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Toxigenic Status of *Staphylococcus aureus* Isolated from Bovine Raw Milk and Minas Frescal Cheese in Brazil

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ABSTRACT

A group of 291 *Staphylococcus aureus* isolates from mastitic cow's milk ($n = 125$), bulk tank milk ($n = 96$), and Minas frescal cheese ($n = 70$) were screened for staphylococcal enterotoxin (SE) genes (*sea, seb, sec, sed, see, seg, seh, sei, selj,* and *sell*) and for the *tst-1* gene encoding staphylococcal toxic shock syndrome toxin 1 by PCR assay. A total of 109 (37.5%) of the isolates were positive for at least one of these 11 genes, and 23 distinct genotypes of toxin genes were observed. Of the *S. aureus* isolates bearing SE genes, 17 (13.6%) were from mastitic cow's milk, 41 (41.7%) were from bulk tank milk, and 51 (72.9%) were from Minas frescal cheese. The occurrence of exclusively more recently described SE genes (*seg* through *sell*) was considerably higher (87 of 109 PCR-positive strains) than that of classical SE genes (*sea* through *see*, 15 strains). The SE genes most commonly detected were *seg* and *sei*; they were found alone or in different combinations with other toxin genes, but in 60.8% of the cases they were codetected. No strain possessed *see*. The *tst-1* gene was found in eight isolates but none from mastitic cow's milk. Macrorestriction analysis of chromosomal DNA from 89 *S. aureus* isolates positive for SE gene(s) was conducted with the enzyme *Sma*I. Fifty-five distinct pulsed-field gel electrophoresis patterns were found, demonstrating a lack of predominance of any specific clone. A second enzyme, *Apa*I, used for some isolates was less discriminating than *Sma*I. The high genotype diversity of potential toxigenic *S. aureus* strains found in this study, especially from Minas frescal cheese, suggests various sources of contamination. Efforts from the entire production chain are required to improve consumer safety.

Staphylococcus aureus is a human and animal pathogen that can produce numerous toxins, including the pyrogenic toxins staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) *(39)*. SEs are a leading cause of gastroenteritis and vomiting resulting from consumption of contaminated food *(22)*. SE-contaminated milk and milk products often are involved in outbreaks. Milk and soft cheese are good substrates for *S. aureus,* and when an enterotoxigenic strain exceeds 10^5 CFU/ml or CFU/g, it may produce sufficient amount of toxin to cause intoxication symptoms *(3)*.

The SEs are a group of heat stable and pepsin resistant exotoxins encoded by genes in the chromosome, pathogenicity island, phages, or plasmids *(10)*. To date, 19 types of SEs, divided into two groups, have been reported and their genes described: the classical SEs (SEA, SEB, SEC, SED, and SEE) and the more recently described SEs, including SE-like (SEl) toxins (SEG, SEH, SEI, SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SElR, SElU, and SElV) *(10–12, 16, 21, 23, 25)*. The SEls are toxins that do not have emetic activity or have not been tested for emetic activity, a defined property of SEs *(17)*. SEF, which was discovered in 1980, was renamed TSST-1 because of the lack of evidence of emetic activity in monkeys *(3)*. TSST-1 causes toxic shock syndrome *(3, 38)*.

S. aureus is ubiquitous; it is usually present on the skin and mucosa of animals and humans and is frequently associated with bovine mastitis *(35)*. Without proper hygienic or sanitary precautions, contamination can occur throughout the milk processing chain. In Brazil, several researchers have reported recovery of *S. aureus* from mastitic bovine milk *(15, 30, 43)* and high counts in raw milk and Minas frescal cheese, with many samples exceeding 10^5 CFU/g *(6, 27, 29)*. Outbreaks of staphylococcal food poisoning associated with consumption of Minas frescal cheese containing classical enterotoxins have been reported *(7, 34)*. Of the more recently described SEs, only SEH has been associated with food poisoning, and one case was associated with cheese consumption in Brazil *(28)*. However, few studies are available concerning the occurrence of the newly described SE genes in *S. aureus* isolated from milk and/or milk products.

