Enterotoxin-producing *Staphylococcus aureus* genotype B as a major contaminant in Swiss raw milk cheese

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ABSTRACT

The objective of this study was to characterize *Staphylococcus aureus* isolates from Swiss raw milk cheeses that had been found to be contaminated with coagulase-positive staphylococci and to estimate the frequency of the various genotypes, in particular the mastitis-associated *Staph. aureus* genotype B (GTB). The isolates were also tested for staphylococcal enterotoxin (SE) genes and other virulence factors. From 623 coagulase-positive staphylococci isolated from 78 contaminated raw milk cheeses, 609 were found to be *Staphylococcus aureus*. Genotyping of all *Staph. aureus* isolates was performed by PCR amplification of the 16S–23S rRNA intergenic spacer region, as this method was used previously to differentiate between mastitis subtypes associated with their clinical outcome. In total, 20 different genotypes were obtained and the 5 most frequently occurring genotypes were distributed in 6.4% or more of the samples. The enterotoxin-producing *Staph. aureus* GTB, known for its high contagiousness and increased pathogenicity in Swiss mastitis herds, was found to be the most abundant subtype at the sample level (71.8%) as well as among the isolates (62.0%). A subset of 107 isolates of the different genotypes were analyzed for the presence of SE genes and revealed 9 different SE gene patterns, with *sed* being most frequently detected and 26% being PCR-negative for SE genes. Almost all isolates of the major contaminant GTB contained the SE gene pattern *sed*, *sej*, *ser*, with half of them additionally carrying *sea*. Production of SE in vitro was consistent with the SE genes detected in most of the cases; however, some isolated GTB did not produce SEA. *Staphylococcus aureus* Protein A (*spa*) typing revealed 30 different subtypes and most GTB isolates belonged to the bovine *spa* type t2953; GTB/t2953 was linked among other subtypes to SE production in cheese and staphylococcal intoxication cases. Furthermore, 1 of the 623 isolates was a methicillin-resistant *Staph. aureus*,

which was an *seh*-carrying *Staph. aureus spa* type t127 (non-GTB). We conclude that control and reduction of enterotoxigenic *Staph. aureus* GTB in dairy herds in Switzerland will not only prevent economic losses at the farm level but also improve the safety of raw milk cheeses; distribution of methicillin-resistant *Staph. aureus* via raw milk cheese is of less concern.

Key words: *Staphylococcus aureus*, subtyping, mastitis, raw milk cheese

INTRODUCTION

Staphylococcus aureus is one of the most important causes of chronic, clinical, or subclinical bovine mastitis worldwide and is associated with great economic losses in dairy. This species is also well known for its causative role in food poisoning outbreaks. The types of food associated with staphylococcal food poisoning (SFP) vary widely between countries and include milk, cream, cheeses, ham, sausages, salads, mushrooms, and cooked meals (Hennekinne et al., 2012). The importance of milk and milk products in SFP has been reviewed by De Buyser et al. (2001). In Switzerland, 7 of the reported 11 SFP outbreaks from 1996 to 2006 were linked to consumption of cheese (Baumgartner, 2008).

Enterotoxin A (SEA) and enterotoxin D (SED) are the most common staphylococcal enterotoxins (SE) observed in association with SFP (Balaban and Rasooly, 2000). These SE, together with the enterotoxins B (SEB), C (SEC), E (SEE) and the toxic shock syndrome toxin-1 (TST), have been well known for many years, whereas the toxins SEG, SEH, SEI, SER, SES, and SET and the enterotoxin-like (SEI) proteins SEIJ to SEIQ and SEIU to SEIV have only been reported more recently, as reviewed by Hennekinne et al. (2012). All SE and SEI proteins have superantigenic properties, resulting in nonspecific polyclonal T-cell expansion followed by massive release of cytokines, which may induce toxic shock. In addition, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER, SES, and SET are all capable of inducing emesis. However, commercial detection kits are available only for the “classical” enterotoxins SEA to SEE. The control of SE production

