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RESEARCH ARTICLE

Food handlers: a bridge in the journey of enterotoxigenic MRSA in food

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Abstract We investigated the presence of enterotoxigenic and methicillin-resistant *Staphylococcus aureus* (MRSA) in a kitchen of a catering firm by collecting 100 food samples and 100 utensil-equipment samples after sanitation. Additional 186 samples in total were collected by swabbing the mouths, noses and hands of 62 kitchen staff members during working hours. The isolation of *S. aureus* was performed using a culture method and verified by using a genetic method (PCR). The presence of toxin genes and the toxic shock syndrome gene (*tst*) was analyzed by PCR. ELISA was used to investigate whether the isolates bearing a toxin gene that produces enterotoxin or not. In this research we obtained 1930 isolates from 386 samples. Using molecular typing by PCR, 842 *Staphylococcus* spp. were found of which 307 (36.4 %) were identified as *S. aureus*. 198 (64.4 %) of the *S. aureus* strains were identified as *CoA* positive and 89 (28.9 %) as MRSA. Our data demonstrate the presence of MRSA and enterotoxigenic *S. aureus* in a catering kitchen providing daycare and hospital refectories. Given the facts that the rate of kitchen staff members contaminated with enterotoxigenic strains was rather high and that the kitchen contained *S. aureus* even after sanitation and that there was uneducated staff and insufficient hygiene practices aroused suspicions of the presence of biofilms and/or multi-resistant strains which could manifest a serious public health concern.

Keywords Food · Food contact surface · Food handlers · *Staphylococcus aureus* · Enterotoxin · MRSA

1 Introduction

Staphylococcus aureus is a common member of the normal microflora of human skin and mucous membranes. Some strains are capable of producing a highly heat-stable protein toxin that causes illness in humans (Anonymous 2012; Bergdoll 1983). Serologically, five main classical staphylococcal enterotoxin (SEs) types (SEA to SEE) have been identified, and SEA and SED are commonly (95 %) responsible for staphylococcal food poisonings. SEG, SEI, SEIH, SEIJ,SEIK, SEIL, SEIM, SEIN, SEIO, SEIU, SEIP, SEIQ, SEIR, TSST have been identified in foods and food handlers, clinical samples, and staphylococcal food poisoning (Bergdoll 1983; Carfora et al. 2015; Mehrotra et al. 2000; Omoe et al. 2002; Vazquez-Sanchez et al. 2012). At present the relationship between these new SEs and human food poisoning is not fully understood. SEs are resistant to proteolysis and are heat-stable, so the presence of SEs presents a significant food safety risk (Omoe et al. 2005).

In this study enterotoxigenic *S. aureus* was isolated from samples collected from the kitchen of a catering

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firm. The presence of the *nuc* and *CoA* genes was investigated by PCR and toxin expression was determined by ELISA. MRSA strains were identified based on *mecA* gene detection by PCR.

2 Materials and methods

2.1 Sampling

We collected samples from an establishment engaged in catering services in Kars, Turkey that prepares and delivers food to dining facilities of a hospital, a daycare, and 3 academic institutions. Samples were obtained from the meat section (meat section staff, cutting and chopping boards, mincing machine, etc.), bakery section (dough kneading machine, dessert production unit, dessert section staff, etc.), vegetable-salad section (salad section staff, salad cutting boards, processing places, etc.), cooked food section (food samples) and food serving section (foods ready to serve, serving staff, service equipment, etc.). All samples were collected on the same day. Sampling from the staff members was performed during their working hours (if gloves were worn, sampling was performed instantly after the gloves were taken off). An hour after food preparation was finished and cleaning completed we took samples by swabbing 100 utensils, equipment surfaces and counters as well as hands, noses and mouths of 62 staff members using sterile physiological saline solution (0.9 % PS) and swabs (Anonymous 2004). Food samples were taken at the time point of food service.

2.2 Isolation and identification of *Staphylococcus aureus*

100 g food sample homogenized with 900 ml PS and decimal dilutions were prepared under aseptic conditions and plated onto Baird-Parker Agar (Oxoid CM0275) with added Egg Yolk Tellurite (Oxoid SR0054). After streaking swab samples directly onto the agar we performed a basic identified test on isolated colonies (Anonymous 1999; Bennett and Lancette 2001; Harrigan 1998).