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TABLE 1. *Primers for the detection of* Staphylococcus aureus *toxin genes*

Gene	Primer sequence (5′ to 3′)	Amplicon (bp)	Reference
sea	ESA1: ACGATCAATTTTTACAGC ESA2: TGCATGTTTTCAGAGTTAATC	544	*31*
seb	ESB1: GAATGATATTAATTCGCATC ESB2: TCTTTGTCGTAAGATAAACTTC	416	*31*
sec	ESC1: GACATAAAAGCTAGGAATTT ESC2: AAATCGGATTAACATTATCCA	257	*31*
sed	ESD1: TTACTAGTTTGGTAATATCTCCTT ESD2: CCACCATAACAATTAATGC	334	*31*
see	ESE1: ATAGATAAAGTTAAAACAAGCAA ESE2: TAACTTACCGTGGACCC	170	*31*
seg	ESG1: ACGTCTCCACCTGTTGAAGG ESG2: TGAGCCAGTGTCTTGCTTTG	400	*31*
seh	ESH1: TCACATCATATGCGAAAGCAG ESH2: TAGCACCAATCACCCTTTCC	357	*31*
sei	ESI1: TGGAACAGGACAAGCTGAAA ESI2: TAAAGTGGCCCCTCCATACA	467	*31*
selj	ESJ1: CAGCGATAGCAAAAATGAAACA ESJ2: TCTAGCGGAACAACAGTTCTGA	426	*31*
sell	SEL-F: CACCAGAATCACACCGCTTA SEL-R: CTGTTTGATGCTTGCCATTG	240	*8*
tst-1	TSST-1: AAGCCCTTTGTTGCTTGCGAC TSST-2: AGCAGGGCTATAATAAGGACT C	250	*36*
femA	GFEMAR-1: AAAAAAGCACATAACAAGCG GFEMAR-2: GATAAAGAAGAAACCAGCAG	132	*19*

S. aureus isolates from milk and milk products can bear one or more enterotoxigenic genes, and some clones may be found. This study was conducted to investigate the distribution of classical SE genes (*sea* through *see*), the more recently described SE genes (*seg* through *sell*), and the TSST-1 gene (*tst-1*) among *S. aureus* isolates from mastitic cow's milk and bulk tank raw milk collected at dairy farms in the Rio de Janeiro and Minas Gerais states, Brazil, and from Minas frescal cheese sold in Juiz de Fora city, Minas Gerais. The enterotoxigenic strains also were characterized by their pulsed-field gel electrophoresis (PFGE) patterns.

MATERIALS AND METHODS

Bacterial strains. A total of 291 *S. aureus* isolates from mastitic cow's milk ($n = 125$), bulk tank milk ($n = 96$), and Minas frescal cheese ($n = 70$) were studied. All isolates were obtained from the bacterial collection of Embrapa Dairy Cattle (Juiz de Fora, Minas Gerais State, Brazil). The mastitic strains and the bulk tank milk strains originated from 40 and 22 dairy herds, respectively, located in the states of Rio de Janeiro and Minas Gerais, Brazil. The cheese strains were isolated from 12 brands of Minas frescal cheese made from pasteurized milk (under municipal, state, or federal inspection services) and sold in Juiz de Fora city, Minas Gerais. The bacteria from milk samples were isolated on mannitol salt agar (Difco, BD, Sparks, MD) and those from cheese samples were isolated on Baird-Parker agar (Difco, BD) *(2)*. The bacterial isolates were first characterized by Gram staining, catalase reaction, hemolytic properties, acetoin production (Voges-Proskauer reaction), and the tube coagulase reaction *(1)*. Isolates were then confirmed as *S. aureus* by PCR assay performed for the species-specific *femA* gene *(19, 40)*.

DNA extraction. Total genomic DNA was obtained from pure cultures of *S. aureus* isolates by the method described by Rosec and Gigaud *(31)* with some modifications. A bacterial culture was incubated overnight in brain heart infusion (BHI, Difco, BD) broth at 37°C, and 2 ml of this culture was centrifuged at 12,000 × *g* for 10 min. The cell pellet was washed two times with TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7.4) and resuspended in 200 μl of TE buffer containing 15 μl of lysostaphin (1 mg/ml; Sigma Aldrich, St. Louis, MO). After 30 min of incubation at 35°C, 10 μl of proteinase K (20 mg/ml) was added, and the suspension was incubated at 60°C for 20 min. The suspension was placed in a boiling bath for 10 min and then centrifuged at 12,000 × *g* for 2 min. The supernatant containing DNA was kept frozen (−20°C), and the DNA was quantified by spectrophotometry (Nanodrop ND-1000, Thermo Scientific Inc., Wilmington, DE) before PCR amplification.

