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Molecular characterisation of *Staphylococcus aureus* from some artisanal Brazilian dairies

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ABSTRACT

The clonal relationships and antimicrobial resistance of *Staphylococcus aureus* isolates from three fresh Minas cheese production lines were investigated. Putative *S. aureus* were confirmed by 16S rRNA gene sequencing and were characterised by multilocus sequence typing (MLST) and antimicrobial susceptibility. Overall, 33 out of 64 samples were contaminated with *S. aureus* (51.6%). The contamination rates of the dairies were 60.8%, 21% and 68.2%. Thirty representative isolates were selected to be typed by MLST and five sequence types (ST) were found (ST1, 97, 126, 3531), including the new ST 3816. The clonal complex 1 (CC1) was dominant. Resistance to commonly used antimicrobials was observed but only one strain was resistant to methicillin. This methicillin resistant *S. aureus* strain belonged to the CC126 that is commonly associated with mastitis in ruminants. The dissemination of zoonotic bacteria through the food chain is not a frequent event but represents a concern to public health.

1. Introduction

Foodborne diseases represent a major issue for public health worldwide and there is a growing number of outbreaks and sporadic cases of disease associated especially with raw and minimally processed food (Jung, Jang, & Matthews, 2014; Klein, Tian, Witmer, & DeWaal, 2009; WHO, 2015).

Staphylococcal food poisoning (SFP) is among the most prevalent foodborne diseases and it is caused by the ingestion of staphylococcal enterotoxins (SE), which are thermostable and resistant to gastrointestinal proteases (Kadariya et al., 2014). *S. aureus* can enter the food chain through contaminated raw material and also by inappropriate handling of processed food, with failure to maintain the cold chain (Argudín, Mendoza, & Rodicio, 2010; Gomes, Franco, & De Martinis, 2013; Kadariya et al., 2014). Moreover, *S. aureus* has the ability to form biofilms on different types of surfaces commonly found in the dairy industry. In biofilms, *S. aureus* can survive in hostile environments and contaminate the final products. Indeed, there are reports of recovery of *S. aureus* from industrial facilities even after standard cleaning and disinfection procedures (Gutiérrez et al., 2012).

Soft cheeses are considered high-risk products for SFP, as they provide excellent nutritional substrate for growth of enterotoxigenic *S. aureus* strains (Carmo et al., 2002; Kümmel et al., 2016; Nunes & Caldas, 2017). Fresh Minas cheese (FMC) is a soft white cheese that is affordable and appreciated in Brazil. It is characterised by low acidity (pH 5.1–5.6), high moisture content (over 55%) and it is lightly salted (1.4–1.6% sodium chloride). FMC is traditionally obtained by enzymatic coagulation of milk with enzymes (rennet) and addition of lactic acid bacteria is optional (Dantas et al., 2016; Teusink & Molenaar, 2017; DOU, 1997). For FMC, Brazilian legislation sets the upper contamination limits at 5×10^2 and 5×10^3 cfu of coagulase-positive staphylococci per gram of cheese for FMC produced with commercial rennet or added lactic acid bacteria starters, respectively; however, no limit for staphylococcal toxin concentration is specified, which is only investigated if a foodborne disease outbreak is suspected (DORFB, 2001; Nunes & Caldas, 2017). Studies have shown, however, that over 70% of *S. aureus* isolates from FMC were enterotoxigenic, which corroborates with

epidemiological data showing the high prevalence of SFP among foodborne diseases reported in Brazil (Carmo et al., 2002; Nunes & Caldas, 2017; Rodrigues et al., 2017; Sabioni, Hirooka, & Souza, 1988). Ideally, FMC should be produced solely with pasteurised milk and starter cultures could be used to increase the microbiological safety of the product (Santos et al., 2009). However, both the pasteurisation process and the presence of starter cultures, could influence FMC sensory quality and consumers greatly appreciate the unique flavours of artisanal products made from raw curd using commercial rennet. In fact, this kind of product is regarded as a traditional heritage in some Brazilian regions (Almeida Filho & Nader Filho 2000; Bulhões & Rossi Júnior, 2002; SAPI, 2002).

