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Antimicrobial resistance profile of *Staphylococcus* spp. isolates in cattle herds from Western Amazon

Perfil de resistência antimicrobiana de *Staphylococcus* spp. isolados em rebanhos leiteiros da Amazônia Ocidental

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Highlights

The evaluated dairy herds showed a high prevalence of *Staphylococcus aureus.*
Penicillin, ampicillin and tetracycline resistance were observed more frequently.
The isolates showed 100% susceptibility to oxacillin, cefalexin, and gentamicin.
In total, 12 resistance, including 6 multiresistance, patterns were identified.

Abstract

Staphylococcus is one of the most frequent etiological agents of bovine mastitis, causing economic losses to milk farming. This study aimed to describe the milk production and management practices in 15 herds located in three microregions of the State of Rondônia and to identify the antimicrobial resistance profile of 97 isolates of the genus *Staphylococcus* from these herds. The isolates were subjected to antimicrobial susceptibility tests using the agar diffusion method. Twenty-nine *S. aureus* isolates were selected to determine their minimum inhibitory concentration (MIC). In the farms studied, the breeding system was semi-intensive, with the Girolando breed being predominant. Milking was performed predominantly using a mechanical milking system (86.7%), twice a day (66.7%), in the presence of the calf (53.8%). The average number of lactating cows in the farms was 24, with an average milk production of 204.9 L d^{-1} and a milk productivity of 10.2 L $animal^{-1}$ d^{-1}. The use of antimicrobials for the treatment and prevention of bovine mastitis was reported for all properties, and therapy for dry cows was adopted in 80% of the herds. The percent susceptibility to antimicrobials ranged from 85.5% to 100% for *S. aureus,* 22.2% to 88.9% for coagulase-positive staphylococci (CPS), and 69.2% to 100% for coagulase-negative staphylococci (CNS), with the highest resistance frequencies for penicillin, ampicillin, and tetracycline. Among the *S. aureus*

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isolates, 100% susceptibility to oxacillin, cefalexin, and gentamicin was observed, and among the CNS isolates, 100% susceptibility to gentamicin was observed. None of the antimicrobials tested showed 100% *in vitro* effectiveness for CPS. CPS and CNS presented lower percentages of susceptibility to penicillin, ampicillin, and tetracycline than the *S. aureus* isolates. Twelve resistance patterns were detected, six of which were multiresistance patterns. The most prevalent resistance patterns were penicillin and ampicillin (PEN-AMP) and penicillin, ampicillin, and tetracycline (PEN-AMP-TET). Results of the MIC assay revealed that all 29 *S. aureus* isolates were susceptible to cephalothin, cefoxitin, tetracycline, and erythromycin, whereas 25 (86.2%) were susceptible to penicillin.
Key words: Agar diffusion test. Bovine mastitis. Minimum inhibitory concentration. *Staphylococcus aureus.*