PCR amplification. PCR amplification was performed for *femA,* 10 SE genes (*sea* through *sell*), and *tst-1* using primers previously described (Table 1). The detection of *femA* was done with both a simple and a multiplex PCR assay. The genes *selj* and *tst-1* were detected only in separate reaction mixtures. The detection of the other SE genes was performed by multiplex PCR assay with five reaction mixtures containing primers for two genes (*seg*+*femA, seh*+*sei, seb*+*sell, sea*+*sed,* and *sec*+*see*). The American Type Culture Collection (ATCC) *S. aureus* strains ATCC 19095 (*sec*, *seh*, *seg*, *sei,* and *sell*), ATCC 23235 (*sed*, *seg*, *sei*, and *selj*), ATCC 13565 (*sea*), ATCC 14458 (*seb*), and ATCC 27664 (*see*) were used as positive controls. Mixtures without DNA was used as negative controls.

The DNA amplification program was 35 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. Amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The reactions were carried out in a 50-μl volume consisting of 1 × PCR buffer, 1.5 mM $MgCl_2$, 10 μM concentrations of each deoxynucleoside triphosphate, 40 pmol of each primer, 100 ng of bacterial

DNA, and 3 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). PCR products were visualized by electrophoresis in a 1.8% agarose gel (wt/vol) stained with ethidium bromide, and gels were photographed under UV light (Eagle Eye II, Stratagene, La Jolla, CA).

Sequencing of amplicons. For each gene (*sea* through *sell* plus *tst-1*), one sample amplicon was sequenced to confirm the results. Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and the both strands were sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare Biosciences, Uppsala, Sweden) on an automated sequencer (DNA MegaBACE 1000, GE Healthcare). The partial sequences were analyzed with the LaserGene package (DNASTAR, Madison, WI). To confirm the results, we performed BLAST searches at the National Center for Biotechnology Information (Bethesda, MD).

PFGE analysis. PFGE typing of *S. aureus* strains bearing enterotoxigenic genes was performed with a modified version of the protocol proposed by Morot-Bizot et al. *(20).* Overnight staphylococcal culture was inoculated into fresh BHI broth and grown until it reached an optical density of 1. After centrifugation, the pellet was resuspended in TEE (10 mM Tris HCl pH 9.0, 100 mM EDTA, 10 mM ethylene glycol tetraacetic acid). The suspension was mixed with an equal volume of 1% low-melting-point agarose (Invitrogen) and 20 μl of 0.5 mg/ml lysostaphin (Sigma, St. Quentin, France). Agarose plugs were incubated in TEE with 5 mg/ml lysozyme (Sigma) and 0.05% sarkosyl for 2 h at 37°C. Lysis was performed overnight in TEE containing 1 mg/ml proteinase K and 1% sodium dodecyl sulfate (Merck, Darmstadt, Germany) at 55°C. The plugs were then washed three times for 60 min each time in TE buffer (1 mM EDTA, 10 mM Tris HCl pH 8.0) containing 20 mM phenylmethylsulphonylfluoride (Sigma). DNA in plugs was digested by 5 U/μl *Sma*I or *Apa*I restriction enzyme (Promega, Lyon Charbonnières, France) overnight at 25°C. Digested DNA was separated in a 1% agarose gel in 0.5× Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio-Rad, Ivry, France). Electrophoretic conditions were 40- to 100-s pulses for 2 h and 5 to 35-s pulses for 22 h at 14°C at a constant voltage of 6 V/cm and an angle of 120°. Lamba DNA concatemers (Promega Corporation, Madison, WI) were used as molecular weight markers. Gels were stained with ethidium bromide, and the patterns were visualized under UV light (Gel DOC 2000, Bio-Rad). *Sma*I DNA restriction bands were analyzed using the GelCompar II—Comparative Analysis of Eletrophoresis Patterns, version 2.0 (Applied Maths, Kortrijk, Belgium) with the Dice coefficient and represented by unweighted pair grouping by mathematical averaging (UPGMA) with 1.5% band tolerance. The isolates were defined as clusters or as being closely related when the PFGE patterns had at least 95% similarity, corresponding to one to three band differences on visual examination *(37).*

RESULTS

Detection of enterotoxin genes. The 291 isolates studied were positive for catalase, hemolysis, coagulase, and acetoin production and were all identified as *S. aureus* by the amplification of the expected 132-bp *femA* PCR product.

