOCOCCUS AUREUS* STRAINS ($N = 154$)

		Number of resistant Staphylococcus aureus *strains*	
Category	*Antibiotics*	R *(%)*	I *(%)*
Critically important	Gentamicin (10 μg)	10 (6.5)	1 (0.6)
	Kanamycin (30 μg)	15 (9.7)	22 (14.3)
	Tobramycin (10 μg)	9 (5.8)	5 (3.3)
	Rifampicin (5 μg)	11 (7.1)	8 (5.2)
	Imipenem (10 μg)	—	—
	Teicoplanin (30 μg)	1 (0.6)	30 (19.5)
	Vancomycin (30 μg)	—	—
	Erythromycin (15 μg)	28 (18.2)	41 (26.6)
	Linezolid (30 μg)	36 (23.4)	—
	Amoxicillin-clavulanic acid (20/10 μg)	7 (4.5)	—
	Penicillin G (10 μg)	110 (71.4)	—
	Levofloxacin (5 μg)	18 (11.7)	7 (4.5)
	Ofloxacin (5 μg)	19 (12.3)	8 (5.2)
Highly important	Cefazolin (30 μg)	1 (0.6)	—
	Cefoxitin (30 μg)	—	—
	Sulfamethoxazole/trimethoprim (23.75–1.25/25 μg)	20 (13)	4 (2.6)
	Tetracycline (30 μg)	24 (15.6)	18 (11.7)
Important	Chloramphenicol (30 μg)	12 (7.8)	6 (3.9)
	Fusidic acid (10 μg)	14 (9.1)	29 (18.8)
	Clindamycin (2 μg)	13 (8.4)	40 (26)
	Oxacilin (1 μg)	—	—

Resistant (R) or intermediate (I) against antibiotics (R, I; in mm): gentamicin <12,13–14; kanamycin <13,14–17; tobramycin <12,13–14; rifampicin <12,13–14; imipenem <13,14–15; teicoplanin <10,11–13; vancomycin <15; erythromycin <13,14–22; linezolid <21; amoxicillin/clavulanic acid <19; penicillin G <28; levofloxacin <15,16–18 ofloxacin <14,15–17; cefazolin <14,15–17; cefoxitin <19; sulfamethoxazole/trimethoprim <10,11–15; tetracycline <14,15–18; chloramphenicol <12,13–17; fusidic acid <15,16–21; clindamycin <14,15–20; oxacillin <10,11–12 (Comité de L'antibiogramme de la Société Française de Microbiologie Communiqué, 1998) [levels for fusidic acid]; CLSI, 2006).