Resumo

O gênero *Staphylococcus* destaca-se como um dos agentes etiológicos mais frequentes da mastite bovina, causando prejuízos econômicos à pecuária de leite. Este estudo teve o objetivo de descrever a produção de leite e práticas de manejo de 15 rebanhos localizados em três microrregiões do Estado de Rondônia, e identificar o perfil de resistência aos antimicrobianos de 97 isolados do gênero *Staphylococcus* provenientes destes rebanhos. Os isolados foram submetidos a testes de suscetibilidade a antimicrobianos, utilizando-se a técnica de difusão em ágar. Foram selecionados 29 isolados de *Staphylococcus aureus* (*S. aureus*) para determinação da concentração inibitória mínima (CIM). Nas propriedades estudadas, o sistema de criação era semi-intensivo, com predomínio da raça Girolando. A ordenha era realizada predominantemente em sistema de ordenha mecânica (86,7%), duas vezes ao dia (66,7%) e com a presença do bezerro (53,8%). A média do número de vacas em lactação das propriedades era de 24 animais, com média de produção de leite de 204,9 L d^{-1} e produtividade de leite de 10,2 L $animal^{-1}$ d^{-1}. O uso de antimicrobianos para o tratamento e prevenção da mastite bovina foi relatado em todas as propriedades, sendo adotada a terapia da vaca seca em 80% dos rebanhos. A percentagem de susceptibilidade aos antimicrobianos variou de 85,5 a 100% para *S. aureus,* 22,2 a 88,9% para *Staphylococcus* coagulase positivos (SCP) e 69,2 a 100% para *Staphylococcus* coagulase negativos (SCN), sendo as maiores frequências de resistência para penicilina, ampicilina e tetraciclina. Entre os isolados de *S. aureus* foi observada 100% de susceptibilidade aos antimicrobianos oxacilina, cefalexina e gentamicina, e entre os isolados de SCN, 100% de susceptibilidade à gentamicina. Nenhum dos antimicrobianos testados apresentou 100% de efetividade in vitro para SCP. SCP e SCN apresentaram menores percentagens de susceptibilidade à penicilina, ampicilina e tetraciclina do que os isolados de *S. aureus.* Foram observados 12 padrões de resistência, sendo seis padrões de multirresistência. Os padrões de resistência mais prevalentes foram resistência à penicilina e ampicilina (PEN-AMP) e à penicilina, ampicilina e tetraciclina (PEN-AMP-TET). A determinação da CIM dos 29 isolados de *S. aureus* mostraram que todos foram susceptíveis à cefalotina, cefoxitina, tetraciclina e eritromicina, e 25 (86,2%) foram susceptíveis à penicilina.
Palavras-chave: Concentração inibitória mínima. Mastite bovina. *Staphylococcus aureus.* Teste de difusão em ágar.

The state of Rondônia is the seventh largest milk producer in Brazil, producing 1.129 billion liters in 2019, representing 50.43% of the production in the northern region and 97.5% in the western Amazon (Instituto Brasileiro de Geografia e Estatística [IBGE], 2021). Milk production in the state is family-based and involves 31,026 producers, with an average daily production of 67.4 liters per farm and productivity of 5.0 L cow^{-1} d^{-1} (Agência de Defesa Sanitária Agrosilvopastoril do Estado de Rondônia [IDARON], personal communication, May 27, 2020).

Bovine mastitis is the most prevalent and economically relevant infectious disease affecting dairy herds. Dias et al. (2021) reported a prevalence of 45.5% of subclinical mastitis (somatic cell count, SCC >200 × 10^3 cells mL^{-1}) in dairy herds located in six microregions of the state of Rondônia, which are characterized by low adoption of technologies and the presence of a few specialized animals for milk production. In a study carried out in 566 refrigeration tanks located in the main milk-producing microregions of the state, SCC averages of 263 × 10^3 cells mL^{-1} in the rainy season and 235 × 10^3 cells mL^{-1} in the dry season were observed, with spatial clustering of higher SCC in the microregion of Ariquemes (Dias, Pacheco, Grego, Faria, & Cruz, 2020).

Among the pathogens that cause intramammary infections in cattle, Staphylococcus is among the most prevalent (Beuron et al., 2014; Taponen, Nykäsenoja, Pohjanvirta, Pitkälä, & Pyörälä, 2016). In Rondônia, studies have shown the low adoption of technologies and practices for the prevention and control of mastitis in herds and the predominance of bacteria of the genus *Staphylococcus* in milk samples (Dias, Antes, & Queiroz, 2015; Dias, Antes, Grego, & Menezes, 2016). Dias et al. (2015) demonstrated that variables related to the technification of farms were associated with an increased probability of occurrence of S. aureus in the herd compared to farms with lower technification.

Bovine mastitis is recognized as the most frequent cause for the use of antimicrobials in dairy herds, and among the problems attributed to the use of antimicrobials in animals are: the occurrence of residues in milk and the potential development of antimicrobial resistance that can become a public health problem (Pol & Ruegg, 2007; Taponen et al., 2016). Studies conducted in different Brazilian states aimed to identify the antimicrobial resistance profiles of bacteria of the genus *Staphylococcus* isolated from milk samples and presented varied results in resistance patterns (Costa et al., 2013; Beuron et al., 2014; Santos et al., 2016).