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is complex and involves various genetic loci, including the quorum-sensing accessory gene regulator (*agr*) operon. This fact may explain the observation that a relatively dense growth of *Staph. aureus* (approximately 10^5 cfu/g) is necessary for SFP. The occurrence of different patterns of SE gene expression and SE protein production depend on the SE type and diverse other factors, including whether the growth of *Staph. aureus* takes place in an artificial laboratory medium or a food matrix such as raw milk or cheese (Derzelle et al., 2009; Duquenne et al., 2010; Pexara et al., 2012).

A recent study from Switzerland (Fournier et al., 2008) isolated *Staph. aureus* from bovine IMI and found that the species is a heterogeneous group in that country. A total of 17 genotypes were detected by ribosomal spacer PCR from 101 epidemiologically independent isolates. Two of the genotypes, B (GTB) and C (GTC), predominated, accounting for 80.2% of the isolates, whereas the other genotypes (OG) were rarely detected (1 to 4% of the isolates). The different genotypes were highly associated with their virulence gene patterns. In particular, GTB was characterized by the presence of the *sea*, *sed*, and *sej*, a long x-region of *spa*, and a GTB-typical SNP in the leukocidin E (*lukE*) gene. In contrast, GTC was positive for *sec*, *seg*, *sei*, and *tst*, a short x-region of *spa*, and no SNP in the *lukE* gene. The OG were heterogeneous in their virulence gene patterns. Likewise, Fournier et al. (2008) found differences in the prevalence of IMI among genotypes. If GTB was isolated, the median cow prevalence in a herd was 47.2%, whereas for GTC and OG the median prevalence was 7.1% ($P < 0.001$) and 6.3% ($P < 0.001$), respectively. Thus, *Staph. aureus* GTB is involved in a herd problem (many cows infected), whereas GTC and OG are associated with individual cow disease (Fournier et al., 2008; Graber et al., 2009).

Raw milk is commonly used for the production of Swiss cheese because the milk enzymes add to the desired taste of the cheese. As a consequence, the bacterial flora present in the milk delivered by the farmers is crucial for cheese quality and safety. This flora is greatly influenced by the milking hygiene of the farmer and the bacterial udder health of a herd. Particularly in the case of *Staph. aureus* GTB, which causes herd problems of mastitis, the delivered milk is expected to be enriched by this subtype, so that increased amounts of *Staph. aureus* may also be present in the dairy products. In addition, *Staph. aureus* present in these products may also originate from other sources, including humans or contaminated equipment (Jørgensen et al., 2005; Kérouanton et al., 2007; Sakwinska et al., 2011).

To date, molecular data on *Staph. aureus* from Swiss raw milk cheeses are largely lacking. The objective of this study was to characterize *Staph. aureus* isolates

from Swiss raw milk cheeses contaminated with coagulase-positive staphylococci (CPS) and to estimate the frequency of the various genotypes, in particular the mastitis-associated *Staph. aureus* GTB. The isolates were also tested for SE genes and other virulence factors. Because methicillin-resistant *Staph. aureus* (MRSA) have been found in milk products and mastitis milk (Normanno et al., 2007; Huber et al., 2010) and because they are considered a major public health concern, we also screened our isolates for the presence of this resistance trait.

MATERIALS AND METHODS

Bacterial Isolates from Raw Milk Cheeses

In total, 623 isolates of CPS were analyzed. These strains (one or more per positive sample) were isolated from 78 bovine raw milk cheeses by different food control laboratories and were sent to our laboratory for further characterization. They originated from cheese curd ($n = 48$) and finished cheese ($n = 30$) produced in 60 dairies located throughout Switzerland.

With the exception of 5 soft cheeses, 1 fresh cheese, and 1 hard cheese, all samples were of semi-hard cheese type. For 63 samples, multiple (up to 10) isolates were available for testing.