DNA from those isolates was examined for the presence of 10 SE genes (*sea, seb, sec, sed, see, seg, seh, sei, selj, sell*) and *tst-1*. Amplicons of the SE genes and *tst-1* were subjected to nucleotide sequencing, and their sequences had 90 to 98% homology with already published DNA sequences of the

TABLE 2. *Genotype profile of* Staphylococcus aureus *isolated from mastitic cow's (MC) milk, farm bulk tank (FBT) milk, and Minas frescal (MF) cheese in Brazil*

Genotype	No. of isolates from: MC milk	FBT milk	MF cheese	Total
Total no. of isolates	125	96	70	291
Positive for one or more toxin genes	17	41	51	109
Positive for at least one classical SE gene	1	2	12	15
sea, *seb*			2	2
seb		1	1	2
sec		1		1
sec, *sed*			1	1
sea, *seg*, *tst-1*			1	1
sea, *seg*, *sei*			1	1
sea, *seb*, *seh*, *selj*			1	1
seb, *seh*			5	5
seb, *seg*, *sei*	1			1
Positive for at least one more recently described SE gene (*seg–sell*) and *tst-1*	16	39	39	94
seg	4	1	5	10
seg, *sei*	2	8	13	23
seg, *seh*	1	4	2	7
seg, *seh*, *sei*		1		1
seg, *sei*, *selj*		1	4	5
seg, *sell*	1			1
seg, *selj*			1	1
seh	1	13	3	17
sei	1	3	7	11
sei, *selj*			2	2
selj	4	2	1	7
sell	2			2
tst-1		5	1	6
tst-1, *sei*		1		1

TABLE 3. *Distribution of SE genes and* tst-1 *among* Staphylococcus aureus *isolates from milk and Minas frescal cheese in Brazil*

Gene	No. (%) of isolates: Mastitic cow's milk (n = 125)	Farm bulk tank milk (n = 96)	Minas frescal cheese (n = 70)	Total (n = 291)
sea			5 (7.1)	5 (1.7)
seb	1 (0.8)	1 (1.5)	9 (12.9)	11 (3.8)
sec		1 (1.5)	1 (1.4)	2 (0.7)
sed			1 (1.4)	1 (0.3)
see				0
seg	9 (7.2)	14 (14.6)	28 (40.0)	51 (17.5)
seh	2 (1.6)	18 (18.7)	11 (15.7)	31 (10.6)
sei	4 (3.2)	13 (13.5)	23 (32.9)	40 (13.7)
selj	4 (3.2)	2 (2.1)	9 (12.9)	15 (5.1)
sell	3 (2.4)			3 (1.0)
tst-1		6 (6.2)	2 (2.9)	8 (2.8)

TABLE 4. *Origin, strain, toxin gene(s), and* Sma*I and* Apa*I PFGE patterns (100% similarity) of 89* S. aureus *strains isolated from milk and Minas frescal cheese in Brazil*