Considering the importance of the genus *Staphylococcus* in the epidemiology of bovine mastitis, this study aimed to describe milk production and practices related to the prevention and control of mastitis in herds with a higher degree of technification located in three microregions of the State of Rondônia and identify the antimicrobial resistance profile of isolates of the genus *Staphylococcus* from these herds.

Data were collected from 15 dairy farms from eight municipalities in the microregions of Ji-Paraná (Jaru, Ouro Preto do Oeste, and Presidente Médici), Rolim de Moura (Castanheiras, Rolim de Moura, and São Felipe do Oeste), Porto Velho (municipality of Porto Velho), and Guajará-Mirim (municipality of Guajará-Mirim), located in the state of Rondônia. An epidemiological questionnaire was administered to each farm to obtain information on the production system,

infrastructure and management, hygiene and maintenance of milking equipment, measures to control and prevent mastitis, and use of antimicrobials. Data and milk samples were collected between February 2013 and July 2014. The registry of access to the genetic heritage in this study received the SisGen AFCD75D code.

From each farm participating in the study, composite milk samples from the four mammary quarters of lactating cows were collected for the isolation of *Staphylococcus* spp., according to the procedures described by the National Mastitis Council [NMC] (2004). The tamis test was performed on the animals prior to the collection of milk samples. Ten to fifteen cows, tamis test negative, were evaluated by farm. To determine the somatic cell count, a representative sample of the total production of each animal was collected during the first milking of the day using vials containing the preservative bronopol. All samples were sent to isothermal boxes containing recyclable ice for analysis at the Milk Quality Laboratory located in Embrapa Rondônia.

SCC was determined by flow cytometry using automated equipment (CombiScope FTIR400 - Delta Instruments) according to the International Organization for Standardization.

Staphylococcus spp. were isolated and identified from the milk samples of cows according to the procedures established by NMC (2004). Gram staining and catalase, coagulase, and acetoin production assays were used for identification. Acetoin-producing coagulase-positive isolates were considered to be *S. aureus,* and non-acetoin-producing coagulase-positive isolates were considered to be coagulase-positive *Staphylococcus* spp. (CPS).

Antimicrobial susceptibility tests of the *Staphylococcus* spp. isolates were performed according to the Clinical and Laboratory Standards Institute [CLSI] (2013a), and the inhibition zones were compared with the parameters established in the CLSI (2013b). The agar diffusion method was used, with discs containing penicillin (10 IU), ampicillin (10 µg), oxacillin (1 µg), cefalexin (30 µg), ceftiofur (30 µg), tetracycline (30 µg), neomycin (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), and enrofloxacin (5 µg). *Staphylococcus aureus* ATCC 25923 was used as the control.

The minimum inhibitory concentration (MIC) of 29 *S. aureus* isolates was determined in Mueller-Hinton agar, according to CLSI (2013a), using the antimicrobials penicillin, cephalothin, cefoxitin, tetracycline, and erythromycin. These isolates were selected from 62 *S. aureus* isolates from seven different herds to maintain their representativeness. A plate without antimicrobials and *S. aureus* ATCC 29213 was included in each test as quality control. The cutoff points for determining susceptibility to antimicrobials were interpreted according to CLSI (2013b).

For the inclusion of data obtained from epidemiological questionnaires and laboratory analyses, as well as for performing descriptive statistical analysis (frequency, average, median), the Epiinfo 7.2.2.16 Program was used.

In the 15 farms evaluated in this study, the lactating animals were grazing and were supplemented with feed. Milking was performed predominantly using a mechanical milking system (86.7%), twice a day (66.7%), in the presence of the calf (53.8%). The average number of lactating cows on the farms was

24, with an average milk production of 204.9 L d^{-1} and milk productivity of 10.2 L $animal^{-1}$ d^{-1}. The predominant breed was the Girolando breed and the Gir and Jersey breeds were also observed. The calf breeding system was predominantly individual (80%), and 50% of the properties reported purchasing females from other properties. In 91% of the farms, the water used for milking came from a well, either simple or artesian. As for good milking practices, 73.3% of the farms reported washing and drying the teats with paper towels. The tamis test and teat antisepsis before milking were performed in 53.3% of the farms, and teat antisepsis after milking in 66.7% of the evaluated herds.