Bacteriological and Molecular Testing

Upon arrival, all isolates were streaked on Baird Parker agar supplemented with rabbit plasma fibrinogen (BioMérieux, Marcy l'Etoile, France), blood agar (Oxoid, Basingstoke, UK) and Chromagar *Staph. aureus* (Chromagar, Paris, France). Subsequently, the CPS were screened by PCR for the presence of the *nuc* gene, which codes for thermonuclease and is specific for *Staph. aureus* (Brakstad et al., 1992). All *Staph. aureus* isolates (*nuc*-positive CPS with typical appearance on the different agars) were then subjected to genotyping (see below). Afterwards, 1 isolate of each genotype from each sample was further characterized. In case of multiple samples per cheese production site, each genotype was further characterized only once, even if it appeared several times.

Nucleic acid extraction; PCR amplification of *nuc*, *coa*, *spa*, *tst*, and *lukE* genes; and genotyping based on PCR amplification of the 16S–23S rRNA intergenic spacer region were performed as described by Fournier et al. (2008). The SE genes *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *ser*, and *sep* were amplified using 2 multiplex PCR protocols (De Buyser et al., 2009). In addition, all samples were further tested with singleplex PCR for *sed*, after Fournier et al. (2008), as weak

sed amplification was occasionally observed with the multiplex reaction. All PCR products were analyzed by the miniaturized electrophoresis system DNA 7500 LabChip (Agilent Technologies, Basel, Switzerland). Furthermore, all CPS were screened for growth on MRSA brilliance agar (Oxoid) and tested by real-time PCR for the presence of the MRSA-specific *mecA* gene, after de Boer et al. (2009), using the Suremaster Probe V Mastermix with an internal amplification control (R-Biopharm, Darmstadt, Germany) on a Rotorgene 6000 cycler (Corbett Research, Mortlake, Australia).

Staphylococcus aureus Protein A (*spa*) typing was performed by amplification of the *spa* gene followed by sequence analysis (Microsynth, Balgach, Switzerland) and assignment using the Ridom Staphtype software (Ridom GmbH, Würzburg, Germany; www.spaserver.ridom.de).

SE Production Tests

Isolates were grown aerobically on blood agar plates for 18 to 24 h. A single colony was transferred into tryptic soy broth (Oxoid) and incubated aerobically at 37°C for 18 to 24 h under continuous shaking at 150 rpm. Testing for enterotoxin production (SEA to SED) was done with the reversed passive latex agglutination assay (SET-RPLA; Oxoid) according to manufacturer's instructions.

RESULTS

In total, 609 of 623 CPS isolates were found to be *Staph. aureus* (*nuc*-positive colonies with typical appearance on the media used). These *Staph. aureus* originated from 77 of the 78 raw milk cheese samples contaminated with CPS. Subsequent genotyping of all *Staph. aureus* isolates was performed by PCR amplification of the 16S–23S rRNA intergenic spacer region, as this method was used earlier to differentiate between mastitis subtypes associated with their clinical outcome (Fournier et al., 2008). The results for each sample are summarized in Table 1. The most frequently found genotypes were GTB with 386 (62.0%), GTI with 72 (11.6%), GTF with 54 (8.7%), GTC with 25 (4.0%), and GTA with 14 (2.2%) among the isolated CPS. In addition to these genotypes, 15 OG with low frequencies were found. The GTB isolates were abundant regardless of whether the isolates came from cheese curd or end products. At the sample level, the most frequently found genotypes—GTB, GTI, GTF, GTC, and GTA—were observed in 56 (71.8%), 27 (35.6%), 15 (19.2%), 9 (11.5%), and 5 (6.4%) of the 78 samples, respectively (Table 2). However, in soft cheeses, the proportion of GTB was lower compared that that of GTI, whereas

the opposite was observed for semi-hard cheeses. In addition, isolates belonging to GTB, GTI, GTF, GTC, and GTA were present in 41 (68.3%), 20 (33.3%), 12 (20.0%), 7 (11.6%), and 5 (8.3%) samples from the 60 different cheese production sites, respectively (data not shown). In 4 of 5 production facilities, where isolates from multiple samples were available and where GTB was present, this genotype persisted up to 27 wk (at least 3 time points measured; data not shown). The most frequently occurring genotypes were observed in samples from all different regions of Switzerland (data not shown).