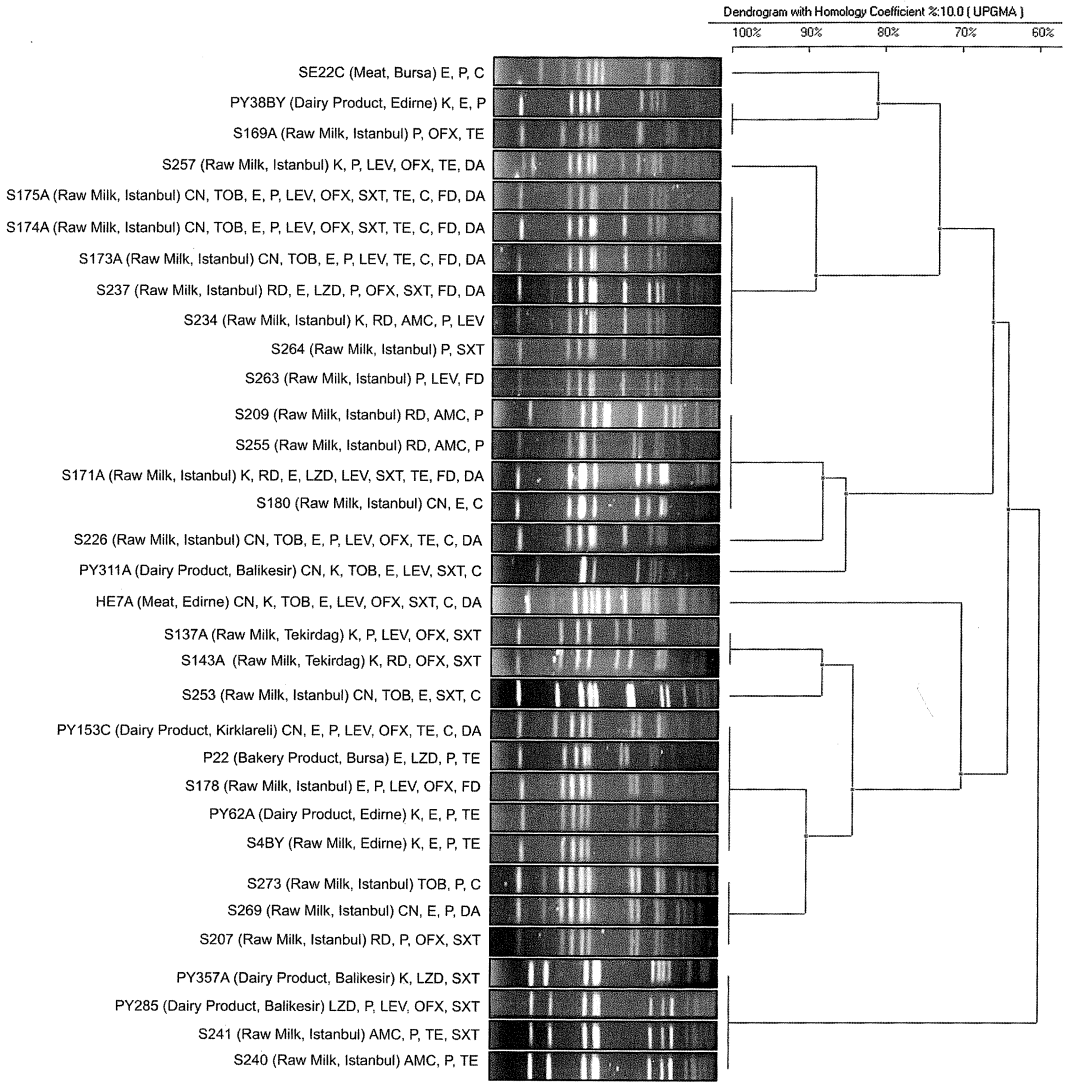

FIG. 1. Dendrogram of pulsed-field gel electrophoresis patterns showing the genetic-relatedness of 33 multidrug-resistant *Staphylococcus aureus* strains. The name of strains, the source of samples, the isolated area of *S. aureus*, and the code of resistant antibiotics were shown in left side. AMC, amoxicillin/clavulanic acid; C, chloramphenicol; CN, gentamicin; DA, clindamycin; E, erythromycin; FD, fusidic acid; K, kanamycin; LEV, levofloxacin; LZD, linezolid; OFX, ofloxacin; P, penicillin G; RD, rifampicin; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline; TOB, tobramycin.

TABLE 3. THE MINIMUM INHIBITORY CONCENTRATIONS OF RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS

	Number of S. aureus *strains MIC (μg/mL)*												
Antibiotics	*Total*	*≤0.025*	*0.026–1*	*2*	*3*	*4*	*6*	*12*	*16*	*24*	*32*	*>32*	*96*	*>256*
Gentamicin (≥16 μg/mL)	10	1	7			1	1							
Vancomycin (≥2 μg/mL)	2		2											
Erythromycin (≥8 μg/mL)	28		22											6
Linezolid (≥4 μg/mL)	36		14	10		12								
Penicillin G (≥0.25 μg/mL)	110	1	9	1	1	1		3	3			91		
Sulfamethoxazole/trimethoprim (≥4/76 μg/mL)	20	7	12									1		
Tetracycline (≥16 μg/mL)	24	3	6		1				6	3	3		2	
Oxacillin (≥4 μg/mL)	3	2	1											

Levels of MIC values against tested antibiotics (CLSI, 2006).
MIC, minimum inhibitory concentration.

