Bovine mastitis: prevalence and antimicrobial susceptibility profile and detection of genes associated with biofilm formation in *Staphylococcus aureus*

Mastite bovina: prevalência, pefil de suscetibilidade aos antimicrobianos e detecção de genes associados à formação de biofilmes em *Staphylococcus aureus*

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Abstract

Brazil currently ranks as one of the world leaders in food production and exportation. This scenario encourages the development of animal and plant health programs to ensure the production of safe food, helping the country to become an international provider of food for excellence. However, some health problems in dairy production, such as mastitis, have garnered increasing concern. This study aimed to estimate the prevalence of bovine mastitis in select properties located in the western Santa Catarina region, to assess the susceptibility profile to antimicrobial agents used for treatment and to check for the presence of genes (*icaA* and *icaD*) associated with biofilm formation in *Staphylococcus aureus*. In 148 milk samples collected, 72.97% had bacterial growth (n = 108). Among the isolated microorganisms, 21.62% (n = 32) were classified as *Staphylococcus aureus*, 18.91% (n = 28) as *Staphylococcus* sp. coagulase negative, 7.43% (n = 11) as *Corynebacterium* sp., 6.76% (n = 10) as *Staphylococcus* sp. coagulase positive, 5.41% (n = 8) as *Nocardia* sp. and 12.83% (n = 19) classified in different bacterial genera. Among the isolates submitted for antimicrobial susceptibility testing, it was observed that 8.95% (n = 6/67) had resistance to amoxicillin, 8.04% (n = 7/87) to ampicillin, 5.88% (n = 5/85) to cephalothin, 3.40% (n = 3/88) to ceftiofur and enrofloxacin, 20.45% (n = 18/88) to streptomycin, 17.04% (n = 15/88) to gentamicin and lincomycin, 31.81% (n = 28/88) to neomycin, 14.94% (n = 13/87) to penicillin and 25% (n = 22/88) to tetracycline. *Staphylococcus* sp. coagulase negative isolates showed higher multidrug resistance when compared to those of *S. aureus* and *Staphylococcus* sp. coagulase positive. Thirty-one strains of *S. aureus* isolates were genotypically tested by polymerase chain reaction (PCR), yielding a positive result for the *icaA* gene in 83.87% of the samples, 80.64% positive for *icaD* and 74.19% of these showed both genes. The results reinforce the importance of controlling this disease by adopting strict hygiene protocols and disinfection of equipment used for milking and the prescription of appropriate therapies to clinical and subclinical cases of mastitis, as well as evaluating not only the susceptibility profile of isolates but also the biofilm formation capacity.
Key words: Antimicrobial resistance. Biofilm. Mastitis. *Staphylococcus aureus*.

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Resumo

O Brasil situa-se hoje como um dos líderes mundiais em produção e exportação de alimentos. Este cenário incita a elaboração de programas de sanidade animal e vegetal que garantam a produção de alimentos seguros, contribuindo para que o país se torne um fornecedor internacional de alimentos por excelência. Entretanto, alguns problemas sanitários na produção leiteira, como a mastite, têm acarretado crescentes preocupações. O presente estudo teve como objetivo estimar a prevalência de mastite bovina em algumas propriedades localizadas na região Oeste de Santa Catarina, avaliar o perfil de suscetibilidade aos antimicrobianos empregados para tratamento e verificar a presença de genes (*icaA* e *icaD*) associados à formação de biofilmes em *Staphylococcus aureus*. Em 148 amostras de leite coletadas, 72,97% apresentaram crescimento bacteriano (n=108). Dentre os micro-organismos isolados, 21,62% (n=32) foram classificados como *Staphylococcus aureus*, 18,91% (n=28) como *Staphylococcus* sp. coagulase negativa, 7,43% (n=11) como *Corynebacterium* sp., 6,76% (n=10) como *Staphylococcus* sp. coagulase positiva, 5,41% (n=8) como *Nocardia* sp. e 12,83% (n=19) classificados em diferentes gêneros bacterianos. Dentre os isolados submetidos ao teste de suscetibilidade aos antimicrobianos, observou-se 8,95% (n=6/67) de resistência à Amoxicilina, 8,04% (n=7/87) à Ampicilina, 5,88% (n=5/85) à Cefalotina, 3,40% (n=3/88) ao Ceftiofur e Enrofloxacina, 20,45% (n=18/88) à Estreptomicina, 17,04% (n=15/88) à Gentamicina e Lincomicina, 31,81% (n=28/88) à Neomicina, 14,94% (n=13/87) à Penicilina e 25% (n=22/88) à Tetraciclina. Ainda, os isolados de *Staphylococcus* sp. coagulase negativa apresentaram maior multirresistência quando comparados aos isolados de *S. aureus* e S*taphylococcus* sp. coagulase positiva. Trinta e uma amostras de *S. aureus* isoladas foram testadas genotipicamente pela técnica de reação em cadeia da polimerase (PCR), obtendo-se resultado positivo em 83,87% das amostras para o gene *IcaA*, 80,64% positivas para *IcaD* e 74,19% dessas apresentaram ambos os genes. Os resultados obtidos demonstram a importância do controle desta enfermidade pela adoção de protocolos rígidos de higiene e desinfecção dos equipamentos utilizados na ordenha e também a prescrição de terapias adequadas aos casos clínicos e subclínicos de mastite, bem como a avaliação não apenas do perfil de suscetibilidade dos isolados, mas também a capacidade de formação de biofilmes.
Palavras-chave: Biofilme. Mastite. Resistência aos antimicrobianos. *Staphylococcus aureus*.

