ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2021.15.1.9427

Biofilm forming antimicrobial-multiresistant *Staphylococcus aureus*

Multirresistência antimicrobiana de Staphylococcus aureus formadores de biofilme

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ABSTRACT: *Staphylococcus aureus* is one of the main agents isolated from bovine mastitis cases, characterized by lower cure rates compared to other pathogens causing this disease. This phenomenon is mainly explained by the multiresistance acquisition to antimicrobials and the ability of *S. aureus* to form biofilms on biotic and abiotic surfaces. In this work 15 samples of *S. aureus* isolated from the automated milking facility were analyzed regarding the resistance profile to antimicrobials, virulence factors (capsule production, hemolysin, and protease) and adhesion capacity under different temperatures (42±1°C, 36±1°C, 25±1°C, 9±1°C, and 3±1°C). All isolates showed methicillin-resistant (MRSA) characteristics and multidrug resistance profile to the antimicrobials tested (penicillin G, chloramphenicol, oxacillin, cephalexin, tetracycline, amoxicillin + clavulanic acid, sulfa + trimetropim, gentamicin, doxycycline, ceftiofur, neomycin, and vancomycin) with an IRMA index between 0.5 and 1.0. Five isolates were resistant to vancomycin (VRSA), two were resistant to all active principles, and the others to at least six of these drugs. Adhesion capacity and biofilm formation were found in 3 of the 5 evaluated temperatures, including the cooling conditions. Regarding the virulence factors, 86.7% of the isolates formed capsules, 60% revealed the presence of protease, 26.7% expressed the α-hemolysin factor, and 13.3% of them presented β-hemolysin. The fact that all isolates presented MRSA characteristics represents a potential risk to those exposed to this agent, and the formation of biofilm in liners even after the use of detergents and sanitization highlights the urgency of searching for alternatives for dispersion of the biofilm by *S. aureus* in the automated milking facility.

KEYWORDS: mastitis, virulence factors, adhesion on surfaces.

RESUMO: O *Staphylococcus aureus* é um dos principais agentes isolados de casos de mastite bovina, caracterizado por menores taxas de cura em comparação com outros patógenos desta enfermidade. Esse fenômeno é explicado principalmente pela aquisição de resistência à antimicrobianos e a capacidade do *S. aureus* formar biofilmes em superficies bióticas e abióticas. Neste trabalho foram utilizadas 15 amostras de *S. aureus* isolados de ordenhadeira, analisados quanto ao perfil de resistência à antimicrobianos, fatores de virulência (produção de cápsula, hemolisina e protease) e capacidade de adesão sob diferentes temperaturas (42±1°C, 36±1°C, 25±1°C, 9±1°C e 3±1°C). Todos os isolados apresentaram perfil de multirresistência aos antimicrobianos testados (penicilina G, cloranfenicol, oxacilina, cefalexina, tetraciclina, amoxicilina + ácido clavulônico, sulfa + trimetropim, gentamicina, doxiciclina, ceftiofur, neomicina e vancomicina) com índice IRMA entre 0,5 a 1,0. Duas cepas foram resistentes a todos os princípios ativos e as demais a pelo menos seis destes fármacos. Os isolados avaliados apresentaram característica de meticilina-resistentes (MRSA) e destes, 33,34% (5/15) foram resistentes à vancomicina (VRSA). Houve capacidade de adesão e formação de biofilmes em 3 das 5 temperaturas avaliadas, incluindo as temperaturas de refrigeração. Em relação aos fatores de virulência, 86,7% dos isolados formaram cápsula, 60% presença de protease, 26,7% expressaram o fator α-hemolisina e 13,3% β-hemolisina. O fato de todos isolados apresentarem característica MRSA representa um risco potencial aos expostos a esse agente. Já a formação de biofilmes em teteiras, mesmo após detergência e sanitização, destacam a urgência de alternativas de dispersão de biofilmes no ambiente de ordenha.

PALAVRAS-CHAVE: mastite, fatores de virulência, adesão nas superfícies.

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Received: 07/01/2020. Accepted: 09/29/2020

INTRODUCTION

Bovine mastitis is an inflammation of the mammary glands that negatively affects the dairy industry, with strong negative consequences for animal welfare, food safety, and productivity, leading to economic losses due to reduced milk production and increased costs of clinical treatment (SCHUKKEN et al., 2011; HEIKKILÄ et al., 2018). *Staphylococcus aureus* is one of the main agents causing subclinical and clinical mastitis in dairy cattle (RALL et al., 2014; BONSAGLIA et al., 2018). However, unlike clinical mastitis, subclinical mastitis shows few visible symptoms in infected cows (VIGUIER et al., 2009; LE MARÉCHAL et al., 2011), and this failure to detect this disease rapidly leads to a high prevalence of *S. aureus* infections in dairy farms (GRUET et al., 2001).

