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Research Letter

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Methicillin-resistant *Staphylococcus aureus* in minas frescal cheese: evaluation of classic enterotoxin genes, antimicrobial resistance and clonal diversity

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One sentence summary: Clones of methicillin-resistant *Staphylococcus aureus*, usually found in the hospital environment, carrying classical enterotoxin genes, were isolated from minas frescal cheese, a ready-to-eat product.

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ABSTRACT

This study aimed to investigate classical enterotoxin (*sea* to *see*) and *mec*A genes, by polymerase chain reaction and anitimicrobial susceptibility, by disk diffusion test of *Staphylococcus aureus* isolated from minas frescal cheese (MFC). All methicillin-resistant *S. aureus* (MRSA) isolates were investigated for the presence of Panton-Valentine leukocidin (PVL) genes and clonal diversity. Thirty-one *S. aureus* were isolated from four MFC samples. Seven (22.6%) *S. aureus* carried *mec*A gene and two of them carried enterotoxin genes *seb*/*sec* and *sea*/*seb*. Five (16.1%) *S. aureus* isolates showed induced resistance to clindamycin and nine (29%) were resistant to multiple –antibiotics (MDR), among these, six were MRSA. No MRSA isolates presented the PVL genes. Four MRSA were grouped into three clones and three isolates were not typable by pulsed field gel electrophoresis. MRSA isolates showed, by multilocus sequence typing, sequence types ST1, ST5, ST72 and ST4304 (new ST) and *S. aureus* protein A (spa type) t127, t568 and t2703. These data suggest that MFC may constitute a risk to the consumer because of its potential for staphylococcal food poisoning; however it might, also, become one of MRSA and MDR strains disseminator, including clones usually found in the hospital environment.

Keywords: coagulase positive staphylococci; MRSA; MDR; enterotoxigenic staphylococci; MLST; spa typing

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INTRODUCTION

Staphylococcus spp. colonizes skin and mucous membranes, especially the nasal-pharyngeal region and gastrointestinal tract of humans and animals (Wendlandt, Schwarz and Silley 2013). Foodborne illness caused by *Staphylococcus aureus* is a poisoning that happens after ingestion of staphylococcal enterotoxins (SE) released in food (Le Loir, Baron and Gautier 2003).

Minas frescal cheese (MFC) is one of the most popular cheeses in Brazil, produced from pasteurized milk, has soft consistency, high water activity, pH around 5.1 to 5.6 and 1.6% NaCl. MFC is highly perishable, providing conditions for the development of many enteropathogenic bacterial species (Gonzalez *et al.* 2000), including *S. aureus* (Arcuri *et al.* 2010).

Staphylococcus aureus releases a variety of extracelular proteins, including several SE, which are resistant to proteolytic enzymes and heat stable, requiring a temperature of 100°C for 5–10 min to be destroyed. Usually, these enterotoxins are not completely inactivated by pasteurization and other usual thermal treatments (Le Loir, Baron and Gautier 2003). Staphylococcal food poisoning generally occurs from strains that produce one or more of the five classical SEs (SEA to SEE) (Viçosa *et al.* 2013).

The production of SE is correlated with proliferation of the bacterium in food, so bacterial colonies count is used to determine the safety of the product. Food poisoning is caused, predominantly, by coagulase-producing strains (Schelin *et al.* 2011). The main coagulase-positive SE-producing species is *S. aureus*; however, some other coagulase-positive species such as *S. intermedius* and *S. hyicus* have also been described in food poisoning (Martins *et al.* 2013). Because of this, Brazilian law (Brasil 2001) establishes the coagulase-positive *Staphylococcus* (a) (colonies count) as quality and safety indicator for different types of food.

Methicillin-resistant *S. aureus* (MRSA) is spread throughout the world (Wendlandt, Schwarz and Silley 2013), adapting to different environmental conditions and modulating its pathogenicity, due to the ability to acquire foreign genes, including resistance genes to multiple antibiotics (Moellering 2012). MRSA have been isolated from animal foods in different countries; however, only a few studies monitor MRSA in food (Wendlandt, Schwarz and Silley 2013), mainly in dairy products (Basanisi *et al.* 2017). Monitoring MRSA from food is important because of the ability of these bacteria to spread multiple antimicrobial resistance genes, turning it into a major public health problem (Pinchuk, Beswick and Reyes 2010).

This study aimed to isolate *S. aureus* from MFC and investigate the presence of *S. aureus* species-specific genes, classical SE encoding genes, the gene that confers resistance to methicillin and investigate antimicrobial resistance profile. In addition, the clonal relationship among MRSA isolates was also determined.