Introduction

Brazil is considered one of the largest producers of milk, and the South and Southeast regions are the main producers. Santa Catarina state is the sixth largest national milk producer, and approximately 70% of Santa Catarina's production is concentrated in the western region (IBGE, 2013), playing an important role for small farms from an economic and social point of view. Milk production is responsible for direct and indirect job creation and income to farmers, thus becoming one of the most important activities related to rural development.

In this context, the quality of milk is essential for the competitiveness of dairy farming; quality is influential in both the manufacture of dairy products and derivatives as well as in consumer preference. Despite advances in the dairy cattle industry, one of the barriers to the development and consolidation of the dairy industry in Brazil is the quality of the milk (FAGUNDES; OLIVEIRA, 2004).

Some health problems in the dairy industry have caused increasing concerns. Mastitis is characterized by an inflammation of the mammary gland, usually infectious in nature, in which bacteria are the main etiological agents. This condition affects dairy cattle and leads to losses resulting from the reduction in milk production, animal value depreciation, limiting the production of derivative products, milk disposal, spending on medicines, veterinary fees, labor augmentation, early disposal of females, among others. Furthermore, the possibility of transmission of pathogenic microorganisms and/or their toxins or transfer of antibiotic residue through the milk pose severe risks to public health (FORSYTHE, 2002).

Staphylococcus aureus is the main agent associated with the contagious bovine mastitis. Many chronic infections caused by this pathogen are related to the formation of biofilms; this feature hinders the action of macrophages, and it also provides resistance to various antibiotics and

encourages the residual persistence of these in the environment (BOYEN et al., 2009). Several genes are involved in the individual capacity of biofilm formation, highlighting the *icaA* and *icaD* genes (MELCHIOR et al., 2009).

Antibiotic use in the treatment of mastitis is frequent; however, there has been an increase in the bacterial resistance index (BRITO et al., 2001). This fact reinforces the importance of adopting appropriate treatment protocols with effective medicines, which must be selected from phenotypic and genotypic testing (COELHO et al., 2007).

This study aimed to estimate the prevalence of bovine mastitis in dairy farms located in the western region of Santa Catarina to determine the main isolated microorganisms, to evaluate the susceptibility front of the main antibiotics used in the treatment of this disease, as well as to check for the presence of genes associated with biofilm formation in *S. aureus* strains.

Material and Methods

Place of execution and collection of material

A total of 148 individual milk samples were collected from 148 animals on 12 farms located in the western region of Santa Catarina. All properties analyzed had adopted the mechanics milking system, and the herds were composed of primiparous and pluriparous cows of different races and different stages of lactation.

All procedures performed in the collection, transportation of samples, isolation and identification of the etiologic agents of mastitis followed the recommendations of National Mastitis Council (2004). Prior to harvest, the ceilings were individually cleaned using water and disposable paper towels, disregarding the three foremilk on black background mug to evaluate for the presence of clinical mastitis. The CMT (California Mastitis Test) was performed to confirm the presence of subclinical mastitis without the evaluation of

different degrees. Prior to taking the samples, the animal teats were disinfected with cotton that was soaked with 70% ethanol. Three milk jets from each teat were collected and stored in sterile vials, and CMT testing was performed on sample from each animal. The vials were opened only at the moment of harvest; they were opened in a horizontal position to avoid contaminants entering into the container. The samples were duly identified and kept under refrigeration (4 to 8 ° C) during transport to the microbiology laboratory for further analysis.