When several isolates per sample were available, up to 5 different genotypes could be detected (Table 1). The GTB isolate, when present in samples with multiple isolates, made up more than half of the isolates in 42 of 52 (80.8%) cases; GTB was present in 14 of 21 of samples containing $>10^5$ cfu/g CPS.

The occurrence and distribution of virulence genes and their properties were studied in a subset of 107 isolates (Table 3). From 20 different genotypes, 14 represented isolates positive for SE genes and, from the most commonly found, only isolates of GTI carried no SE genes. Isolates of GTB and 4 OG contained at least one of the “classical” SE genes (*sea* to *see*). Neither *seb* nor *see* could be detected, and *sea* occurred exclusively in *Staph. aureus* GTB. In GTB isolates, the SE gene patterns *sea*, *sed*, *sej*, *ser*, *sed*, *sej*, *ser*; and *sea* were observed in 21 (50.0%); 20 (48.0%); and 1 (2%) of the 42 cases. All but 2 isolates of OG being PCR-positive for SE genes contained one or more of the “new” SE genes, and in isolates representing 4 genotypes (GTC and 3 OG), the *tst* gene was present. The latter gene was always associated with the presence of *sec* or *seg*, *sei*. Several SE gene patterns were restricted to one distinct genotype each (all SE gene patterns in GTB; *seh*; *sed*, *seg*, *sej*, *sep*, *ser*; and *sed*, *seg*, *sei* in 3 different OG), whereas others (*seg*, *sei*; *sei*) were found in more than one genotype. Between some of the isolates representing genotypes with the same SE gene pattern, differences were observed when additional tests on the *luk* and *coa* gene loci were applied (Table 3).

Sixty isolates carrying the classical SE genes were assessed in regard to their potential to produce enterotoxin in vitro. All strains produced SE, which was detected by the reversed passive latex agglutination test. However, in 6 GTB isolates with the *sea*, *sed*, *sej*, *ser* gene pattern, SEA production was not observed (Table 3).

Thirteen of 78 (16.6%) cheese samples contaminated with CPS were free from isolates carrying SE, whereas 58 of 78 (75.6%) contained isolates with the classical SE genes *sea* to *see* (mainly from GTB), and 7 contained isolates that contained exclusively new SE genes *seg*, *seh*, *sei*, *sej*, *ser*, and *sep* (data not shown).

Table 1. Distribution of coagulase-positive staphylococci (CPS) genotypes among Swiss raw milk cheese samples