Several characteristics contribute to the pathogenesis and spread of *S. aureus*, including virulence factors, host, and environment (ACOSTA et al., 2018). Its ability to adhere and form biofilms on biotic and abiotic surfaces significantly increases the persistence of this pathogen in equipment and facilities, providing a physiological advantage as an etiological agent of diseases caused by food consumption, especially through the consumption of milk and its derivatives (MERGHNI et al., 2015; WANG et al., 2018; REN et al., 2020)

The formation of biofilms contributes to the resistance to antimicrobials (MELCHIOR et al., 2006; RAZA et al., 2013). Moreover, the excessive use of antimicrobials has been shown to favor the emergence and selection of resistant bacteria, altering the structure of bacterial communities and inducing a rapid evolution, with unpredictable consequences for human and animal health (VAZ-MOREIRA; NUNES; MANAIA, 2014). Thus, the prevention and control of staphylococcal mastitis are vital for the entire milk production chain, since this disease requires an increased use of antimicrobials in dairy herds (STEVENS et al., 2018).

In this context, 15 samples of *S. aureus* isolated from the automated milking environment were analyzed regarding the antimicrobial resistance profile, virulence factors, and adhesion ability under different temperatures.

MATERIALS AND METHODS

Microbiological tests were performed at the Laboratory of Bacteriology and Veterinary Mycology of the Veterinary Hospital of the Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo (FAMV/UPF)

We analyzed 15 samples of *S. aureus* isolated from different points of the automated milking facility on a dairy farm in northern Rio Grande do Sul state, Brazil. The sample identification and the site of isolation are described in Table 1.

For isolating *S. aureus*, 25 mL of the samples were homogenized in 225 mL of Buffered Peptone Water (BPW, Laborclin®1) and, after a series of decimal dilutions, a 0.1 mL aliquot of each sample was seeded on plates containing Agar Baird-Parker (ABP, Laborclin®1), which were incubated at 36±1°C for 48 h (EVANCHO et al., 2001). S. aureus compatible colonies were submitted to Gram staining and biochemical catalase, coagulase, DNAse, and Voges-Proskauer testing. The results were expressed as log10 UFC.cm^2.

In order to evaluate sensitivity to antimicrobials by disc-diffusion, pure colonies of S. aureus were incubated in Brain Heart Infusion broth (BHI, HiMedia®3) at 36±1°C for 16

Table 1. Identification of samples of *S. aureus* isolated from different points of the milking facility.

Sample	Bacteria species	Points at the milking facility	Place of collection
ST1	*S. aureus*	Liners with milk residues	Rio Grande do Sul
ST2	*S. aureus*	Water from the 1st rinse of the CIP process	Rio Grande do Sul
ST3	*S. aureus*	Milk from the cluster	Rio Grande do Sul
ST4	*S. aureus*	Liners after detergency	Rio Grande do Sul
ST5	*S. aureus*	Liners after sanitizing and detergency	Rio Grande do Sul
ST6	*S. aureus*	CIP water after sanitizing and detergency	Rio Grande do Sul
ST7	*S. aureus*	Liners after sanitizing	Rio Grande do Sul
ST8	*S. aureus*	Rinse water from CIP piping, after sanitizing	Rio Grande do Sul
ST9	*S. aureus*	Liners with milk residues	Rio Grande do Sul
ST10	*S. aureus*	Water from the 1st rinse of the CIP process	Rio Grande do Sul
ST11	*S. aureus*	Milk from the milking cluster	Rio Grande do Sul
ST12	*S. aureus*	Tank with residues after removing milk from the cluster	Rio Grande do Sul
ST13	*S. aureus*	Tank after detergency	Rio Grande do Sul
ST14	*S. aureus*	Liners after detergency	Rio Grande do Sul
ST15	*S. aureus*	CIP water after detergency	Rio Grande do Sul

CIP: *Clean-in-Place*. Internal cleaning process of a part or equipment without relocation or disassembly, automatically recirculating the detergent and rinsing solutions.