Isolation and bacterial identification

The cultivation of the samples was performed on blood agar supplemented with 5% defibrinated sheep blood, MacConkey agar and Sabouraud agar. The plates were incubated at 36°C for 24 to 48 hours, the microorganisms were identified according to the morphological and staining characteristics according to the description presented by Markey et al. (2013). After incubation, the colonies were evaluated according to the morphological characteristics and hemolysis patterns. Gram-staining and catalase tests were performed, the latter separating the likely *Staphylococcus* and *Micrococcus* strains (catalase-positive) from *Streptococcus* and *Enterococcus* strains (catalase-negative); this also assisted in the identification of strains of *Corynebacterium* sp. The isolates that were positive for the catalase test were submitted to the coagulase test tube, acetoin production and growth on agar salt mannitol. The isolates that were negative in the catalase test underwent the esculin test, bile esculin test, Pyrrolidonyl arylamidase test, hippurate, CAMP and NaCl 6.5% to characterize the species. For isolates that presented as Gram-negative, the tests used were oxidase, OF, motility, indole, VM, VP, citrate, urea, gelatin, TSI, among others.

Antimicrobial susceptibility testing

Once the strains had been isolated and identified, susceptibility testing to antibiotics was performed by

the disk diffusion method of Kirby-Bauer modified in accordance with the recommendations of the veterinary CLSI (2008) to evaluate the susceptibility profile of the front of these microorganisms compared to antimicrobials commonly used in treatment of mastitis. The following antimicrobials classes were tested: beta-lactams – penicillin (10 IU), amoxicillin (10μg), ampicillin (10μg), oxacillin (1μg), cephalothin (30μg) and ceftiofur (30μg); aminoglycosides – streptomycin (10μg), gentamicin (10μg) and neomycin (10μg); lincosamides – lincomycin (2μg); fluoroquinolones – enrofloxacin (5μg); sulfonamides – sulfazotrim (25μg) and tetracycline – tetracycline (30μg). All isolates were stored on nutrient agar and then stored at –20 ° C in BHI broth and glycerol in a 8:2 ratio, respectively.

Presence of genes associated with biofilm formation

Isolates of *Staphylococcus aureus* were characterized by the presence of genes associated with the formation of biofilms (*icaA* and *icaD*) by PCR. The DNA extraction was performed according to the description of Rademaker and De Bruijn (1997).

For PCR execution, 4 μl of DNA suspension were applied in 11μl of mix containing 20 pmol/μl of each primer, 2.5 mM of the deoxyribonucleotides, 1.5μL 10 × PCR buffer (750 mM Tris HCl (pH 8.8) 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20) and 0.3 μL Taq DNA Polymerase 5 U / uL. This mixture was brought to the thermocycler with cyclical conditions suggested by Vasudevan et al. (2003). Modifications were as follows: initial denaturation at 94 ° C for 2 min, 30 cycles of denaturation at 94 ° C for 45 s, annealing at 49.8 ° C for 45 s, 1 min extension at 72 ° C and a final extension of 7 min at 72 ° C. In all reactions, negative control samples were used (without target DNA), and the positive control was *S. aureus* strain ATCC 25923. The banding patterns were evaluated using a 100 bp molecular weight marker.

The results of the PCRs were checked on 1.5% agarose gel, stained with ethidium bromide (2 μg/ ml, Sigma, St. Louis, USA), visualized on a transilluminator, and the image was captured and digitized by the Kodak 2200 system (Rochester, New York, USA). The primers for detecting *icaD* and *icaA* genes in *S. aureus* isolates are described in Table 1.

Table 1. Oligonucleotide primers used for detection of *icaD* and *icaA* genes in *Staphylococcus aureus* isolates.

