Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Enterotoxin gene profiles of *Staphylococcus aureus* isolated from milk and dairy products in Italy

D.M. Bianchi[1], S. Gallina[1], A. Bellio[1], F. Chiesa[2], T. Civera[2] and L. Decastelli[1]

1 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy
2 Dipartimento di Scienze Veterinarie, Università di Torino, Grugliasco, Torino, Italy

Significance and Impact of the Study: The analyses targeted 11 staphylococcal enterotoxins genes and 35 different enterotoxin gene profiles were distinguished among the isolates. A total of 255 *Staph. aureus* isolates were positive for one or more SE genes while *ser* gene was the most prevalent. In 93% of the isolates bearing genes located on the enterotoxin gene cluster (n = 89), both *seg* and *sei* genes were present.

Keywords
dairy products, foodborne disease, milk, mobile genetic elements, staphylococcal enterotoxins, *Staphylococcus aureus*.

Correspondence
Francesco Chiesa, Department of Veterinary Science, University of Turin, 10095, Grugliasco (TO), Italy.
E-mail: francesco.chiesa@unito.it

2013/1006: received 21 May 2013, revised 15 October 2013 and accepted 16 October 2013

doi:10.1111/lam.12182

Abstract

Staphylococcal foodborne intoxication, occurring after consumption of staphylococcal enterotoxins (SEs) in food, is considered one of the most common forms of bacterial foodborne outbreaks worldwide. Milk and dairy products account for 5% of all the incriminated foods in staphylococcal outbreaks, referring to Europe. The distribution of genes encoding for enterotoxins in *Staphylococcus aureus* strains is highly variable, with some carried on stable regions of the chromosome and others carried on mobile genetic elements. The aim of this study was to analyse the distribution of genes encoding for SEs in *Staph. aureus* strains isolated from milk and dairy products. In the period from January 2010 to June 2011, a total of 1245 dairy samples (848 of raw milk and 397 of dairy products) were collected and analysed for detection of genes encoding for 11 SEs and SE*l*s (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER SE*l*J and SE*l*P) according to the procedures of the Italian National Reference Laboratory for coagulase-positive *Staphylococci* including *Staph. aureus*. *Staphylococcus aureus* strains were isolated in 481 (39%) samples. Of the 481 isolates of *Staph. aureus* tested, 255 (53%) were positive for one or more SE genes, and thirty-five different enterotoxin gene profiles were distinguished among the isolates. *ser* gene, found in 134 (28%) of the isolates, was the most frequent, followed by *sed* (25%) and *selj* genes (25%). The identification of new SEs increased the isolation frequency of enterotoxigenic staphylococci, thus suggesting that the pathogenic potential of *Staph. aureus* may be of greater importance than previously thought. Further studies are needed to quantify the expression of these new enterotoxins, and to assess their contribution to foodborne disease burden.

Introduction

Staphylococcal foodborne intoxication (SFP) is considered one of the most common forms of bacterial foodborne outbreaks worldwide. According to the outbreak reports from 15 European countries, milk and dairy products represented 1–9% (mean 4·8%) of all the incriminated foods in staphylococcal outbreaks (Balaban and Rasooly 2000). SFP is characterized by gastrointestinal symptoms and occurs after consumption of staphylococcal enterotoxins (SE) in food. SEs are produced by enterotoxinogenic strains of coagulase-positive staphylococci (mainly *Staph. aureus)* while coagulase negative staphylococci have never been reported as cause foodborne outbreaks. SFPs

are not always notified in all Member States, therefore increasing the possibility of under reporting of cases (SCVPH 2000).

Coagulase-positive staphylococci are facultative anaerobic bacteria which can grow over a relatively wide range of pH (4–10, with the optimum being 6–7), temperature (7–48°C) and water activity (minimum Aw is 0·83, provided that all other conditions are optimal), while ranges for SE production are somewhat narrower (Hennekinne *et al.* 2011). The adaptability of the organism permits its growth in many types of food, leading to enterotoxins production and subsequent possibility of causing food poisoning. However, not all coagulase-positive staphylococci are SE producers and even so, SE production will not occur in every food. Environmental factors and a conspicuous combinations of parameters of the food can influence and contribute to the formation of SE, such as water activity, pH, redox potential and temperature; besides, bacterial antagonism is known to play an important role (Genigeorgis 1989; Hennekinne *et al.* 2011; Schelin *et al.* 2011).

