



Phenotypic and Genotypic of Antimicrobial Resistance Profile of *Staphylococcus* spp. Isolated from Raw Milk and Artisanal Cheese

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Abstract

Staphylococcus aureus and a few species of coagulase negative are frequently associated with food poisoning. Raw milk and dairy products are among the foods usually associated with outbreaks due to staphylococcal intoxication. This study aimed to determine phenotypic and genotypic antimicrobial resistance profiles to beta-lactam drugs in *Staphylococcus* coagulase positive (CoPS) and negative (CoNS) isolates. A total of 58 CoPS and 45 CoNS isolates recovered from raw milk and artisanal cheese from Santa Catarina were analyzed. All isolates ($n = 103$) were subjected to antimicrobial susceptibility testing. High levels of resistance to penicillin (41% of CoPS and 31% of CoNS), amoxicillin (40% CoPS), ampicillin (36% CoPS), and sulfamethoxazole-trimethoprim (35% CoNS) were observed. Twenty six percent of the isolates (18 CoPS and 9 CoNS) exhibited multiresistance profile; which means, they were resistant to at least three different classes of the antimicrobial drugs. Detection of resistance genes (*mecA*, *mecC*, and *blaZ*) was performed using multiplex polymerase chain reaction. Twelve isolates (9 CoPS and 3 CoNS) were positive for *mecA*, whereas 10 strains (4 CoPS and 6 CoNS) were positive for *blaZ*. The detection of resistant and multidrug resistant isolates emphasizes the necessity to develop strategies to better comply with good manufacturing practices and health care guidelines.

Keywords: antimicrobial susceptibility, genotypic characterization, multidrug resistance

Introduction

Serrano artisanal cheese is a widely distributed product in the mountainous region of Santa Catarina State and is manufactured with raw milk. Small-dairy farmers produce their cheese, and many of them still commercialize their products informally. In other words, the products do not go through a rigorous inspection process, which ensures the quality and safety of the product for consumers (Ide and Benedet., 2001; Melo et al., 2013). Food safety is a public health concern since products available for commercialization might pose a risk to the consumer's health (Guimarães et al., 2012).

Consumption of unpasteurized milk and raw dairy products is usually associated with staphylococcal food poisoning in humans, and the contamination is associated with poor quality of raw materials and failures in following good manufacturing practices (Santana et al., 2010).

Staphylococcus aureus is considered one of the three most important foodborne pathogens that can cause diseases in humans (Al-Nabulsi et al., 2020). In 2024, the World Health Organization (WHO) updated and refined the prioritization of antibiotic-resistant bacterial pathogens to address the evolving challenges of antibiotic resistance. This Bacterial Priority Pathogens List covers

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24 pathogens, among these is present the *Staphylococcus aureus* (WHO, 2024).

The *Staphylococcus* have a particular relevance in raw milk cheeses once they can contaminate the milk due to poor hygienic milking conditions, conservation, transport, and mastitis occurrence (Aguilar et al., 2021). Recent studies revealed that the isolation of *Staphylococcus aureus* from raw milk and raw milk cheese was above 10^3 UFC/g, which is considered the maximum value allowed by the current legislation (Melo et al., 2013; Pontarolo et al., 2017).

Raw milk and raw milk cheeses are often contaminated with enterotoxigenic *Staphylococcus* spp. and are considered potentially dangerous foods for enterotoxins transmission (Titouche et al., 2019; Leroy et al., 2020). Staphylococcal enterotoxins are considered factor linked to food poisoning by the *Staphylococcus* genus, and these toxins are generally found in milk and dairy products (Aguilar et al., 2021).

The genus *Staphylococcus* is subdivided into two groups according to their ability to produce coagulase, an enzyme enrolled in the plasma coagulation cascade (Podkowik et al., 2013), coagulase-positive staphylococci (CoPS), and coagulase-negative staphylococci (CoNS). CoNS are frequently isolated from human and animal clinical samples, but their importance is related to epidemiological data and suspicion of resistance to glycopeptides (Santana et al., 2010). Interestingly, *S. aureus* is considered the most important species among CoPS since it harbors several virulence factors that are responsible for a variety of infections (Santana et al., 2010).