Primer	Sequence (5´-3´)	Fragment length	Reference
IcaA F	CCT AAC TAA CGA AAG GTA G	1315 bp	Vasudevan et al., 2003
IcaA R	AAG ATA TAG CGA TAA GTG C	1315 bp	Vasudevan et al., 2003
IcaD F	AAA CGT AAG AGA GGT GG	381 bp	Vasudevan et al., 2003
IcaD R	GGC AAT ATG ATC AAG ATA C	381 bp	Vasudevan et al., 2003

Statistical analysis

The descriptive statistics analysis (absolute and relative frequencies) was realized using Statistical Package for the Social Sciences (SPSS) software version 20.

Results

Of the 148 samples analyzed, 72.97% (n = 108) showed bacterial growth. Of these, 21.62% (n = 32) were classified as *S. aureus*, followed by *Staphylococcus* sp. coagulase negative (CNS), *Corynebacterium* sp., *Staphylococcus* sp. coagulase positive (SCP), and other agents described in Table

2. There were no microbiological growth in forty samples taken (27.03%), and only one sample showed growth of yeast cells in Sabouraud agar.

The highest resistance rates were observed for the antibiotics neomycin (31.81%, n = 28/88), tetracycline (25%, n = 22/88), streptomycin (20.45%, n = 18/88), gentamicin and lincomycin (17.04%, n = 15/88) and penicillin (14.94%, n = 13/87). The bacterial isolates expressed greater sensitivity to ceftiofur and enrofloxacin principles (96.59% susceptibility, n = 3/88) and cephalothin (94.11%, n = 80/85).

Table 2. Frequency and percentage (%) of microorganisms isolated in milk samples collected from cows with positive CMT.

Isolated microorganisms	Frequency	%
No growth	40	27. 03%
Staphylococcus aureus	32	21. 62%
Staphylococcus sp. coagulase negative (CNS)	28	18. 92%
Corynebacterium sp.	11	7. 43%
Staphylococcus sp. coagulase positive (SCP)	10	6. 76%
Nocardia sp.	8	5. 41%
Streptococcus equinus	5	3. 38%
Streptococcus uberis	4	2. 70%
Enterobacter aerogenes	3	2. 03%
Yeast growth	1	0. 68%
Enterobacter sp.	1	0. 68%
Enterococcus sp.	1	0. 68%
Escherichia coli	1	0. 68%
Streptococcus alactolyticus	1	0. 68%
Streptococcus dysgalactiae	1	0. 68%
Streptococcus acidominimus	1	0. 68%
Total	148	100%

All isolates of *Staphylococcus aureus* presented sensitivity to ampicillin, cephalothin and ceftiofur; higher levels of resistance were observed in the principles neomycin (37.5%, n = 12/32), gentamicin (28.12%, n = 9/32) and tetracycline (12.5%, n = 4/32), which are principles used frequently in systemic and topical treatments.

Although *S. aureus* isolates are among the most prevalent and pathogenic in this study, CNS isolates showed greater resistance to the total number of classes of antimicrobials evaluated when compared to *S. aureus* and SCP (Table 3).

Multidrug-resistant isolates in this study were defined as those isolates that were resistant to three or more antimicrobial classes, according to Schwarz et al. (2010). One *Enterococcus* sp. isolate, nine CNS and three *S. aureus* presented multidrug resistance profiles to antibiotics, and some strains expressed resistance to five evaluated classes. Multidrug resistance profiles are detailed in Table 3.

Of the 31 *S. aureus* isolates evaluated for the presence of *icaA* and *icaD* genes associated with formation of biofilm, 83.87% had the *icaA* gene, 80.64% *icaD* the gene and 74.19% were positive for both genes. The DNA extraction of one isolate could not be performed; thus, it was not submitted for PCR analysis.

Table 3. Multidrug resistance profiles to antimicrobial presented by microorganisms in milk samples.

Microorganism	Multidrug resistance profile	Isolates (n)	Resistant classes
Enterococcus sp.	STR/LIN/NEO/PEN/SUT/TET	1	5
	AMO/AMP/CEF/CTF/ENR/STR/LIN/PEN/TET	1	5
	CTF/EST/GEN/LIN/NEO/TET	1	5
	AMO/AMP/CEF/STR/LIN/PEN/TET	1	4
	CEF/CTF/EST/GEN/NEO/TET	1	4
S. coagulase negative (CNS)	NEO/PEN/SUT/TET	1	4
	AMO/AMP/LIN/PEN/TET	1	3
	AMO/CEF/LIN/PEN/TET	1	3
	ENR/GEN/NEO/SUT	1	3
	LIN/NEO/OXA	1	3
	AMO/SUT/TET	1	3
S. aureus	ENR/GEN/TET	1	3
	STR/LIN/TET	1	3
S. coagulase positive (SCP)	AMP/CEF/LIN/OXA/PEN	1	3

AMO=Amoxicillin, AMP= Ampicillin, CEF= Cefalotin, CTF= Ceftiofur, ENR=Enrofloxacin, STR= Streptomycin, GEN=Gentamicin, LIN= Lincomycin, NEO=Neomycin, PEN= Penicillin, SUT= Sulfazotrim, TET= Tetracycline.