The use of antimicrobials for the treatment and prevention of bovine mastitis was reported in all farms, and dry cow therapy was adopted in 80% of the herds. The diagnosis of subclinical mastitis by the CMT test was performed in 53.8% of the farms, and the milking line was adopted in only three farms. In 80% of the herds, treatment for mastitis was recommended by a veterinarian, and the protocol was established in seven farms. The most commonly used active compounds for the treatment of mastitis were gentamicin (60%), neomycin (46.3%), cefquinome (40%), tetracycline (20%), and sulfadiazine + trimethoprim (20%). Only one farm reported a microbiological diagnosis to identify the pathogens that cause mastitis.

The dairy herds evaluated in this study had a higher production median (L d^{-1}), higher milk productivity (L cow^{-1} d^{-1}), and a higher frequency of adoption of technologies and practices for the prevention and control of mastitis compared to the herds evaluated from the microregion of Ji-Paraná (Dias et al., 2016), from the municipality of Nova Brasilândia do Oeste (Benatti et al., 2020) and dairy farms that supply family agroindustries in the state (Dias et al., 2021), demonstrating a greater degree of technification.

Considering the variables related to mastitis control strategies, it was observed that the frequency of cow culling resulting from chronic mastitis in the present study was lower than that observed by Dias et al. (2016) (46.7 vs. 72.6%) in herds with a lower degree of technification. In regional conditions, according to the data obtained in these studies, this type of culling is related to clinical mastitis conditions that are unresponsive to treatment. Some factors may have contributed to the lower culling of cows from the herds evaluated in this study, such as the higher probability of diagnosis of clinical and subclinical mastitis in the field (tamis test and CMT), greater opportunity for immediate treatment of clinical cases, and higher frequency of use of dry cow therapy. These farms also showed a higher frequency of antimicrobial prescription by veterinarians (80% vs. 20%) and mastitis treatment protocols (46.7% vs. 0.4%), when compared to the study conducted by Dias et al. (2016).

From the microbiological analyses of 161 milk samples, 97 bacterial isolates, 62 *S. aureus,* nine CPS and 26 CNS were obtained. *Staphylococcus* spp. isolates in milk correlated with somatic cell counts in the analyzed milk samples. An SCC average of 637.8 x 10^3 cells mL^{-1} was observed for CPS (including *S. aureus*) and 375.2 x 10^3 cells mL^{-1} for CNS. Cows that did not show bacterial growth showed fewer somatic cells than cows with pathogens of the genus *Staphylococcus* ($P < 0.05$), the counts being 107.1 x 10^3 cells mL^{-1} and 580.1 x 10^3 cells mL^{-1}, respectively. Among the isolated pathogens, *S. aureus*

and CPS were responsible for the highest elevations in SCC ($P < 0.05$), compared to CNS.

Staphylococcus aureus was the most prevalent mastitis pathogen and was isolated from all evaluated farms. In a previous study, Dias et al. (2015) reported the isolation of *S. aureus* from 36% of the total milk samples from 266 dairy cattle herds in the microregion of Ji-Paraná, the largest milk producer in the northern region. The results of the present study confirm the importance of this pathogen in the epidemiology of bovine mastitis in Rondônia.

The antimicrobial resistance patterns of the *Staphylococcus* isolates are presented in Table 1. The percentages of susceptibility to antimicrobials ranging from 85.5% to 100% were verified for *S. aureus,* 22.2% to 88.9% for CPS, and 69.2% to 100% for CNS. The highest resistance indices were observed for penicillin, ampicillin, and tetracycline. Among the *S. aureus* isolates, 100% susceptibility to the antimicrobials oxacillin, cephalexin and gentamicin was observed, and 100% susceptibility to gentamicin among the CNS isolates. None of the antimicrobials tested showed 100% *in vitro* effectiveness against CPSs. CPS and CNS presented lower percentages of susceptibility to penicillin, ampicillin, and tetracycline than the *S. aureus* isolates.