CPS (cfu/g)	Samples (no.)	Matrix type (no.)	<i>Staph. aureus</i> genotypes and non- <i>Staph. aureus</i> CPS ¹ isolates per sample (no.) ²	Isolates (no.)
<10 ³	5	Curd (3) Cheese (2) Curd (12)	B (7); B (5), I (3); B (7), F (3) B (7), OG (3) ; B (10) ³ B (5), I (2), OG (2), F (1); B (10); B (10); B (7), OG (1); OG (6), I (2), C (1), OG (1); B (8), C (2); B (10); B (10); B (9), C (1); B (9), F (1); C (4), I (3), OG (2), B (1) NSA (10); B (10); B (3); I (2), OG (2), F (2), A (1); F (10); B (10)	25 20 118
10 ³ to <10 ⁴	17	Curd (12)	B (9); OG (9); B (6); B (10); B (8), F (2); B (5), F (4), I (1); B (7), I (2); B (8), F (2); B (9), A (1); B (10); B (7), F (2), C (1); F (5), C (4), NSA (1); B (7), A (2), NSA (1); B (8), OG (1), NSA (1); B (9), OG (1); B (3), C (1); C (9), B (1); F (6), B (4)	50 186
10 ⁴ to <10 ⁵	35	Curd (20) Cheese (5)	I (10); OG (1); B (6), I (4); F (10); OG (10); B (9), OG (1); B (10); B (1); B (1); I (1); I (1), OG (1) B (10); B (8), I (1), OG (1); B (9); I (9), B (1); B (10); B (10); B (9), F (1); B (10); B (8), I (2) OG (1); B (1); OG (1) ; B (1); I (1) B (9), OG (1); I (10); I (5), B (4), NSA (1); A (9), B (1) F (5), B (2), I (2), A (1); OG (1) ; I (1) B (315), I (48), F (27), C (25), A (12), OG (27), NSA (4) B (71), I (24), F (27), C (0), A (2), OG (31), NSA (10) Total: B (386), I (72) F (54), C (25), A (14), OG (58), NSA (14)	78
10 ⁵ to <10 ⁶	14	Curd (9) Cheese (5)	B (10); B (8), I (1), OG (1); B (9); I (9), B (1); B (10); B (10); B (9), F (1); B (10); B (8), I (2) OG (1); B (1); OG (1) ; B (1); I (1)	89 5
>10 ⁶	7	Curd (4) Cheese (3)	B (9), OG (1); I (10); I (5), B (4), NSA (1); A (9), B (1) F (5), B (2), I (2), A (1); OG (1) ; I (1)	40 12
Total	78	Curd (48) Cheese (30)	B (315), I (48), F (27), C (25), A (12), OG (27), NSA (4) B (71), I (24), F (27), C (0), A (2), OG (31), NSA (10) Total: B (386), I (72) F (54), C (25), A (14), OG (58), NSA (14)	458 155 623

¹B, I, F, C, A = *Staph. aureus* genotypes B, I, F, C, A; OG = other genotypes; NSA = non-*Staph. aureus* CPS.
²Genotypes found in one sample are separated by colon; samples are separated by semicolon; genotypes in bold were involved in an outbreak or sporadic case of intoxication.
³Sample positive for staphylococcal enterotoxin (SEA to SEE).

Additionally, we applied *spa* typing as a second subtyping scheme to our subset of *Staph. aureus* isolates (Table 3). The 20 different genotypes were split into 30 *spa* types. With the exception of *spa* types t1773 and t2094, which occurred in 2 different groups of genotypes, we found no overlap between *spa* types and genotypes found. The GTB isolate comprised 6 known *spa* types and 3 that have not been described before. However, t2953 clearly dominated within the isolates belonging to GTB, as it was present in 34 of 42 cases (81.0%). Outside the GTB cluster, *spa* type t524 was predominant.

All 617 CPS isolates were screened by real-time PCR for the presence of the *mecA* gene, and 1 isolate was found to be positive. This isolate grew well on MRSA plates and was a *Staph. aureus* carrying the *seh* gene and belonging to *spa* type t127. The MRSA phenotype of this isolate was confirmed at the Institute for Infectious Diseases IFIK (University Hospital of Berne, Switzerland).

