SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2022v43n1p141

# Classification of dairy properties according to the geometric mean of the somatic cell count and its antimicrobial resistance profile

# Classificação de propriedades leiteiras de acordo com a média geométrica da contagem de células somáticas e seu perfil de resistência aos antimicrobianos

Mauricio Fanin[1]*; Isabela Carvalho dos Santos[2]; Geysiane Moreira Gerotti[3]; Camila de Cuffa Matusaiki[4]; Daniela Dib Gonçalves[5]; Benito Guimarães de Brito[6]; Kelly Cristina Tagliari de Brito[6]; Jessika Fernanda Boeira[6]; Luciana Kazue Otutumi[5]

## Highlights

High somatic cell count negatively affects constituents present in milk.
*Mec*A gene was not detected in any of the coagulase-negative *Staphylococcus* isolates.
Antibiotic resistance to *Enterobacter* isolates is higher in terms of good traits.
Dairy farm classification differs in antibiotic resistance against *Staphylococcus* spp.

## Abstract

Milk and its derivatives are highly consumed foods worldwide, with recognized nutritional importance. The search for the production of products with superior quality is constant. For the present work, 26 milk-producing properties were selected, with a total of 506 milk samples collected during the period from October 2019 to May 2020 being evaluated. The objective of this study was to evaluate the quality of milk produced in dairy properties in the region west Paraná, classified as good or bad based on the results of the Somatic Cell Count (SCC) and through sampling (n = 10) to evaluate the resistance profile of enterobacteria and *Staphylococcus* spp. isolated from milk samples, in addition to the presence of the *mec*A gene in strains of *Staphylococcus*

[1] Doctor in Animal Science wit Emfasis on Boactive Products, Universidade Paranaense, UNIPAR, Umuarama. PR, Brazil. E-mail: fanin@hotmail.com
[2] Student of the Doctoral Course of the Postgraduate Program in Animal Science with Emfasis on Bioactive Products, UNIPAR, Umuarama, PR, Brazil. E-mail: isacarsan@gmail.com
[3] Student of Master Course of the Postgraduate Program in Animal Science with Emfasis on Bioactive Products, UNIPAR, Umuarama, PR, Brazil. E-mail: geysianegerotti66@gmail.com
[4] Student of Veterinary Medicine Course, UNIPAR, Umuarama, PR, Brazil. E-mail: camila_cuffa@hotmail.com
[5] Profas Dras, Postgraduate Program in Animal Science with Emfasis on Bioactive Products, UNIPAR, Umuarama, PR, Brazil. E-mail: danieladib@prof.unipar.br; otutumi@prof.unipar.br
[6] Researcher, Secretaria da Agricultura, Pecuária e Desenvolvimento Rural do Rio Grande do Sul, Instituto de Pesquisas Veterinárias Desidério Finamor, IPVDF, Eldorado do Sul, RS, Brasil. E-mail: benitobrito@gmail.com; kelly_tagliari@hotmail.com; jessikaboeira@yahoo.com.br
* Author for correspondence

Received: June 18, 2021 - Approved: Oct. 20, 2021

spp. resistant to oxacillin. There were significant differences between the good and bad properties for the levels of lactose, SCC (cell/mL), and Standard Plate Count (SPC) (CFU/mL). The strains of *Staphylococcus* spp. showed differences in the percentage of resistance in relation to the good and bad properties for antibiotics: tetracycline, ciprofloxacin, oxacillin, amikacin, clindamycin, gentamycin, and erythromycin. The *mec*A gene was not detected in any of the coagulase-negative *Staphylococcus* isolates that showed resistance to oxacillin. For enterobacteria, the isolated species differed in relation to the classification of properties, with predominance for *Escherichia coli* (40%) for properties classified as bad and *Hafnia alvei* (40%) for those classified as good. The percentage of antibiotic resistance compared to enterobacteria isolates was higher in properties classified as good. Monitoring through microbial culture and antibiogram is extremely important, favoring the correct choice for the treatment of animals with a reduced selection of resistant strains.
**Key words:** Milk quality. *Staphylococcus* spp. Enterobacteria. SCC. SPC.

## Resumo

O leite e seus derivados são alimentos altamente consumidos em todo mundo, com importância nutricional reconhecida. A busca pela produção de produtos com qualidade superior é constante. Para o presente trabalho foram selecionadas 26 propriedades produtoras de leite, sendo avaliado um total de 506 amostras de leite colhidas durante o período de outubro de 2019 a maio de 2020. O objetivo deste estudo foi avaliar a qualidade do leite produzido em propriedades leiteiras da região oeste do Paraná, classificadas como boas ou ruins com base nos resultados da Contagem de Células Somáticas (CCS) e por meio de amostragem (n=10) avaliar o perfil de resistência das enterobactérias e *Staphylococcus* spp. isolados das amostras de leite, além da presença do gene *mec*A em cepas de *Staphylococcus* spp. resistentes à oxacilina. Houve diferenças significativas entre as propriedades boas e ruins para os teores de lactose, CCS (cél./mL) e Contagem Padrão em Placas (CPP) (UFC/mL). As cepas de *Staphylococcus* spp. apresentaram diferenças no percentual de resistência em relação às propriedades boas e ruins para os antibióticos: tetraciclina, ciprofloxacina, oxacilina, amicacina, clindamicina, gentamicina e eritromicina. Não foi detectada a presença do gene *mec*A em nenhum dos isolados de *Staphylococcus* coagulase negativa que apresentaram perfil de resistência à oxacilina. Para as enterobactérias as espécies isoladas diferiram em relação à classificação das propriedades, com predomínio para *Escherichia coli* (40%) para as propriedades classificadas como ruins e *Hafnia alvei* (40%) para as classificadas como boas. O percentual de resistência aos antibióticos frente aos isolados de enterobactérias foi maior nas propriedades classificadas como boas. É extremamente importante o monitoramento por meio de cultura microbiana e antibiograma, favorecendo a correta escolha para o tratamento dos animais com redução da seleção de cepas resistentes.
**Palavras-chave:** Qualidade do leite. *Staphylococcus* spp. Enterobactérias. CCS. CPP.

## Introduction

Milk is a food of great nutritional importance, with proteins, fats, vitamins, and minerals in its composition (Salvador, Burin, Frias, Oliveira, & Faila, 2012). Due to this fact, it becomes an excellent culture medium for the development of deteriorating microorganisms or with pathogenic potential (Perin, Pereira, Bersot, & Nero, 2019).