2.3 Molecular typing of bacterial isolates

Genomic DNA was extracted from 24 h cultures in Brain Heart Infusion Broth (Oxoid CM 225) using a DNA extraction kit (Sentro-pure, Turkey) following the manufacturer's instructions. 100 ng of genomic DNA was used for PCR. The reaction mixture also contained 1× Taq buffer (Vivantis, S buffer with $MgCl_2$ 17.5 mM), 1 U taq DNA polymerase (Vivantis, PL1202 5U/μl), 0.2 mM of dNTP mix (Vivantis, NP2406), 0.6 mM of forward and reverse primers (Sentromer DNA Technologies, Istanbul, Turkey) and sterile Hypure water up to final volume of 25 μl. The sequence of the forward and reverse primer for *Staphylococcus* spp. was 5′-CCA CCT TCC TCC GGT TTG TCA CC-3′ (Staph750R) and 5′-AAC TCT GTT ATT AGG GAA GAA CA–3′ (Staph756F), for *S. aureus* 5′–TGA AGT CAA ATA AAT CGC TT GC-3′(Nuc2F) and 5′–CCC TTT TCC ACT AAT TCC TTA TT GT-3′ (NucR) and for coagulase activity 5′–ACC ACA AGG TAC TGA ATC AA CG-3′(CoA1) and 5′–TGC TTT CGA TTG TTC GAT GC-3′ (CoA2) (Perillo et al. 2012; Veras et al. 2008; Zhang et al. 2004) (Table 1).

We applied the method suggested by Perillo et al. (2012) with modification in order to identify members of the genus *Staphylococcus* (16S rRNA) and to detect the *S. aureus nuc* and coagulase activity (*CoA*) genes. PCR was performed in a Biometra Thermocycler using the following conditions: 95 °C for 2 min (initial cycle), followed by 30 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. Control strains for PCR-based analysis included the following *S. aureus* strains: ATCC 13565, ATCC 14458, ATCC 19095, ATCC 23235, ATCC 51811, ATCC 27664, ATCC 43300, and ATCC 51650. PCRs received Hypure water instead of template DNA as negative control. The PCR products were loaded onto a 2 % agarose gel containing ethidium bromide followed by electrophoresis using a 100 bp DNA ladder in TAE buffer at 90 V for 60 min following visualization by an UV illuminator.

2.4 Detection of toxin genes

The presence of 12 toxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sel, seq, sek*) and the toxic shock syndrome toxin gene (*tst*) was investigated by PCR. Nucleotide sequences of PCR primers and respective amplified products are listed in Table 1. Each master mixture contained 100 ng genomic DNA, 1× Taq buffer, 1 U Taq polymerase, 0.2 mM of dNTP mix, 1 mM of forward and reverse primers (Sentromer) and sterile Hypure water up to final volume of 25 μl. The cycling parameters were: 95 °C for 2 min (initial), followed by 25 cycles of 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 30 s. For *sea* and *sed*, an initial step of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s was used (Perillo et al. 2012).

Table 1 PCR primers used in this study

Gene target	Primer	Sequence 5′–3′	References
sea	SeA-1	CATTGCCCTAACGTGGACAACAAG	Perillo et al. (2012)
	SeA-2	ATCCCCTCTGAACCTTCCCATC	
Seb	Seb1	TCGCATCAAACTGACAAACG	Becker et al. (1998)
	Seb4	GCAGGTACTCTATAAGTGCCTGC	
Sec	Sec3	CTCAAGAACTAGACATAAAAGCTAGG	Becker et al. (1998)
	Sec4	TCAAAATCGGATTAACATTATCC	
Sed	SedF	GTGGTGAAATAGATAGGACTGC	Pereira et al. (2009)
	SedR	ATATGAAGGTGCTCTGTGG	
See	SeeF	TACCAATTAACTTGTGGATAGAC	Pereira et al. (2009)
	SeeR	CTCTTTGCACCTTACCGC	
Seg	SegF	CGTCTCCACCTGTTGAAGG	Pereira et al. (2009)
	SegR	CCAAGTGATTGTCTATTGTCG	
She	SehF	CAACTGCTGATTTAGCTCAG	Pereira et al. (2009)
	SehR	GTCGAATGAGTAATCTCTAGG	
Sei	SeiF	CAACTCGAATTTTCAACAGGTACC	Pereira et al. (2009)
	SeiR	CAGGCAGTCCATCTCCTG	
Sej	SejF	CATCAGAACTGTTGTTCCGCTAG	Pereira et al. (2009)
	SejR	CTGAATTTTACCATCAAAGGTAC	
sek	SeK-1	TAGGTGTCTCTAATAATGCCA	Omoe et al. (2005)
	SeK-2	TAGATATTCGTTAGTAGCTG	
Sel	SeL-F	GCTTTCTGGAAGACCGTATCCTGTG	Perillo et al. (2012)
	SeL-R	GGCGATGTAGGTCCAGGAAACCT	
Seq	SeQ-1	AATCTCTGGGTCAATGGTAAGC	Omoe et al. (2005)
	SeQ-2	TTGTATTCGTTTTGTAGGTATTTTCG	
Tst	TstF	GCTTGCGACAACTGCTACAG	Pereira et al. (2009)
	TstR	TGGATCCGTCATTCATTGTTAT	