to 18 h and a suspension equivalent to MacFarland8 scale obtained by dilution was used for inoculation of test bacteria in Agar Mueller-Hinton (MH, Oxoid®2). After seeding the plate, a commercial paper disc (Laborclin®1) containing the defined concentration of the antimicrobial being evaluated was applied on the agar. The disc-diffusion test was performed according to the recommendations of CLSI (CLSI, 2019) and BrCAST (BRCAST, 2017) against the following active principles: Cephalexin (CFE) 30 µg, Gentamicin (GEN) 10 µg, Tetracycline (TET) 30 µg, Oxacillin (OXA) 1 µg, Neomycin (NEO) 30 µg, Sulfa + Trimetropim (SUT) 25 µg, Penicillin G (PEN) 10 U, Amoxicillin + Clavulonic Acid (AMC) 30 µg, Vancomycin (VAN) 30 µg, Chloramphenicol (CLO) 30 µg, Doxycycline (DOX) 30 µg and Ceftiofur (CTF) 30 µg.

After incubation at 36±1°C for 24 h the inhibition halos were read and interpreted according to a specific table. We used the criterion for multi-drug resistance of the National Antimicrobial Resistance Monitoring System (NARMS, 2012) which cites multi-drug resistance as a resistance to three or more classes of antimicrobials and also by specific phenotypes.

The data obtained by disc-diffusion were submitted to Analysis of Variance (ANOVA), and the means of halos were compared by the Tukey test, both at 5% significance. The multiple antimicrobial resistance index (IRMA) for each sample was calculated according to KRUMPERMAN (1983), through the ratio between the number of resistant antibiotics and the total number of antibiotics tested.

The ability of S. aureus to produce capsules as a presumptive test for biofilm formation was determined by the Congo Red Agar (CRA, Difco™[4]) plate culture described by FREEMAN; FALKINER; KEANE (1989). CRA[4] was prepared from 37g.L-1 Brain Heart Infusion broth (BHI, HiMedia*[3]), 50 g.L-1 sucrose (Difco™[4]), 15 g.L-1 Base Agar (DifcoTM4) and 0.8 g.L-1 Congo Red Dye (Difco™[4]). S. aureus colonies obtained overnight in Soya Triptone Broth (TSB, Merck[5]) were inoculated in CRA[4] and incubated at 36°±1°C for 24 h. Samples considered to be capsule-producing showed black coloring and those regarded as not producing present a red color.

Hemolysin production was evaluated according to the methodology described by DIAS et al. (1994). After 24 h of incubation in Agar Triptone Soy (TSA, Difco™[4]) bacterial cultures were inoculated in Blood Agar plates (TSA with 5% sheep blood, Laborclin*[1]) and incubated at 36°±1°C for 48 h. The presence of circular zones of total (β) or partial (α) hemolysis around the colonies indicated positive results for the test.

Protease production was determined in Agar Milk (Milk HiVeg™Agar, HiMedia*[3]) according to BUDI et al. (2001). After incubating the bacterial cultures in TSB5 broth at 36°±1°C for 24 h, the colonies were inoculated into Agar Milk[3] plates and incubated at 36°±1°C for 48 h. The presence of degradation, visualized as a clear halo around the colonies, indicated positive results for the assay.

The ability to adhere under different temperatures and the detection of biofilm formation on polystyrene surface was performed by the microplate (MP) method described by RODRIGUES et al. (2010). The strains were incubated on TSA[4] agar with 4% glucose at 36±1°C for 24 h. Then, they were grown in 3 mL of TSB[5] broth with 4% glucose and incubated at 36°±1°C for 24 h. Each sample was then diluted in 1 mL of TSB[5] broth with 4% glucose to the score of MacFarland scale[8]. Each sample was inoculated 200 µL of bacterial suspension in three separate wells into 96-well flat-bottomed polystyrene plate, with each plate incubated at 42±1°C, 36±1°C, 25±1°C, 9±1°C, and 3±1°C for 24 h. As a negative control, non-inoculated TSB[5] broth was used, also in triplicate.

After the period, the bacterial suspension of each well was aspirated and washed three times with 250 µL sterile 0.9% sodium chloride solution. After that, the bacterial cells were fixed with 200 µL of methanol p. a. for 15 min with subsequent removal. The plates were dried at room temperature and stained with 200 µL of Hucker's 2% violet crystal solution for 5 min, then washed and dried again at room temperature. The procedures were performed in duplicate and each sample yielded 6 replicates for reading, at each evaluated temperature.