Another important factor regarding *Staphylococcus* species is the emergence of resistant and/or multidrug-resistant strains (Fleming et al., 2010). The consumption of raw food products without proper processing or preservation could lead to a substantial risk of bacterial antibiotic resistance transfer to the human gastrointestinal microbiota (intra and interspecies, pathogenic and nonpathogenic bacteria). Thus, the detection of resistant and/or multidrug bacteria in food of animal origin raises concerns about public health.

Resistance to beta-lactams has been associated mainly with two mechanisms. The first mechanism involves the production of an extracellular β -lactamase enzyme (encoded by the *blaZ* gene), while the second one includes the production of a low-affinity penicillin (PEN) protein (PBP2a), which is encoded by the *mecA* gene (Lowy, 2003). The *mecC* gene was detected in bovine *S. aureus* strains and also in human *S. aureus* specimens. Although this gene exhibited 70% nucleotide homology to *mecA*, little is known about its functionality (Pichon et al., 2012).

In this study, we investigated the phenotypic and genotypic antimicrobial resistance profiles of *Staphylococcus* spp. isolates recovered from raw milk and artisanal cheese samples produced and commercialized in Santa Catarina, Brazil.

Materials and Methods

Bacterial collection

A total of 103 *Staphylococcus* spp. isolates (58 CoPS and 45 CoNS), coming from milk ($n = 33$) and artisanal Serrano cheese with 14 and 28 days of maturation ($n = 70$), produced by 31 farms belonging to the Association of Serrano Artisanal Cheese Producers of the State of Santa Catarina were used in the present study. The isolates for analysis were

obtained from a previous study carried out in 2015 by our research team (Pontarolo et al., 2017). These specimens are currently part of the bacterial collection at the State University of Santa Catarina (UDESC). All isolates were identified as *Staphylococcus* spp. according to NI 62 of the Ministry of Agriculture, Livestock, and Supply (MAPA, 2003) and confirmed by standard conventional laboratory methods, through the macroscopic characteristics of the colonies and Gram, catalase and coagulase tests with lyophilized rabbit plasma. The samples were kept at -20°C in brain heart infusion broth with 20% glycerol.

Antimicrobial susceptibility testing

The susceptibility of all isolates to a panel of 18 antibiotics was defined using the Kirby–Bauer disk diffusion method in Mueller Hinton agar according to M02-A12 of CLSI (2015). The *S. aureus* reference strain ATCC 25923 was used as a quality control. The antimicrobial agents tested were cefoxitin 30 μg (CFO), PEN G 10 U, amikacin 30 μg (AMK), neomycin 30 μg (NEO), streptomycin 10 μg (STP), tobramycin 10 μg (TOB), gentamicin 10 μg (GEN), enrofloxacin 05 μg (ENO), norfloxacin 10 μg (NOR), ciprofloxacin 5 μg (CIP), levofloxacin 5 μg (LVX), azithromycin 15 μg (AZM), erythromycin 15 μg (ERY), clindamycin 2 μg (CLI), doxycycline 30 μg (DOX), tetracycline 30 μg (TET), rifampicin 5 μg , and trimethoprim-sulfamethoxazole 23.75/1.25 μg (TMP-SXT). All antibiotic disks were purchased from Laborclin (Vargem Grande, PR, Brazil). After incubation, the zones of growth inhibition around each antibiotic disk were measured and interpreted using the criteria established by the CSLI for human (CLSI, 2024b) and animal (CLSI, 2024a) specimens.

Phenotypic detection of multidrug-resistant strains of public health significance was performed by disk diffusion with CFO 30 μg to methicillin-resistant *Staphylococcus* (MRS). All isolates were performed in accordance with CLSI M100-34ED (2024), where halos ≤ 21 mm were considered resistant for CoPS and halos ≤ 24 mm for CoNS. Confirmation of the isolate as MRS was performed using the polymerase chain reaction (PCR) technique for the *mecA* and *mecC* genes.