Thus, several prophylactic hygiene measures are important to mitigate the effects of microbial contamination, always seeking a better-quality product and increased herd productivity (Langoni, 2013). In addition, several problems caused by lack of hygiene in milking compromise the production and microbiological quality of milk, with bovine mastitis being one of the most important problems reported (Menezes et al., 2015).

Cows affected by mastitis usually need to be treated with antimicrobials, which can cause bacterial resistance, generating an alert for One Health, in addition to the damage caused to lactation (Artursson, Söderlund, Liu, Monecke, & Schelin, 2016) and as a consequence to quality of milk (Pereira & Scussel, 2017).

Oxacillin resistance in *Staphylococcus aureus* is mediated by the production of a supplemental penicillin-binding protein (PBP 2 or PBP 2a), which shows a low affinity for penicillins and is encoded by the *mec*A gene. Therefore, when the *mec*A gene is present, the cell is able to grow in the presence of oxacillin and other beta-lactams (Cunha, 2017).

In this sense, the objective of the present work was to classify, as good or bad, 26 dairy farms in the western region of the state of Paraná, Brazil, based on the results of geometric means of somatic cell count (SCC). Then, evaluate, through sampling, five rural properties classified as good and five rural properties classified as bad in relation to the resistance profile of enterobacteria and *Staphylococcus* spp. isolated from milk samples and evaluate the presence of the *mec*A gene in isolates of oxacillin-resistant *Staphylococcus* spp.

## Material and Methods

### *Animals and study site*

Data regarding milk quality control from 26 dairy farms, which belong to a cooperative located in the western region of Paraná, were organized in tables. The data and characterization of the properties were provided by the veterinarian responsible for the cooperative's milk development. After tabulating the data and previous analysis of the results of the arithmetic mean of the geometric mean of the somatic cell count (SCC), obtained from milk samples from dairy farms in the period from October 2019 to May 2020, the rural properties were classified as good or bad, being considered good those with SCC less than 500,000 cells per mL and bad those with a value greater than 500,000 cells per mL.

The median of production and milk quality data for each rural property was calculated. Subsequently, they were analyzed in relation to this classification. In addition, 10 properties were randomly chosen, five classified as good and five as bad in relation to the parameters previously described, for subsequent collection of milk samples in order to a) isolate enterobacteria and *Staphylococcus* spp., b) to evaluate the antibiotic resistance profile and c) to detect the presence of the *mec*A gene from *Staphylococcus* spp isolates that were identified as resistant to oxacillin.

### *Sample collection*

Samples of raw bovine milk refrigerated at 4 °C in sterile flasks were collected. After collecting the milk samples,

the biological materials were kept and sent under refrigeration to the Laboratory of Preventive Veterinary Medicine and Public Health of the Postgraduate Program in Animal Science with Emphasis on Bioactive Products at Universidade Paranaense (UNIPAR) for processing.

### *Bacterial culture, isolation, and identification*

Milk samples were streaked onto plates containing Blood Agar Base enriched with defibrinated blood (8%) and kept in an oven for 24 hours at 37 °C. Subsequently, each isolate was submitted to the analysis of macroscopic characteristics of the colonies, microscopic (Gram stain), and biochemical tests (Koneman, Allen, Janda, Schreckenberger, & Winn, 2008). Gram-positive catalase-positive cocci were submitted to the coagulase test for classification into coagulase-positive *Staphylococcus* (CoPS) or coagulase-negative *Staphylococcus* (CoNS). Gram-negative bacilli were submitted to biochemical tests to identify enterobacteria.

### *Biochemical identification of bacterial isolates*

Bacteria of the Enterobacteriales order were biochemically identified using an enterobacteria kit (NewProv®, Paraná, Brazil) according to the manufacturer's recommendations (Quinn, Markey, Cater, Donnely, & Leonar, 2005).

### *Phenotypic assays of antimicrobial sensitivity*

Assays were performed according to the Clinical and Laboratory Standards Institute [CLSI] (2013). The agar diffusion method was performed after standardization, in BHI broth, of the inoculum according to the 0.5 McFarland standard. The results obtained were registered considering the interpretation of the Brazilian Committe on Antimicrobial Susceptibility Testing [BrCAST] (2020) for bacteria isolated from animals and the measurement of the size of the inhibition zones, in millimeters (mm). The antimicrobials evaluated against Gram-positive cocci were ampicillin (10 µg), oxacillin (1 µg), amoxicillin (30 µg), cefoxitin (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), meropenem (10 µg) and ceftriaxone (30 µg).

In turn, the gram-negative isolates were tested against ampicillin (10 µg), amoxicillin (30 µg), amoxicillin + clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), aztreonam (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), amikacin (30 µg) and sulfazotrim (25 µg).

### *Molecular tests*

#### *mecA gene search*

DNA from oxacillin-resistant Staphylococcus spp. isolates was extracted using the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer's information and polymerase chain reactions (PCR) were performed using Platinum PCR SuperMix (Invitrogen, Carlsbad, California, USA); primer *mec*A1 (AAAATCGATGGTAAAGGTTGG) and *mec*A2 (AGTTCTGCAGTACCGGATTTG) at

5 µM; DNA from the isolates and nuclease-free water totaling 25 µL. For amplification, a thermocycler, Thermo, model Px2, was used under the parameters described by Murakami et al. (1991).

The amplification of the products was visualized by 2% agarose gel electrophoresis stained with GelRed (Uniscience, Osasco, São Paulo, Brazil), and the products were visualized as a single band of 533 bp. As a positive control for the *mec*A gene, *S. aureus* ATCC 33591 was used and for the negative control, *S. aureus* ATCC 25923 was used.

### *Identification of Staphylococcus aureus*

The polymerase chain reaction (PCR) was performed for samples classified as CoPS to verify which of these isolates were *Staphylococcus aureus.* The DNA was extracted with the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, California, USA), according to the manufacturer's information, and the reactions were performed using the primer Sa442-1 (5'- AAT CTT TGT CGG TAC ACGATA TTC TTC ACG- 3') and the primer Sa442-2 (5'-CGT AAT GAGATT TCA GTA GAT AAT ACA ACA-3'), following the methodology of Martineau, Picard, Roy, Ouellette and Bergeron (1998). For DNA amplification, an Applied Biosystems™ Veriti™ 96-Well Thermal Cycler was used.

Amplification of products was visualized by 2% agarose gel electrophoresis stained with GelRed (Uniscience, Osasco, São Paulo, Brazil) using a 100 bp molecular marker, and the products were visualized as a single 241 bp band. As a positive control, *S. aureus* ATCC 33591 was used.