TABLE 4. RESISTANCE OF *STAPHYLOCOCCUS AUREUS* STRAINS BASED ON THE FOOD SOURCE

		Meat (n = 16)		*Meat products* (n = 8)		*Raw milk* (n = 64)		*Dairy products* (n = 54)		*Bakery products* (n = 10)		*Ready-to-eat food* (n = 2)		*Total*	
	Antibiotics	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R (%)*	*I (%)*
Critically important	Gentamicin	1	—	—	—	7	1	2	—	—	—	—	—	10 (6.5)	1 (0.6)
	Kanamycin	1	—	1	1	7	14	6	7	—	—	—	—	15 (9.7)	22 (14.3)
	Tobramycin	1	—	—	—	6	4	1	1	1	—	—	—	9 (5.8)	5 (3.3)
	Rifampicin	—	1	1	—	7	5	3	2	—	—	—	—	11 (7.1)	8 (5.2)
	Imipenem	—	—	—	—	—	—	—	—	—	—	—	—	0	0
	Teicoplanin	—	5	—	2	1	7	—	10	—	4	—	2	1 (0.6)	30 (19.5)
	Vancomycin	—	—	—	—	—	—	—	—	—	—	—	—	0	0
	Erythromycin	3	1	—	2	19	18	4	15	2	4	—	1	28 (18.2)	41 (26.6)
	Linezolid	2	—	2	—	22	—	7	—	3	—	—	—	36 (23.4)	0
	Amoxicillin/clavulanic acid	—	—	—	—	7	—	—	—	—	—	—	—	7 (4.5)	0
	Penicillin G	12	—	8	—	53	—	27	—	8	—	2	—	110 (71.4)	0
	Levofloxacin	1	—	1	—	12	4	3	3	1	—	—	—	18 (11.7)	7 (4.5)
	Ofloxacin	1	—	2	—	12	6	4	1	—	1	—	—	19 (12.3)	8 (5.2)
Highly important	Cefazolin	—	—	—	—	1	—	—	—	—	—	—	—	1 (0.6)	0
	Cefoxitin	—	—	—	—	—	—	—	—	—	—	—	—	0	0
	Sulfamethoxazole/ trimethoprim	2	—	—	—	12	3	6	—	—	1	—	—	20 (13.0)	4 (2.6)
	Tetracycline	2	1	—	1	12	12	8	4	2	—	—	—	24 (15.6)	18 (11.7)
Important	Chloramphenicol	2	—	—	1	7	3	3	—	—	2	—	—	12 (7.8)	6 (3.9)
	Fusidic acid	—	2	—	1	12	20	1	4	1	2	—	—	14 (9.1)	29 (18.8)
	Clindamycin	1	1	—	1	9	23	2	11	—	3	1	1	13 (8.4)	40 (26.0)
	Oxacillin	—	—	—	—	—	—	—	—	—	—	—	—	0	0

foods and bakery products showed high level of resistance to penicillin G (Table 4), most probably due to ineffective heat treatment of these foods.

S. aureus has developed multidrug resistance worldwide, with wide diversity in prevalence rate in different regions (Gundogan *et al.*, 2005; Chao *et al.*, 2007; Normanno *et al.*, 2007; Andre *et al.*, 2008). Normanno *et al.* (2007) reported that 9.6% (12/125) and 4% (5/125) of the *S. aureus* strains had resistance to three and four of the tested antibiotics, respectively. In addition, Chao *et al.* (2007) found a high level (79%) (69/87) of multidrug resistance amongst isolates. In this study, 25.3% (39/154) of *S. aureus* strains showed multidrug resistance (Fig. 1) primarily to penicillin G (28/39), erythromycin (19/39), and sulfamethoxazole/trimethoprim (14/39). The results demonstrated that a large proportion of resistant strains were isolated from raw milk and dairy products, indicating a higher incidence of MDR *S. aureus* in dairy farms.