Extraction of bacterial DNA

Genomic DNA extraction was performed according to the protocol described by Doyle and Doyle (1987), with some modifications. An aliquot of 200 μL of each inoculum was added to 500 μL of chloroform:isoamyl alcohol (24:1), and this mixture was incubated for 30 min at 56°C . After that, samples were centrifuged at 12,000 rpm for 10 min. The recovered supernatants were transferred to new sterile microtubes, and 600 μL of cold 70% alcohol were added. Subsequently, microtubes were centrifuged for 20 min at 13,500 rpm. Then, the supernatants were discarded, and the pellets were allowed to air-dry. DNA samples were carefully resuspended and homogenized with 200 μL of sterile ultrapure water and stored at -20°C .

PCR amplification of beta-lactam resistance genes

We used the multiplex-PCR technique to identify the *blaZ*, *mecA*, and *mecC* genes; the protocol by Nakadomari et al. (2019) was followed. Amplification reactions were performed

in a final volume of 25 μ L of PCR mixture prepared for each isolate, containing final concentrations of 2 μ L of extracted DNA, 0.4 pmol of each primer are shown in Table 1 (*blaZ*, *mecA*, *mecC*, and 16S), 200 mM of each dNTP, 1 $\times$ PCR buffer, 2 mM MgCl₂, and 1.25 U of Platinum Taq DNA polymerase. The PCR program was carried out as follows: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 95°C for 1 min; annealing of the primer at 54°C for 1 min; and 1 min of extension at 72°C. The last cycle extension was at 72°C for 7 min. The amplified fragments were subjected to electrophoresis with 2% agarose gel (100 V, 300 mA) for 1 h using GelRed™ and visualization in a transilluminator. Reference strains ATCC 43300 (*mecA*) and BAA-2313 (*mecC*) were used as positive controls. The 16S gene (amplification product of 228 bp) and ultrapure water (no-template) samples were used as internal and negative controls, respectively.

Results and Discussion

The current practice of indiscriminate use of antimicrobial drugs has been triggering some complications, such as the emergence of antibiotic bacterial resistance (Guimarães et al., 2012). The emergence of resistant strains is a natural phenomenon. It occurs when microorganisms are exposed to antibacterial drugs. It is an intrinsic feature of the microorganism that ensures its survival in unfavorable environments (Umber and Bender., 2009). Moreover, antibiotic resistance might be transferred by bacterial chromosomes or plasmids (Rapini et al., 2004).

The present study demonstrated that higher resistance rates were observed to PEN (41% of CoPS and 31% of CoNS). Similarly, Rola et al. (2016) investigated 122 isolates recovered from raw milk, artisanal cheese, and handler hand swabbing samples in Poland. The authors observed that 50.8% ($n = 62$) were also resistant to PEN. Another study conducted in Italy observed that out of the 160 isolates of *S.*

aureus retrieved from bovine milk and dairy products, six (3.57%) were resistant to PEN (Normanno et al., 2007).

Interestingly, a study carried out in Brazil also reported a high resistance rate (56%) in isolates from foodborne bacterial diseases (Peresi et al., 2006). Most research concerning antibiotic resistance from food focuses on the *Staphylococcus aureus* species, and less attention is paid to the group of CoNS. For many years, the CoNS were considered nonpathogenic, but the recent research suggest that CoNS can display a wide range of antimicrobial resistances, and thus may potentially serve as a reservoir of resistance genes.

Lamaita et al. (2005) observed the production of enterotoxins in positive and negative *Staphylococcus* coagulase strains isolated from refrigerated raw milk samples. They also found a higher percentage of strains producing enterotoxins coagulase negative, a worrying fact since this parameter of *Staphylococcus* coagular negative are not considered by the legislation in force in Brazil (BRASIL, 1996). Figures 1 shows the results of the antimicrobial susceptibility testing for this study. The highest resistance rates were observed for PEN (41% CoPS and 31% CoNS) and TMP-SXT (35% CoNS). Fourteen CoPS isolates (24%) and seven CoNS isolates (15%) were sensitive to all antimicrobial agents tested. Isolates (both CoPS and CoNS) exhibited a similar pattern of susceptibility (>90%) against NEO, TOB, GEN, NOR, CIP, LVX, and DOX. In addition, CoPS were also sensitive (>90%) to ENO and TMP-SXT. With the exception of sulfazotrim, ENO, LVX, and DOX, CoPS showed greater resistance to most antimicrobials than CoNS.