### *Statistical analysis*

Descriptive data analysis was performed by determining the mean and median of the milk quality parameters and the characteristics of the properties in relation to their classification as good or bad. For the resistance results, the absolute and relative (%) frequencies were determined. Continuous variables were analyzed for data normality (Lilliefors test) and differences between the classification of properties were analyzed by Student's t-test for independent samples when data were normally distributed or by Mann-Whitney U test when the data were not normally distributed. Characterization of properties was analyzed by Fisher's exact test. The analysis was performed using the BioEstat 5.0 software (Ayres, Ayres, Ayres, & Santos 2007) and for all tests, the significance level of 5% was considered.

## Results and Discussion

The milk quality parameters must be considered independent of the production system, the size of the properties, or the socio-economic level of the producer, given that hygiene is the main practice to be adopted (Langoni, 2013). To assess the quality of raw milk, in addition to the physicochemical analysis, the health of the mammary gland is measured by evaluating the SCC and the hygienic-sanitary quality by determining the standard plate count (SPC).

Currently, Brazilian legislation, through normative instruction No. 76, provides that SPC geometric means must have a maximum limit of 300,000 colony-forming units (CFU) per mL and 500,000 somatic cells per mL of milk for SCC (Instrução Normativa n. 76 de 26 de novembro de 2018.).

In Table 1, the descriptive statistics of the properties classified as good and bad can be observed in detail, as a function of the geometric mean of the SCC. The average monthly milk production was higher in properties classified as good, with higher fat content (Table 1). It should be noted that higher production was also observed in the average milk yield per animal in properties classified as good.

Rural properties classified as bad had higher averages in protein and casein contents in milk and higher levels of urea nitrogen in milk when compared to good properties (Table 1).

The results were significant for the mean percentage of lactose present in milk, which was 4.49% and 4.33% for the good and bad properties, respectively. Miller and Nesi (2012) report the increase in lactose levels as production increases, corroborating the results of this research.

The concentration of lactose linearly decreased from the increase in the count of somatic cells present in the milk of animals with different genetic patterns (Ludovico, Trentin, & Rêgo, 2019). Studies show that this decrease occurs due to the increase in the microbial load in milk, where microorganisms use lactose as a substrate, reducing the synthesis of this compound by the mammary gland (Araújo et al., 2018).

For SCC, significant results were found. The averages were 345.86 (x 1000 cells/mL) for good properties and 991.47 (x 1000 cells/mL) for bad properties (Table 1). Higher levels of SCC can decrease production and change its composition (Lima, Coelho, Bueno, & Neves, 2016), which corroborates the present study. Although not significant, the production averages of good properties were higher than those classified as bad.

The increase in SCC resulted in a linear decrease in milk production in different breeds evaluated (Ludovico et al., 2019). According to Miller and Nesi (2012), SCC significantly reduced as production increased.

Elevated SPC is associated with ineffective hygiene in the milking process, due to dirt present in the mammary gland, inadequate cleaning of equipment and utensils used in milking management, and subsequent failures in the milk cooling process (Scabin, Kozusny-Andreani,, & Frias, 2012). In the SPC evaluation, the results showed significantly lower averages, that is, 130 (x 1000 CFU/mL) for the good properties and 177.83 (x 1000 CFU/mL) for the bad ones.

In Brazil, numerous characteristics are present in dairy properties, whether in land area, level of technology used, production systems, socioeconomic and cultural factors. Based on these particularities, studies seek to correlate these variables with the quality of the milk produced (Miller & Nesi, 2012; Eckstein et al., 2014; Reis et al., 2020). However, it is known that the greater the specialization of the production system, the better the results of milk production per animal, and lower levels of somatic cell counts are found in milk (Simioni, Baretta, Stefani, Lopes, & Tizziani, 2013).

According to Taffarel et al. (2015), milk from properties with volumes greater than 15 thousand liters per month has lower levels of SPC and somatic cell count, and the variations in SPC are smaller in properties that have milking with a piped system. In the search to find possible variables correlated with the quality of the milk produced, it was decided to characterize the properties classified as good and bad (Table 2), evaluating the facilities, production system, and some management techniques.

**Table 1**
**Descriptive statistics of the classification of bovine milk-producing properties classified as good and bad based on the geometric mean of the Somatic Cell Count (SCC) (n=506)**

| Parameters | Dairy farm classification | |
|---|---|---|
| | Good | Bad |
| Average milk production (liters/month)* | 22681.82 | 14866.67 |
| Median milk production (liters/month) | 16.000 | 13.000 |
| Average number of lactating animals*** | 29.00 | 22.93 |
| Median number of lactating animals | 22.00 | 21.00 |
| Average number of employees*** | 2.45 | 2.47 |
| Median number of employees | 2.00 | 2.00 |
| Average fat percentage* | 3.70 | 3.64 |
| Average percentage of protein* | 3.15 | 3.26 |
| Average percentage of lactose** | 4.49[a] | 4.33[b] |
| Average percentage of total solids*** | 12.11 | 12.12 |
| Average SCC (*1000 cells/mL)** | 345.86[b] | 991.47[a] |
| Median SCC (*1000 cells/mL) | 352.00 | 780.50 |
| SPC Average (CFU/mL)**** | 130.00[b] | 177.83[a] |
| Median SPC (CFU/mL) | 42.00 | 153.50 |
| Average Urea Nitrogen in milk (mg/dL)** | 9.62 | 11.34 |
| Average percentage of casein*** | 2.51 | 2.60 |

*Not significant by Student's t-test
**Means followed by different letters differ by Student's t-test (P<0.01)
***Not significant by Mann-Whitney U test
****Means followed by different letters differ by the Mann-Whitney U test (P=0.032).

**Table 2**
**Characteristics of bovine milk-producing properties classified as good and bad based on Somatic Cell Count (SCC)**

| Variable | Response | Dairy farm classification | |
|---|---|---|---|
| | | Bad | Good |
| Milker's sex | Male | 1 (20%) | 1 (20%) |
| | Female | 1 (20%) | 1 (20%) |
| | Both | 3 (60%) | 3 (60%) |
| Milker** | Owner | 4 (80%) | 5 (100%) |
| | Employee | 1 (20%) | 0 |
| Milker's age | Up to 30 years old | 0 | 1 (20%) |
| | 31 to 45 years old | 4 (80%) | 2 (40%) |
| | Above 45 years old | 1 (20%) | 2 (40%) |

continue...

continuation...