The use of antimicrobials as feed additives has been one of the major concerns in antibiotic-resistant food-related bacteria (Sørum and L'Abée-Lund, 2002). In a recent study, Pereira *et al.* (2009) isolated 148 foodborne *S. aureus* strains and found that 73%, 70%, 38%, 5%, 3%, and 1% of *S. aureus* strains were resistant to penicillin G, ampicillin, oxacillin, erythromycin, gentamicin, and tetracycline, respectively. Plasmidic penicillin resistance spreads rapidly among strains and remains the most frequently detected in foodborne *S. aureus*. In previous studies concerning penicillin resistance, Peles *et al.* (2007) found the penicillin resistance to be 88.9% with 20% of the strains recovered from mastitic milk and bulk tank milk; and Moon *et al.* (2007) determined that 90.2% and 89% of *S. aureus* strains were resistant to penicillin G and ampicillin, respectively; Guven *et al.* (2010) showed that penicillin resistance was the highest (92.7%) among *S. aureus* food isolates. It was found to be 71.4% in this study, taking up most of the tested strains.

Broad-spectrum tetracyclines are widely used as growth factors in veterinary medicine for livestock rearing as well as in the treatment of bacterial infections occurring in plants, agriculture, and human medicine (Ardic *et al.*, 2005). Tetracycline resistance rate in foodborne *S. aureus* was previously reported to be 19.1%, 24.7%, and 50% (Chao *et al.*, 2007; Pesavento *et al.*, 2007; Andre *et al.*, 2008); and in the current study, it was found to be 15.6%, which was lower comparing to preliminary studies from Turkey (55.3% and 39.6%) (Bayhun, 2008; Unal and Istanbulluoglu, 2009).

In the current study, two *S. aureus* strains showed 14 mm zone diameter against vancomycin, but they were determined as susceptible by *E*-test and negative for *vanA*-PCR. Vancomycin has been commonly used in methicillin-resistant *S. aureus* infection cases. Since the first discovery of clinical vancomycin intermediate *S. aureus* in 1997 (Hiramatsu *et al.*, 1997), *S. aureus* with reduced sensitivity to vancomycin had been reported worldwide (CDCP, 2002). Up to date, few studies concern vancomycin-resistant *S. aureus* strains in food; and as an exception, Manie *et al.* (1998) reported that 7% *Staphylococcus* isolates ($n = 190$) from chickens were resistant to vancomycin in South Africa.

Prevalence of methicillin-resistant *S. aureus* strains in food samples is low; and in the current study, MICs of oxacillin-resistant strains were low and the presence of 2 out of 714 *S. aureus* isolates from retail raw chicken meat in Japan was found to be *mecA* positive (Kitai *et al.*, 2005). In another study from Turkey, Unal and Istanbulluoglu (2009) reported 3.1% resistance to oxacillin. Although Pereira *et al.* (2009) found that 38% of the *S. aureus* strains were resistant to oxacillin (≥6 μg/mL), only 0.68% (1/148) of the strains contained *mecA*. In our study, MIC levels of oxacillin-resistant strains were very low (1 μg/mL or lower), and the presence of *mecA* was not detected in these strains.

PFGE is "gold standard" technique in determination of genetic-relatedness of the bacteria, especially in outbreaks due to its high discriminatory power. According to the PFGE patterns (Fig. 1), some strains (PY38BY and S169A; PY153C, P22, S178, PY62A and S4BY; PY357A, PY285, S241 and S240) from different sources were found highly homologous, and no endemic clone was detected among MDR strains. The existence of a variety of genetically diverse *S. aureus* strains and lack of predominance of an endemic clone observed in this study, which had been previously observed (Andre *et al.*, 2008), indicated the possible contamination by *S. aureus* from different sources and routes (Peles *et al.*, 2007).