The "One Health Initiative" (http://www.onehealthinitiative.com/) reinforces the concept that animal, environmental, and human isolates of bacterial pathogens are closely related and that epidemiological surveys are crucial to monitor bacterial diseases worldwide. In this sense, it is noticeable that there are limited studies on the molecular patterns and monitoring of antimicrobial susceptibility of foodborne pathogens found in dairies and dairy products in Brazil (Dittmann et al., 2017), which is a country with continental dimensions and one of the largest suppliers of food and commodities in the world (Gomes et al., 2013). In this paper, we report on the clonal relationships and antimicrobial resistance profiles of *S. aureus* isolates from some Brazilian artisanal dairy processing lines.

2. Materials and methods

2.1. Dairy plants, sampling procedures and bacterial isolations

In Brazil, the production of milk and its derivatives is mainly concentrated in the Midwest, Southeast and South regions of the country that stand out in the agribusiness of milk and dairy products. From November 2014 to May 2015, three small artisanal dairy plants located in the Midwest (Goiás state) were sampled (A, B and C). Those dairies produced between 20 and 50 pieces of FMC per day, processing about 150 to 250 litres of cows' milk a day, from single herds raised in situ. All cheeses were

produced from unpasteurised milk with commercial rennet.

S. aureus contamination was investigated in the processing environments, raw materials, and food products. The samples were collected along the cheese production chain and included, in total: raw material (e.g., milk, curd, whey, brine, n = 16), food contact surfaces (e.g., milk transport gallons, buckets, ladle, processing tanks, jar, freezer, hand handler, cloth mould, n = 26), non-food contact surfaces (e.g., floor, sinks, n = 5) and individual ready-to-eat cheese (n = 17).

Environmental sampling was performed according to Oxaran et al. (2017) using sponges or swabs, depending on the accessibility of the site, and transferred to sterilised bags. Ready-to-eat cheese and raw material samples (100 g or 100 mL) were collected depending on availability and, to improve pathogen detection rates, samples from the lateral and upper surfaces of FMC were also obtained (Barancelli et al., 2011). Samples were stored on ice and analysed immediately upon arrival at the laboratory. Isolation of putative *S. aureus* was done by surface plating on Baird Parker agar as described by Dittmann et al. (2017). Three to five presumptive *S. aureus* colonies were purified on tryptic soy agar supplemented with 0.6% yeast extract and subjected to Gram staining and catalase testing. At least one colony from each sample was further investigated for coagulase (Coagu-plasma; Laborclin, Marabá, Brazil) and/or for clumping test (Staphiclin; Laborclin), following the manufacturers' recommendations. Selected putative *S. aureus* isolates were stored at -20 °C in brain heart infusion (BHI) broth containing 20% glycerol (Fluka Analytical, Sigma-Aldrich, St. Louis, MO, USA). All culture media used in this study were from Oxoid (Basingstoke, UK), unless otherwise noted. 116

2.2. Molecular identification of bacterial isolates

All putative *S. aureus* isolates were confirmed by PCR amplification followed by DNA sequencing of the 16S rRNA gene, using primers 27_F (5´-AGAGTTTGATCMTGGCTCAG-3´) and 1492R (5´-GCTTACCTTGTTACGACTT-3´) according to Devereux and Wilkinson (2004). Genomic DNA was extracted with the Illustra Bacteria Genomic Prep Mini Spin Kit (GE Life Sciences, Sweden).

Amplicons were purified with Illustra™ GFX™ PCR DNA (GE Healthcare, USA) and sequenced on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). Nucleotide sequences were compared with those available in GenBank using the BLAST algorithm 126 (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Multilocus sequence typing

Multilocus sequence typing (MLST) was performed based on the DNA sequences of seven conserved housekeeping genes, *arcC* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol kinase), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase) and *yqi* (acetyl coenzyme A acetyltransferase) which were amplified using specific primers as described by Enright, Day, Davies, Peacock, and Spratt (2000). PCR amplification was performed in 50 µL final volume *per* reaction using JumpStart™ Taq DNA Polymerase (Sigma-Aldrich), according to the manufacturer's instructions with approximately 100 ng of genomic DNA from each bacterial isolate and 1 µmol L^{-1} of each primer. The PCR assay was conducted following the conditions suggested by the MLST website (http://saureus.mlst.net/) except to the annealing temperature, as follows: initial denaturation 94 °C for 7 min, 30 cycles at 94 °C for 1 min, annealing temperature 56 °C (*aroE*), 57 °C (*glpF*) or 55 °C (remaining genes) for 1 min, 72 °C for 1 min and an additional extension at 72 °C for 5 min. The detailed methodology (including the description of primers used) is available at the MLST database site for *S. aureus*.