| | | | |
|---|---|---|---|
| Only source of income?** | Yes | 2 (40%) | 2 (40%) |
| | No | 3 (60%) | 3 (60%) |
| Does the farm have technical assistance?** | Yes | 3 (60%) | 2 (40%) |
| | No | 2 (40%) | 3 (60%) |
| Time in activity** | 5 to 10 years | 0 | 1 (20%) |
| | More than 10 years | 5 (100%) | 4 (80%) |
| Milking system** | Pipeline | 2 (40%) | 3 (60%) |
| | No pipeline | 3 (60%) | 2 (40%) |
| Are pre- and post-dipping done?** | Yes | 5 (100%) | 3 (60%) |
| | No | 0 | 2 (40%) |
| Is the strip cup mastitis test performed as a routine?** | Yes | 4 (80%) | 5 (100%) |
| | No | 1 (20%) | 0 |
| Are paper towels used?** | Yes | 2 (40%) | 2 (40%) |
| | No | 3 (60%) | 3 (60%) |
| Is the CMT done?** | Once a month | 2 (40%) | 2 (40%) |
| | Never | 3 (60%) | 3 (60%) |
| Milking Machine Maintenance** | Once a year | 1 (20%) | 1 (20%) |
| | Only when necessary | 4 (80%) | 4 (80%) |
| Is dry cow therapy done?** | Yes | 5 (100%) | 3 (60%) |
| | No | 0 | 2 (40%) |
| Production system | Extensive | 1 (20%) | 2 (40%) |
| | Semi-intensive | 4 (80%) | 2 (40%) |
| | Intensive | 0 | 1 (20%) |
| Does the milker participate in courses and lectures? | No | 0 | 1 (20%) |
| | Once a year | 5 (100%) | 3 (60%) |
| | Twice or more a year | 0 | 1 (20%) |
| Are culture and antibiotic sensitivity testing performed on the milk?** | Yes | 0 | 1 (20%) |
| | No | 5 (100%) | 4 (80%) |
| What treatment is used for mastitis?** | Others | 1 (20%) | 2 (40%) |
| | Systemic and intramammary treatment* | 4 (80%) | 3 (60%) |
| Owner's income (reais/month) | Below 10.000 | 1 (20%) | 1 (20%) |
| | 10.000 to 20.000 | 3 (60%) | 3 (60% |
| | Above 20.000 | 1 (20%) | 1 (20%) |

*Not significant by Student's t-test
**Means followed by different letters differ by Student's t-test (P<0.01)
***Not significant by Mann-Whitney U test
****Means followed by different letters differ by the Mann-Whitney U test (P=0.032).

Among the variations found in the characterization of properties, for those classified as good, the person responsible for milking was the owner himself and most of them had a milking pipeline system. According to Taffarel, Costa, Oliveira, Braga and Zonin (2013), milk obtained by the milking pipeline system has better microbiological quality.

In all properties classified as good, the strip cup mastitis test is routinely used, which according to Gonçalves, Tomazi and Santos (2017) has a high correlation and reliability to prove the existence of clinical mastitis in animals.

For properties classified as bad, most have technical assistance, indicating that failures may be occurring and/or the producers or employees may not be following the indications suggested by the specialized professionals. This result is justified since studies indicate the improvement of milk quality after the adoption of prophylactic management measures and good practices in milking hygiene (Eckstein et al., 2014; Picoli et al., 2014; Werncke et al., 2016).

Some important variables were found in all rural properties evaluated (data not shown), being recommended in several situations to improve the health of the property, such as the non-use of gloves during the milking process, the order of the animals at the time of milking following the health standards of the mammary gland and, finally, the non-treatment of the water used in the properties.

The treatment of water used in rural areas is currently still rudimentary (Orwa, Matofary, & Muliro, 2017). The use of contaminated water can act as a vehicle for disease transmission, compromising the hygiene of milking equipment and coolers, affecting the quality of milk (Satake, Assunção, Lopes, & Amaral, 2012). In the present study, all dairy farms did not have treatment for the water used, which could contribute to water contamination and as a consequence of milk.

From each sample collected, one strain of *Staphylococcus* spp. was isolated, being eight strains of CoNS and two strains of CoPS. The results of the antibiotic resistance analysis showed differences in the percentage of resistance in relation to properties classified as good and bad based on the geometric mean of the SCC for the antibiotics: tetracycline, ciprofloxacin, oxacillin, amikacin, clindamycin, gentamicin, and erythromycin (Table 3), and for ampicillin there was 100% sensitivity.

Differing from the results found in this study, E. R. Silva, Pereira, Moraes, Santoro and Silva (2012) and Pati and Mukherjee (2016) found a high profile of resistance to ampicillin in *Staphylococcus aureus* isolated from animals with bovine mastitis, with percentages of 88 and 93 %, respectively.

Some antibiotics that are commonly used in dairy farms for therapeutic purposes, such as gentamicin and tetracyclines, showed 60% resistance levels in bad properties, very similar results found by Noel, Motta, Francisco, Almeida and Soares (2016) in isolates of *Staphylococcus* spp. in milk, which obtained resistance of 56% to tetracyclines, and Pati and Mukherjee (2016), evaluating *Staphylococcus aureus* from animals with mastitis, showed resistance of 57% to gentamicin and 54% to tetracycline. However, in a study carried out in the northeast region of Brazil, lower levels of resistance were found for the same classes, resulting in 6% for tetracyclines and 18% for gentamicin, suggesting less use of these drugs in the sampled herds (E. R. Silva, et al., 2012).

**Table 3**
**Absolute and relative frequencies (%) of the antibiotic resistance profile against *Staphylococcus* spp. isolates from bovine milk samples from producers classified as good and bad in relation to Somatic Cell Count (SCC)**

| Class | Antibiotics | Dairy farm classification | |
|---|---|---|---|
| | | Bad | Good |
| Beta lactams | Ampicillin | --- | --- |
| | Amoxicillin | 2/5 (40%) | 2/5 (40%) |
| | Oxacillin | 3/5 (60%) | 1/5 (20%) |
| | Cefoxitin | 2/5 (40%) | 2/5 (40%) |
| | Ceftriaxone | 2/5 (40%) | 2/5 (40%) |
| | Imipenem | 2/5 (40%) | 2/5 (40%) |
| | Meropenem | 2/5 (40%) | 2/5 (40%) |
| Aminoglycosides | Tobramycin | 2/5 (40%) | 2/5 (40%) |
| | Amikacin | 3/5 (60%) | 1/5 (20%) |
| | Gentamicin | 3/5 (60%) | 2/5 (40%) |
| Tetracyclines | Tetracycline | 3/5 (60%) | 2/5 (40%) |
| Quinolones | Ciprofloxacin | 0/5 (0%) | 1/5 (20%) |
| Lincosamides | Clindamycin | 2/5 (40%) | 1/5 (20%) |
| Macrolides | Erythromycin | 1/5 (20%) | 2/5 (40%) |

As for the good properties, erythromycin and ciprofloxacin showed higher resistance, 40%, and 20% respectively, showing results close to those found by Pati and Mukherjee (2016), with 66% for erythromycin and 37% resistance for ciprofloxacin. The results differ from those found by Bitencourt et al. (2018), who demonstrate a sensitivity profile of 95% for erythromycin and 100% for ciprofloxacin, reporting a high profile of resistance to penicillin, favoring the development of a higher prevalence of samples resistant to this active principle, reducing the selection of resistance for other classes of pharmaceuticals.