Table 1
Susceptibility to antimicrobials (no. of isolates and percentage) of *Staphylococcus aureus,* coagulase-positive *Staphylococcus* spp. (CPS) and coagulase-negative *Staphylococcus* spp. (CNS) isolated from the milk samples of cows from herds located in the municipalities of the microregions of Ji-Paraná, Porto Velho and Rolim Moura, in the State of Rondônia, 2013-2014

Group	Antimicrobials	Micro-organisms			Total = 97
		S. aureus (n=62)	CPS (n=9)	CNS (n=26)	n (%)
Betalactam	Penicillin	53 (85.5)	3 (33.3)	18 (69.2)	74 (76.3)
	Ampicillin	53 (85.5)	2 (22.2)	18 (69.2)	73 (75.3)
	Oxacillin	62 (100.0)	8 (88.9)	25 (96.2)	95 (97.9)
Cephalosporin	Ceftiofur	61 (98.4)	8 (88.9)	25 (96.2)	94 (96.9)
	Cefalexin	62 (100.0)	8 (88.9)	25 (96.2)	95 (97.9)
Tetracycline	Tetracycline	58 (93.5)	6 (66.7)	20 (76.9)	84 (86.6)
Aminoglycoside	Neomycin	56 (90.3)	6 (76.8)	23 (88.5)	85 (87.6)
	Gentamicin	62 (100.0)	8 (88.9)	26 (100.0)	96 (98.9)
Macrolide	Erythromycin	56 (90.3)	8 (88.9)	25 (96.2)	89 (91.7)
Lincosamide	Clindamycin	61 (98.4)	8 (88.9)	24 (92.3)	93 (95.9)
Fluoroquinolone	Enrofloxacin	61 (98.4)	8 (88.9)	25 (96.2)	94 (96.9)

The most prevalent resistance patterns were penicillin and ampicillin (PEN-AMP) and penicillin, ampicillin, and tetracycline (PEN-AMP-TET). Six isolates (6.2%), one *S. aureus,* two CPS, and three CNS, presented multiresistance, which is the resistance observed in three or more classes of antimicrobials. The minimum inhibitory concentrations of penicillin, cephalothin, cefoxitin, tetracycline, and erythromycin were determined for the 29 *S. aureus* isolates (Table 2). All the isolates were susceptible to cephalothin, cefoxitin, tetracycline, and erythromycin, and 86.2% were susceptible to penicillin. The MIC_{50} and MIC_{90} columns showed the lowest concentrations (μg mL^{-1}), which inhibited 50% and 90% of *S. aureus* isolates, respectively. Evaluation of the antimicrobial susceptibility of the *Staphylococcus* isolates demonstrated higher resistance frequencies for penicillin, ampicillin, and tetracycline. These results are consistent with those reported by Costa et al. (2013) and Santos et al. (2016). According to Van Boeckel et al. (2019), penicillin, tetracyclines, and sulfonamides are the most commonly used antimicrobials in animal production worldwide. In general, *S. aureus* isolates presented higher percentages of susceptibility to antimicrobials than CPS and CNS isolates, as observed in other studies (Santos et al., 2016; Taponen et al., 2016).

Table 2
Minimum inhibitory concentration (MIC) of five antimicrobials for 29 *Staphylococcus aureus* isolates from dairy herds of the microregions of Ji-Paraná, Rolim de Moura and Porto Velho, Rondônia, 2013-2014

Antimicrobial	Cutoff point (μg mL^{-1})	Variation (μg mL^{-1})	MIC_{50} [1] (μg mL^{-1})	MIC_{90} [2] (μg mL^{-1})	Susceptibility (%)
Penicillin	≥ 0.25	0.003 - 2	0.03	0.5	86.2
Cephalothin	≥ 32	0.0625 - 0.25	0.125	0.25	100
Cefoxitin	≥ 8	1 - 4	2	2	100
Tetracycline	≥ 16	0.007 - 0.125	0.125	0.125	100
Erythromycin	≥ 8	0.25 - 0.50	0.5	0.5	100

[1]MIC_{50} - Minimum inhibitory concentration for 50% of *S. aureus* tested samples; [2]MIC_{90} - Minimum inhibitory concentration for 90% of *S. aureus* tested samples.