The PCR products were purified with the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Life Sciences, Lund, Sweden) and the purified products were sequenced using an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, CA, USA). Sequencing reactions were done using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA), according to the manufacturer's recommendations, using the same primers used for the PCR reactions, at the Centre for Studies on Human Genome, Biomedical Sciences Institute, University of São Paulo, Brazil.

The sequences obtained were analysed by Chromas Pro software (Technelysium, Brisbane, QLD, Australia). For each locus, the sequences from all isolates were compared and each sequence was assigned with an allele number. For each isolate, the alleles of each of the seven loci defined the allelic profile to which each sequence type (ST) corresponds. The alleles and STs of each isolate were determined using the platform available on the website.

The determination of the clonal and epidemiological relationships, as well as the formation of clonal complexes (CC), were performed by analysing a genetic similarity diagram constructed using the program eBURSTv3 (Feil, Li, Aanensen, Hanage & Spratt, 2004; http://eburst.mlst.net/). This diagram of genetic similarity allows demonstration of the genetic relationship among the bacteria, through the analysis of their respective ST, which can be grouped in single locus variant (SLV), double locus variant (DLV) and triple locus variant (TLV) or present in isolation (singletons). The diagram also allows to verify the grouping of STs, representing CC, when they exist.

2.4. Antimicrobial susceptibility tests

Confirmed *S. aureus* isolates submitted to MLST analysis were also screened for susceptibility to penicillin 10 U (PEN); cefoxitin 30 µg (CFO); gentamicin 10 µg (GEN); erythromycin 15 µg (ERM); tetracycline 30 µg (TET); ciprofloxacin 5 µg (CIP); clindamycin 2 µg (CLIN); trimethoprim–sulfamethoxazole 1.25/23.75 µg (SXT) and chloramphenicol 30 µg (CLO). Thistest was performed by disk diffusion (Sensifar, Brazil) at 35 °C on Mueller Hinton agar strictly following CLSI (2015) guidelines, which fully describe the level of each antimicrobial to be tested. The Minimal Inhibitory Concentration (MIC) of vancomycin (VAN, Sigma-Aldrich) was determined by macrodilution method using Mueller Hinton broth at 35 °C (CLSI, 2015). Susceptibility patterns were scored as sensitive, resistant and intermediate (CLSI, 2015). *S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls for antimicrobial tests. These experiments were conducted as biological duplicates.

3. Results

3.1. Staphylococcus aureus *isolation*

From 64 evaluated samples, 33 were contaminated with *S. aureus* (51.6%, Table 1) of which 66 *S. aureus* isolates were identified with phenotypic tests and confirmed by 16S rRNA gene sequencing (GenBank accession numbers from MF158055 to MF158084 and from MF423380 to MF423415).

S. aureus was most frequently isolated from ready-to-eat cheese (82%), followed by raw material (50%), non-food contact surfaces (40%) and food contact surfaces (34.6%), as shown in Table 1. At dairy plant A, the presence of *S. aureus* was confirmed in 14 out of 23 samples (60.8%), namely unpasteurised milk (1), curd (1), brine (1), processing tank (1), curd basket (1), cloth mould (1), fresh Minas cheese (4), and fresh Minas cheese surface (4). At dairy plant B, there was no final product available for analysis, but the pathogen was confirmed in 4 out of 19 (21%) samples analysed, as follows: brine (1), curd (1), processing tank (1), and refrigerated milk storage tank (1). At dairy plant C, 15 out of 22 samples (68.2%) were positive for *S. aureus*: milk transport gallon (1), unpasteurised milk (1), unpasteurised milk strainer (1), curd with whey (1), whey (1), curd (1), fresh Minas cheese (3), fresh Minas cheese surface (3), jar (1), freezer (1), and sink (1).

3.2. MLST analysis

Among the 66 confirmed identified *S. aureus*, MLST analysis was done on 30 isolates, selected to represent all sampling categories (food contact surfaces, non-food contact surfaces, raw material, and ready-to-eat products) (Table 2). However, housekeeping genes from isolates SABRC16, SABRC23 and SABRC28 were not amplified and these isolates were reported as non-typeable (Table 2).

Among the 27 typed isolates, five different STs were found, including four STs previously described (1, 97, 126, 3531) and a novel allele combination (ST3816). ST1 accounted for the majority of isolates (n = 13), seven isolates were assigned to ST97, four to ST126, two to ST3531, and one to

ST3816.