Evaluating 29 samples of *Staphylococcus* spp. from cows with mastitis, the highest percentages of resistant samples were penicillin (34.48%), oxacillin (20.69%), and erythromycin (17.24%), with greater sensitivity to gentamicin (96.55%) and tetracycline (82.76%) (Kurosawa, Cézar, Marques, Oriani, & Moraes, 2020).

Antimicrobial resistance was evaluated in *Staphylococcus* spp. strains isolated from milk samples by Ceniti et al. (2017), demonstrating full sensitivity to gentamicin and oxacillin in all isolates. The same authors report resistance to ampicillin (34%), clindamycin (15.62%), tetracycline (9%), and erythromycin (6%).

In most cases, the multi-resistance of disease-causing pathogens occurs through the indiscriminate, inappropriate, and/or mistaken use of drugs, resulting in the possibility of selecting resistant bacteria (Zimermann & Araújo, 2017).

Eight species of enterobacteria were isolated from milk samples collected from farms located in western Paraná. However, the isolated species differed in relation to the classification of properties, with a predominance of *Escherichia coli* (40%), for properties classified as bad, and *Hafnia alvei* (40%), for those classified as good (Figure 1).

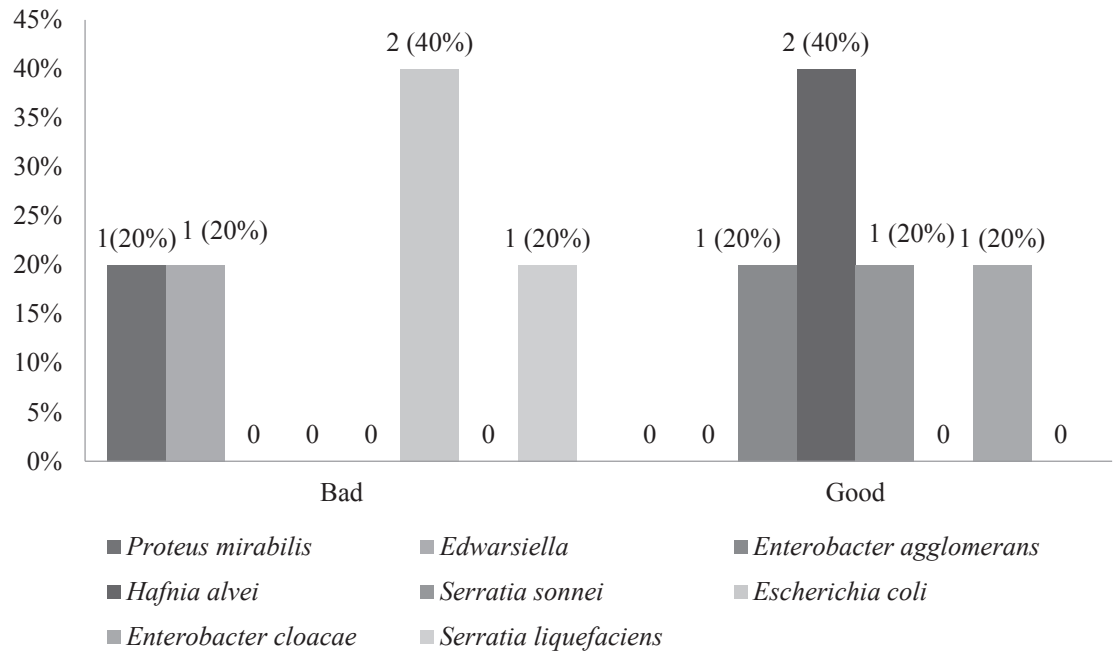


**Figure 1.** Species of enterobacteria isolated from bovine milk samples from dairy farms located in western Paraná, classified as good or bad according to the Somatic Cell Count (SCC).

*Escherichia coli* is one of the main environmental pathogens found in milk, being considered an opportunistic pathogen and the most isolated species in cases of environmental mastitis (Kempf, Slugocki, Blum, Leitner, & Germon, 2016). The isolation of *E. coli* reveals contamination carried by animal feces, water, and other environmental substrates, demonstrating the need for greater rigor in the cleaning of the environment, especially animal litter, avoiding the occurrence of contamination by environmental pathogens (Rodenburg, 2012).

In a work carried out by Salina et al. (2016), the authors evaluated the occurrence of enterobacteria in milk samples from different states and found that the most frequently isolated pathogen was *Escherichia coli* (74.6%), followed by *Hafnia alvei* (5.1%), corroborating the results of this research.

The isolation of *Hafnia alvei* strains from raw milk has been described and considered multiresistant to various classes of antimicrobials, showing 100% sensitivity to gentamicin (R. T. Silva, Lopes, Oliveira, Ribeiro, & Beloti, 2019), results very close to those found in the present study, in which 80% and 100% sensitivity to gentamicin was verified for the good and bad properties, respectively.

There were differences between the properties classified as good and bad in relation to the antibiotic resistance profile against the enterobacterial isolates (Table 4), and the antibiotic resistance profile was higher in the properties classified as good. The highest rates were presented for drugs belonging to the beta-lactam class, such as amoxicillin (80%) and ampicillin (60%).

Studies have demonstrated *in vitro* microbial resistance of strains isolated from cow's milk of 30% to the drug ampicillin (Bolaños et al., 2014; Siqueira et al., 2014).

It was described by R. T. Silva et al. (2019) the percentage of antimicrobial resistance against bacteria isolated from raw milk, demonstrating resistance of 33.33% to amoxicillin and 16.67% to ampicillin.