Discussion

Of all the obtained samples, 108 showed bacterial growth (72.97%), which reveals a high prevalence of mastitis in the sampled herds. As reported in Zanela et al. (2006), the high mastitis levels are directly linked to hygienic conditions of the property and animal health, including flaws in the cleaning of pickets, management problems and milking environment, as well as failures in nutrition of animals.

Of the 148 isolates, 40 (27.03%) showed no microbial growth. Although bacterial isolation is considered the gold standard technique for characterizing the etiology of mastitis, some samples of milk with clinical and subclinical mastitis can present negative results (MAKOVEC; RUEGG, 2003). This fact can be justified due to the intermittent elimination of the agent in chronic infections or from their reduced presence in milk (SEARS et al., 1990).

S. aureus was the most prevalent agent (21.62%) in this study. Similar data were described by Ferreira et al. (2007) and Martins (2010) in which both observed a 21.5% of prevalence of *S. aureus* strains isolated in cases of subclinical mastitis. In both studies, the evaluated properties had adopted similar managements as the use of mechanical milking at different stages of lactation. They were unable to evaluate whether there was any pattern in temporal distribution, such as seasonality between the isolated microorganisms, as the samples were taken at different times of the year.

S. aureus is considered to be the most important microorganism in contagious mastitis in cattle. Despite nearly a 22% prevalence, this percentage may be determined to be low when compared to the ratios reported in other studies. Unakal and Kaliwal (2010) evaluated 105 samples of milk from different dairy farms and reported a prevalence of approximately 65% for this agent; other authors also reported a high prevalence (WAAGE et al., 1999).

The second most prevalent agent was the CNS, which highlights the importance of this microorganism as the etiologic agent of mastitis in this study. Amaral et al. (2003) emphasize the importance of infection in the mammary gland as a

result of CNS, which may be responsible for long-term processes that compromise the composition and milk production. In similar studies, the authors found CNS as the most prevalent agent (FREITAS et al., 2005; TENHAGEN et al., 2006). A similar result was found by Marques et al. (2013), who analyzed 272 samples of milk from eight properties of the South Fluminense region of the state of Rio de Janeiro and found 250 isolates of *Staphylococcus* spp., from which 58% (145/250) were CNS and 42% (105/250) *Staphylococcus* sp. coagulase positive. Of these, 36.2% (38/105) were identified as *S. aureus*.

In relation to antimicrobial susceptibility testing, high bacterial resistance rates were observed when evaluating antibiotics neomycin, tetracycline, streptomycin, gentamicin, lincomycin and penicillin. Although some studies show higher resistance rates (RABELLO et al., 2005; ZAFALON et al., 2008; UNAKAL; KALIWAL, 2010), this may be related to the more frequent use of these agents and/or the presence of genes associated with biofilm formation (MELCHIOR et al., 2006a).

Oliveira (2002) showed a high percentage of resistance of *Staphylococcus* sp. samples isolated from animals with subclinical mastitis; the resistance was especially present in penicillin and tetracycline. These authors noted the incorrect and indiscriminate use of these antimicrobials was responsible for this situation. However, in the present study, there was a higher percentage of *in vitro* resistance to neomycin, gentamicin and streptomycin. These antibiotics are widely used in dairy cattle to treat various conditions (NETTO et al., 2005), especially uterine infections, often in under-dosing due to the application in the form of infusion. In addition, several drugs of intramammary application have these three antimicrobials in their compositions, which may have contributed to the increase in resistance rates, especially when therapy is administered incorrectly.