Growth of *Staph. aureus* and SE production in dairy products can be prevented during food processing by heat treatment of milk, or inhibited using starter cultures, antagonistic effect of natural flora, concentration of salt, drop of pH, low temperature of processing and storage of cheese and/or minimizing the pressing time. SEs, however, are much more resistant to environmental effects and food-processing procedures than the staphylococcal bacterial cells; thus, although the bacteria are eliminated, the toxins will remain and can cause SFP (SCVPH 2000; Hennekinne *et al.* 2011; Schelin *et al.* 2011).

Several SEs are designated as SE-like (SE*l*) toxins because they lack the emetic properties or still have not been tested (Lina *et al.* 2004; Omoe *et al.* 2004). Thus, to date, a total of 21 SEs and SE*l*s toxins have been reported. In addition to the five well-characterized classical staphylococcal enterotoxins SEA, SEB, SEC, SED and SEE, 16 new types of SEs (SEG, SEH, SEI, SER, SES, SET) and SE*l*s (SE*l*J, SE*l*K, SE*l*L, SE*l*M, SE*l*N, SE*l*O, SE*l*P, SE*l*Q, SE*l*U and SE*l*V) have been introduced (Argudin *et al.* 2010).

The distribution of genes encoding for enterotoxins in *Staph. aureus* strains is highly variable, with some carried on stable regions of the chromosome (e.g. enterotoxigenic gene cluster—EGC) associated with particular lineages and others carried on mobile genetic elements (MGEs). MGEs are segments of DNA that encode enzymes and other proteins that confer their ability to move horizontally between bacterial cells (Frost *et al.* 2005). In *Staph. aureus*, the major MGEs are bacteriophages, *Staph. aureus* pathogenicity islands (SaPIs), plasmids, transposons and staphylococcal cassette chromosomes (SCCs). All have been reported to carry SE genes, except SCCs which typically carry antibiotic resistance genes, including *mecA* (Lindsay 2011). Most MGEs can move at high frequency between *Staph. aureus* isolates, including during the course of infection (Goerke and Wolz 2004; Lindsay and Holden 2006; Lindsay 2011).

The aim of this study was to analyse the distribution of genes encoding for SEs in *Staph. aureus* strains isolated from milk for human consumption and from dairy products. Strains were analysed for detection of genes encoding for 11 SEs and SE*l*s (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER SE*l*J and SE*l*P) according to the procedures of the Italian National Reference Laboratory for coagulase-positive *Staphylococci* including *Staph. aureus*.

Results and discussion

Staphylococcus aureus strains were isolated in 481 (39%) of 1245 milk and cheese samples, with *Staph. aureus* positive samples accounting for 40% (343/848) and 35% (138/397), respectively. Table 1 shows the results of molecular tests for the detection of genes encoding the toxins SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SE*l*J, SE*l*P and SER. Of the 481 strains of *Staph. aureus* tested, 255 (53%) were positive for one or more SE genes and 35 different enterotoxin gene profiles were distinguished among the isolates (Table 1). None of the isolates was positive for *seb* and *see* genes, while *ser* gene, found in 134 (28%) of the isolates, was the most frequent, followed by *sed* (25%) and *selj* (25%) genes. The genes encoding for SED, SER and SE*l*J are carried on the same plasmid and among all the profiles, *sed-ser-selj* (15%) was the most common, followed by *seg-sei* (12·3%). These latter genes are likely to be carried together on the EGC. SE genes carried on plasmid and EGC were the most frequently present within the isolates bearing SE genes: genes known to be carried by those elements were present in 142 (30%) and 89 (19%) isolates, respectively (Table 1, Figure 1).