**Table 4**
**Absolute and relative frequencies (%) of antibiotic resistance profile against enterobacterial isolates from bovine milk samples from producers classified as good and bad in relation to Somatic Cell Count (SCC)**

| Class | Antibiotics | Dairy farm classification | |
|---|---|---|---|
| | | Bad | Good |
| Beta lactams | Ampicillin | 1/5 (20%) | 3/5 (60%) |
| | Amoxicillin | 2/5 (40%) | 4/5 (80%) |
| | Amoxicillin + clavulanic acid | 2/5 (40%) | 2/5 (40%) |
| | Cefotaxime | 0/5 (0%) | 1/5 (20%) |
| | Ceftazidime | 1/5 (20%) | 1/5 (20%) |
| | Ceftriaxone | 0/5 (0%) | 1/5 (20%) |
| | Imipenem | 0/5 (0%) | 1/5 (20%) |
| | Aztreonam | 2/5 (40%) | 2/5 (40%) |
| Quinolones | Ciprofloxacin | 2/5 (40%) | 0/5 (0%) |
| Aminoglycosides | Amikacin | 1/5 (20%) | 1/5 (20%) |
| | Gentamicin | 0/5 (0%) | 1/5 (20%) |
| Sulfonamides | Sulfazotrim | 1/5 (20%) | 1/5 (20%) |

Some bacteria have intrinsic resistance to certain drugs, not being related to the use of antimicrobials, characteristic of the structure of some bacteria since their emergence without acquiring resistance genes (Cox & Wright, 2013).

According to Song et al. (2017), *Hafnia alvei* strains show natural resistance to several classes of antibiotics, such as tetracyclines, penicillins, and cephalosporins. The resistance found to quinolones, such as ciprofloxacin, may not be associated with gene transfer, but with the intrinsic capacity of *E. coli* to develop resistance when exposed to a selective environment (Bhatnagar & Wong, 2019).

The high rate of resistance of the isolates to beta-lactam antibiotics can be explained by the routine use of these drugs

for the treatment of bovine mastitis for many years (Kowalski et al., 2015). The incorrect and indiscriminate use of antimicrobials represents dangers to human and animal health resulting from therapeutic failures with increased rates of bacterial resistance (Costa et al., 2013).

The demonstrated resistance to antibiotics of the beta-lactam class, such as ampicillin and amoxicillin, may be related to the presence of bacterial strains with the production of beta-lactamases or penicillinases (Costa et al., 2013). However, the growing number of strains resistant to antimicrobials, often related to the inefficient use of drugs, indicates that one of the alternatives for infections caused by multi-resistant bacteria will be through alternative treatments with revolutionary therapies (Fanin et al., 2020).

The presence of a higher resistance profile for the good properties may be related to the production volume, the number of animals present in the herd, and, consequently, the increased use of antibiotics. In a study conducted by Redding, Bender and Baker (2019), the authors found a statistically significant difference in the incidence of treatment and duration of therapy by farm size.

Methicillin resistance in staphylococci occurs through the production of an anomalous penicillin-binding protein (PBP 2a) present in the cell wall, which has a reduced affinity for beta-lactams. This altered protein is encoded by a chromosomal gene called *mec*A, resulting in the presence of intrinsic resistance of staphylococci to methicillin and beta-lactams (Cunha, 2017).

The *mec*A gene was not detected in any of the coagulase-negative *Staphylococcus* isolates that showed resistance to oxacillin. Among the four *Staphylococcus* CoNS isolates, one was from a property classified as good and three classified as bad.

It is suggested that dairy farms with the intention of producing milk as a quality raw material make use of management technologies and monitoring of the health of the herd's mammary gland, performing routine microbiological analyzes in order to identify the pathogens present on the farm. It is also recommended to perform antibiotic sensitivity testing to monitor bacterial resistance for the correct choice of antimicrobials in the treatment of animals affected by diseases, implementation of good prophylactic management practices to mitigate the effects of contamination on milking, and equipment. Without a serious program in place at the dairy farm with routine monitoring, the challenges to reach the minimum standards required by current legislation will hardly be met.

## Conclusions

It is concluded that, due to the large microbiological variability present in raw milk in the different properties, demonstrating variations in the bacterial resistance profile, monitoring through microbial culture and antibiotic sensitivity testing is vital, favoring the correct choice for the treatment of animals with a reduced selection of resistant strains.

## References

Araújo, T. P. M., Rangel, A. H. N., Lima, G. F. C., Peixoto, M. G. C. D., Urbano, S. A., & Bezerra, J. S. (2018). Gir and Guzerat cow milk production and composition according to lactation stage, somatic cell count,

physiological state, and body condition. *Acta Scientiarum Animal Sciences, 40*(1), 339-352. doi: 10.4025/actascianimsci.v40i1.39352

Artursson, K., Söderlund, R., Liu, L., Monecke, S., & Schelin, J. (2016). Genotyping of Staphylococcus aureus in bovine mastitis and correlation to phenotypic characteristics. *Veterinary Microbiology, 193*(1), 156-161. doi: 10.1016/j.vetmic.2016.08.012

Ayres, M., Ayres, J. R. M., Ayres, D. L., & Santos, A. S. (2007). *BioEstat 5.0-Aplicações estatísticas nas áreas das ciências biológicas e médicas: Sociedade Civil Mamirauá, Belém.* Brasília: CNPq.

Bhatnagar, K., & Wong, A. (2019). The mutational landscape of quinolone resistance in *Escherichia coli. PLoS ONE, 14*(11), e0224650. doi: 10.1371/journal.pone.0224650

Bitencourt, L. L., Leite, M. C. T., Cazaroti, E. P. F., Botelho, F. N., Giuberti, K. C., & Silva, V. B. P. (2018). Identificação e sensibilidade antimicrobiana de *Staphylococcus aureus* isolados do leite de vacas com mastite. *Revista Ifes Ciência, 4*(10), 133-142. Recuperado de https://ojs2.ifes.edu.br/index.php/ric/article/view/965/653

Bolaños, C. A. D., Pantoja, J. C. F., Alves, A. C., Risseti, R. M., Listoni, F. J. P., & Ribeiro, M. G. (2014). Qualidade do leite de vacas criadas no sistema silvipastoril no Vale do Cauca, Colômbia. *Pesquisa Veterinária Brasileira, 32*(2), 134-140. doi: 10.1590/S0100-736X2014000200007

Brazilian Committe on Antimicrobial Susceptibility Testing (2020). *Tabela de pontos de corte clínicos BrCAST 2020-01-05-2020,* Versão 10.

Ceniti, C., Britti, D., Santoro, A. M. L., Musarella, R., Ciambrone, L., Casalinuovo, F., & Costanzo, N. (2017). Phenotypic antimicrobial resistance profile of isolates causing clinical mastitis in dairy animals. *Italian Journal of Food Safety, 6*(2), 6612. doi: 10.4081/ijfs.2017.6612

Clinical and Laboratory Standards Institute (2013). *Performance standards for antimicrobial susceptibility testing* (23nd informational supplement, CLSI document M100-S23). Wayne, PA: Clinical and Laboratory Standards Institute.

