Classical enterotoxins of coagulase-positive Staphylococcus aureus isolates from raw milk and products for raw milk cheese production in Ireland

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ORIGINAL PAPER

Classical enterotoxins of coagulase-positive *Staphylococcus aureus* isolates from raw milk and products for raw milk cheese production in Ireland

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Abstract Toxin-producing *Staphylococcus aureus* can be present in raw milk and therefore in cheese made from raw milk. To determine the number and type of toxin producers in raw milk used for raw milk cheese production in Ireland, 117 samples of raw milk and related products from five raw milk suppliers, to four raw milk cheesemakers in the South of Ireland, were analysed for coagulase positive *S. aureus*. Enumeration, using ISO 688-2 and plating on Baird Parker Rabbit Plasma Fibrinogen selective agar showed samples were within limits set by EC regulations. Isolates (151 from 81 positive samples) were characterised for production of staphylococcal enterotoxins (SEs) SEA, SEB, SEC and SED by reverse passive latex agglutination (SET-RPLA) and by multiplex polymerase chain reaction for the *sea*, *seb*, *sec*, *sed* and *see* genes. The results showed 83.2% of the isolates did not contain the *se* genes or the toxin producing capability tested for. From only one supplier, 26 isolates contained the *sec* gene and produced SEC. Within these 26 isolates, there were only two PFGE types. One SEC-producing isolate showed no toxin production when grown in sterile 10% reconstituted skim milk at 10 °C and 12 °C for 96 and 74 h, respectively. Low concentrations of SEC were produced at 14 °C and 16 °C after 74 and 55 h, respectively. The results of this survey indicate that milk used for raw milk cheese production in Ireland poses a limited risk to public health, although further studies on occurrence of toxin producing *S. aureus* should be undertaken.

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爱尔兰生乳及其产品中金黄色葡萄球菌毒素和毒素基因的检测

摘要 ： 生乳及由其生产的干酪中可能存在产毒素的金黄色葡萄球菌。为了检测在爱尔兰用于生产干酪制品的生乳中产毒素菌的金黄色葡萄球菌的类型和数量，从爱尔兰南部地区5个原料奶的供应商和4个生乳干酪生产厂家收集了117个生乳及其相关产品，采用贝尔德派克兔血浆纤维蛋白原选择性琼脂平板计数法(ISO 688-2)法测定了凝血酶阳性葡萄球，结果表明，样品中凝血酶阳性葡萄球菌菌数在欧盟法规规定的范围之内。采用反向被动乳胶凝集试验(SET-RPLA)测定了金黄色葡萄球菌(从81个阳性样本中分离出的151株菌)产生的肠毒素型SEA-SED，以及应用多重聚合酶链反应(PCR)对sea、*seb*、*sec*、*sed*和*see*基因进行扩增，实验结果表明83.2%的金黄色葡萄球菌株不含有产肠毒素(SE)的基因或者经检测不具有产肠毒素的能力。仅从1个原料乳供应商所提供的原料乳中分离出的26株金黄色葡萄球菌中检测到了*sec*基因和具有产肠毒素C的能力，而且这26株分离菌株属于2个不同PEGE基因型。一株产SEC的金黄色葡萄球菌在10%的无菌还原脱脂乳培养基中分别在10 °C和12 °C下培养96和74 h时后，没有肠毒素产生；而在14 °C和16 °C下培养74和55h后，产生了低浓度的SEC。调查结果表明，尽管爱尔兰用于生产干酪的生乳对公众健康影响的风险较低，但仍有必要进一步研究产肠毒素的金黄色葡萄球菌对食品安全带来的潜在危害。

Keywords *Staphylococcus aureus* · Raw milk · Enterotoxins · SE genes · SEC production

关键词 金黄色葡萄球菌 · 生乳 · 肠毒素 · 葡萄球菌肠毒素基因 - 产葡萄球菌肠毒素C

1 Introduction

Coagulase positive *Staphylococcus aureus* is a Gram-positive, facultative anaerobe that is ubiquitous in nature. Some, but not all strains produce pyrogenic, pepsin-resistant staphylococcal enterotoxins (SEs) which are potent emetic agents causing staphylococcal food poisoning (SFP; Pinchuk et al. 2010; Le Loir et al. 2003). SFP has recently been reported to have a low hospitalisation rate of 6.4% (Scallan et al. 2011); however, due to the nature of the toxin the effect of symptoms are rarely severe, leading to high levels of under-reporting.