2.5 Detection of methicillin resistance

The presence of the *mecA* gene was investigated by PCR. Sequences of the forward and reverse primers were: 5′-ACT GCT ATC CAC CCT CAA AC-3′ (GMECAR-1) and 5′-CTG GTG AAG TTG TAA TCT GG–3′ (GMECAR-2) (Mehrotra et al. 2000). The PCR parameters were an initial denaturation at 94 °C for 5 min, followed by 35 cycles of amplification at 94 °C for 2 min, 57 °C for 2 min, 72 °C for 1 min and 72 °C for 7 min (Mehrotra et al. 2000).

2.6 Detection of toxin production in isolates possessing enterotoxin genes

To detect toxin production in strains identified having at least one of the classical Staphylococcus toxin genes "sea-seb-sec-sed and see" we used an ELISA test kit (r-biopharm-Ridascreen R4101 SET). The test was performed in accordance with the instructions of the manufacturing company and the plates were measured with SpectraMax plus 384 at 450 nm, and the results were evaluated according to the company's instructions.

3 Results

We detected 842 *Staphylococcus* spp. out of 386 samples of which 307 (36.4 %) were identified as *S. aureus* strains (Table 2), 198 (64.4 %) were *CoA* positive and 89 (28.9 %) carried the mecA gene. All mecA-positive strains were also *CoA* positive. *S. aureus* was isolated from 49 out of 62 staff members. 32 (from 17 surfaces) of 500 isolates from 100 surface samples and 36 (from 22 food samples) out of 500 isolates from 100 food samples contained *S. aureus*.

All of the 307 isolates had at least 3 toxin genes. One strain had 7 toxin genes and 22 had 6 toxin genes. All *S. aureus* isolates had *sek* (100 %), 302 (98.3 %) of them harbored *seg*, 293 (95.4 %) of them had *seq*, and 276 (89.9 %) of them had *sea.* 39 different genotypes were identified by considering the distribution of toxin genes. Among these, the most

Table 2 Distribution of the isolates according to the samples

	Staff	Food-contacting surfaces, utensils-equipment	Food	Total
Number of samples	186	100	100	386
Number of isolates	930	500	500	1930
Staphylococcus spp.	624	124	94	842
S. aureus	239 (49 staff members)	32 (17 surface)	36 (22 food)	307
CoA positive	163	13	22	198
MRSA	71	2	4	89

Staff (nose, mouth, hands), utensils-equipment (fork, spoon, knife, meat cleaver, tray, plate, meat board, salad board, dough kneading machine, dessert counter), food (salad, rice, meat stew, vegetable-meat stew, dessert, soup, kebab)

Table 3 Genotype distribution of the toxin genes according to the sample groups

Toxin genes	Food (n = 36)	Food-contacting surfaces, utensils-equipment (n = 32)	Staff (n = 239)	Total
Sea	36	27	213	276
Seb	3	1	2	6
Sec	19	14	84	117
Sed	2	1	9	12
See	1	2	5	8
Seg	35	31	236	302
She	6	3	36	45
Sei	1	1	6	8
Sej	1	–	5	6
Sel	–	–	4	4
Sek	36	32	239	307
Seq	34	29	230	293
Tst	–	–	9	9

commonly observed was the *"sek-seg-seq-sea"*-group detected in 113 isolates. The genotype distribution of the toxin genes in the strains are listed in Table 3.

ELISA was performed to identify at least one of the classical toxin genes (*sea-seb-sec-sed-see*) produced by *S. aureus* (Table 4). While 285 of the isolates had at least one gene from the *sea-seb-sec-sed-see* group, according to ELISA test results, only 240 out of the 285 isolates produced toxin.