The majority of CoPS isolates (76%) were resistant to at least one class of antimicrobial agents, while 31% of them exhibited resistance to at least three different classes of drugs. Similarly, most CoNS isolates (84%) were resistant to at least one class of antimicrobial agent, whereas 20% of them were classified as multidrug resistant.

The second-highest antimicrobial resistance rate was detected for TMP-SXT (35.56%) in CoNS isolates. In

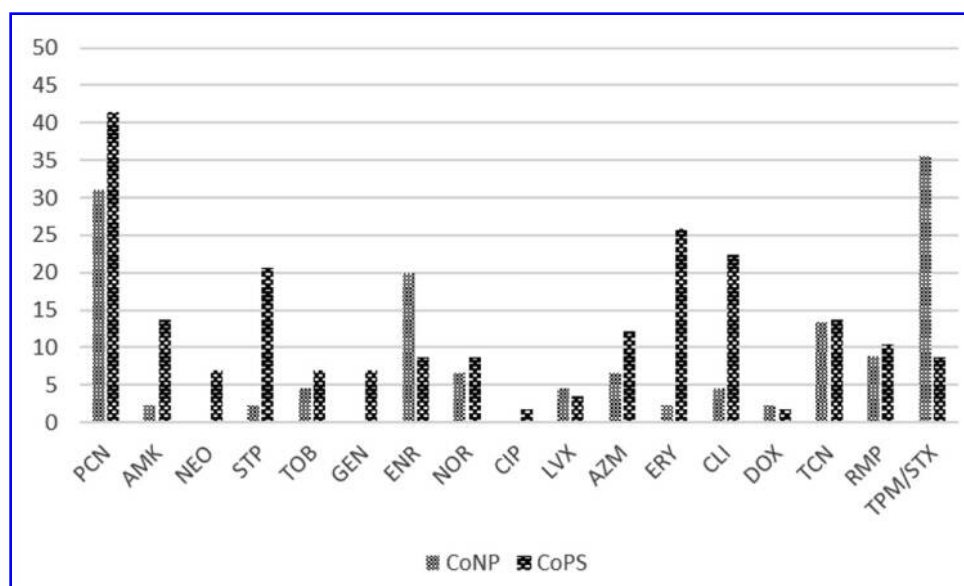


FIG. 1. Resistance profile of CoPS and CoNS isolates recovered from raw milk and serrano artisanal cheese produced in the mountainous region of Santa Catarina, Brazil. CoPS, coagulase-positive *Staphylococcus*; CoNS, coagulase-negative *Staphylococcus*.

addition, another study revealed 26.7% resistance to this same antimicrobial drug in *Staphylococcus* spp. isolates from curdling cheese (Rapini et al., 2004). However, Peresi et al. (2006) reported a resistance percentage of only 4% in relation to TMP-SXT.

The emergence of antibiotic-resistant *S. aureus* may be mainly associated with the selective pressure put into place by the irrational use of antimicrobials to treat infections such as bovine mastitis (Aguar et al., 2021). Research conducted by Guimarães (2011) identifies that the presence of genes encoding enterotoxins, factors of virulence, and resistance to antimicrobials was higher in CoNS than in *S. aureus*. The results of the study demonstrated the importance of CoNS not only as etiological agents of mastitis but also for representing a serious risk to the health of the consumer of milk and dairy products.

The study was to do detect the presence of genes responsible for the production of enterotoxins in CoNS in cheese samples. It was concluded that CoNS need to be included in bacterial foodborne disease research because the genes responsible for the production of toxins were detected and CoNS should be considered as a target of importance in the epidemiology of staphylococcal intoxications (Nascimento et al., 2020).

It is plausible that the excessive use of TMP-SXT against bovine mastitis accelerated the rate of bacterial resistance in this region. For that reason, it is important to perform antimicrobial susceptibility testing in order to prevent treatment failure and the emergence of resistant and/or multidrug-resistant microorganisms.