S. aureus strains produce a wide range of toxins, and at present 21 SEs or enterotoxin-like proteins have been identified (Schlievert and Case 2007; Thomas et al. 2006). Of these toxins, SEA and SED are most frequently associated with food (De Buyser et al. 2001; Kérouanton et al. 2007), and are therefore most clinically relevant, while SEC is associated with animal origins (Pinchuk et al. 2010); more recently SEE was associated with SFP (Ostyn et al. 2010). Outbreak investigations in Austria with pasteurised milk had SEA and SED (Schmid et al. 2009), and in Japan SEA was the cause of an outbreak associated with pasteurised milk (Asao et al. 2003). Other examples of SFP caused by milk and dairy products are listed by Cretenet et al. (2011).

One of the major causes of bovine mastitis is *S. aureus*, which can then easily contaminate raw milk, particularly in cases of sub-clinical mastitis (Boynukara et al. 2008; Fagundes et al. 2010). With no heat treatment prior to manufacture, and milk being an ideal growth medium for bacteria, bacterial counts of *S. aureus* can increase (Charlier et al. 2009; Even et al. 2009). With subsequent growth opportunities during cheesemaking, it is possible that *S. aureus* counts can be relatively high in cheese.

Approximately 10% of cheese in Europe is made from raw milk (Beuvier and Buchin 2004), presenting a considerable potential risk to public health. In Ireland, a recent study showed that 96% of farmhouse cheese was within EU specifications for *S. aureus* (O'Brien et al. 2009). In Scotland, *S. aureus* was found to be the most frequent pathogen in raw milk cheese (Williams and Withers 2010). In France, a study of foodborne disease outbreaks from 1992 to 1997 found that *S. aureus* was the most frequent pathogen associated with milk related outbreaks (De Buyser et al. 2001). Other *S. aureus* studies of raw milk and raw milk products showed that the majority of raw milk supplied to artisan cheesemakers had high microbiological quality (D'Amico and Donnelly 2010).

Bovine raw milk cheese is considered a high-risk food in relation to the pathogen *S. aureus* (André et al. 2008). Where raw milk and cheese are concerned, there is a need for good hygiene both in the dairy and in the cheesemaking facilities (Little et al. 2008). Bacterial counts of *S. aureus* need to reach 10^5 to 10^8 cfu.mL^{-1} before sufficient toxin is produced to cause illness (Pinchuk et al. 2010). As SE toxins are heat resistant (and not affected by pasteurisation), bacterial counts of *S. aureus* in raw milk cheese can be misleading, where counts of *S. aureus* are high. Toxin can be produced, and if the *S. aureus* cells are inactivated, by pasteurisation for example, the toxin can remain in the milk and can cause SFP at levels of between 20 and 100 ng.mL^{-1} (Asao et al. 2003).The need for measurement of these toxins produced from the *S. aureus* in dairy products has been identified (Cretenet et al. 2011).

Not all coagulase positive *S. aureus* produce toxin. In Italy, 55% of food isolates were positive for classical SEs (Normanno et al. 2007), while in Norway 48% of isolates from bovine raw milk and raw milk products were identified as SE producers (Loncarevic et al. 2005). Studies of cheese from dairy farms in Sweden (Rosengren et al. 2010), including both bovine and ovine milk sources, showed that about 70% of cheese isolates carried one or more s*e* genes, where s*ec* and *sea* were the most common, therefore highlighting a risk with cheese produced by on-farm dairies. The phenotypic characterisation of *S. aureus* SE producing isolates includes the production of its specific toxin, while their genotypic characteristic includes the presence of the specific toxin genes. Polymerase chain reaction (PCR) methods have been developed to study gene occurrence in correlation with toxin production (Boerema et al. 2006).

In Ireland, there are approximately 60 farmhouse cheese-producing facilities, which use milk from a variety of mammals including cows, sheep and goats. The farmhouse cheese production is approximately 1,000 tonnes per annum, of which approximately 110 tonnes are from raw milk. In this study, the suppliers produce approximately 80 tonnes of bovine raw milk cheese per annum, representing over 70% of the national farmhouse bovine raw milk cheese production.