Conclusions

The present study demonstrated that the most *S. aureus* food isolates were found to be resistant to tested antibiotics, especially in the critically important category, with a large percentage of MDR isolates, which raised a great concern for the risk of consuming foods contaminated by resistant bacteria. Further, PFGE analysis indicated a high genetic diversity showing that bacterial contaminations of food products could come from numerous sources such as processing environments, personnel, and farms.

Acknowledgments

The authors thank Biotechnology and Bioengineering Central Research Laboratory (Izmir Institute of Technology) and Res. Assist. Funda YILMAZ (Istanbul University) for their invaluable assistance. Further, Assist. Prof. Dr. Faith Critzer (Univeristy of Georgia, Center for Food Safety) is acknowledged for critical reading of the manuscript. This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project number: 107T266). A part of this study was presented in 3rd National Veterinary Food Hygiene Congress (May 14–16, 2009) in Bursa–Turkey.

Disclosure Statement

No competing financial interests exist.

References

Aires-De-Sousa M, Boye K, De Lencastre H, *et al.* High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. J Clin Microbiol 2006;44:619–621.

Andre MCDPB, Campos MRH, Borges LJ, *et al.* Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following *Sma*I digestion. Food Control 2008;19:200–207.

Ardic N, Ozyurt M, Sareyyupoglu B, *et al.* Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. Int J Antimicicrob Agents 2005;26:213–218.

Bayhun S. Beta-lactamase activities and antibiotic resistance comparison of *Staphylococcus aureus* isolated from clinic and food material [M.Sc. Thesis]. Gazi University, Institute of Science and Technology, 2008, p. 126.

Budak O. Isolation and identification of *S. aureus*, detect these bacteria enterotoxins and determine number of somatic cells and antibiotic from Mastitic Cows' milk in Kocaeli Region and enviromental effects in the milk [M.Sc. Thesis]. Gebze Institute of Technology, Institute of Science and Engineering 2008, p. 99.

[CDCP] Centre for Disease Control and Prevention. *Staphylococcus aureus* resistant to Vancomycin–United States, 2002. MMWR 2002;51:26.

Chao G, Zhou X, Jiao X, *et al.* Prevalance and antimicrobial resistance of foodborne pathogens isolated from food products in China. Foodborne Pathog Dis 2007;4:277–284.

[CLSI] Clinical Laboratory Standards Institute. Performance standards for antimicrobial disc susceptibility testing. Sixteenth International Edition. Document M100-S16. PA, 2006, p. 188.

Clark NC, Cooksey RC, Hill BC, *et al.* Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 1993;37:2311–2317.

Collignon P, Powers JH, Chiller TM, *et al.* World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clin Infect Dis 2009;49:132–141.

Comité de L'antibiogramme de la Société Française de Microbiologie Communiqué. Pathol Biol 1998;46:1–16.

De Buyser ML, Dufour B, Maire M, *et al.* Implication of milk and milk products in foodborne diseases in France and in different industrialised countries. Int J Food Microbiol 2001; 67:1–17.

Gundogan N, Citak S, Yucel N, *et al.* A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. Meat Sci 2005;69:807–810.

Guven K, Mutlu BM, Gulbandilar A, *et al.* Occurence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. J Food Saf 2010; 30:196–212.

Hiramatsu K, Hanaki H, Ino T, *et al.* Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. Antimicrob Agents Chemother 1997;40:135–136.

Hookey V, Edwards V, Cookson BD, *et al.* PCR–RFLP analysis of the coagulase gene of *Staphylococcus aureus*: application to the differentiation of epidemic and sporadic methicillin-resistant strains. J Hosp Infect 1999;42:305–312.