In spite of the great discrepancy in data concerning the prevalence of enterotoxigenic *Staph. aureus* isolates found in the literature, which is attributable to different types of foods and biovars involved, SEA is the most frequently observed enterotoxin in enterotoxigenic strains of *Staph. aureus* (Normanno *et al.* 2005). Asao *et al.* (2003) reported an outbreak of foodborne disease in Kansai, Japan, where 13 420 people were affected after ingesting skimmed milk and yogurt (prepared with powdered milk) contaminated with 0·38 ng ml^{-1} and 3·7 ng g^{-1} of SEA, respectively.

Several studies have investigated the amount of ingested SE required to initiate symptoms: indeed, doses are supposed to change in relationship with SE types and the health status and age of patients.

Table 1 Enterotoxin gene profiles with putative MGEs location. The number at the end of each line represents the number of isolates bearing a specific enterotoxin gene profile. The number at the bottom of each column represents the number of isolates bearing a specific enterotoxin gene

Phage		SaPI		Plasmid		EGC		Transp.	
sea	*selp*	*sec*	*sed*	*selj*	*ser*	*seg*	*sei*	*seh*	No of isolates
			X	X	X				72
						X	X		59
X			X	X	X				27
		X							23
X				X	X				10
								X	10
		X				X	X		7
			X	X	X	X	X		6
			X						4
X					X				4
			X		X				3
X							X		3
X									3
			X		X	X	X		2
X					X	X	X		2
				X					1
				X	X				1
					X				1
							X		1
	X		X		X				1
X			X		X				1
X				X					1
			X			X	X		1
X						X			1
X						X	X		1
	X					X	X		1
X			X			X	X		1
X			X		X	X	X		1
X			X	X	X	X	X		1
	X								1
X								X	1
		X			X				1
						X	X	X	1
X		X				X	X		1
X		X			X				1
59	3	33	120	119	134	85	88	12	

One of the first trials demonstrated that ingestion of 20–25 μg of SEB (*c.* 0·4 μg kg^{-1} of body weight) is able to cause emesis (Raj and Bergdoll 1969). However, the average dose of SEA ingested by students in an outbreak caused by milk chocolate in the United States resulted to be 144 $\pm$ 50 ng (Evenson *et al.* 1988). Furthermore, an outbreak in Japan caused by low-fat milk contaminated with SEA showed that the total intake of SEA per individual was estimated to be *c.* 20–100 ng (Asao *et al.* 2003). More recently, in a French outbreak caused by contaminated cheese, doses of SEE ingested by symptomatic persons were estimated to be about 90 ng, based on the mean weight of the cheese portion (about 200 g) and the total amount of SEE of food sample (0·45 ng g^{-1}) (Ostyn *et al.* 2010). In this study, the SEA gene was found in 59 isolates (12%), which is in contrast with previous findings, where SEA gene was predominant (Lawrynowicz-Paciorek *et al.* 2007; Morandi *et al.* 2009; Ostyn *et al.* 2012). The most frequent SE gene found in the isolates has been SER, together with SED and SE*l*J, carried on the same plasmid (Rall *et al.* 2008). As shown in Fig. 1, in our research, these genes were found together in 106 (22%) isolates and in 35 (7%) separately. SED was previously reported as the most frequently isolated toxin type, after SEA, in staphylococcal food-poisoning outbreaks involving dairy products (Lawrynowicz-Paciorek *et al.*