Costa, G. M., Barros, R. A., Custódio, D. A. C., Pereira, U. P., Figueiredo, D. J., & Silva, N. (2013). Resistência a antimicrobianos em *Staphylococcus aureus* isolados de mastite em bovinos leiteiros de Minas Gerais, Brasil. *Arquivos do Instituto Biológico, 80*(3), 297-302. doi: 10.1590/S1808-16572013000300006

Cox, G., & Wright, G. D. (2013). Intrinsic antibiotic resistance: Mechanisms, origins, challenges, and solutions. *International Journal of Medical Microbiology, 303*(6), 287-292. doi: 10.1016/j.ijmm.2013.02.009

Cunha, M. L. R. S. (2017). *Staphylococcus aureus: infections, treatment, and risk assessment.* New York: Nova Biomedical.

Eckstein, I. I., Pozza, M. S. S., Zambom, M. A., Ramos, C. E. C. O., Tsutsumi, C. Y., Fernandes, T., Busanello, M. (2014). Qualidade do leite e sua correlação com técnicas de manejo de ordenha. *Scientia Agraria Paranaensis - SAP, 13*(2), 143-151. doi: 10.18188/sap.v13i2.7071

Fanin, M., Balestrin Fanin, E. L. B., Santos, I. C., Lima, J. S., Gonçalves, A. P. P., & Martins, L. A. (2020). Métodos alternativos no tratamento de infecções causadas por *Staphylococcus aureus. Medicina Veterinária (UFRPE), 14*(1), 24-32. doi: 10.26605/medvet-v14n1-3711

Gonçalves, J. L., Tomazi, T., & Santos, M. V. (2017). Rotina de ordenha eficiente para a produção de leite de alta qualidade. *Revista Acadêmica Ciência Animal, 15*(2), 9-14. doi: 10.7213/acadêmica.15.S02.2017.A02

Instrução Normativa n. 76 de 26 de novembro de 2018. *Regulamentos Técnicos que fixam a identidade e as características de qualidade que devem apresentar o leite cru refrigerado, o leite pasteurizado e o leite pasteurizado tipo A, na forma desta Instrução Normativa e do Anexo Único.* Diário Oficial [da] União, Brasília, 26 nov. 2018

Kempf, F., Slugocki, C., Blum, S. E., Leitner, G., & Germon, P. (2016). Genomic comparative study of bovine mastitis *Escherichia coli. PLoS ONE, 11*(1), e0147954. doi: 10.1371/journal.pone.0147954

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, J. R. (2008). *Diagnóstico Microbiológico.* Rio de Janeiro: Meds.

Kowalski, A. P., Maboni, G., Espindola, J. P., Foletto, A., Bandeira, G., Pötter, L. Jr., Vargas, A. C. (2015). Temporal profile of antimicrobial resistance exhibited by strains of *Staphylococcus* spp. isolated from cases of bovine mastitis for 20 years (1992-2011). *Ciência Rural, 45*(6). doi: 10.1590/0103-8478cr20140928

Kurosawa, L. S., Cézar, L. M. L., Marques, F. A., Oriani, M. R. G., & Moraes, M. E. (2020). Perfil de susceptibilidade antimicrobiana de *Staphylococcus* spp. associados a mastite bovina. *PUBVET, 14*(5), 1-6. doi: 10.31533/pubvet.v14n5a563.1-6

Langoni, H. (2013). Qualidade do leite: utopia sem um programa sério de monitoramento da ocorrência de mastite bovina. *Pesquisa Veterinária Brasileira, 33*(5), 620-626. doi: 10.1590/S0100-736X2013000500 012

Lima, B. L., Coelho, K. O., Bueno, C. P., & Neves, R. B. S. (2016). Contagem celular somática nos grandes constituintes do leite. *PUBVET, 10*(8), 604-607. Recuperado de http://www.pubvet.com.br/artigo/2937/contagem-celular-somaacutetica-nos-grandes-constituintes-do-leite

Ludovico, A., Trentin, M., & Rêgo, F. C. A. (2019). Fontes de variação da produção e composição do leite em vacas Holandesa, Jersey e Girolando. *Archivos de Zootecnia, 68*(262), 236-243. doi: 10.21071/az.v68I262.4142

Martineau, F., Picard, F. J, Roy, P. H., Ouellette, M., & Bergeron, M. G. (1998). Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus. Journal of Clinical Microbiology, 36*(3), 618-623. doi: 10.1128/JCM.36.3.618-623.1998

Menezes, I. R., Almeida, A. C., Morão, R. P., Reis, S. V., Santos, C. A., & Lopes, I. L. (2015). Qualidade microbiológica do leite cru produzido no Norte de Minas Gerais. *Revista Brasileira de Ciência Veterinária, 22*(1), 58-63. doi: 10.4322/rbcv.2015.321

Miller, E. A., & Nesi, C. N. (2012). Prevalência de agentes causadores de mastite, qualidade do leite e conformidade com a IN n. 51. *Unoesc & Ciência - ACET, 3*(2), 195-204. Recuperado de https:// portalperiodicos.unoesc.edu.br/acet/article/view/1096

Murakami, K., Minamide, W., Wada, K., Nakamura, E. Teraoka, H., & Watanabe, S. (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *Journal of Clinical Microbiology, 29*(10), 2240-2244. Retrieved from https://jcm.asm.org/content/29/10/2240.short

Noel, C. C., Motta, F. S., Francisco, N. L. S. G., Almeida, N. R., & Soares, L. C. (2016).

Perfil de suscetibilidade antimicrobiana e produção de "slime" de isolados de *Staphylococcus* spp. provenientes de casos de mastite bovina na região sul-fluminense. *Revista de Saúde, 7*(1), 22-26. doi: 10.21727/rs.v7i1.82

Orwa, J. D., Matofary, J. W., & Muliro, P. S. (2017). Handling practices and microbial contamination sources of raw milk in rural and peri-urban smallholder farms in Nakuru County, Kenya. *International Journal of Livestock Production, 8*(1), 5-11. doi: 10.5897/IJLP2016.0318

Pati, B. K., & Mukherjee, R. (2016). Characterization of *Staphylococcus aureus* isolates of bovine mastitis origin and antibiotic sensitivity pattern from northern plains of India. *Journal of Veterinary Research and Animal Husbandry, 1*(1), 105.