MATERIAL AND METHODS

MFC samples and *Staphylococcus aureus* isolation

In total, 30 samples of MFC have been analyzed, from different brands, sold in the city of Niteroi, during the period comprised between July and September 2012. All MFC samples analyzed showed the Federal Inspection Service (SIF) registration.

Twenty-five grams of each sample were homogenized in 225 mL of 0.1% peptone water (w/v) (DIFCO). Dilutions were plated onto Baird-Parker Agar (BP) (DIFCO), supplemented with egg emulsion and potassium tellurite (Laborclin, Brazil), and incubated at 35±1°C for 24–48 h. Suspect colonies of *Staphylococcus* (black colonies surrounded by clear zone) were counted, at least five colonies were selected and inoculated onto Brain Heart Infusion (BHI) broth (DIFCO) and BHI agar (DIFCO) for confirmation and subsequent calculation of the CFU of CoPS per gram of MFC, as recommended by the Compendium of Methods for the Microbiological Examination of Foods (Lancette and Bennet 2001). The colonies confirmed as CoPS were stored at –20°C in triptcase soy broth (TSB) (DIFCO) supplemented with 20% (v/v) glycerol (KASVI) (TSB-G).

DNA extraction

A total of 10 μL of the bacterial culture in TSB-G has been inoculated in 5 mL of BHI broth and incubated at 35°C for 18 h. DNA was extracted using the QIAamp DNA Minikit (Qiagen, Hiden, Germany), according to the manufacturer's protocol, adding 30 μL of lysozyme (20 mg mL^{-1}) and 3 μL of lysostaphin (10 mg mL^{-1}).

Polymerase chain reaction

DNA was used as template to search for 16S rRNA gene, specific for the genus *Staphylococcus* (Zhang *et al.* 2004); *nuc* gene, encoding the thermonuclease enzyme specific for *S. aureus* species (Gandra *et al.* 2011); *sea, seb, sec, sed* and *see* genes, encoding for the classical SE (Becker, Roth and Petres 1998); *mec*A gene for methicillin resistance (Oliveira and Lencastre 2002); and *luk*-S-PV and *luk-F*-PV genes (Lina *et al.* 1999) by polymerase chain reaction (PCR). Genes used, oligonucleotides (Invitrogen, São Paulo, Brazil), cycling conditions, primer concentration and base pair size are described in Table 1. *Staphylococcus aureus* ATCC 25923 was used as positive control for 16S rRNA and *nuc* genes; *S. aureus* ATCC 13565, 14458, 19095, 23235 and 27664 for *sea, seb, sec, sed* and *see* genes, respectively; and *S. aureus* HU25 (BEC) for *mec*A. *Escherichia coli* ATCC 25922 and *S. epidermidis* ATCC 12228 were used as negative control for 16S rRNA and *nuc* genes, respectively.

Antimicrobial susceptibility testing

All isolates were evaluated through disk diffusion test on Muller-Hinton agar. The investigated antimicrobial agents were erythromycin (15 μg), clindamycin (2 μg), sulfamethoxazole-trimethoprim (25 μg), rifampicin (5 μg), chloramphenicol (30 μg), linezolid (30 μg), ciprofloxacin (5 μg), penicillin G (10 U), oxacillin (1 μg), tetracycline (30 μg), gentamicin (10 μg) and cefoxitin (30 μg) (Sensibiodisc, CECOM, Brazil) (Fig. S1, Supporting Information). The isolates resistant to erythromycin and sensitive to clindamycin were evaluated for resistance induced by clindamycin through D-Test (CLSI 2014) (Fig. S2, Supporting Information).

Pulse-field gel electrophoresis

DNA of all MRSA isolates was digested with five units of *Sma*I (Invitrogen) restriction endonuclease for 18 h at 30°C. Restriction fragments were separated by 1% (w/v) agarose gel (Molecular Biology Certified agarose, Bio-Rad Laboratories, Richmond, CA, USA) in a pulsed-field gel electrophoresis (PFGE) system (CHEF DR-III, BioRad), with an initial pulse time of 1 s and final of 30 s for 25 h at 11.3°C in a voltage gradient of 6 V per cm and angle of 120°. Fragmentation profiles were subjected to visual analysis considering the criteria suggested by Tenover *et al.* (1995). Similarity profiles were analyzed using the Dice correlation coefficient (Dice 1945) conducted by the UPGMA (unweighted pair

Table 1. Genes, primers, cycling conditions, primer concentration, base pair size, references.