The review by Borges et al. (2008) cites cheese contamination attributed mainly to the use of raw milk in its manufacture or to failures during processing, mainly in pasteurization, post-processing handling, or in the distribution of the product.

Our results show an alarming number of multidrug resistance in both CoPS (31%) and CoNS (20%) isolates. Similarly, prior studies demonstrated a significant increase in bacterial antimicrobial resistance in isolates from food and, in addition, observed a high multiresistance *S. aureus* samples retrieved from hands of food handlers (Martins et al., 2009).

In contrast, Fleming et al. (2010) observed that two cheese samples commercialized in Rio de Janeiro State (Brazil) exhibited a multidrug resistance profile. Therefore, such a bacterial resistance boost could lead to therapy failure and, consequently, the development of severe infections.

Rodrigues et al. (2017) found dairy-related staphylococcal strains resistant to PEN, CFO, oxacillin, clindamycin, erythromycin, TET, TOB, GEN, ciprofloxacin, and chloramphenicol and reported the presence of community-acquired-methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in raw milk and cheese samples from Brazil.

Milk and milk products can be an important source of foodborne pathogens, including multidrug-resistant bacteria such as MRSA. In recent decades, the emergence of microorganisms resistant to antibiotics, among which *S. aureus* stands out as methicillin-resistant (MRSA), has been observed. The presence of MRSA in foods and food-producing animals, including milk and milk products, has been frequently reported worldwide, raising public health concerns because of the wide spectrum of infections (Titouche et al., 2022).

The review conducted by Oniciuc et al. (2017) indicated the presence of MRSA along the food chain, and the potential of food-producing animals and associated foodstuffs for

the transmission of MRSA was evidenced by an increasing prevalence of MRSA.

In the review conducted by Titouche et al. (2022), the prevalences ranged between 0.34% and 8.3% and in other studies, the probable role of milk and milk products in the transmission of MRSA strains through the food chain was also examined.

Another worrying factor regarding contamination by *S. aureus* associated with food handlers is related to the transmission of methicillin-resistant strains, known by the acronym MRSA.

Various studies were observed during the review by Titouche et al. (2022) to assess the presence of MRSA in milk and milk products. Studies from Greece revealed prevalences of 8.3–11.1% in bovine bulk tank milk and a prevalence of 4.4%. In Germany, MRSA contamination level was 28.6% in the Czech Republic, and there was a prevalence of 8.1% in bulk tank milk in Portugal. In America, 4% of MRSA-positive samples came from 3.3% of Minas frescal cheese in Brazil; in Mexico, an incidence of 14% was observed in dairy products. The African milk samples revealed the presence of MRSA in 0.8–20.3% of different samples. Low prevalence rates were observed, ranging from 4.1% to 6%.

Multiple properties confer resistance to *Staphylococcus* spp. Regarding the molecular characterization, we observed that nine CoPS isolates harbored the *mecA* gene, and seven of them revealed a phenotypic resistance profile as well. Four CoPS samples harbored the *blaZ* gene, and three of them exhibited a phenotypic resistance profile. Simultaneous amplification of both genes was not observed.

Molecular characterization of resistance β -lactamase genes revealed that nine CoPS isolates (15.5%) harbored *mecA*, while four of them (6.9%) were positive for the *blaZ* gene. Regarding CoNS samples, three (6.7%) isolates harbored the *mecA* gene, while six (13.3%) of them were positive for *blaZ*. None of the CoPS and CoNS samples harbored the *mecC* gene (Table 2). The antimicrobial resistance of CoPS and CoNS that presented the *mecA* gene is shown in Tables 3 and 4. The results revealed in this study were not dependent on the biological source of the samples.

The work of de Dias et al. (2011) identified that 11% of samples had *S. aureus* carrying the *mecA* gene resistant to methicillin, representing a potential risk to public health. For that reason, we point out the importance of implementing antimicrobial susceptibility testing methods not only in veterinary clinics but also in healthcare facilities.