4 Discussion

We investigated the presence of *S. aureus* in the kitchen of a catering company in this study. In total 1930 isolates out of 386 samples collected from the kitchen were examined, and 842 *Staphylococcus* spp. colonies were obtained in 36.4 % (n = 307) identified as enterotoxigenic *S. aureus*. Enterotoxigenic *S. aureus* isolation was performed at a ratio of 38.2 % (n = 36) out of food samples ready for consumption. Considering the fact that the catering company rendered service to hospitals and daycares including people who may have impaired or suppressed immune systems, this ratio seems rather high and is a serious risk in terms of food intoxication. There was no statistical difference among the food groups in terms of enterotoxigenic *S. aureus* presence. There is a lot of research reporting various rates of *S. aureus* and/or enterotoxigenic *S. aureus* isolation in food (Aydin et al. 2011b; Can and Çelik 2012; Carfora et al. 2015; Kamal et al. 2013; Vazquez-Sanchez et al. 2012). There are many reasons behind these varying rates expressed in the study results, such as the sensitivity of analysis methods, the food process being analyzed, selling conditions and the form of presentation to consumption. Certainly, many contamination sources are involved in the phases of which food products go through, from production to consumption.

As far as staphylococci are concerned, the most important contamination source is the food handlers. In this research, the carriage rate of enterotoxigenic *S. aureus* in food handlers was found to be as high as 79 % (49 out of 62 staff members were positive). This poses a serious risk in terms of food safety and public health. Previous research available on the carriage of *Staphylococcus* spp. in food handlers and the studies performed indicate that different amounts of *Staphylococcus* spp. can be found on mouths, noses and hands of food handlers (Asghar et al. 2006; El-Shenawy et al. 2013; Ho et al. 2014; Kamal et al. 2013; Uzunoviç et al. 2013). Differences in results could be based on various factors such as health conditions of food handlers, their immune systems, cleaning

Table 4 Comparison of PCR and ELISA results in terms of enterotoxin (SEA-SEE) production ability of the *S. aureus* isolates

Group of samples	The genes detected by PCR					SEs detected by ELISA				
	sea	*seb*	*Sec*	*sed*	*See*	SEA	SEB	SEC	SED	SEE
Food (n = 36)	36	3	19	2	1	24	–	10	2	1
Food-contacting surfaces, utensils-equipment (n = 32)	27	1	14	1	2	13	1	9	–	–
Staff (n = 239)	213	2	84	9	5	192	–	71	5	2
Total (n = 307)	276	6	117	12	8	229	1	90	7	3

n number of *S. aureus* isolates having enterotoxin gene

habits, level of education, the state of raw-cooked food processing, the hygiene of utensils-equipment and the environment, the state of development of the country the food handler is working in and the regulations on inquest (El-Shenawy et al. 2013; Ho et al. 2014; Sivaraman et al. 2009).

Obviously all surfaces, utensils and equipment used for food preparation and servicing in kitchens may also be sources of *S. aureus* contamination. A total of 100 utensil-equipment and surface samples, enterotoxigenic *S. aureus* was isolated in 17 % of the samples. Considering that we took samples from cleaned surfaces, this high rate indicates the presence of a serious contamination. Additionally, a more serious problem may exist, as this high rate may be indicative of the presence of strains resistant against the detergents or disinfectants used, suggesting biofilm formation. The most important factors in assessing hygiene in food establishments are the type and number of microorganisms. The contamination of food with microorganisms on utensils, equipment and food-contacting surfaces is inevitable. Hence, it is very important to maintain hygienic conditions, practice good food manufacturing applications and implement HACCP in the establishment.

Schlegelova et al. (2010) collected samples at intervals from the surfaces of four different dairy and meat establishments after the standard cleaning processes were completed and investigated the presence of certain pathogens. They found contamination with *Staphylococcus* spp. forming a multi-resistant biofilm particularly in equipment used for dairy. Moretro et al. (2013) performed sampling on meat processing surfaces after sanitation at an abattoir and examined the presence of various pathogens. They determined that Staphylococci formed biofilm and were sensitive to desiccation but resistant to disinfectants. This condition, which is a very common and a serious problem in the food industry, may be caused by the lack of effective/sufficient cleaning and the presence of multi-resistant microorganisms and/or biofilm-forming microorganisms in the establishments (Brooks and Flint 2008; Sharma and Anand 2002).