		Cycling conditions[a]								
		D[b]		A		E[c]				
Gene	Primer (5′→ 3′)	T	t	T	t	T	t	Primer[d]	bp	Reference
sea	CCT TTG GAA ACG GTT AAA ACG TCT GAA CCT TCC CAT CAA AAA C	95	60	55	60	72	120	0,5	127	Becker, Roth and Petres (1998)
seb	TCG CAT CAA ACT GAC AAA CG GCA GGT ACT CTA TAA GTG CCT GC	95	60	55	60	72	120	0,5	477	Becker, Roth and Petres (1998)
sec	CTC AAG AAC TAG ACA TAA AAG CTA GG TCA AAA TCG GAT TAA CAT TAT CC	95	60	55	60	72	120	0,5	271	Becker, Roth and Petres (1998)
sed	CTA GTT TGG TAA TAT CTC CTT TAA ACG TTA ATG CTA TAT CTT ATA GGG TAA ACA TC	95	60	55	60	72	120	0,5	319	Becker, Roth and Petres (1998)
see	CAG TAC CTA TAG ATA AAG TTA AAA CAA GC TAA CTT ACC GTG GAC CCT TC	95	60	55	60	72	120	0,5	178	Becker, Roth and Petres (1998)
16S *rRNA*	AAC TCT GTT ATT AGG GAA GAA CA CCA CCT TCC TCC GGT TTG TCA CC	94	30	50	30	72	60	20	756	Zhang *et al.* (2004)
nuc	ATGAAGTCAAATAAATCGCT TTTGGTGAAAAATACTTCTC	95	120	50	120	72	120	20	458	Gandra *et al.* (2011)
mecA	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	94	30	55	30	72	60	20	162	Oliveira and Lencastre (2002)
spa	AGACGATCCTTCGGTGAGC GCTTTTGCAATGTCATTTACTG	95	30	55	30	72	45	20	556	Shopsin *et al.* (1999)
luk-PV	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAATSGTATTGGATAGCAAAGC	94	60	55	60	72	60	20	433	Lina *et al.* (1999)
arcC	TTGATTCACCAGCGCGTATTGTC AGGTATCTGCTTCAATCAGCG	95	60	55	60	72	60	20	456	Enright *et al.* (2000)
aroE	ATCGGAAATCCTATTTCACATTC GGTGTTGTATTAATAACGATATC	95	60	55	60	72	60	20	456	Enright *et al.* (2000)
glpF	CTAGGAACTGCAATCTTAATCC TGGTAAAATCGGCATGTCCAATTC	95	60	55	60	72	60	20	465	Enright *et al.* (2000)
gmk	ATCGTTTTATCGGGACCATC TCATTAACTACAACGTAATCGTA	95	60	55	60	72	60	20	429	Enright *et al.* (2000)
pta	GTTAAAATCGTATTACCTGAAGG GACCCTTTTGTTGAAAAGCTTAA	95	60	55	60	72	60	20	474	Enright *et al.* (2000)
tpi	TCGTTCATTCTGAACGTCGTGAA TTTGCACCTTCTAACAATTGTAC	95	60	55	60	72	60	20	402	Enright *et al.* (2000)
yqiL	CAGCATACAGGACACCTATTGGC CGTTGAGGAATCGATACTGGAAC	95	60	55	60	72	60	20	516	Enright *et al.* (2000)

[a]30 cycles; [b]preceded by an initial denaturation step for 5 min; [c]preceded by a final extension step for 5 min; [d]primer concentration (pmol/μl).
D: denaturation; A: annealing; E: elongation; T: temperature in celsius degree; t: time in seconds; bp: size of the amplicon in base pairs.

group method using arithmetic) average included in the MEGA 6 program (Tamura *et al.* 2013).

Multilocus sequence typing

MRSA isolates were typed through multilocus sequence typing (MLST) as described previously (Enright *et al.* 2000). We conducted MLST analysis by sequencing fragments of the PCR product of seven house-keeping genes (*arcC, aroE, glpF, gmk, pta, tpi* and *yqiL*) (Table 1). DNA sequences of PCR product from each of the seven genes were assembled in SeqMan (Lasergene 8, DNASTAR, Madison, WI, USA), and allele number and sequence types (ST) were assigned by using the *S. aureus* MLST website (https://pubmlst.org/saureus/).

Staphylococcal protein A (*spa*) typing

We conducted PCR using oligonucleotides (Invitrogen) for *spa* (staphylococcal protein A gene) typing (Table 1) of all MRSA isolates. DNA sequencing was performed according to Shopsin *et al.* (1999). Sequences were grouped in Software SeqMan Pro (Lasergene-Dnastar, Winsconsin), and Ridom *spa* types were assigned by using the SpaServer website (http://spaserver2.ridom.de).