[ISO] International Organization for Standardization. EN ISO 6881-1 Microbiology of food and animal feeding stuffs: horizontal method for the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird Parker agar medium, ISO, Geneva, Switzerland, 1999.

JETACAR. The use of antibiotics in food-producing animals: antibioticresistant bacteria in animals and humans. 1999. Available at www.health.gov.au/internet/main/Publishing.nsf/Content/2A8435C711929352CA256F180057901E/$File/jetacar.pdf, accessed December 15, 2009. (Online.)

Kitai S, Shimizu A, Kawano J, *et al.* Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J Vet Med Sci 2005;67:107–110.

Lem P, Spiegelman J, Toye B, *et al.* Direct detection of *mecA*, nuc and 16S rRNA genes in BacT/Alert blood culture bottles. Diagn Microbiol Infect Dis 2001;41:165–168.

Lin J, Yeh K-S, Liu H-T, *et al. Staphylococcus aureus* isolated from pork and chicken carcasses in Taiwan: prevalence and antimicrobial susceptibility. J Food Prot 2009;72:608–611.

Manie T, Khan S, Brözel VS, *et al.* Antimicrobial resistance bacteria isolated from slaughtered and retail chickens in South Africa. Lett Appl Microbiol 1998;26:253–258.

Moon JS, Lee AR, Jaw SH, *et al.* Comparison of antibiogram, staphylococcal enterotoxin productivity, and coagulase genotypes among *Staphylococcus aureus* isolated from animal and vegetable sources in Korea. J Food Prot 2007;70:2541–2548.

Normanno G, La Salandr G, Dambrosio A, *et al.* Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int J Food Microbiol 2007;115:290–296.

Peles F, Wagner M, Varga L, *et al.* Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. Int J Food Microbiol 2007;118:186–193.

Pereira V, Lopes C, Castro A, *et al.* Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol 2009;26:278–282.

Pesavento G, Ducci B, Comodo N, *et al.* Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: a research for methicillin resistant *Staphylococcus aureus* (MRSA). Food Control 2007;18:196–200.

Sørum H and L'Abe'e-Lund TM. Antibiotic resistance in food related bacteria—a result of interfering with the global web of bacterial genetics. Int J Food Microbiol 2002;78:43–56.

Sudagidan M and Aydin A. Screening virulence properties of staphylococci isolated from meat and meat products. Wien Tieraerztl Monat 2009;96:128–134.

Sudagidan M and Aydin A. Virulence properties of methicillin-susceptible *Staphylococcus aureus* food isolates encoding Panton-Valentine Leukocidin gene. Int J Food Microbiol 2010; 138:287–291.

Sudagidan M, Çavuşoğlu C, Bacakoğlu F. Investigation of the virulence genes in methicillin-resistant *Staphylococcus aureus* strains isolated from biomaterial surfaces. Mikrobiyoloji Bülteni 2008;42:29–39.

Unal N and Istanbulluoglu E. Phenotypic and genotypic features of *Staphylococcus aureus* strains isolated from cattle and humans. Ankara Univ Vet Fak Derg 2009;56:119–126.

[WHO] World Health Organization. Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: scientific assessment. Geneva: WHO, 2003. Available at www.who.int/foodsafety/publications/micro/nov2003/en/, accessed December 9, 2009. (Online.)

[WHO] World Health Organization. Critically important antibacterial agents for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to nonhuman use. Report of the second WHO Expert Meeting, Copenhagen, 29–31 May 2007. Geneva: WHO, 2007. Available at www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf, accessed December 9, 2009. (Online.)

Address correspondence to:
Ali Aydin, D.V.M., Ph.D.
Department of Food Hygiene and Technology
Faculty of Veterinary Medicine
Istanbul University
34320 Avcilar
Istanbul
Turkey

E-mail: aliaydin@istanbul.edu.tr