We investigated the presence of 13 toxin genes in this study. All isolated *S. aureus* strains contained at least 3 toxin genes. The ones identified at the highest rates were *sek* (100 %), *seg* (98.3 %), *seq* (95.4 %) and *sea* (89.9 %). We detected 39 different genotypes among which the *"sek-seg-seq-sea"*-group was the most common (n = 113). Nine (2.9 %) out of 307 isolates of *S. aureus* carried the *tst* gene responsible for toxic shock syndrome and they all originated from kitchen staff members (3.7 % of 239 staff isolates). The presence of the *tst* gene from clinical samples, milk with mastitis and various foods has been investigated previously with different results (Udo et al. 2009; Cha et al. 2006; Aydin et al. 2011b). In the studies conducted on toxin genes of enterotoxigenic *S. aureus*, it was determined that a strain usually contained one or more toxin genes. In particular *sea-see*-genes were investigated by various different methods like PCR or ELISA, and other toxin genes and their genotypes were detected (Carfora et al. 2015; El-Shenawy et al. 2013; Kamal et al. 2013). The results from other studies (Ho et al. 2014; Omoe et al. 2002; Schlegelova et al. 2010; Vazquez-Sanchez et al. 2012) confirm our results showing that enterotoxigenic *S. aureus* can be commonly found on hands, noses and mouths of food handlers, on surfaces that are in contact with food, utensils and equipment, and that this may exhibit a serious public health concern.

The ELISA test was performed to identify whether the *S. aureus* strains possessing toxin genes produced toxin or not. While 285 of the isolates had one gene from the group *sea-seb-sec-sed-see*, according to the ELISA test results only 240 out of 285 isolates produced one or more of the classical *Staphylococcus* toxins (*sea-seb-sec-sed-see*). Omoe et al. (2002) tested the toxin production of toxin gene-containing *S. aureus* by using sandwich ELISA. Although there were isolates causing food poisoning among the isolates

that had been analyzed, they were unable to identify the toxin production of most of the isolates having toxin gene using ELISA. Carfora et al. (2015) detected toxin production by RPLA in toxin gene-containing isolates which they had identified by PCR. They could detect toxin production in 50 % of the toxin gene-containing isolates. Even though the researchers identified the presence of toxin genes by PCR and the toxin production by various methods like ELISA, VIDAS and RPLA, the results indicate a similarity. The inability to identify toxin production in strains having a toxin gene has been linked to various reasons. Toxin production may not be possible even when there are enterotoxin genes in the environment, because they may be subject to expression depending on various factors; the gene may not be detected even when there is enterotoxin as a result of various factors; the amounts of toxin secretion by a strain under laboratory conditions or in food environment may differ; the toxin may not have been produced in detectable amounts; or there may be sensitivity and specificity differences between the methods (Aydin et al. 2011b; Omoe et al. 2002; Pereira et al. 2009).

A substantial number of researches exist on antibiotic resistance which has reached a critical level in recent years. The World Health Organization (WHO) published a report on the irrational use of antibiotics, existing antibiotic resistance, health concerns that antibiotic resistance may cause in the future and the measures to be taken (Avorn et al. 2001). In our study, 28.9 % of the enterotoxigenic *S. aureus* strains were detected to be MRSA. The results of our and other studies intended to determine the antibiotic resistance of *S. aureus* isolated from food, staff, utensils and equipment indicate that resistance is a commonly encountered issue and that the MRSA rates of isolates may vary (Aydin et al. 2011a; Can and Çelik, 2012; Carfora et al. 2015; Mehrotra et al. 2000; Perillo et al. 2012).

Data obtained in this study indicate the existence of MRSA and enterotoxigenic *S. aureus* in a catering kitchen. Because this kitchen serves food particularly to daycare and hospital refectories, it exhibits a serious public health concern. Staff carrying enterotoxigenic strains at rather high levels and the kitchen containing *S. aureus* even after sanitation are indicative of uneducated staff and insufficient hygiene practices. More significant than that, the presence of enterotoxigenic *S. aureus* and MRSA after sanitation gives rise to suspicions that biofilms and/or multi-resistant strains may exist. The most crucial measures to be taken to achieve the desired success from the protection and control measures implemented for public health are the education of staff, the prevention of cross contamination, practicing good food manufacturing applications, the prevention of irrational use of antibiotics, successful hygiene and sanitation implementations as well as the maintenance of continuous and effective audits. We believe that the results of this study will illuminate forthcoming studies and will contribute to the protection from and the control of food-borne diseases.

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