RESULTS AND DISCUSSION

Staphylococcus aureus in the MFC

Staphylococcal food poisoning is caused predominantly by strains producing the enzyme coagulase (Schelin *et al.* 2011). In view of this, Brazilian legislation (Brasil 2001), as well as European legislation (European Union 2005), establishes CoPS count as an indicator of quality and safety for cheese. From 30 MFC samples analyzed, CoPS were identified in four (13.3%) samples (Q5, Q18, Q24 and Q28), of different manufacturers, acquired in different commercial establishments. Three (10%) samples (Q5, Q24 and Q28) showed CoPS above the standard (5.0×10^2 CFU/g) established by Brazilian law (Brasil 2001). *Staphylococcus aureus* is very vulnerable to destruction by heating and sanitizing agents, so its presence in processed foods is usually indicative of poor hygiene and/or deficient pasteurization (Le Loir, Baron and

Table 2. Characteristics of MRSA isolated from MFC, sample Q28.

	PCR									
Isolates	16S rRNA	*nuc*	*se*	*mecA*	*luk*-PV	oxa/cfx[a]	D test[b]	PFGE[c]	ST[d]	spa[e]
CH271	+	+	–	+	–	R	CR	A	72	t2703
CH273	+	+	–	+	–	R	CR	A	72	t2703
CH275	+	+	–	+	–	S	+	NT	72	UN
CH276	+	+	*seb/sec*	+	–	R	CR	B	1	t127
CH278	+	+	–	+	–	S	+	NT	4304	UN
CH279	+	+	–	+	–	S	+	NT	5	UN
CH280	+	+	*sea/seb*	+	–	R	CR	C	5	t568

[a]Phenotypic test using oxa (oxacillin) and cfx (cefoxitin): R (resistance); S (sensitive).
[b]Induced resistance to clindamycin test: CR (constitutive resistance to clindamycin); + (induced resistance to clindamycin).
[c]PFGE, pulsed-field gel electrophoresis: NT (non-typeable).
[d]Sequence type by multilocus sequence typing (MLST).
[e]spa, DNA sequencing of the repeat region of *Staphylococcus* protein A gene type: UN (unknown type).

Gautier 2003). Furthermore, it is already known that human skin and nasal mucosa act as reservoirs of *S. aureus*, resulting as the main source of food contamination (Wendlandt, Schwarz and Silley 2013). MFC is a much-manipulated cheese and the samples analyzed in this study were produced from pasteurized milk. Thus, we suggest that the contamination of MFC may have occurred as result of failure in the pasteurization of milk, poorly cleaned equipment or mainly manipulation hygiene failure.

Thirty-one CoPS were isolated and all isolates were confirmed as *S. aureus* by 16S rRNA and *nuc* gene PCR (Table S1, Supporting Information). The *nuc* gene encodes the thermonuclease enzyme and has been used as a valuable genetic marker for rapid identification of *S. aureus*.

Staphylococcal enterotoxins

The main importance of staphylococci in foods is its ability to produce SE, leading to food poisoning; therefore, its search is important, regarding the risk for human health (Pinchuk, Beswick and Reyes 2010). In Brazil, staphylococcal food poisoning is one of three most common foodborne diseases (Brasil 2017). Most cases of staphylococcal food-poisoning outbreaks occur due to production of classical SE (Viçosa *et al.* 2013). In this study, two (6.5%) isolates of *S. aureus*, CH276 and CH280, presented the SE genes *seb/sec* and *sea/seb*, respectively (Table 2; Table S1). Higher percentages of enterotoxigenic *S. aureus* isolated from dairy products have been described in Brazil (Arcuri *et al.* 2010) and other countries (Rosengren *et al.* 2010). MFC is a ready-to-eat-food, which raises concerns, as this fact enhances the likelihood of food poisoning. SEA seems to be the most common SE implicated in staphylococcal food poisoning (Rosengren *et al.* 2010). Studies have shown the relationship between the source of contamination and the type of SE produced by *S. aureus*. SEA and SEB are associated with contamination of human origin, whereas SEC and SED are related to contamination from animals (Pinchuk, Beswick and Reyes 2010). However, *S. aureus* SEC-producing isolates have also been related to human contamination. In the USA, Jones *et al.* (2002) identified a handler colonized by MRSA as a source of contamination of a food-poisoning outbreak in which the strains involved were characterized as SEC producers. The results presented in this study allow us to speculate that contamination of the MFC sample by *S. aureus* might have had human origin.