The percentages of *S. aureus* susceptibility in the present study were higher than those reported by Beuron et al. (2014) and Silveira et al. (2014) in *S. aureus* isolated from the dairy herds in the states of São Paulo and Pernambuco, respectively. The percentages of susceptibility to penicillin and ampicillin in *S. aureus* from this study were also higher than those reported by Costa et al. (2013) in the dairy herds in Minas Gerais, although the percentages of susceptibility to ceftiofur, gentamicin, and enrofloxacin were similar. In the present study, a single multiresistant *S. aureus* strain was identified, at a low

percentage compared to those reported by Costa et al. (2013) (18%) and Silveira et al. (2014) (63%, in two herds).

The high susceptibility of *S. aureus* to antimicrobials in this study was also confirmed by the determination of MIC, which detected 100% susceptibility of *S. aureus* to cephalothin, cefoxitin, erythromycin, and tetracycline. The percentage of penicillin susceptible *S. aureus* was similar to that observed in the diffusion tests. No isolates were resistant to cefoxitin, indicating the absence of methicillin-resistant *S. aureus* (MRSA), as phenotypic susceptibility to cefoxitin has been used to predict methicillin susceptibility (CLSI, 2013a).

The percentage of susceptibility observed in *S. aureus* in this study by the MIC test for penicillin and tetracycline was higher than that reported in the studies of Brito, Brito, Silva and Carmo (2001) and Santos et al. (2016) in *S. aureus* isolated from the herds of different Brazilian states. The MIC variation range found in this study was also lower than those reported by Brito et al. (2001) and Santos et al. (2016) for penicillin and tetracycline. This suggests that the selective pressure caused by the use of antimicrobials to treat mastitis in the studied herds was lower, resulting in the selection of a smaller number of *S. aureus* resistant to the analyzed antimicrobials.

Identification of resistance patterns in *Staphylococcus* isolates demonstrated a higher frequency of resistance to penicillin and ampicillin (PEN-AMP) and penicillin, ampicillin, and tetracycline (PEN-AMP-TET). Several resistance patterns were observed in the same farm located in the microregion of Porto Velho that had a history of recurrent clinical mastitis in the herd, use of different antimicrobials without technical guidance, and a low cure rate. The farm was characterized by failure to adopt practices for the prevention and control of mastitis, including non-performance of the tamis test and CMT.

Beuron et al. (2014) evaluated the association between the management practices and procedures used in the treatment of bovine mastitis and the susceptibility of *S. aureus* to antimicrobials. The non-performance of microbiological examination and susceptibility tests and treatment of the cow in the dry period were considered risk factors for the resistance of *S. aureus* to penicillin and enrofloxacin, respectively. Pol and Ruegg (2007) analyzed the relationship between the use of antimicrobials on farms and the results of tests for the susceptibility of mastitis pathogens to antimicrobials. The use of penicillin has been associated with the reduced susceptibility of *S. aureus* and CNS to this antimicrobial agent. In the present study, the antimicrobials that showed the highest resistance were penicillin, ampicillin, and tetracycline, but only tetracycline was cited as an antimicrobial widely used in herds.

The results of this study show the importance of *Staphylococcus* as an etiological agent of bovine mastitis in farms with a higher degree of technification, considering the predominant production characteristics of the state of Rondônia. A higher frequency of Staphylococcus spp. resistance to penicillin, ampicillin, and tetracyclines, antibiotics widely used in dairy cattle, was observed, indicating the importance of resistance monitoring as an auxiliary tool in the choice of the antimicrobial. Efforts are needed so that farmers have access to technical assistance and laboratory diagnostics. The adoption of good milking practices and mastitis control, as well as the correct identification of the pathogens and

the rational use of antimicrobials, are essential tools for greater effectiveness in the treatment of bovine mastitis, minimizing the selection of resistant microorganisms, and retaining the effectiveness of the drugs currently in use.