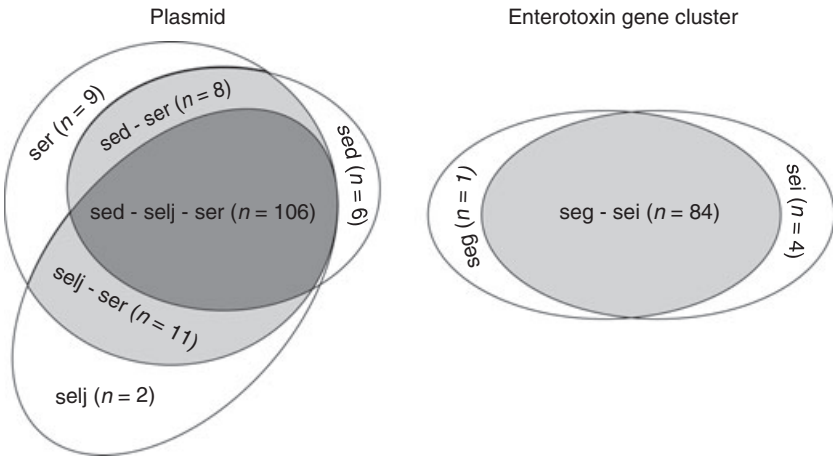

Figure 1 Number of isolates bearing SE genes and SE genes profiles located on Plasmid and EGC.

2007; Morandi *et al.* 2009). The SEG gene was observed in 85 (18%) isolates of *Staph. aureus*; just in one of the cases, it was not associated with SEI (Fig. 1). Similar values were reported by Rosec and Gigaud (2002), who observed that SEG and SEI were associated in 80·6% of 155 strains. These genes are frequently found together because they are within the same genetic cluster (EGC), in a 3·2-kb DNA fragment (Jarraud *et al.* 2001). The small percentage of strains carrying only one of these two enterotoxin genes could be explained by point mutations in *seg* or by variations in the cluster where these two genes are located (Jarraud *et al.* 2001). With PCR and enzyme-linked immunosorbent assays, Omoe *et al.* (2005) showed that most of the *seg*-positive *Staph. aureus* isolates, and about 60% of the SEI-positive isolates, did not produce detectable levels of SEG or SEI, while reverse transcription-PCR showed the presence of mRNA generated from *seg* and *sei* genes. The gene products of *seg, sei, selm, seln* and *selo* may therefore be produced in small quantities only, resulting in a minor or negligible role in staphylococcal food poisoning (Bystron *et al.* 2010), although other authors suggested a relevant role for strains producing SEG and SEI in foodborne severe neonatal enteropathy (Naik *et al.* 2008) and toxic shock syndrome (Jarraud *et al.* 1999; Holtfreter *et al.* 2004).

The recent identification of new SEs has considerably increased the isolation frequency of enterotoxigenic staphylococci, thus suggesting that the pathogenic potential of *Staph. aureus* may be greater than previously thought. Little is known, however, about the role of these new enterotoxins by *Staph. aureus* in the occurrence of SFPs, and this is particularly true for SEs encoded by genes located on the EGC (*seg* and *sei*). Further studies are needed to quantify the expression of these, and to assess their contribution to foodborne disease burden. Moreover, due to the involvement of recently described SEs, commercially available kits able to detect these toxins should also be developed, as, to date, available kits are only able to detect SEA to SEE toxins (Hennekinne *et al.* 2010). In particular, this could be the case with SEH, which has been responsible for milk-based food-poisoning outbreaks (Jørgensen *et al.* 2005; Ostyn *et al.* 2012).

Materials and methods

In the period from January 2010 to June 2011, a total of 1245 dairy samples were collected in Turin area (north-western Italy) and carried to local public laboratory (Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta, Italy) at a controlled temperature +1/+8°C according to ISO 7218:2007 (Anonymous 2007). In total, 848 samples of raw cow milk, derived from unpasteurized milk dispensers, and 397 samples of unpasteurized cheese products were analysed for CPS isolation and for *Staph. aureus* identification.

For coagulase-positive staphylococci isolation, serial dilutions of each sample homogenate were plated on Baird–Parker agar + rabbit plasma fibrinogen (BP-RPF) (Liofilchem srl, Roseto degli Abruzzi - TE, Italy) and incubated at 37 ± 1°C for 48 h. At least one characteristic colony per sample was tested with commercial biochemical identification kits API® ID32 STAPH (bioMérieux, Marcy l'Etoile, France) to identify *Staph. aureus* strains.