Subsequent eBURST analysis (Fig. 1) showed that, except for ST126, all other isolates presented STs with very close clonal relationships, SLV, DLV or TLV. Four divergent loci from each other were found for ST97 and ST3531. The STs were distributed in three clonal complexes, as follows: CC1, CC97, and CC126.

3.3. Antimicrobial susceptibility

The thirty strains analysed by MLST were subjected to antibiotic susceptibility tests (Table 2). No resistance, or intermediate tolerance, to gentamicin, chloramphenicol and vancomycin were observed. Four strains showed intermediate tolerance to one antimicrobial (either ciprofloxacin, clindamycin or erythromycin), but were sensitive to the other antibiotics tested. Resistance towards only one antibiotic was observed for penicillin (8 strains), tetracycline (9 strains) and trimethoprim–sulfamethoxazole (one strain). One strain was tolerant to penicillin and intermediate tolerant to ciprofloxacin and, another strain was tolerant to tetracycline and intermediate tolerant to erythromycin. Simultaneous resistance towards two antibiotics was observed for one strain (penicillin and tetracycline). One strain was intermediate tolerant to clindamycin and simultaneously resistant to cefoxitin, trimethoprim– sulfamethoxazole and to penicillin, being a methicillin-resistant *S. aureus* (MRSA). This strain was recovered from brine at dairy B.

4. Discussion

Dairy processing environments are particularly susceptible to the entry of *S. aureus* and contamination rates ranging from 0 to 50% have been reported (André et al., 2008; Medeiros et al., 2013; Rola, Czubkowska, Korpysa-Dzirba, & Osek, 2016). In Brazil, FMC is one of the most popular dairy products and *S. aureus* has been isolated from both the product and environment of processing plants, which is in agreement with the results obtained in this study (Freitas, Brito, Nero, & Carvalho,

2013; Rodrigues et al., 2017).

In this research, the three evaluated processing lines presented high prevalence of contamination by *S. aureus*, from 21 to 68.2%. *S. aureus* was isolated from samples of all categories available in each dairy (food contact surfaces, non-food contact surfaces, raw material and ready-to-eat product). Dairy A was previously sampled (five months before) and analysed by Dittmann et al. (2017) and a high prevalence of contamination was also reported (63.3%). Milk contamination with *S. aureus* is usually related to bovine mastitis or to human carriers (nasal cavities and hands), leading to contamination of finished products if good animal husbandry and food handling practices are not in place (Fagundes, Barchesi, Nader Filho, Ferreira, & Oliveira, 2010; Schmidt, Kock, & Ehlers, 2017).

Particularly for raw milk products, literature reports that *S. aureus* can occur at frequencies varying from 5% to 100% (Almeida Filho & Nader Filho 2000; Lee et al. 2012; Verraes et al., 2015). In this survey, *S. aureus* was confirmed in 82% of final ready-to-eat-products (FMC). Quantitative data and toxin testing on *S. aureus* are not available for the present study because it was focused on gathering inedit epidemiological data on this foodborne pathogen. Nonetheless, all strains were coagulase positive, which is a phenotypic marker of the potential for production of SE and, only coagulase positive staphylococci strains have been evidenced in food poisoning incidents (Hennekinne, De Buyser, & Dragacci, 2012).

The high contamination rates observed in the dairies evaluated should increase the awareness of producers, consumers, and inspection agents to improve good manufacturing practices for artisanal FMC, especially at production stages that may be particularly vulnerable to contamination, such as reception and storage of raw milk, heat treatment and storage of the finished product (Cusato et al., 2013). To type *S. aureus* isolated from the three dairy plants studied, multilocus sequence typing (MLST) analysis was performed for selected strains, representative of the four samples categories. MLST is a tool developed to overcome problems related to methodologies that, although being successfully used to compare *S. aureus* isolates from outbreaks, do not always present interlaboratory reproducibility, such as phage typing and pulsed-field gel electrophoresis (PFGE) (Rabelo et al., 2007; Smith et al., 2005; Zadoks et al., 2002).