Although *S. aureus* was the most frequently isolated agent, a result of extreme importance in this study was that CNS multidrug-resistant isolates compared to *S. aureus* and SCP. Rajala-Schultz et al. (2004), in a study evaluating 202 bacterial agents, showed a prevalence of 78% of CNS, and of these, 44% were resistant to more than one antibiotic. Similarly, Medeiros et al. (2009) also found CNS isolated from cows with subclinical mastitis with resistance profiles for more than eight different antimicrobial compounds. This finding reinforces the importance of this agent in cases of mastitis because the multidrug resistance to treatment may be associated with persistence of agents in the herd, leading to cases of chronic infection as well as predisposing to further infection.

Human diseases caused by *S. aureus* multi-resistant strains from animals are found worldwide, which makes it essential to assess the susceptibility of this isolated microorganism in relation to antibiotics. The increasing number of globally distributed multi-drug resistant isolates from mastitis cases is a serious problem that causes increases in treatment costs and a greater likelihood of transmission of this disease due to the persistence of bacteria in the herds (SABOUR et al., 2004; FAGUNDES; OLIVEIRA, 2004). The importance of carrying out accurate diagnosis through isolation and identification of agents in laboratories and *in vitro* analysis of antimicrobial susceptibility is emphasized, mainly to prescribe the most appropriate therapy and so reduce the risk of bacterial multidrug resistance.

Most *S. aureus* isolates evaluated demonstrated the presence of one or both genes associated with biofilm formation. The ability of the bacterial agents to form a biofilm is directly related to microbial adhesion to a variety of surfaces, allowing it to be found on the milking environment, including the udder and teats of the animals, blowers, milking machines and utensils used in the cleaning before and after milking. These factors make it difficult to control mastitis in dairy farms (LEE et al., 2012). In the biofilm, bacteria remain less susceptible to the action of antibiotics and innate host response by preventing phagocytosis, as well as favoring

the maintenance and the persistence of the bacteria in the environment. Melo (2012) investigated the presence of *icaA* and *icaD* (genes responsible for the synthesis of the biofilm) in 94 *S. aureus* strains isolated from milk samples derived from cattle with subclinical mastitis and confirmed that 95.7% had both genes. Vasudevan et al. (2003) also analyzed *S. aureus* strains isolated from bovine milk samples with subclinical mastitis and found 100% were positive for *icaA* and *icaD,* thus confirming the potential of these genes as virulence factor in the pathogenesis of mastitis of ruminants. The high presence of *icaA* and *icaD* genes in *S. aureus* isolates emphasizes the importance in controlling mastitis caused by this agent because once formed the biofilm, the infection is likely to become chronic and difficult to treat.

Despite the high presence of *icaA* and *icaD* genes, only three *S. aureus* isolates showed a multidrug resistance profile with the disk diffusion test. Detection of the *icaA* and *icaD* genes in most tested isolates demonstrates that these strains may come to form biofilm once activation occurs. Thus, even when the isolates presented sensitivity to various antibiotics, this microorganism can remain protected from the action of these by biofilms. The discrepancy between the results of *in vitro* antimicrobial susceptibility and the *in vivo* cure obtained by antimicrobial therapies highlights the importance of biofilm formation *in vivo* by *S. aureus*, which in turn has resulted in disappointing cure rates, especially in long term infections (MELCHIOR et al., 2006b). Melchior et al. (2011) evaluated a collection of *S. aureus* isolates from bovine mastitis cases by antimicrobial susceptibility extended to biofilm test (EBS) and found the duration of therapy as the most important factor for the bovine mastitis cure rate. However, this same article emphasized that treatment should be recommended only in recent *S. aureus* infections.

Conclusion

Despite the role that the high prevalence of bovine mastitis directly plays in the sustainability and viability of dairy cattle, the occurrence of multidrug resistance coupled with gene expression capacity that is associated with the formation of biofilms reinforces the importance of controlling this disease by adopting strict protocols of hygiene and disinfection of equipment used in milking. Furthermore, it also aids in prescribing therapies appropriate to the clinical and subclinical mastitis, especially in *S. aureus* infections.

Although high levels of susceptibility have been found in *S. aureus*, the observed resistance to neomycin, gentamicin, and tetracycline antibiotics is of concern because they are frequently used in treatment protocols for mastitis. Furthermore, the presence of *icaD* and *icaA* genes in most isolates emphasizes the importance to assess both the susceptibility profile of the isolates and the biofilm formation capacity.

The presence of nine CNS strains with multidrug resistance profiles is extremely important not only for the control of bovine mastitis but also with regards to public health because milk is a major source of income for many producers; it is also an indispensable food for much of the population.

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