Genomic DNA was obtained from *Staph. aureus* strains using InstaGeneTM Matrix (Bio-Rad, Milano, Italy). Each colony was mixed in 100 μl of matrix buffer, incubated for 60 min at 56°C and then for 45 min at 95°C. Finally, the suspension was centrifuged for 5 min at 20 000 ***g***, and supernatant was used for PCR amplification.

To detect SEs genes, two multiplex PCR protocols were used according to European Union Reference Laboratory for Coagulase-Positive Staphylococci (EU-RL CPS) methods (Kérouanton *et al.* 2007). The eleven primer pairs

Table 2 Assay conditions for *sea, seb, sec, sed, see, ser* genes amplification tests

Gene	Primer	Sequence (5′–3′)	Concentration (μmol l^{-1})	Size (bp)	Reference
sea	GSEAR-1	GGT TAT CAA TGT GCG GGT GG	0·2	102	Mehrotra *et al.* (2000)
	GSEAR-2	CGG CAC TTT TTT CTC TTC GG	0·2		
seb	GSEBR-1	GTA TGG TGG TGT AAC TGA GC	0·2	164	Mehrotra *et al.* (2000)
	GSEBR-2	CCA AAT AGT GAC GAG TTA GG	0·2		
sec	GSECR-1	AGA TGA AGT AGT TGA TGT GTA TGG	0·2	451	Mehrotra *et al.* (2000)
	GSECR-2	CAC ACT TTT AGA ATC AAC CG	0·2		
sed	GSEDR-1	CCA ATA ATA GGA GAA AAT AAA AG	0·8	278	Mehrotra *et al.* (2000)
	GSEDR-2	ATT GGT ATT TTT TTT CGT TC	0·8		
see	SA-U	TGT ATG TAT GGA GGT GTA AC	0·6	213	Sharma *et al.* (2000)
	SA-E rev	GCC AAA GCT GTC TGA G	0·6		
ser	SER 1	AGA TGT GTT TGG AAT ACC CTA T	0·2	123	Chiang *et al.* (2008)
	SER 2	CTA TCA GCT GTG GAG TGC AT	0·2		

Table 3 Assay conditions for *seg, seh, sei, selj, selp* genes amplification tests

Gene	Primer	Sequence (5′–3′)	Concentration (μM)	Size (bp)	Reference
seg	SEG-F	GTT AGA GGA GGT TTT ATG	0·6	198	Bania *et al.* (2006)
	SEG-R	TTC CTT CAA CAG GTG GAG A	0·6		
seh	SEH-F	CAA CTG CTG ATT TAG CTC AG	0·4	173	Bania *et al.* (2006)
	SEH-R	CCC AAA CAT TAG CAC CA	0·4		
sei	SEI-F	GGC CAC TTT ATC AGG ACA	0·8	328	Bania *et al.* (2006)
	SEI-R	AAC TTA CAG GCA GTC CA	0·8		
selj	SEJ-F	GTT CTG GTG GTA AAC CA	1·0	131	Bania *et al.* (2006)
	SEJ-R	GCG GAA CAA CAG TTC TGA	1·0		
selp	SEP-F	TCA AAA GAC ACC GCC AA	0·8	396	Bania et al. (2006)
	SEP-R	ATT GTC CTT GAG CAC CA	0·8		

designed for PCR targeting genes (Tables 2 and 3) were purchased from Invitrogen (Carlsbad, CA, USA). Each PCR contained 1 U of FastStart Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany), 1× FastStart Buffer without $MgCl_2$ (Roche Diagnostics), 2·5 mmol l^{-1} $MgCl_2$ (Roche Diagnostics), 0·2 mmol l^{-1} dNTPs (Fermentas, Vilnius, Lithuania), primers (concentration reported in Tables 2 and 3) and 2 μl of DNA samples. The final volume was adjusted to 25 μl by adding sterile ultrapure water.

Reference strains of *Staph. aureus*, FRI S6 (SEA, SEB), FRI 137 (SEG, SEH, SEI), FRI 326 (SEE), FRI 361 (SEC, SED, SER), HMPL 280 (SEG, SEI, SE*l*J, SE*l*P), were used as positive controls (provided by EU-RL of CPS).