The aim of this study was to determine the number of toxin-producing coagulase positive *S. aureus* in raw bovine milk and raw milk cheese products made from that raw milk. Strains isolated were characterised further with regard to the type of SE produced, the identity of the toxin genes present, and the comparison of the strains using pulsed field gel electrophoresis (PFGE). SE production by SE-producing isolates grown in 10% reconstituted skim milk (RSM) at low to moderate temperatures of 10–16 °C was measured. Relatively low temperatures were used in order to identify the risk associated with toxin production at these temperatures.

2 Materials and methods

2.1 Sample collection

A total of 117 samples at various stages in cheese manufacture were aseptically collected from five bovine raw milk suppliers and from four raw milk cheese producers in the south of Ireland, from February to April 2010 (Table 1). Three of the cheese producers used raw milk from their own dairy herd, while the remaining cheese producer obtained milk from suppliers 4 and 5. The samples included raw milk from the bulk storage tank, the same raw milk after it had been added to the cheese vat and samples of the cheese (curds, fresh and ripened cheese) made from that milk. Raw milk and curds and whey were sampled from the farm dairy bulk tank or the cheese vat using a 50-mL sterile dipper and transferred to a sterile container. Fresh and ripened cheese samples were placed directly into a sterile container. All samples were kept at 4 °C, transported to the laboratory and analysed within 24 h of receipt.

2.2 Enumeration of *S. aureus* from raw milk and raw milk products

Coagulase positive *S. aureus* were enumerated using the method for raw milk and raw milk cheese, ISO 688-2-1999 on Baird Parker Rabbit Plasma Fibrinogen selective agar (BP-RPF; Oxoid). The limit of detection for raw milk is 10 cfu.mL^{-1} and for curds and cheeses is 100 cfu.g^{-1}.

2.3 Isolates of *S. aureus* from raw milk and raw milk products

When samples were positive for coagulase positive *S. aureus*, at least two colonies were selected and purified by streaking on tryptic soy agar (TSA). The isolates were frozen at −80 °C in cryovials containing 15% glycerol.

2.4 Analysis of staphylococcal enterotoxin production by the isolates

After growth in tryptone Soya broth (TSB), the 151 isolates were analysed in duplicate by reverse passive latex agglutination (SET-RPLA; Oxoid product Code: SET TD0900)

Table 1 Numbers of raw milk and associated product samples from five raw milk suppliers to four raw milk cheesemakers

Product	Supplier/ manufacturer 1	Supplier/ manufacturer 2	Supplier/ manufacturer 3	Supplier/ manufacturer 4	Supplier 5
Bulk raw milk	10 (10)	10 (3)	11 (9)	10 (10)	10 (10)
Vat raw milk	0	10 (6)	2 (1)	0	0
Curds	0	10 (9)	6 (6)	2 (2)	0
Whey	0	10 (0)	6 (0)	2 (2)	0
Fresh cheese	4 (2)	5 (5)	2 (0)	5 (4)	0
Ripe cheese	0	0	0	2 (0)	0

The number of coagulase positive S. *aureus* samples is shown in brackets

following the manufacturer's instructions. This SE test is for detection of presence or absence of SEA, SEB, SEC and SED. Since toxin production in food can be different than toxin production in laboratory media (Schelin et al. 2011), strains positive for enterotoxin in TSB were also analysed for toxin production in RSM.

2.5 Analysis by multiplex PCR

The primers used for gene detection of the four classical *S. aureus* enterotoxins (*sea*, *seb*, *sec*, *sed*) and *see* were all used previously in a PCR multiplex assay (Gonano et al. 2009; Mehrotra et al. 2000). The DNA extract from four *S. aureus* strains (from Lund University Sweden), known to contain these five enterotoxins was used as a positive control. DNA from the 151 isolates sampled was extracted using Prepman ultra™ (Applied Biosciences code 4318930) and tested in a multiplex PCR assay. The PCR master mix contained the following: 1 μL of a 0.5 μM solution of each primer set, 2 μL DNA, 12.5 μL ImmoMix™ Red (Bioline BIO25021) made up to 25 μL total reaction volume with PCR grade water. Amplicons were produced by subjecting samples to the following amplification conditions; initial heat treatment of 95 °C for 10 min; 30 cycles of 94 °C for 30 s, 54 °C for 45 s and 72 for 30 s; a final extension step of 72 °C for 10 min. The amplicons were separated by electrophoresis conditions at 80 V for 40 min and visualised in UV light with the addition of SyBr® Green (Invitrogen™). For comparative purposes, a 1-kb molecular weight marker (Bioline Hyperladder IV) and a positive control (DNA from four *S. aureus strains containing all five genes) were used in all gels.*

2.6 Analysis by pulsed field gel electrophoresis

Enterotoxin producing strains were analysed by PFGE using a PulseNet Method (CDC PulseNet), as previously described (McDougal et al 2003). This method was modified by adding 2 μL bovine serum albumen (BSA) to the digest mix. The addition of 50 $\mu mol.L^{-1}$ thiourea to both the agarose gel and the running buffer for PFGE improved band resolution.