Pereira, M. N., & Scussel, V. M. (2017). Resíduos de antimicrobianos em leite bovino: fonte de contaminação, impactos e controle. *Revista de Ciências Agroveterinárias, 16*(2), 170-182. doi: 10.5965/223811711622017170

Perin, L. M., Pereira, J. G., Bersot, L. S., & Nero, L. A. (2019). The microbiology of raw milk. In L. A. Nero & A. F. Carvalho (Eds.), *Raw milk: balance between hazards and benefits* (pp. 45-64). Amsterdam: Academic Press.

Picoli, T., Zani, J. L., Bandeira, F. S., Roll, V. F. B., Ribeiro, M. E. R., Vargas, G. D.,... Fischer, G. (2014). Manejo de ordenha como fator de risco na ocorrência de micro-organismos em leite cru. *Semina: Ciências Agrárias, 35*(4), 2471-2480. doi: 10.5433/1679-0359.2014v35n4Suplp2471

Quinn, P. J., Markey, B. K., Cater, M. E., Donnely, W. J., & Leonar, F. C. (2005). *Microbiologia veterinária e doenças infecciosas.* Porto Alegre: Artmed.

Redding, L. E., Bender, J., & Baker, L. (2019). Quantification of antibiotic use on dairy farms in Pennsylvania. *Journal of Dairy Science, 102*(2), 1494-1507. doi: 10.3168/jds.2018-15224

Reis, E. M. B., Vieira, J. A., Lopes, M. A., Demeu, F. A., Bruhn, F. R. P., Vicente, F. H. Pereira, A. B.; & Simões, L. M. Fo. (2020). Diagnóstico de propriedades leiteiras e fatores associados à qualidade higiênico sanitária do leite. *PUBVET, 14*(2), 1-15. doi: 10.31533/pubvet.v14n2a508.1-15

Rodenburg, J. (2012). *Mastitis prevention for dairy cattle: environmental control.* Ontario, CA: Factsheet.

Salina, A., Dalanezi, F. M., Junqueira, N. B., Oliveira, G. C., Joaquim, S. F., Vasconcelos, C. G. C.,... Langoni, H. (2016). Participação de enterobactérias na etiologia de mastites bovinas em rebanhos de estados do Sul, Sudeste e Centro-oeste do Brasil. *Revista de Educação Continuada em Medicina Veterinária e Zootecnia do CRMV-SP, 14*(3), 88. Recuperado de https://www.revistamvez-crmvsp.com.br/index.php/recmvz/article/view/34959

Salvador, F. C., Burin, A. S., Frias, A. A. T., Oliveira, F. S., & Faila, N. (2012). Avaliação da qualidade microbiológica do leite pasteurizado comercializado em Apucarana-PR e região. *Revista F@pciência, 9*(5), 30-41. Recuperado de http://www.fap.com.br/fap-ciencia/edicao_2012/005.pdf

Satake, F. M., Assunção, A. W. A, Lopes, L. G., & Amaral, L. A. (2012). Qualidade da água em propriedades rurais situadas na bacia hidrográfica do córrego rico, Jaboticabal-SP. *Ars. Veterinaria, 28*(1), 48-55. doi: 10.15361/2175-0106.2012v28n1p048-055

Scabin, K. E. M., Kozusny-Andreani, D. I., & Frias, D. F. R. (2012). Microbiological quality of milk in nature during the process of obtaining and after cooling. *Revista CES Medicina Veterinaria y Zootecnia, 7*(1), 11-21. Retrieved from https://www.redalyc.org/articulo.oa?id=321428107002

Silva, E. R., Pereira, A. M. G., Moraes, W. S., Santoro, K. R., & Silva, T. R. M. (2012). Perfil de sensibilidade antimicrobiana in vitro de Staphylococcus aureus isolado de mastite subclínica bovina. *Revista Brasileira de Saúde e Produção Animal, 13*(3), 701-711. Recuperado de https://periodicos.ufba.br/index.php/rbspa/article/view/40898/22657

Silva, R. T., Lopes, J. B. A., Oliveira, K. L. L., Ribeiro, J. C., Jr., & Beloti, V. (2019). Perfil de sensibilidade a antimicrobianos de bactérias patogênicas humanas isoladas de leite cru. *Revista do Instituto de Laticínios Cândido Tostes, 74*(3), 185-194. doi: 10.14295/2238-6416.v74i3.743

Simioni, F. J, Baretta, C. R. D. M., Stefani, L. M., Lopes, L. S., & Tizziani, T. (2013). Qualidade do leite proveniente de propriedades com diferentes níveis de especialização. *Semina: Ciências Agrárias, 34*(4), 1901-1912. doi: 10.5433/1679-0359.2013v34n4p1901

Siqueira, A. K., Alves, T. S., Ferraz, M. M. G., Franco, M. M. J., Motta, R. G., Listoni, F. J. P.,... Leite, D. S. (2014). Resistência antimicrobiana em coliformes totais isolados de tanques de refrigeração de leite bovino. *Atas de Saúde Ambiental - ASA, 2*(3), 2-15.

Song, H. S., Kim, J. Y., Kim, Y. B., Jeong, M. S., Kang, J., Rhee, J. K.,... Roh, S. W. (2017). Complete genome sequence of a commensal bacterium, Hafnia alvei CBA7124, isolated from human feces. *Gut Pathogens, 9*(41). doi: 10.1186/s13099-017-0190-0

Taffarel, L. E., Costa, P. B., Oliveira, N. T. E., Braga, G. C., & Zonin, W. J. (2013). Contagem bacteriana total do leite em diferentes sistemas de ordenha e de resfriamento. *Arquivos do Instituto Biológico, 80*(1), 7-11. doi: 10.1590/S1808-16572013000100002

Taffarel, L. E., Costa, P. B., Tsutsumi, C. Y., Klosowski, E. S., Portugal, E. F., & Lins, A. C. (2015). Variação da composição e qualidade do leite em função do volume de produção, período do ano e sistemas de ordenha e de resfriamento. *Semina: Ciências Agrárias, 36*(3), 2287-2300. doi: 10.5433/1679-0359.2015v36n3Supl1p2287

Werncke, D., Gabbi, A. M., Abreu, A. S., Felipus, N. C., Machado, N. L., Cardoso, L. L.,... Thaler, A., Neto. (2016). Qualidade do leite e perfil das propriedades leiteiras no sul de Santa Catarina: abordagem multivariada. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 68*(2), 506-516. doi: 10.1590/1678-4162-8396

Zimermann, K. F., & Araújo, M. E. M. (2017). Mastite bovina: agentes etiológicos e susceptibilidade a antimicrobianos. *Revista Campo Digital, 12*(1), 1-7.