Antimicrobial resistance profile

There were *S. aureus* isolates presenting a wide spectrum of antimicrobial resistance profile. All isolates of *S. aureus* were resistant to penicillin, 21 (67.74%) to erythromycin, 12 (38.71%) to ciprofloxacin, 4 (12.9%) to clindamycin, 4 (12.9%) to oxacillin and cefoxitin, 2 (6.45%) to rifampicin, 1 (3.23%) to chloramphenicol and 1 (3.23%) to tetracycline. All isolates were susceptible to trimethoprim-sulfamethoxazole, gentamicin and linezolid (Table S1). *Staphylococcus aureus* showed a high rate of resistance to penicillin (Yucel, Citak and Bayhün 2011). However, *S. aureus* penicillin-sensitive has been isolated from food (Oliveira *et al.* 2016). In general, a great percentage of erythromycin and ciprofloxacin resistance is observed (Yucel, Citak and Bayhün 2011).

MRSA isolates

CLSI (2014) establishes, for *S. aureus*, the use of cefoxitin disk (30 μg) to detect oxacillin resistance mediated by *mec*A gene. However, this method is often not sufficiently sensitive or specific; therefore, the detection of the *mec*A gene is applied and considered the gold standard for MRSA identification (CLSI 2014). Seven (22.6%) *S. aureus* presented the *mec*A gene, being classified as MRSA, were isolated from one (3.3%) MFC sample (Q28), but not all isolates showed oxacillin and cefoxitin resistance phenotype (Table 2, Table S1). Several studies have shown varying accuracies in the identification of MRSA strains by disk diffusion method using cefoxitin and oxacillin, and this event is attributed to the heterogeneous expression of resistance (Mohanasoundaram and Lalitha 2008). Thus, we suggest the inclusion of *mec*A gene search for identification of MRSA from food.

MRSA have been isolated from animal, raw milk and raw meat in different countries, including Brazil (Wendlandt, Schwarz and Silley 2013; Oliveira *et al.* 2016; Basanisi *et al.* 2017); however, few studies have been conducted to MRSA detection from dairy products, including cheese made from pasteurized milk. Normanno *et al.* (2007), in Italy, isolated MRSA from 1.3% of dairy products. Rodríguez-Lázaro *et al.* (2015) investigated 195 samples of different foods confiscated from passengers flying to the USA, where they identified MRSA in 9.1% of the samples (one cheese). In another study, Basanisi *et al.* (2017) reported 8.3% of strains of *S. aureus* isolated from milk and dairy products were MRSA. In Brazil, Silveira-Filho *et al.* (2014), studying milk and dairy products, did not find any MRSA strain.

Table 3. Antimicrobial resistance profile of *S. aureus* isolated from samples of MFC.

MFC sample	isolates	Antimicrobial resistance profile	D-test (no of isolate)
Q5, Q24	CH42, CH43, CH44, CH49, CH50, CH231, CH232, CH233, CH235, CH236	pen	–
Q5, Q18, Q24, Q28	CH41, CH45, CH46, CH48, CH176, CH180, CH234, CH277, CH279[a,b]	pen, eri	+(1)
Q24, Q28	CH239, CH272[a], CH274[a], CH278[a,b,c]	pen, eri, cip	+(3)
Q24	CH237, CH240	pen,eri, cip, rif[d]	–
Q24	CH238	pen,eri, cip,clo[d]	–
Q28	CH275[a,b]	pen, eri, cip, tet[d]	+(1)
Q28	CH271[b], CH273[b], CH276[b], CH280[b]	pen, eri, cip, clin, oxa, cfo[d]	CR

pen: penicillin G, eri: erythromycin, cip: ciprofloxacin, rif: rifampicin, clo: chloramphenicol, tet: tetracycline, clin: clindamycin, oxa: oxacillin, cfo: cefoxitin.
[a]Induced resistance to clindamycin.
[b]Methicillin-resistant *Staphylococcus aureus* (MRSA).
[c] Multidrug-resistant (MDR) to carrying the *mecA* gene.
[d]MDR.
CR: constitutive resistance to clindamycin.

D-test

Five (16.1%) *S. aureus* isolates presented induced resistance to clindamycin, observed by the D-test; among these, three were MRSA (*mecA*-positive, oxacilin- and cefoxitin-sensitive) (Table 3, Table S1). The increase in infections caused by *Staphylococcus* spp. and the change in resistance profiles of these isolates has led to the use of clindamycin for the treatment of MRSA and MSSA (methicillin-sensitive *S. aureus*) infections. D-test is an important laboratory test to aid in the effective use of clindamycin for staphylococcal infections that present the MLSBi (macrolides, lincosamides and streptogramin resistance inducible pattern) phenotype, and thus avoid therapeutic failures in clinical practice (Martinez-Aguilar *et al.* 2003).