Origin[a]	Strain	Gene(s)	*Sma*I PFGE	*Apa*I PFGE
MC/F8	5481	*sell*	1	
MFC/D	7835	*sell*, *selj*	2	
BT19	7730	*seh*	3	
BT3	4713	*seg*	4	P1
BT3	4712	*seg*, *seh*, *sei*	4	P1
MFC/D	7255	*seg*, *seh*, *sei*	5	
MFC/F	7834	*sea*, *seg*, *tst-1*	6	
MFC/N	7838	*seg*, *sei*	7	
MFC/K	7855	*sei*	8	P4
MFC/K	7862	*seg*, *sei*	8	P4
MFC/K	7465	*seg*, *sei*	9	
MFC/H	7281	*seg*	10	
MFC/N	7325	*sei*	11	P4
MFC/N	7964	*seg*, *sei*	11	P4
MFC/N	7556	*sei*	12	P4
MFC/I	7582	*seg*	13	
MC/F10	3007	*seg*, *seh*	14	
BT19	7735	*seg*, *sei*	15	P6
MC/F16	5595	*seg*, *sell*	15	P6
BT22	7913	*seh*	16	P6
BT22	7915	*seg*, *seh*	16	P6
BT22	7917	*seg*, *seh*	16	
BT22	7919	*seg*, *seh*	16	
BT22	7920	*seg*, *seh*	16	P6
BT22	7921	*seh*	16	
BT22	7922	*seg*, *sei*	16	
BT22	7923	*seg*, *sei*	16	
BT4	4726	*seg*, *sei*	17	P6
BT7	5161	*seg*, *sei*	17	P6
MC/F6	2242	*seg*, *sei*	18	
BT1	4692	*seg*, *sei*	18	
MC/F19	5607	*seb*, *seg*, *sei*	18	
MFC/I	7825	*seg*	19	
BT19	7734	*seg*, *sei*	19	
BT19	7736	*seg*, *sei*	19	
MFC/J	7598	*seg*, *sei*	20	
MFC/B	7810	*seg*	21	
MFC/B	7815	*seg*	21	
MFC/H	7271	*seg*, *sell*, *selj*	22	
MFC/H	7275	*seg*, *sell*, *selj*	22	
MFC/J	7605	*sei*	23	
MFC/N	7846	*sea*, *seg*, *sei*	24	
MFC/D	7837	*sell*, *selj*	25	P11
MFC/J	7509	*seg*, *selj*	25	P11
BT21	7904	*seh*	26	P12
MC/F4	2014	*seg*	27	
MFC/F	7779	*seb*	28	
BT13	5181	*seh*	29	
MFC/I	7580	*seh*	30	P2
MFC/K	7848	*seh*	30	P2
MFC/K	7830	*seh*	30	
MC/F10	3006	*seh*	31	
MFC/H	7279	*selj*	32	
MFC/L	7505	*seg*, *seh*	33	
MFC/L	7592	*seg*, *seh*	33	
MFC/D	7234	*sea*, *seb*, *seh*, *selj*	34	
MFC/F	7818	*sea*, *seb*	35	P5
MFC/F	7832	*sea*, *seb*	35	
MFC/G	7780	*seb*, *seh*	35	
MFC/G	7782	*seb*, *seh*	35	P5
MFC/G	7784	*seb*, *seh*	35	
MFC/G	7786	*seb*, *seh*	35	
MFC/G	7788	*seb*, *seh*	35	
BT11	4793	*tst-1*	36	
MFC/B	7932	*tst-1*	37	
BT19	7731	*seh*	38	
BT19	7732	*seh*	38	
BT21	7733	*tst-1*	39	
BT21	7908	*seh*	39	
MC/F6	7909	*seh*	39	
BT14	5482	*seh*	40	P8
BT19	4927	*sell*	40	
BT20	7892	*seh*	41	P7
BT20	7893	*seh*	41	
BT20	7890	*seh*	42	
BT12	5278	*sec*	43	
MC/F3	1999	*selj*	44	
BT15	5229	*selj*	45	
MC/F5	2221	*sei*	46	
BT10	4779	*tst-1*	47	
BT6	4752	*seb*	48	
MFC/I	7590	*sec*, *sed*	49	
BT1	4687	*sell*, *tst-1*	50	
BT1	4688	*sei*	51	P3
BT13	5183	*sei*	51	P3
MC/F5	1621	*selj*	52	
BT18	5175	*tst-1*	53	
BT1	4693	*sei*	54	
MFC/L	7294	*sei*	55	

[a] MC, mastitic cow/farm number; MFC, Minas frescal cheese/cheese brand; BT, bulk tank milk number.

respective genes (GenBank no. 1004003, 3237776, 5317214, M28521.1, 8614569, 2862465, 8614573, AB075606.1, 5560343, 3795130).

Of the 291 *S. aureus* isolates, 109 (37.5%) were positive for one or more toxin genes (Table 2). Classical SE genes were found in combination or alone in 15 (5.2%) of the strains: 1 from mastitic cow's milk, 2 from farm bulk tank milk, and 12 from Minas frescal cheese (Table 2). Of the remaining 94 positive strains (32.3%), 87 carried only recently described SE genes (*seg* through *sell*) and 7 carried *tst-1* alone or in combination with *sei* (Table 2).

Twenty-three distinct genotypes of the toxin genes were observed (Table 2). Of the 125 *S. aureus* isolates from the mastitic cow's milk, 17 (13.6%) were grouped in nine genotypes. The *seg* and *sej* genes predominated; *seg* was found in nine strains alone or in association with other enteroxin genes, and *sej* was found in four strains. Of the 96 *S. aureus* isolates from farm bulk tank milk, 41 (42.7%) were grouped in 12 genotypes with predominance of *seh*. Among the 70 isolates from Minas frescal cheese, 51 (72.9%) were grouped in 16 genotypes, and *seg*+*sei* was the prevalent genotype.