2.7 SEC production

An SEC-producing isolate (isolated from this study) was inoculated into RSM at a concentration 10^7 $cfu.mL^{-1}$.This inoculated milk was incubated at 10 °C, 12 °C, 14 °C and 16 °C for 96, 74, 74 and 55 h, respectively, to determine the minimum temperature at which toxin will be produced and thus assess the risk of toxin in the milk prior to cheesemaking. As a control, an SEAD strain (isolated from boiled ham in a food poisoning outbreak; SA45 obtained from Swedish Institute for Food and Biotechnology, Gothenburg, Sweden) was tested under the same conditions. During incubation, five samples at 10 °C and ten samples at 12 °C, 14 °C and 16 °C were enumerated for *S. aureus*, using TSA plates (Merck). Initial and final samples of inoculated milk were collected and analysed for SE using 3 M™ Tecra™ Staph Enterotoxin Visual Immunoassay (SETVIA96; NZFSA validated) according to the manufacturer's instruction. SETVIA96 is an enzyme-linked immunosorbent assay (ELISA) method which relates SE concentration to absorbance (measured using a Synergy HT Multi-Mode Microplate Reader Gen 5).

Table 2 Enumeration results of *S. aureus* in samples of milk and products manufactured from the milk

Product	Supplier/ manufacturer 1	Supplier/ manufacturer 2	Supplier/ manufacturer 3	Supplier/ manufacturer 4	Supplier 5
Bulk raw milk	80–900 (260)	<10–35 (8)	10–110 (20)	140–700 (300)	15–330 (80)
Vat raw milk	–	<10–60 (14)	<10	–	–
Curds	–	<100–3,100 (700)	17–800 (300)	780–7,200 (4,000)	–
Whey	–	<1	<1	24–38	–
Fresh cheese	<100(40)	<100–6,700 (4200)	–	1,800–14,000 (2,200)	–
Ripe cheese	–	–	–	<10	–

The range of values (in cfu.mL^{-1} or cfu.g^{-1}) is shown with the average number in brackets

3 Results

3.1 Enumeration of *S. aureus* from raw milk and raw milk products

Table 1 shows the number of samples taken from each of the five raw milk suppliers. The bacterial count of coagulase positive *S. aureus* samples is shown in brackets. Supplier 1 only made cheese occasionally during the sampling period and therefore only four fresh cheeses were obtained for analysis. A total of 151 *S. aureus* isolates were obtained from the 81 positive samples. Table 2 shows enumeration results in cfu per millilitre. All samples were within the regulatory limits of <10^5 cfu.g^{-1} for cheese set by the EU (Commission Regulation [EC] No. 2073/2005; Commission Regulation [EC] No. 1441/2007): there are no EU regulations for raw milk. From supplier 4, two of the curd samples resulted in higher *S. aureus* counts, than the associated fresh cheeses; when the fresh cheese was ripened, bacterial counts were below the limit of detection.

3.2 Characterisation of isolates using SET-RPLA and multiplex PCR

All isolates were grown in TSB and tested for toxin production by SET-RPLA. Twenty-six of the isolates produced SEC. No other toxins were produced by any of the strains (Table 3). The SEC-producing isolates were also tested for SEC production

Table 3 Phenotypic and genotypic characterisation of 151 *S. aureus* isolates

Enterotoxin	No samples tested	Phenotypic (SET-RPLA[a])	Genotypic (multiplex PCR[b])
SEA	151	0	0
SEB	151	0	0
SEC	151	26	26
SED	151	0	0
SEE	151	Not determined	0

SET-RPLA staphylococcal enterotoxin reverse passive latex agglutination, *PCR* polymerase chain reaction

Table 4 Sample type from which staphylococcal enterotoxin C (SEC) producing isolates were obtained

Product	Total no. samples tested[a]	SEC-producing isolates[b]
Bulk raw milk	51	1
Vat raw milk	12	6
Curds	18	14
Whey	18	0
Fresh cheese	16	5
Ripe cheese	2	0

[a] As described in Table 1

[b] All positive isolates were from the same source, supplier/manufacturer 2

at 37 °C in RSM, and all were shown to produce SEC (data not shown). All SEC producing isolates were obtained from the same supplier/manufacturer (Table 4).