MLST analyses relies on the sequencing of seven housekeeping genes for each *S. aureus* isolate. The sequences are compared using an online database (http://www.mlst.net) that attributes the allelic profile and performs the concatenation leading to the sequence type (ST). MLST results are more accurate and reproducible among laboratories and over time, giving detailed information on the overall epidemiology of the organism (Rabelo et al., 2007; Xie et al., 2011). STs are grouped in clonal complexes (CCs) of isolates that share five to seven alleles with another ST in the group (Smith et al., 2005). In our study, four previously known STs (ST 1, 97, 126, 3531) and a novel ST (ST 3816) were found and these were clustered in three CCs (1, 97, 126) (Fig. 1). At dairy A, the STs 1 and ST3531, both belonging to CC1, were identified. In a previous study conducted at the same dairy by Dittmann et al. (2017), besides ST 3531, the STs 398 and 3540 (respectively from CC398 and CC1) were also identified, but the ST1 was not detected. These results may be indicative that ST 3531 is either persistent or it re-entered dairy A, while ST1 was introduced later in this environment. At dairy B, two different STs (1, 126), belonging to CC1 and CC126, co-existed. At dairy C, STs 97 and 3816, from CC97 and CC1/CC97 respectively, were identified.

CC1 is a major *S. aureus* complex that harbours several community-associated methicillin-resistant *S. aureus* (ca-MRSA) and it has been often implicated in animal disease and in human clinical infections (Alba et al., 2015; Dabul & Camargo, 2014; Rabello et al., 2007; Sobral et al., 2012). The ST1 isolates, belonging to this lineage, have been incriminated in SFP cases in South Korea and China (Yan et al., 2012). The ST3531 was first described in the same dairy factory (Dittmann et al., 2017), and there are, to date, no other reports of this ST in food products or cases of disease.

The CC126 is the smallest clonal complex. Strains belonging to CC126 have been linked to mastitis in ruminants and have a rather limited distribution, being mainly reported in Brazil (Rabello et al., 2007; Silva et al., 2013). CC97 is an important *S. aureus* lineage whose strains, including several MRSA, are mainly associated with animals, especially livestock (Budd et al., 2015; Monecke et al., 2011; Schmidt et al., 2017; Smith et al., 2005). Also, strains from CC97 have been previously implicated in SFP (Sobral et al., 2012). Interestingly, the novel ST3816, described in this study, joined, for the first time, the clonal complexes CC1 and CC97, indicating a linkage of the isolate to both CC

(Fig. 1).

Although some of the *S. aureus* isolates evaluated from CC1 and CC97 showed resistance or intermediate resistance towards one or even two antibiotics, multidrug resistance or MRSA strains were not identified for these lineages (Table 2). However, one of the strains from CC126, isolated from brine at dairy B, was a multidrug resistant MRSA. Interestingly, another strain, belonging to CC1 and resistant to tetracycline, was also found in the same sample of brine (Table 2) demonstrating that the same sample can carry strains with different antimicrobial profiles.

To our knowledge, it is the first time that a strain from CC126 is described as a MRSA. Being such a versatile pathogen, *S. aureus* can rapidly adapt to a wide range of environmental conditions and it has the ability to develop antibiotic resistance to practically all antibiotics (Mccallum, Berger, Achi, & Senn, 2010). Therefore, the presence of multidrug resistant *S. aureus* strains in cheese processing lines is concerning given the strong evidence that handling and consumption of food of animal origin contaminated with MRSA represent a potential vehicle for transmission to humans (Ogata et al, 2012). Also, a high genetic similarity between *S aureus* isolates, both methicillin-susceptible (MSSA) or resistant (MRSA), from animals and humans has already been demonstrated for strains belonging to CC1 and CC97, among others, which is indicative that either human strains have adapted to ruminants (or vice versa) or that these strains share a common ancestor (Alba et al., 2015; Franco et al., 2011; Schmidt et al., 2017; Spoor et al., 2013).

Zoonotic spread of MRSA strains to humans, resulting in severe infections have been reported, clearly highlighting animals as potential reservoirs of MRSA (Girardini et al., 2016; Harrison et al., 2013; Juhász-Kaszanyitzky et al., 2007; Silva et al., 2013). Furthermore, a recent study based on a high-resolution phylogenetic approach, reported the emergence of human epidemic ca-MRSA, resulting from the adaptation of CC97 strains from bovine to human hosts (Feltrin et al., 2016; Spoor et al., 2013). This reinforces that the knowledge of epidemiology of *S. aureus* is important to control the spread of the bacterium throughout the food chain (Haveri, Taponen, Vuopio-Varkila, Salmenlinna, & Pyörälä, 2005; Haveri, Hovinen, Roslöf, & Pyörälä, 2008) and encourages further characterisation of this foodborne pathogen.