Multidrug resistant

Isolates presenting resistance to three or more antimicrobial classes were classified as multidrug resistant (MDR; Magiorakos *et al.* 2012). A total of nine (29%) isolates were classified as MDR (Table 3, Table S1). MRSA strains are usually MDR pathogens (Pinchuk, Beswick and Reyes 2010); however, MRSA CH279 isolate showed only resistance to penicillin and erythromycin (Table 3, Table S1), not being classified as MDR, which is a common feature found among isolates classified as CA-MRSA (Vandenesch *et al.* 2003). Multiresistant bacteria are a major public health problem, and food is a possible transmitter of these microorganisms to humans and environment (Pinchuk, Beswick and Reyes 2010). The presence of antibiotic resistance in potentially pathogenic bacteria isolated from foods of animal origin justifies further research into the role of antimicrobial agents.

Panton-Valentine leukocidin toxin

MRSA isolates were investigated for the presence of the *luk-S* and *luk-F-PV* genes. Panton-Valentine leukocidin (PVL) genes were not observed in any MRSA isolates (Table 2). These genes, encoding PVL toxin, are usually observed in CA-MRSA (community-associated MRSA) (Vandenesch *et al.* 2003). However, data indicate that PVL is not always present in CA-MRSA isolates (Zhang *et al.* 2008), especially in Brazil (Carvalho, Berezin and Mimica 2012).

Clonal diversity

MRSA *mecA*-positive oxacillin/cefoxitin-resistant isolates presented three different PFGE profiles (Fig. S3, Supporting Information) and were grouped into three clonal groups: A, B and C. MRSA *mecA*-positive oxacillin/cefoxitin-susceptible isolates were non-typeable by PFGE (Table 2). Using the criteria established by Tenover *et al.* (1995), isolates belonging to clone A have 82% similarity with clone B isolates (CH273), whereas clones A and B have 79% similarity with clone C (CH280) (Fig. S4, Supporting Information).

According to MLST, MRSA isolates showed four different sequence types (ST1, ST5, ST72 and ST4304) (Table 2, Table S2, Supporting Information). Four isolates were grouped into three different *spa* typing (t127, t568 and t2703) and three isolates presented unknown spa typing (Table 2). MRSA ST1, ST5 and ST72 have been isolated in hospitals in Brazil (Schuenck *et al.* 2009; Matos *et al.* 2016), MRSA ST4304 is a new allele deposited in the *S. aureus* MLST database (https://pubmlst.org/saureus/), with ID32918. In Korea, nasal MRSA ST72 was predominant in patients newly admitted to hospitals (Park *et al.* 2016). Based on the genotyping methods used, six different clones were identified among the seven MRSA isolates from the same cheese. Isolates with the same ST were differentiated through spa typing. Song *et al.* (2015) reported that spa typing seems to present better resolution than MLST. The PFGE and spa typing methodologies presented correlation (Table 2).

MRSA isolates belonging to clones B (CH276; ST1, t127) and C (CH280; ST5, t568) were also enterotoxigenic (Table 2). Isolates showing ST1 and ST5 have been related to staphylococcal food poisoning (Fetsch *et al.* 2014). In China, *S. aureus* ST5 was prevalent among enterotoxin-producing strains isolated from different foods, including MRSA (Chang *et al.* 2016). These authors suggest strains of *S. aureus* ST5 tend to have great ability to produce enterotoxins, in addition to present increased antimicrobial resistance.

Isolation of MRSA from MFC indicates that health service inspection should give special attention to this food, since *S. aureus* are known to cause serious systemic infections. Even though rarely reported, food can be a vehicle for systemic staphylococcal infections (Yucel, Citak and Bayhün 2011). Kluytmans *et al.* (1995) described a severe outbreak of MRSA infection in immunocompromised individuals, who consumed contaminated food by the handler. During this specific outbreak, five deaths occurred. The treatment of MRSA infections is difficult and costly, thereby effective control to the production of MFC must be performed to prevent contamination, and thus the spread of these strains through food.