Figure 1 is a representative gel of the results obtained. Of the isolates tested, 26 had only the *sec* gene while the remaining isolates had none of the toxin genes tested for (data not shown).

3.3 Analysis of toxin-producing isolates by PFGE

The 26 SEC-producing isolates (which all came from the same supplier/manufacturer) were characterised as two distinct PFGE types, A and B, with three band differences between the two types (Fig. 2). There were nine isolates of type A and 17 isolates of type B. Type A was found in milk and subsequently in the cheese products associated with that milk; however, type B was only found in the vat milk and the cheese products.

3.4 Analysis of SEC production at different temperatures

The SEC-producing isolates were grown in RSM for 4 days at 10 °C, 3 days at 12 °C and 14 °C and 55 h at 16 °C. Figure 3 shows the results of end-point analysis for toxin. No absorbance increase was seen at 10 °C and 12 °C (indicating no toxin production), while low absorbance values were measured at 14 °C and 16 °C (indicating some toxin production). Results are compared to the SEAD control strain, which showed production of enterotoxin at all four temperatures, under the same

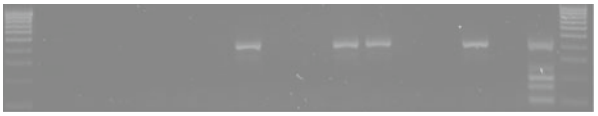

Fig. 1 Agarose gel electrophoresis profile, showing multiplex PCR of 14 *S. aureus* isolates. *Lanes 1 and 18* represent the 1-kb molecular weight marker; *lanes 8, 11, 12 and 15* are strains showing staphylococcal enterotoxin C (*sec*) gene. *Lane 16* negative control containing no DNA; *lane 17* positive control containing DNA from four *S. aureus* strains containing *sea* (102 bp), *seb* (164 bp), *sec* (451 bp), *sed* (278 bp) and *see* (209 bp) genes. All other lanes contained strains with no enterotoxin genes

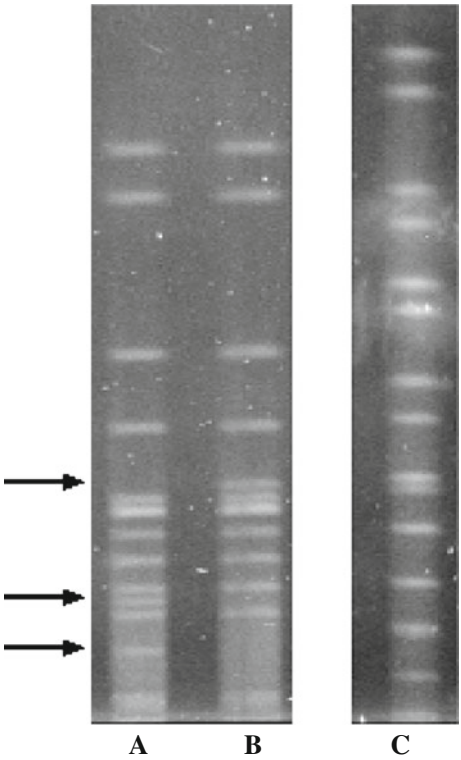

Fig. 2 PFGE profiles of two staphylococcal enterotoxin C PFGE types found (A, nine strains; B, 17 strains) showing three band differences (arrows showing A has one less band and two additional bands when compared to B) and *Salmonella* standard strain C

conditions. Higher absorbance values were observed for the SEAD producing strain, indicating the possible production of higher concentrations of toxin, although this would need to be measured by a quantitative assay.

4 Discussion

There are no EU specifications for bacterial counts of coagulase positive *S. aureus* in raw milk, but all of the cheeses tested were within the EU specifications for cheese (EC regulation 2073/2005, as amended by EC regulation 1441/2007).