DISCUSSION

Our study of the enterotoxigenic properties and genotyping of *Staph. aureus* isolates from Swiss raw milk cheese revealed substantial diversity in the population of this pathogenic species. Up to 5 different genotypes were observed in an individual cheese, which is similar to the findings of a Norwegian study, where isolates with 6 different pulsed-field gel electrophoresis patterns were described in a particular cheese sample (Loncarevic et al., 2005). The same study found less diversity in raw milk, which can be explained by the fact that, for *Staph. aureus*, contamination sources other than raw milk exist. Potential sources are the production environment and food handlers. Most of the collected isolates in our study came from cheese curd in which the highest numbers of CPS are expected to occur during production. Therefore, samples taken at this stage of production are tested for whether the process complies with the requirements of the Ordinance of Hygiene (Federal Department of Home Affairs, 2014), which is identical to the European Commission (EC) regulation 2073/2005. In 22 of 61 (36%) samples with multiple isolates of *Staph. aureus*, only 1 genotype was found. However, in an early processing step where curd is formed, up to 4 different genotypes were observed (in 2 samples; Table 1). In several cases, toxigenic and nontoxigenic isolates representing different genotypes were detected in the same samples. Therefore, we recommend testing multiple isolates from curd or cheese sampled in case of SFP outbreak investigations.

A diversity of 20 different genotypes has been identified in this study. However, only 5 genotypes oc-

Table 2. Distribution of coagulase-positive staphylococci (CPS) genotypes among samples of different cheese types

Cheese type	Number (%) of samples with a specific genotype ¹						
	B	I	F	C	A	OG	NSA
Fresh (n = 1)	0	0	0	0	0	1	0
Soft (n = 5)	2	5	1	0	1	1	0
Semi-hard (n = 71)	53 (74.7)	22 (31.0)	14 (19.7)	9	3	24 (33.8)	5
Hard (n = 1)	1	0	0	0	1	1	0
Total (n = 78)	56 (71.8)	27 (35.6)	15 (19.2)	9	5	27 (34.6)	5

¹*Staph. aureus* genotypes B, I, F, C, A, and other genotypes (OG); NSA = non-*Staph. aureus* CPS.

curred in 6 to 72% of the samples, with GTB clearly dominating. Other studies also described high diversity but few dominating clusters of *Staph. aureus* isolates in milk or dairy products using different typing procedures such as random amplification of polymorphic DNA (RAPD)-PCR, pulsed-field gel electrophoresis, or amplified fragment length polymorphism (Villard et al., 2005; Boerema et al., 2006; Morandi et al., 2009). These authors showed associations between typing clusters and SE gene patterns in many but not all of the cases, a finding confirmed by our study.

We found 9 different SE gene patterns occurring in the subset of isolates, whereas 26% were SE gene negative (Table 3). The frequencies of SE gene negative isolates from dairy products vary remarkably between different reports; for example, 32% (Loncarevic et al., 2005) and 52% (Rosec and Gigaud, 2002). In our study, SE gene patterns *seg*, *sei*, *sea*, *sed*, *sej*, *ser*, and *sed*, *sej*, *ser* dominated among our isolates, with 22, 20, and 19%, respectively. At the single SE gene level, *sed* was most abundant (42%). These findings are in contrast to studies from Japan, Austria, France, Norway, and Turkey, where *sec* was most commonly detected in milk and raw milk cheeses (Katsuda et al., 2005; Loncarevic et al., 2005; Villard et al., 2005; Gonano et al., 2009; Kav et al., 2011). However, several Italian studies reported a situation similar to our data with dominance of *sed* often associated with *sea* and *sej* (Cremonesi et al., 2007; Normanno et al., 2007; Morandi et al., 2009). These data possibly indicate a different prevalence of certain *Staph. aureus* subtypes in different geographical regions. In Switzerland, the SE genes *sea* and *sed* predominate because of the abundant occurrence of the mastitis-associated *Staph. aureus* GTB in Swiss dairy herds. Indeed, this subtype was found in 30% of milk samples submitted for diagnostic purposes (Fournier et al., 2008). Furthermore, this pathogen is contagious (Graber et al., 2009) so that, on average, half of the cows of a herd are infected (Fournier et al., 2008). These cows normally shed large amounts of this subtype ($>5 \times 10^4$ cfu/mL) in the milk (Studer et al., 2008), leading to a high content of *Staph. aureus* GTB in the milk

delivered to the dairy. As Swiss cheese-making facilities are normally small, using milk of only a limited number of different herds, it is not surprising that, under these circumstances, *Staph. aureus* GTB may become the predominant staphylococcal subtype in Swiss raw milk cheese, as found in the present study. This situation may become a food safety concern if the *Staph. aureus* GTB bacteria further grow in the processed milk because of inappropriate milk storage and hygiene. Under these conditions, they may produce SE (Hennekinne et al., 2012), which may lead to food intoxication, as was observed in the present study (Table 1).