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References

Benatti, H. N., Pazdiora, R. D., Ferreira, D. S., Fuzari, P. V. M., Pazdiora, B. R. C. N., Ferreira, E.,... Ziemniczak, H. M. (2020). Diagnosis of milk production in the western state of Rondônia, Brazil. *Research, Society and Development, 9*(7), 1-21. doi: 10.33448/rsd-v9i7.4182

Beuron, D. C., Cortinhas, C. S., Botaro, B. G., Macedo, S. N., Gonçalves, J. L., Brito, M. A. V. P., & Santos, M. V. (2014). Risk factors associated with the antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis. *Pesquisa Veterinária Brasileira, 34*(10), 947-952. doi: 10.1590/S0100-736X2014 001000004

Brito, M. A. V. P., Brito, J. R. F., Silva, M. A. S., & Carmo, R. A. (2001). Concentração mínima inibitória de dez antimicrobianos para amostras de *Staphylococcus aureus* isoladas de infecção intramamária bovina. (2001). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 53*(5), 531-537. doi: 10.1590/S0102-09352001 000500003

Clinical and Laboratory Standards Institute (2013a). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals* (4nd ed. Rev). (CLSI Document VET01-A4). Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute (2013b). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals* (2nd Informational Supplement). (CLSI Document VET01-S2). Wayne, PA: CLSI.

Costa, G. M., Barros, R. A., Custódio, D. A. C., Pereira, U. P. 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.

Dias, J. A., Antes, F. G., & Queiroz, R. B. (2015). Prevalência e fatores de risco associados à ocorrência de *Staphylococcus aureus* em amostras de leite total de rebanhos da microrregião de Ji-Paraná, Rondônia. *Anais do Congresso Brasileiro de Qualidade do Leite,* Curitiba, PR, Brasil, 6.

Dias, J. A., Antes, F. G., Grego, C. R., & Menezes, C. A. (2016). *Spatial distribution and risk factors associated with the occurrence of antimicrobial residues in dairy herds*

of the microregion of Ji-Paraná, State of Rondônia. (Documentos, 163). Porto Velho, RO: EMBRAPA Rondônia.

Dias, J. A., Constantino, N. A. S., Oliveira, A. M., Aprígio, M. E. G., Tenório, T. E. C., & Faria, G. V. (2021). Prevalência e fatores de risco associados à mastite subclínica em rebanhos fornecedores de agroindústrias familiares de Rondônia. *Anais do Congresso Internacional de Pecuária Leiteira da Amazônia Ocidental,* Rio Branco, AC, Brasil, 2.

Dias, J. A., Pacheco, I. F., Grego, C. R., Faria, G. V., & Cruz, P. G. (2020). Spatial characterization of hygienic-sanitary indicators of refrigerated raw milk from three micro regions of the Rondônia state. *Semina: Ciências Agrárias, 41*(5), 2195-2208. doi: 10.5433/1679-0359.2020v41n5supl1p2195

Instituto Brasileiro de Geografia e Estatística (2021). *Pesquisa da pecuária municipal - PPM.* Recuperado de https://sidra.ibge.gov.br/home/leite/brasil

National Mastitis Council (2004). *Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality.* Verona: National Mastitis Council.

Pol, M., & Ruegg, P. L. (2007). Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens *Journal of Dairy Science, 90*(1), 262-273. doi: 10.3168/jds.S0022-0302(07)72627-9

Santos, F. F., Mendonça, L. C., Reis, D. R. L., Guimarães, A. S., Lange, C. C., Ribeiro, J. B., Brito, M. A. V. P. (2016). Presence of mecA-positive multidrug-resistant *Staphylococcus epidermidis* in bovine milk samples in Brazil. *Journal of Dairy Science, 99*(2), 1374-1382. doi: 10.3168/jds.2015-9931

Silveira, W. M., Fº., Luz, I. S., Campos, A. P. F., Silva, W. M., Barros, M. P. S., Medeiros, E. S.,... Leal-Balbino, T. C. (2014). Antibiotic resistance and molecular analysis of *Staphylococcus aureus* isolated from cow's milk and dairy products in Northeast Brazil. *Journal of Food Protection, 77*(4), 583-591. doi: 10.4315/0362-028X.JFP-13-343

Taponen, S., Nykäsenoja, S., Pohjanvirta, T., Pitkälä, A., & Pyörälä, S. (2016). Species distribution and in vitro antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis milk. *Acta Veterinaria Scandinavica, 50*(12), 1-13. doi: 10.1186/s13028-016-0193-8

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G.,... Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animal in low- and middle-income countries. *Science, 365*(6459), 19-44. doi: 10.1126/science.aaw1944.