The bacterial counts of coagulase positive *S. aureus* in the raw milk varied from <1 to 900 cfu.mL^{-1}. Of the 51 milk samples, 78% were positive for *S. aureus*, although the majority of the samples had <200 cfu.mL^{-1}. Samples in this study were collected from late winter to early spring — a time period considered 'worst case' with respect to *S. aureus* counts (Rea et al. 1992). In Vermont, the majority of samples had *S. aureus* at <1 cfu.mL^{-1} (D'Amico et al. 2008). In this current study, there was no physical indication of mastitis in the herds. S. *aureus* enumeration of milk samples from supplier/manufacturer 3 (11 milk samples tested) was relatively lower than other suppliers tested. This may be as a result of the low pressure milking system used by this supplier.

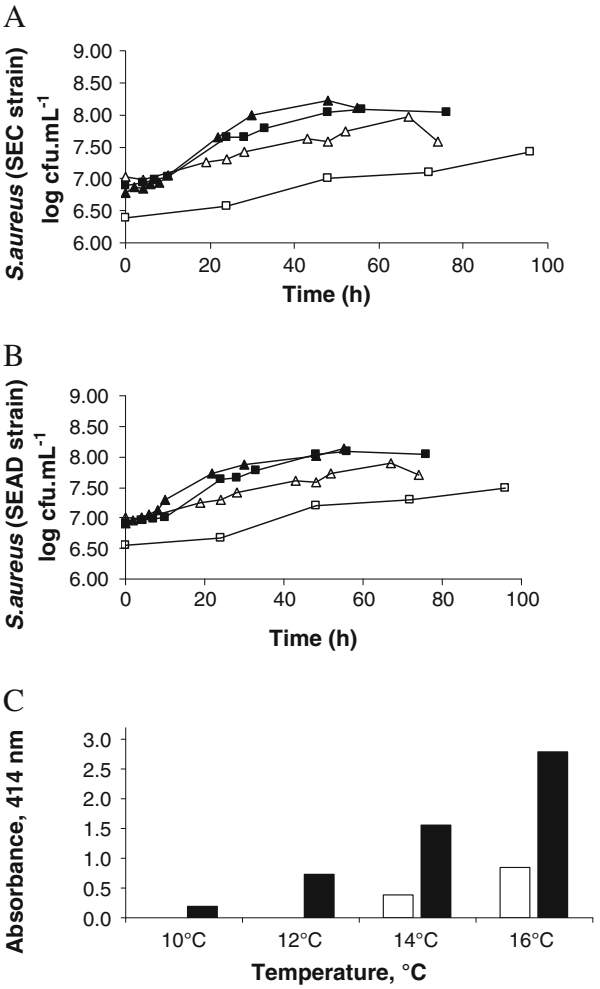

Fig. 3 *S. aureus* growth, measured in log cfu.mL^{-1} of **A** an SEC-producing strain and **B** an SEAD producing strain, at temperatures of 10 °C (*empty square*), 12 °C (*empty triangle*), 14 °C (*filled square*) and 16 °C (*filled triangle*). **C** Absorbance measurements of SEC (*empty square*) and SEAD (*filled square*) production after growth in reconstituted skim milk for 96 h at 10 °C, 74 h at 12 °C, 14 °C and 55 h at 16 °C

The presence of coagulase-positive *S. aureus* in milk, although it may be an indicator of a food safety issue, is not the most important factor. Enterotoxin-producing *S. aureus* must have the opportunity to grow during cheesemaking in order to produce sufficient toxin to cause illness. In general, cheesemaking does provide the opportunity for growth, although the manufacturer can take steps to reduce the amount of growth. For example, coagulase positive *S. aureus* do not grow at 4 °C and are poor competitors; therefore, proper temperature control and timely addition of starter culture can help to control growth (Charlier et al. 2009; Even et al. 2009). In addition, different starter cultures used during cheesesmaking, have been shown to be an important factor in the survival of *S. aureus* (Lindqvist et al. 2002; Bachmann and Spahr 1995). All cheeses in the current study were within the

specifications of $<10^5$ cfu.g^{-1} established by the EU, indicating that adequate control measures were taken by the manufacturers. Only the cheese manufactured from supplier 4 had relatively high *S. aureus* counts in the fresh cheese. Although only two ripe cheeses from supplier/manufacturer 4 were tested, this and other studies with this cheese type (Hunt and Jordan unpublished) have shown that coagulase positive *S. aureus* counts decrease as the cheese ages. The study of Williams and Withers (2010) reported a lower percentage of cheeses having coagulase positive *S. aureus*; however, that study was on ripened cheese, compared to the current study where the majority of cheeses were fresh cheese.