5. Conclusions

Artisanal cheese dairy products in Brazil represent a high risk food category with regard to *S. aureus*, especially considering the possibility of environmental contamination by bovine MRSA and detection of bacterial clones previously associated with human or animal diseases. This study reinforces the need for a better characterisation of an important microbiological hazard related to soft cheeses and highlights that implementation of food safety practices are important to reduce the risk of transmission of antimicrobial resistant bacteria throughout the food chain.

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Figure legend

Fig. 1. eBURST diagram generated with the MLST data, representing the five different sequence types (STs) obtained in this study (indicated by red arrows). Clonal complexes 1 (CC1) and 97 (CC97) are linked by the novel ST3816, described for the first time in this study. Blue dotted lines separate CC1 and CC97. CC126 is the smaller clonal complex; the six STs relative to it are visible. STs related to CC1 and CC97 were omitted to facilitate their visualisation. Each dot represents an ST. In pink, STs found in the *S. aureus* isolates of this study; in blue, primary ST founder of clonal complexes; yellow, STs subgroup founders of the clonal complexes; in black, other STs.

Table 1

Distribution of positive samples per sample category contaminated with *Staphylococcus aureus* collected along fresh Minas cheese artisanal processing lines located in the State of Goiás, Brazil.[a]

Sample category	Positive samples/total number of samples in dairy and total			
	A	B	C	Total (%)
Raw material	2/5	2/6	4/5	8/16 (50)
Ready-to-eat product	8/11	0/0	6/6	14/17 (82)
Food contact surface	3/5	2/13	4/8	9/26 (34.6)
Non-food contact surface	1/2	0/0	1/3	2/5 (40)
Total (%)	14/23 (60.8)	4/19 (21)	15/22 (68.2)	33/64 (51.6)

[a] Raw materials are milk, curd, whey, brine; ready-to-eat product is fresh Minas cheese.

Table 2

Characteristics of the 30 *S. aureus* isolates from three fresh Minas Cheese artisanal processing lines located in the State of Goiás, Brazil. [a]

Dairy factory	Source	*S. aureus* strain	Antibiotic resistance profile			Sequence type (ST)	Clonal complex (CC)
			Resistance	Intermediate	Sensitive		
A	FMC	SABRC1			S	3531	1
	raw milk	SABRC2	PEN	CIP		3531	1
	cloth mould	SABRC3			S	1	1
	cloth mould	SABRC4			S	1	1
	sink	SABRC5			S	1	1
	sink	SABRC6			S	1	1
	sink	SABRC7			S	1	1
	processing tank	SABRC8		CIP		1	1
	curd basket	SABRC9			S	1	1
	curd basket	SABRC10			S	1	1
	FMC	SABRC11	SXT			1	1
	FMC	SABRC12			S	1	1
B	brine	SABRC13	CFO*/PEN/SXT	CLIN		126	126
	brine	SABRC14		ERM		1	1
	brine	SABRC15	TET			1	1
	curd	SABRC16	PEN			NT	-
	curd	SABRC17	PEN			126	126
	curd	SABRC18	PEN			126	126
	processing tank	SABRC19	PEN			1	1
	milk storage tank	SABRC20	PEN			126	126
C	FMC	SABRC21	TET			97	97
	FMC	SABRC22	TET			3816**	CC1/CC97***
	FMC	SABRC23	PEN/TET			NT	-
	FMCS	SABRC24	TET			97	97
	whey	SABRC25	TET			97	97
	FMC	SABRC26	TET			97	97
	milk transport gallon	SABRC27	TET			97	97
	curd	SABRC28	TET			NT	-
	whey+ curd	SABRC29	TET			97	97
	FMCS	SABRC30	TET			97	97

[a] Multilocus sequence types (ST) and clonal complexes (CC) are described as well as the antibiotic susceptibility profile classified as resistance/intermediate/sensitive for each isolate. Abbreviations are: FMC, fresh Minas cheese; FMCS, fresh Minas cheese surface; PEN, penicillin; CFO, cefoxitin; ERM, erythromycin; TET, tetracycline; CIP, ciprofloxacin; CLIN, clindamycin; SXT, trimethoprim– sulfamethoxazole; S, sensitive to all tested antimicrobials according to CLSI (2015); NT, non typeable. Asterisks indicate: *, MRSA strain; **, new ST with alleles unpublished combination; ***, new ST3816 linked the clonal complexes CC1 and CC97 - therefore, it could not be related to a specific clonal complex (isolated).