Table 3 shows the distribution of each toxin gene in the *S. aureus* isolates. Overall, the most frequently observed gene was *seg* (found in 51 of the isolates) followed by *sei* (40 isolates), *seh* (31 isolates), and *selj* (15 isolates). Among the classical SE genes (*sea* through *see*), *seb* was the most frequent (11 isolates) followed by *sea* (5 isolates), *sec* (2 isolates), and *sed* (1 isolate); *see* was not found in any isolate. The *tst-1* gene was found in eight isolates but none from mastitic cow's milk.

Diversity of the strains. The *Sma*I macrorestriction analysis of the 89 *S. aureus* isolates carrying toxin genes revealed 55 distinct PFGE patterns, 36 of which were found in only one isolate (Table 4 and Fig. 1). Only four clusters were observed by employing a cutoff similarity value of 95%, each one including two patterns (10/11, 18/19, 25/26, and 50/51). A large diversity of PFGE patterns was thus found among the isolates.

Nineteen *Sma*I patterns included more than one isolate. The strains with the same *Sma*I profile had the same *Apa*I profile (Table 4 and Fig. 2). *Apa*I was less discriminative; for example, the strains with *Sma*I profiles 15, 16, and 17 had the same *Apa*I profile, P6 (Table 4). The largest group with the same PFGE pattern consisted of eight isolates, all from the same origin (bulk milk tank 22), but these isolates had three distinct enterotoxin genotypes. The second largest group with the same pulsotype consisted of seven isolates from Minas frescal cheeses but from two brands of cheese, and two enterotoxin genotypes were found in samples from each brand. Very few isolates that had the same pulsotype had the same enterotoxin profile, suggesting that the genes carrying enterotoxins were on mobile elements.

DISCUSSION

In previous studies of SE genes and *tst-1* in *S. aureus* strains, a wide range of prevalences of toxigenic strains (27.1 to 80.7%) and great variation in the distribution of the toxin genes have been reported *(5, 26, 29, 41)*. In our study, the toxin genes were carried by 37.5% of all tested strains, but these genes were more prevalent in strains from Minas frescal cheese (72.9%, 51 of 70 isolates) and bulk tank milk (42.7%, 41 of 96 isolates) than from mastitic cow's milk (13.6%, 17 of 125 isolates). A greater diversity of toxin gene combinations also was identified in isolates from Minas frescal cheese and bulk tank milk than in isolates from mastitic cow's milk. These results suggest that the sources of bacterial contamination of bulk tank milk and Minas cheese were multiple, such as raw milk collected from several cows, production and processing environments, equipment, and personnel.

The occurrence of exclusively recently described SE genes (*seg* through *sell*) was considerably higher (87 of 109 PCR-positive strains) than that of the previously characterized SE genes (15 strains). The increase in the number of potentially enterotoxigenic *S. aureus* isolates in relation to detection of new SE genes in addition to the classical SE genes has been reported for milk and/or milk product isolates by other authors *(18, 29, 31, 44)*.