It is considered that in order to pose a threat to food safety, the counts of coagulase positive *S. aureus* in food must reach $>10^5$ cfu.g^{-1}, and must also have the ability to produce toxin. Sufficient toxin to cause illness varied depending on the toxin type, as little as 20–100 ng SEA has been reported to induce a toxic response (Asao et al. 2003). Therefore, the ability and type of strains to produce toxin is a relevant criterion. In this study, 151 isolates were obtained from the samples collected. These represented 20–40 isolates from each of the suppliers/manufacturers. Isolates were characterised for the presence of classical enterotoxins, and associated genes, including the *see* gene. None of the isolates from four of the five raw milk suppliers produced SEA, SEB, SEC or SED. From one manufacturer, 26 of the isolates produced SEC in milk or laboratory media, but no SEA, SEB or SED were produced in either medium. Among these toxins, SEC is the least important as it is rarely implicated in outbreaks of food poisoning (Pinchuk et al. 2010), being mostly associated with bovine strains (Jørgensen et al. 2005; Stephan et al. 2001). In other studies, 55%, 53% and 70% of isolates tested positive for SEs (Normanno et al. 2007; Loncarevic et al. 2005; Rosengren et al. 2010).

SET-RPLA is a recognised frequently used qualitative test for SE analysis by *S. aureus* isolates with a sensitivity of 0.25–0.5 ng.mL^{-1} (Rose et al. 1989; Cretenet et al 2011), equivalent to commercial ELISA or PCR for detection of SEs (McLauchlin et al. 2000). It has been used extensively in studies of SE production (Cenci-Goga et al. 2003; Wieneke 1991).We used a multiplex PCR assay to support the results of the SET-RPLA test. There was 100% correlation between the phenotypic and genotypic methods used. None of the strains tested contained the genes for SEA, SEB or SED and the SEC-producing strains only had the genes for SEC production. It is possible that a strain could contain the genes for toxin production but under the conditions of milk/cheese no toxin was produced. In different conditions toxin could be produced. That the strains tested did not harbour additional toxin producing genes is an additional risk reduction factor as toxin could not be produced under any conditions. In previous studies characterising *S. aureus* strains isolated from raw milk and cheese samples, SEC-producing isolates were also prevalent (Jørgensen et al. 2005; Normanno et al. 2007; Williams and Withers 2010).

From the 26 SEC-producing strains isolated, there were two PFGE types detected: A and B. Type A was found in the bulk tank milk and subsequently in the cheese vat milk and the cheese. Type B was only detected in the cheese vat milk and subsequent cheese. It is possible that type B could have been in the bulk tank milk but not detected as *S. aureus* counts may have been below the limit of detection (10 cfu.mL^{-1}). It is also possible that type B was a contaminant in the milk lines between the milk transport lines between the bulk milk tank and the cheese vat, or it

may be a human contaminant during cheesemaking (Williams and Withers 2010). Further characterisation of these strain types would be required to determine the source.

The SEC production in RSM is more relevant to assessing the risk of toxin in the bulk tank environment when compared to that in broth based assays (Schelin et al. 2011). The guidelines for storage of on-farm bulk milk are <4 °C, and reduction to <4 °C in 3 h when fresh milk is added to the tank. The results of this study indicate that even if milk was stored at an abuse temperature of 14 °C, very little toxin (based on the absorbance values obtained) would be produced even in 3 days. It would be expected that in raw milk toxin production would be even less as *S. aureus* are poor competitors (Charlier et al. 2009; Even et al. 2009). This result would need to be validated with other strains, including strains isolated from human sources and strains producing other SEs.

5 Conclusions

This study shows that only 17% (26 isolates) of coagulase positive *S. aureus* isolates were enterotoxigenic although within these strains there were only two PFGE types. These 26 strains were isolated from the same supplier, were all SEC producers, and were found both in milk and cheese samples. From the samples collected, no isolates harboured the genes encoding SEA, SEB or SED. One of the SEC-producing strains isolated showed no toxin production at 10 °C or 12 °C in milk. Therefore, the risk to public health from toxin-producing *S. aureus* in dairy products can be quantified to some extent and can be managed via temperature control.

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