The application of suitable hygiene procedures, with the adoption of Good Manufacturing Practices Program, in the production of food, is essential to promote satisfactory results

regarding the quality of the final product. In conclusion, MFC can offer potential risk to consumers and community health. *Staphylococcus aureus* isolates showed different antimicrobial resistance profiles. MRSA and MDR in MFC indicate the spread of resistant strains to various environments; in addition, the presence of strains carrying SE shows the pathogenic potential of these bacteria. Currently, the displacement of people and food between different regions is easy and fast. People who are contaminated with enterotoxigenic *S. aureus* or MDR or MRSA strains, through the MFC consumed in Brazil, can, in less than 24 hours, take this strain to any part of the planet. Thus, globalization can make the contamination of a regional food into a global health problem. MFC samples used in this study have been contaminated by different clones, including those related to hospital environment, indicating the contamination from different sources, or even at different times of processing. In order to develop strategies to prevent staphylococcal food poisoning, as well as to control food contamination by strains resistant to multiple antimicrobials, it is important to investigate *S. aureus* in food, especially in ready-to-eat dairy products.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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REFERENCES

Arcuri EF, Angelo FF, Guimarães MF *et al.* Toxigenic status of *Staphylococcus aureus* isolated from bovine raw milk and minas frescal cheese in Brazil. *J Food Protect* 2010;**73**:2225–31.

Basanisi MG, La Bella G, Nobili G *et al.* Genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and dairy products in South Italy. *Food Microbiol* 2017;**62**: 141–6.

Becker K, Roth R, Petres G. Rapid and specific detection of toxigenic *S. aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of *Staphylococcal* enterotoxin genes, esfoliative toxin genes, and toxic syndrome toxin-1 gene. *J Clin Microbiol* 1998;**36**:2548–53.

Brasil. RDC no 12. Aprova o regulamento técnico sobre padrões microbiológicos para Plimentos. January 2nd, 2001. In: *Diário Oficial da União, Ministério da Saúde, Agência Nacional de Vigilância Sanitária*. Brasília, DF.

Brasil, Ministério da Saúde, Secretaria de Vigilância Sanitária. 2017 http://portalarquivos.saude.gov.br/images/pdf/2017/maio/29/Apresentacao-Surtos-DTA-2017. (4 October 2017, date last accessed).

Carvalho RLB, Berezin EN, Mimica MJ. Avaliação da presença dos genes lukS-PV e lukF-PV em cepas pediátricas de *Staphylococcus aureus*. *Arq Med Hosp Fac Ciên Méd Santa Casa de São Paulo* 2012;**57**:59–62.

Chang Y, Gao H, Zhu Z *et al.* High prevalence and properties of enterotoxin-producing *Staphylococcus aureus* ST5 strains of food sources in China. *Foodborne Pathog Dis* 2016;**13**: 386–90.

CLSI (Clinical and Laboratory Standards Institute), 2014. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement*. M100-S24, CLSI, Wayne.

Dice LR. Measures of the amount of ecological association between species. *Ecology* 1945;**26**:297–302.

Enright MC, Day NP, Davies CE *et al.* Spratt, B.G. Multilocus sequence typing for characterization of methicillin-resistant and methicillin susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;**38**:1008–15.

European Union. Commission Regulation (EC) N° 2073 of 15 November 2005. Microbiological criteria for foodstuffs. *Official Journal of the European Union 338* 2005, 1–26.

Fetsch A, Contzen M, Hartelt K *et al. Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. *Int J Food Microbiol* 2014;**187**:1–6.

Gandra EA, Fernandez MA, Silva JA *et al.* Standardization of multiplex PCR for the identification of coagulase-positive *Staphylococcus*. *Food Sci Technol* 2011;**31**:946–9.

Gonzalez AGM, Rosa ACP, Andrade JRC *et al.* Enteropathogenicity markers in *E. coli* strains isolated from soft White cheese and poultry in Rio de Janeiro, Brazil. *Food Microbiol* 2000;**17**: 321–8.

Jones TF, Kellum ME, Porter SS *et al.* An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 2002;**8**:82–84.

Kluytmans J, Van Leeuwen W, Goessens W *et al.* Food-initiated outbreak of methicillin-resistant *Staphylococcocus aureus* analyzed by pheno and genotyping. *J Clin Microbiol* 1995;**33**: 1121–8.

Lancette GA, Bennet RW. *Staphylococcus aureus* and *Staphylococcal* enterotoxins. In: Downes FP, Ito K (eds). *Compendium of Methods for the Microbiological Examination of Foods*. Washington: Sheridan, 2001, 387–404.

Le Loir YL, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. *Gen Mol Res* 2003;**2**:63–76.

Lina G, Piémont Y, Godail-Gamot F *et al.* Involvement of Panton-Valentine leukocidin producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;**29**:28–32.

Magiorakos AP, Srinvasan A, Carey RB *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bactéria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;**18**:268–81.

Martinez-Aguilar G, Hammerman WA, Mason EO *et al.* Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children. *Ped Infect Dis J* 2003;**22**:593–8.