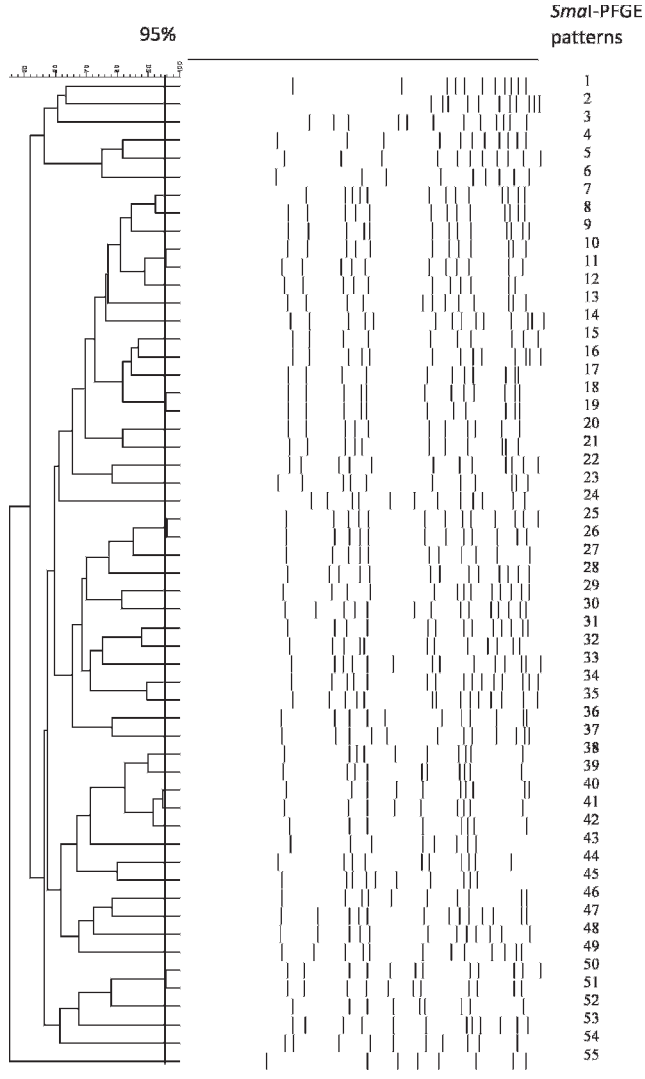

FIGURE 1. *Dendogram and the* Sma*I restriction patterns identified in a set of 89* Staphylococcus aureus *isolates bearing toxin genes. The strains corresponding to the* Sma*I PFGE patterns are shown in Table 4.*

The toxin genes most commonly detected in this study were *seg* and *sei*. These genes were found alone or in different combinations, but in 60.8% of these cases they occurred together. The association between *seg* and *sei* has been attributed to their location within the same gene cluster (*egc*) in genomic island type II vSaβ *(12)*. Rosec and Gigaud *(31)* found *seg* and *sei* predominantly and systematically together. However, other authors have observed them in different combinations, in agreement with our findings *(14, 18, 44)*. The occurrence of strains harboring *seg* or *sei* alone may be explained by mispriming due to a point mutation in one of these genes or the existence of variants in the *egc* cluster *(4)*, combinations of toxin gene–containing mobile elements such as plasmids and genomic islands in the same strain, or even a new type of genetic mobile element *(24, 41)*.

None of the strains in our study harbored *see*, in agreement with other investigations of isolates from milk and milk products in Norway *(13)* and from bulk tank milk in the Czech Republic *(44)*.

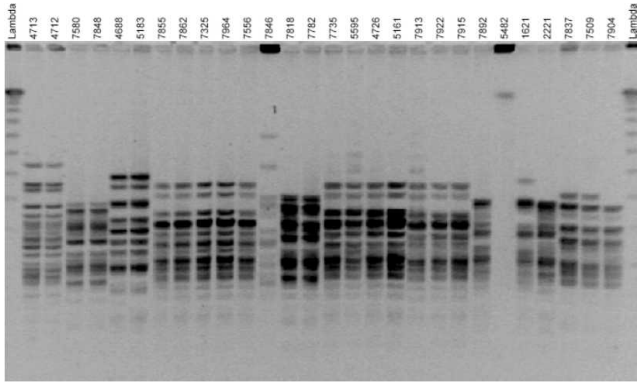

FIGURE 2. *PFGE of* Apa*I macrorestriction fragments of* Staphylococcus aureus *strains isolated from milk and Minas frescal cheese in Brazil.*

Among the methods for molecular typing of *S. aureus* isolates, PFGE is often considered the ''gold standard'' because of its accuracy and reproducibility *(42)*. In relation to PFGE, the PCR-based techniques, such as repetitive element sequence PCR, have less discriminatory power and poorer laboratory-to-laboratory reproducibility *(9, 32)*. In our study, analysis of *Sma*I macrorestriction of genomic DNA of 89 *S. aureus* isolates bearing toxin genes revealed 55 PFGE patterns. Růžičková et al. *(33)* found 20 distinct PFGE profiles for 28 seh^+ *S. aureus* strains. Significant genomic variability also was reported by Boerema et al. *(5)*, who found 65 PFGE patterns ranging from 55 to 100% similarity for 92 *S. aureus* isolates, including 62 enterotoxigenic strains.

In conclusion, the *S. aureus* isolates from mastitic cow's milk, bulk tank milk, and Minas frescal cheese have a diverse enterotoxigenic potential. The more recently described SE genes (*seg* through *sell*) predominated, with *seg* and *sei* the most common. Extensive variation in PFGE patterns also were found among the potentially toxigenic *S. aureus* isolates. Further investigations are needed to evaluate the production of these new toxins in milk and cheese and their significance for consumer safety.

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