PCR were performed on a GeneAmp System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Two thermal profiles were set according to EU-RL of CPS protocols (Tables 2 and 3).

Reaction products were separated by standard gel electrophoresis using 10 μl of the PCR product mixture on 2·5% agarose gels in TAE buffer (0·1 mol l^{-1} Tris, 0·1 mol l^{-1} acetic acid and 0·002 mol l^{-1} Na_2EDTA) adding Gel Green (Biotium, Hayward, CA, USA). Molecular marker (Bio-Rad) was used as molecular weight standard. The gels were visually inspected under a UV transilluminator (GelDoc, Bio-Rad).

Conflict of interest

The authors have no conflict of interests to declare.

References

Anonymous. (2007) Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations. (ISO 7218:2007).

Argudin, M.A., Mendoza, M.C. and Rodicio, M.R. (2010) Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins (Basel)*. **2**, 1751–1773.

Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K., Nakazawa, H. and Kozaki, S. (2003) An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiol Infect* **130**, 33–40.

Balaban, N. and Rasooly, A. (2000) Staphylococcal enterotoxins. *Int J Food Microbiol* **61**, 1–10.

Bania, J., Dabrowska, A., Bystron, J., Korzekwa, K., Chrzanowska, J. and Molenda, J. (2006) Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. *Int J Food Microbiol* **108**, 36–41.

Bystron, J., Podkowik, M., Piasecki, T., Wieliczko, A., Molenda, J. and Bania, J. (2010) Genotypes and enterotoxin gene content of *S. aureus* isolates from poultry. *Vet Microbiol* **144**, 498–501.

Chiang, Y.C., Liao, W.W., Fan, C.M., Pai, W.Y., Chiou, C.S. and Tsen, H.Y. (2008) PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. *Int J Food Microbiol* **121**, 66–73.

Evenson, M.L., Ward Hinds, M., Bernstein, R.S. and Bergdoll, M.S. (1988) Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. *Int J Food Microbiol* **7**, 311–316.

Frost, L.S., Leplae, R., Summers, A.O. and Toussaint, A. (2005) Mobile genetic elements: the agents of open source evolution. *Nat Rev Micro.* **3**, 722–732.

Genigeorgis, C.A. (1989) Present state of knowledge on staphylococcal intoxication. *Int J Food Microbiol* **9**, 327–360.

Goerke, C. and Wolz, C. (2004) Regulatory and genomic plasticity of *Staphylococcus aureus* during persistent colonization and infection. *Int J Med Microbiol* **294**, 195–202.

Hennekinne, J.-A., Ostyn, A., Guillier, F., Herbin, S., Prufer, A.-L. and Dragacci, S. (2010) How should staphylococcal food poisoning outbreaks be characterized? *Toxins.* **2**, 2106–2116.

Hennekinne, J., De Buyser, M. and Dragacci, S. (2011) *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* **36**, 815–836.

Holtfreter, S., Bauer, K., Thomas, D., Feig, C., Lorenz, V., Roschack, K., Friebe, E., Selleng, K. *et al.* (2004) egc-encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. *Infect Immun* **72**, 4061–4071.

Jarraud, S., Cozon, G., Vandenesch, F., Bes, M., Etienne, J. and Lina, G. (1999) Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. *J Clin Microbiol* **37**, 2446–2449.

Jarraud, S., Peyrat, M.A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F. *et al.* (2001) egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens *in Staphylococcus aureus*. *J Immunol.* **166**, 669–677.

Jørgensen, H.J., Mørk, T., Caugant, D.A., Kearns, A. and Rørvik, L.M. (2005) Genetic variation among *Staphylococcus aureus* strains from Norwegian Bulk milk. *Appl Environ Microbiol* **71**, 8352–8361.

Kérouanton, A., Hennekinne, J., Letertre, C., Petit, L., Chesneau, O., Brisabois, A. and De Buyser, M. (2007) Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *Int J Food Microbiol* **115**, 369–375.