Our data on the presence of SE, including the association with SFP, show that *Staph. aureus* belonging to GTB, among other genotypes, can be the source of intoxication followed by illness (Table 1). In one case, the incriminated cheese originated from a production site where *Staph. aureus* GTB was widespread in the associated milk-producing herd. The majority of isolates from this sample belonged to GTB/t2953, carrying *sea* and *sed*, and therefore had the potential to produce SEA and SED in vitro. Furthermore, the incriminated cheese was reported to be positive for SE (A to E). A second cheese sample, from which only GTB/t2953 could be isolated, was found to be SE positive (A to E), but no SFP was reported in this case. In another report, *Staph. aureus* t2953 was described as the causative agent in an SFP outbreak due to contaminated milk (Schmid et al., 2009). Whether the abundant occurrence of GTB/t2953 in CPS-positive Swiss cheeses leads to an increased number of SFP remains to be elucidated and is complicated by the fact that SFP is known to be underreported. This could be especially true for Switzerland with its large number of small production facilities. In addition, our results show that GTB strains do not always produce SEA protein in vitro. Whether the potential of *Staph. aureus* GTB to cause SFP is reduced in such cases has to be examined in further studies.

In addition to its problematic role in food safety, *Staph. aureus* GTB causes high costs in Swiss dairy herds because antibiotic treatment of cows with mas-

Table 3. Distribution of virulence genes and their properties, enterotoxin production, methicillin resistance, and *spa* types among *Staph. aureus* genotypes B, I, F, C, A, and other genotypes (OG)

Genotype, isolates (no.)	Isolates positive for a particular gene, enterotoxin production, polymorphism, or <i>spa</i> type ¹ (n)								
	Toxin gene pattern	SET RPLA	<i>lukE</i>	<i>lukEB</i>	<i>lukEC</i>	<i>spa</i>	<i>coa</i>	<i>mecA</i>	<i>spa</i> type
B (42)	<i>sea, sed, sej, ser</i> (21)	A, D (16); D (5)	+	+	–	+	+	–	t2953 (15), t5268 (2), t334 (1), t3802 (1), t5271 (1), unknown (1)
	<i>sed, sej, ser</i> (20)	D (20)	+	+	–	+	+	–	t2953 (19), t5268 (1)
	<i>sea</i> (1)	A (1)	+	+	–	+	+	–	unknown (1)
I (20)	— (20)	NA	+	+	–	+	–	–	t524 (20)
F (13)	<i>seg, sei</i> (13)	NA	+	–	+	+	+	–	t164 (8), t2094 (2), t1987 (1), t2736 (1), unknown (1)
C (7)	<i>seg, sei</i> (6), <i>tst</i> (1)	NA	+	–	+	+	+	–	t529 (6)
	<i>sei</i> (1)	NA	+	–	+	+	+	–	unknown (1)
A (5)	<i>seh</i> (4)	NA	+	+	–	+	+	–	t127 (3), t2207(1)
	<i>seh</i> (1)	NA	+	+	–	+	+	+	t127 (1)
OG ² (20)	— (8) ³	NA	+	Var.	–	–	–	–	t4201 (1), t5428 (1), t156 (1), t3904 (3), t1773 (1), t2678 (1)
	<i>seg, sei</i> (4)	NA	Var.	Var.	Var.	Var.	Var.	–	t330 (1), t1544 (1), t015 (1), t2094 (1)
	<i>sec, tst</i> (2)	C (2)	+	Var.	Var.	–	–	–	t544 (1), t1773 (1)
	<i>sed, seg, sei, sej, sep, ser</i> (2)	D (2)	+	+	–	+	–	–	t442 (2)
	<i>sed, seg, sei</i> (2)	D (2)	+	–	+	+	+	–	t7013 (2)
	<i>sei</i> (1)	NA	–	+	–	+	+	–	t246 (1)
	<i>seg, sei, tst</i> (1)	NA	–	–	–	+	–	–	t021 (1)