Martins PD, Almeida TT, Basso AP *et al.* Coagulase-positive Staphylococci isolated from chicken meat: pathogenic potential and vancomycin resistance. *Foodborne Pathog Dis* 2013;**10**:771–6.

Matos PD, de Oliveira TL, Cavalcante FS *et al.* Molecular markers of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* SCCmec IV presenting different genetic backgrounds. *Microbial Drug Resist* 2016;**22**:700–6.

Moellering RC, Jr. MRSA: the first half century. *J Antimicrob Chemoth* 2012;**67**:4–11.

Mohanasoundaram KM, Lalitha MK. Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. *Indian J Med Res* 2008;**127**:78–84.

Normanno G, Corrente M, La Salandra G *et al*. Methicillin-resistant Staphylococcus aureus (MRSA) in foods of animal origin product in Italy. *Int J Food Microbiol* 2007;**117**:219–22.

Oliveira CJB, Tiao N, de Sousa FGC *et al*. Methicillin-resistant *Staphylococcus aureus* from Brazilian dairy farms and identification of novel sequence types. *Zoonoses Public Hlth* 2016;**63**:97–105.

Oliveira DC, Lencastre H. Multiplex PCR strategy for rapidy identification of structural types and variants of the *mec* element in methicylin-resistant *S. aureus*. *Antimicrob Agents Ch* 2002;**46**:2155–61.

Park SY, Chung DR, Yoo JR *et al*. Sequence type 72 community-associated meticillinresistant *Staphylococcus aureus* emerged as a predominant clone of nasal colonization in newly admitted patients. *J Hosp Infect* 2016;**93**:386–9.

Pinchuk IV, Beswick EJ, Reyes VE. *Staphylococcal* enterotoxins. *Toxins* 2010;**2**:2177–97.

Rodríguez-Lázaro D, Ariza-Miguel J, Diez-Valcarce M *et al*. Foods confiscated from non-EU flights as a neglected route of potential methicillin-resistant *Staphylococcus aureus* transmission. *Int J Food Microbiol* 2015;**209**:29–33.

Rosengren A, Fabricius A, Guss B *et al*. Occurrence of foodborne pathogens and characterization of Staphylococcus aureus in cheese produced on farm-dairies. *Int J Food Microbiol* 2010;**144**:263–9.

Schelin J, Wallin-Carlquist N, Cohn MT *et al*. The formation de *S. aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2011;**2**:580–92.

Schuenck RP, Nouér SA, Winter CO *et al*. Polyclonal presence of non-multiresistant methicillin-resistant *Staphylococcus aureus* isolates carrying SCC*mec* IV in health care-associated infections in a hospital in Rio de Janeiro, Brazil. *Diagn Micr Infec Dis* 2009;**64**:434–41.

Shopsin B, Gomez M, Montgomery SO *et al*. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999;**37**:3556–63.

Silveira-Filho VM, Luz IS, Campos APF *et al*. Antibiotic resistance and molecular analysis of *Staphylococcus aureus* from cow's milk and dairy products in Northeast Brazil. *J Food Protect* 2014;**77**:583–91.

Song M, Bai Y, Xu J *et al*. Genetic diversity and virulence potential of *Staphylococcus aureus* isolates from raw and processed food commodities in Shanghai. *Int J Food Microbiol* 2015;**195**:1–8.

Tamura K, Stecher G, Peterson D *et al*. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;**30**:2725–9.

Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233–9.

Vandenesch F, Naimi T, Enright MC *et al*. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003;**9**:978–84.

Viçosa GN, Le Loir A, Le Loir Y *et al*. *egc* characterization of enterotoxigenic *Staphylococcus aureus* isolates obtained from raw milk and cheese. *Int J Food Microbiol* 2013;**165**:227–30.

Wendlandt S, Schwarz S, Silley P. Methicillin-resistant *Staphylococcus aureus*: a food-borne pathogen? *Annu Rev Food Sci T* 2013;**4**:117–39.

Yucel N, Citak S, Bayhün S. Antimicrobial resistance profile of *Staphylococcus aureus* isolate from clinical samples and foods of animal origin. *Foodborne Pathog Dis* 2011;**8**:427–31.

Zhang K, McClure JA, Elsayed S *et al*. Novel Multiplex PCR assay for simultaneous identification of community-associated methicillin-resistant *Staphylococcus aureus* strains USA300 and USA400 and detection of mecA and Panton-Valentine Leukocidin genes, with discrimination of *Staphylococcus aureus* from coagulase-negative *staphylococci*. *J Clin Microbiol* 2008;**46**:1118–22.

Zhang K, Sparling J, Chow BL *et al*. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative *Staphylococci*. *J Clin Microbiol* 2004;**42**:4947–55.