The most commonly reported antimicrobial resistance is the one to PEN (Sfaciotte et al., 2015b). According to Daka et al. (2012) and Xu et al. (2014), the prevalence of beta-lactam resistance is frequent in *S. aureus* isolates retrieved from raw milk and raw dairy products.

It was found that 50% of the isolated *S. aureus* strains produced beta-lactamase enzymes (Carvalho et al., 2018). The result of beta-lactamase production by *S. aureus* strains isolated from raw milk and the standard strain for BPC (antimicrobial combination based on PEN, STP, isoniazid, and prednisolone) and MPP (antimicrobial combination based on PEN and STP) showed a percentage of 55.55% of the strains positive for both antibiotics (Carvalho et al., 2018).

Several mechanisms are involved in resistance to beta-lactams, such as production of penicillinase encoded by the

TABLE 1. PRIMERS USED FOR DETECTION OF BETA-LACTAM ANTIMICROBIAL RESISTANCE GENES IN *STAPHYLOCOCCUS* SPP. ISOLATES

Primer	Sequence 5'–3'	Amplicon Length	Reference
<i>mecA</i>	F-GAT GAT ACC TTC GTT CCA R-GTA TGT GCG ATT GTA TTG	316 bp ^a	Sfaiotote et al, 2015a
<i>mecC</i>	F-GAT TTA AAG TAG TAG ACG GC R-TTT CAC CGA TTC CCA AAT CT	138 bp	Pichon et al, 2012
<i>blaZ</i>	F-AGA GAT TTG CCT ATG CTT R-CTT GAC CAC TTT TAT CAG C	516 bp	(Asfour and Darwish, 2011 (modified))
16S	F-AGG TGG CAA GCG TTA TCC R-CGC ACA TCA GCG TCA G	228 bp	Asfour and Darwish, 2011 (modified)

^aBase pairs.

blaZ gene and alteration in the PEN-binding target protein encoded by the *mecA* gene (Sfaiotote et al., 2015b).

In the Colombia study, MRSA and methicillin-resistant NEC isolated from bovine milk were identified by detection of the *mecA* gene, which was identified in 27% of the strains evaluated (Velasquez et al., 2020).

The frequent use of these antimicrobials in cattle production systems could explain why more than 50% of the *Staphylococcus* strains characterized in this study were carriers of the *blaZ* gene, associated with resistance to PEN and/or ampicillin (Velasquez et al., 2020).

The results showed that 23% of the isolates demonstrated resistance to some antibiotics and 5.5% MRS (Aguilar et al., 2021). The genotypic analysis for beta-lactam resistance genes revealed that 15% of CoPS and 7% of CoNS were positive for the *mecA* gene, whereas 7% of CoPS and 13% of CoNS were positive for the *blaZ* gene. These results support the high phenotypic resistance index observed in this study against beta-lactam antibiotics.

Interestingly, a study carried out in Minas Gerais State (Brazil) observed that 33.78% of *Staphylococcus* spp. isolates recovered from milk samples (animals with recurrent mastitis history) harbored the *mecA* gene (Asfour and Darwish, 2011). In Japan, Lee et al. (2004) observed that 3.56% of bovine mastitis *S. aureus* were positive for the same gene. Moreover, an Egyptian study detected the presence of *mecA* and *blaZ* genes in *S. aureus* strains retrieved from meat product samples (Ammar et al, 2016). On the other hand, samples recovered from raw milk and raw dairy products manufactured in the Northeast region of Brazil did not harbor the *mecA* gene (Silveira Filho et al., 2014).

The PCR results presented 42.6% positive strains for the presence of the *blaZ* gene, and 7.4% were resistant to

methicillin using phenotypic testing. The *blaZ* gene was found in 59.4% of the Islamians and the *ermC* gene in 62.3%; the port of these genes is associated with resistance to lactams and macrolides (Velasquez et al., 2020).

The presence of bacteria with antimicrobial resistance genes in food raises great concern.