¹*sea, sec, sed, seg, seh, sei, sej, ser, sep, tst* = staphylococcal enterotoxin genes and toxic shock syndrome toxin-1 gene; SET RPLA = in vitro production of staphylococcal enterotoxins A to D detected by reversed passive latex agglutination assay; *lukE, lukEB, lukEC* = polymorphisms of different leukocidin gene components; *spa* = gene of protein A (amplicon size ≥250 bp); *coa* = coagulase gene (amplicon size 640 bp); *mecA* = presence of methicillin resistance gene (MRSA); *spa* type = *spa* type according to Ridom database; NA = not assessed; Var. = variable result.

²Other sporadically appearing genotypes (15) were summarized as OG.

³Isolates being PCR-negative for SE genes came from 5 different OG.

titis caused by *Staph. aureus* is inefficient (Gruet et al., 2001) and most such cows need to be slaughtered. It would be highly desirable, therefore, to minimize *Staph. aureus* GTB at the country level, although this would require a suitable diagnostic assay. Such a test was recently developed by Boss et al. (2011) and was evaluated in a field study (Syring et al., 2012). It is based on real-time quantitative PCR and is highly sensitive and specific for *Staph. aureus* GTB such that bulk tank milk may be tested, which greatly simplifies the sampling logistics.

We applied *spa* typing to a subset of our *Staph. aureus* isolates, as this scheme has been used successfully in both clinical and food microbiology investigations (Koreen et al., 2004; de Boer et al., 2009; Hata et al., 2010). The *spa* typing revealed that the majority of *Staph. aureus* GTB belonged to *spa* type t2953 (Table 2). This type, and the other frequently appearing *spa* types from our study [t529 (GTC); t164, t2094, t1987 (GTF); t524 (GTI)], have been well described in bovine isolates from different countries worldwide (Ikawaty et al., 2009; Hasman et al., 2010; Hata et al., 2010; Hwang et al., 2010; Johler et al., 2011; Sakwinska et al., 2011).

Other *spa* types grouped into clonal complexes known from studies in humans; for example, t127 and t015. This indicates that several isolates from this study seemed to be of human origin. Among these, one isolate was found to be a MRSA and this is, to our knowledge, the first MRSA isolated from a ready-to-eat food item in Switzerland. The MRSA prevalence within isolates reported here was very low (0.002%), but it is in concordance with another Swiss study (Huber et al., 2010) and in contrast to reports on MRSA in meat (12%) or cheese (7%) from other countries (de Boer et al., 2009; Kav et al., 2011).

CONCLUSIONS

Several subtypes (genotypes and *spa* types) of *Staph. aureus* with different pathogenic and epidemiological properties can be found in Swiss raw milk cheese. Of these subtypes, strains of the mastitis-associated *Staph. aureus* GTB were most frequently found in cheeses contaminated with coagulase-positive staphylococci. Because these strains carry the SE genes *sea*, *sed*, *sej*, *ser* or *sed*, *sej*, *ser*, they can be linked to toxin production in cheese and food poisoning outbreaks. Therefore, control and reduction of *Staph. aureus* GTB in dairy herds will not only prevent economic losses at the farm level but also improve safety of raw milk cheeses. The distribution of MRSA via Swiss raw milk cheese is of less concern.

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