Lawrynowicz-Paciorek, M., Kochman, M., Piekarska, K., Grochowska, A. and Windyga, B. (2007) The distribution of enterotoxin and enterotoxin-like genes in *Staphylococcus aureus* strains isolated from nasal carriers and food samples. *Int J Food Microbiol* **117**, 319–323.

Lina, G., Bohach, G.A., Nair, S.P., Hiramatsu, K., Jouvin-Marche, E. and Mariuzza, R. (2004) Standard nomenclature for the superantigens expressed by *Staphylococcus*. *J Infect Dis* **189**, 2334.

Lindsay, J.A. (2011) Genomics of Staphylococcus. In *Genomics of Foodborne Bacterial Pathogens* ed. Wiedmann, M. and Zhang, W. pp. 237–265. New York, NY: Springer.

Lindsay, J. and Holden, M.G. (2006) Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. *Funct Integr Genomics* **6**, 186–201.

Mehrotra, M., Wang, G. and Johnson, W.M. (2000) Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* **38**, 1032–1035.

Morandi, S., Brasca, M., Andrighetto, C., Lombardi, A. and Lodi, R. (2009) Phenotypic and Genotypic Characterization of *Staphylococcus aureus* Strains from Italian Dairy Products. *J Microbiol Int* **2009**, Article ID 501362.

Naik, S., Smith, F., Ho, J., Croft, N.M., Domizio, P., Price, E., Sanderson, I.R. and Meadows, N.J. (2008) Staphylococcal enterotoxins G and I, a cause of severe but reversible neonatal enteropathy. *Clin Gastroenterol Hepatol.* **6**, 251–254.

Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Decastelli, L., Mioni, R. *et al.* (2005) Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *Int J Food Microbiol* **98**, 73–79.

Omoe, K., Imanishi, K., Hu, D.-L., Kato, H., Takahashi-Omoe, H., Nakane, A., Uchiyama, T. and Shinagawa, K. (2004) Biological properties of Staphylococcal enterotoxin-like toxin type R. *Infect Immun* **72**, 3664–3667.

Omoe, K., Hu, D., Takahashi-Omoe, H., Nakane, A. and Shinagawa, K. (2005) Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiol Lett* **246**, 191–198.

Ostyn, A., De Buyser, M.L., Guillier, F., Groult, J., Felix, B., Salah, S., Delmas, G. and Hennekinne, J.A. (2010) First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. *Euro Surveill* **15**.

Ostyn, A., Buyser, M., Guillier, F., Krys, S. and Hennekinne, J. (2012) Benefits of the combined use of immunological- and PCR-based methods for determination of staphylococcal enterotoxin food safety criteria in cheeses. *Food Anal Methods* **5**, 173–178.

Raj, H.D. and Bergdoll, M.S. (1969) Effect of enterotoxin B on human volunteers. *J Bacteriol* **98**, 833–834.

Rall, V.L.M., Vieira, F.P., Rall, R., Vieitis, R.L., Fernandes, A. Jr, Candeias, J.M.G., Cardoso, K.F.G. and Araújo, J.P. Jr (2008) PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. *Vet Microbiol* **132**, 408–413.

Rosec, J. and Gigaud, O. (2002) Staphylococcal enterotoxin genes of classical and new types detected by PCR in France. *Int J Food Microbiol* **77**, 61–70.

Schelin, J., Wallin-Carlquist, N., Thorup Cohn, M., Lindqvist, R. and Barker, G.C. (2011) The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence.* **2**, 580–592.

SCVPH. (2000) (Scientific Committee on Veterinary Measures relating to Public Health) Opinion on staphylococcal enterotoxins in milk products, particularly cheeses. [online]. Available from: http://ec.europa.eu/food/fs/sc/scv/out61_en.pdf.

Sharma, N.K., Rees, C.E.D. and Dodd, C.E.R. (2000) Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. *Appl Environ Microbiol* **66**, 1347–1353.