The human gastrointestinal tract is a favorable environment for antimicrobial resistance transfer among gut microbiota and pathogenic species (Rapini et al., 2004). Since artisanal cheese is an economical source for many small producers and is widely consumed in the region, the presence of multidrug resistant CoPS and CoNS isolates pose a risk for human and animal health. What could be easily managed

TABLE 3. COAGULASE-POSITIVE *STAPHYLOCOCCUS* WITH THE PRESENCE OF THE *MECA* GENE AND THE RESISTANCE PROFILE OF EACH OF THEM

Samples	Phenotypic Resistance (CFO ^a)	<i>mecA</i>	<i>blaZ</i>	Resistance Profile
1	R ^b	+	–	STP ^d /TOB ^c /AZM ^f /ERY ^g /CLI ^h /RMP ⁱ /TMP-STX ^j
2	R	+	–	AMK ^k /NEO ^l /STP/TOB/GEN ^m /ENO ⁿ /ERY/CLI/TET ^o /TMP-STX
3	R	+	–	NEO/STP/TOB/GEN/ERY/CLI/RMP
4	S ^c	+	–	AMK
5	S	+	+	—
6	R	+	–	—
7	S	+	–	—
8	S	+	+	—
9	S	+	–	—

^aCFO (cefexitin).^bR (resistance).^cS (sensitive).^dSTP (streptomycin).^eTOB (tobramycin).^fAZM (azithromycin).^gERY (erythromycin).^hCLI (clindamycin).ⁱRMP (rifampicin).^jTM^p-STX (trimethoprim-sulfamethoxazole).^kAMK (amikacin).^lNEO (neomycin).^mGEN (gentamicin).ⁿENO (enrofloxacin).^oTET (tetracycline).TABLE 2. PHENOTYPIC RESISTANCE TO BETA-LACTAMS AND PRESENCE OF RESISTANCE GENES *MECA*, *MECC* AND, *BLAZ* IN *STAPHYLOCOCCUS* SPP. ISOLATES RECOVERED FROM RAW MILK AND ARTISANAL CHEESE PRODUCED IN SANTA CATARINA, BRAZIL

	Phenotypic Resistance (CFO ^a)	<i>mecA</i>	<i>mecC</i>	<i>blaZ</i>
CoPS ^b (n = 58)	24.1%	15.5%	0	6.9%
CoNS ^c (n = 45)	6.7%	6.7%	0	13.3%

^aCFO (cefexitin).^bCoPS (coagulase-positive *Staphylococci*).^cCoNS (coagulase-negative *Staphylococci*). Values are expressed in percentage.

TABLE 4. COAGULASE-NEGATIVE *STAPHYLOCOCCUS* WITH THE PRESENCE OF THE *MECA* GENE AND THE RESISTANCE PROFILE OF EACH OF THEM

Samples	Phenotypic Resistance (CFO ^a)	mecA	blaZ	Resistance Profile
1	S ^b	+	–	AZM ^d /CLI ^c
2	R ^c	+	–	TET ^f
3	S	+	–	—

^aCFO (cefoxitin).

^bS (sensitive).

^cR (resistance).

^dAZM (azithromycin).

^eCLI (clindamycin).

^fTET (tetracycline).

with standard therapy becomes a potential health threat. Therefore, we point out that the use of antibiotics in food animals ought to be very cautious, in order to prevent human cross-infections. Nonetheless, the emergence and dissemination of resistant pathogens can be prevented by following good manufacturing practice guidelines during artisanal cheese production.

Antimicrobial resistance was detected in both CoPS and CoNS samples. This points out that CoNS isolates might also play a role in the dissemination of bacterial resistance. The results from this study and previous ones show the need to revise the current health policy and establish new guidelines for the management of CoNS detected in food samples. Furthermore, the isolation of MRS and the detection of beta-lactam resistance genes in foods shown in our work is a public health problem and requires greater attention that goes from the beginning of the food production chain to the final product that arrives for the consumer.

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Authors' Contributions

F.D.M., H.P., R.A.P.S., M.F.S., and L.P. participated in all stages of the research. F.D.M., H.P., and R.A.P.S. wrote original draft and elaborated the content. S.R.W., U.M.C., and S.M.F. acted as advisors, reviewers, and supervisors.

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No competing financial interests exist.

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