The absorbance reading was performed in a ELISA reader at 550 nm. The absorbance value of each sample (DOa) was obtained from the arithmetic mean of the values of the 6 wells and compared with the absorbance mean of the non-inoculated TSB[5] (DO). To determine the degree of adhesion the following classification was used: non-adherent: DOa ≤ DO; low adhesion: DO < DOa ≤ 2.DO; moderately adherent: 2.DO < DOa ≤ 4.DO; strongly adherent: 4.DO < DOa

RESULTS AND DISCUSSION

In the disc-diffusion antimicrobial sensitivity test, all analyzed *S. aureus* isolates showed a pattern of multidrug resistance to antimicrobials tested with Multiple Antimicrobial Resistance (MAR) Index ≥ 0.5, being resistant to three or more classes of antimicrobials (Table 2).

According to KRUMPERMAN (1983), MAR Antimicrobial Resistance indices ≥ 0.2 reveal the phenomenon of multidrug resistance (MDR), indicating a risk to public health since it impairs the disease treatment. The results found in this study were superior than those reported by DA COSTA et al. (2013), in which the MAR index for *S. aureus* varied from 0 to 0.26. However, the author pointed out that 18.15% of the samples presented MAR index ≥ 0.2, characterizing multiresistance. MDR bacteria develop resistance in response to the antibiotics used and the same strains can colonize animals and humans, being easily disseminated among bacterial species or phylogenetically related clones (MANYI-LOH, CHRISTY et al., 2018).

Since 2013, the use of antibiotics as an additive has been suspended by the European Community based on the precautionary principle (HUYGHEBAERT; DUCATELLE; VAN

IMMERSEEL, 2011). To meet international requirements, Brazil has gradually established, through various legal regulations, a stricter use of antibiotics and other performance-enhancing additives. Thus, the following drugs were banned: avoparcin in 1998; antimony compounds in 2002; chloramphenicol and nitrofurans (including veterinary clinical use) in 2003; olaquindox in 2004; carbadox in 2005; amphenicols, tetracyclines, beta-lactams (benzylpenicillin and cephalosporins), quinolones and sulfonamides in 2009; spiramycin and erythromycin in 2012; and colistin in 2016 (RABELLO et al., 2020). Recently, tylosin, lincomycin, and tiamulin additives were banned in Brazil, although virginiamycin and bacitracin are still allowed (BRAZIL, 2020).

All the strains analyzed for antimicrobial susceptibility showed characteristics of methicillin-resistant strains (MRSA), being 100% resistant to penicillin G; 93.34% to oxacillin (MRSA) and the association amoxicillin + clavulanic acid; and 33.34% of these also showed resistance to vancomycin (VRSA).

The recent debate on the high resistance of *S. aureus* MRSA strains, particularly in human medicine, has increased concern about the use of antimicrobials in dairy cow therapy. Even with methicillin out of production, due to the use of more stable penicillin such as oxacillin, the term MRSA is still used for penicillin-resistant *S. aureus*. Penicillin-stable penicillin (PSSP), of which methicillin is a prototype, are semi-synthetic drugs developed to treat infections caused by *S. aureus*, a beta-lactamase producer (BASSETTI et al., 2019). *S. aureus* MRSA strains are resistant to all beta-lactam agents except new fifth-generation cephalosporins, and are often MDRs, which can result in higher costs, prolonged treatment times, and higher rates of hospitalization and comorbidities (KHAN & KHAN, 2015). The fact that all the isolates in this study exhibited MRSA characteristics in the milking environment represents a potential risk to workers, veterinarians, and animals exposed to this agent (MENEGOTTO & PICOLI, 2007).

Although glycopeptides are not commonly used in the treatment of bovine mastitis, isolates have been evaluated for vancomycin susceptibility due to their importance in the treatment of human infections, reported to be the most effective antibiotic for gram-positive bacteria, including MRSA strains (LUNDSTROM & SOBEL, 2000). However, increased use of vancomycin led to the emergence of vancomycin-resistant *S. aureus* (HIRAMATSU et al., 1997). This fact corroborates the findings of this study, in which an emerging 33.34% resistance to vancomycin was identified. These isolates also formed biofilms, with *S. aureus* being an effective antagonist to vancomycin, erfloxacin, and teicoplanin; acting as a barrier to these compounds or even interfering in their action on cell membrane (SOULI & GIAMARELLOU, 1998).

RESENDE et al. (2012) found that ciprofloxacin showed a 100% action on milk isolates from cows with mastitis, followed by gentamicin (95.45%), sulfazotrim (86.36%), and chloramphenicol (86.36%). The highest resistance rates were verified for cefepime (95.45%) and penicillin G (81.82%). These results differ from those found in our study, in which the highest resistance rates were found for chloramphenicol (100%), sulfa+trimetropim (93.33%), and gentamicin (80%).

Clindamycin, gentamicin, and ciprofloxacin are commonly used in the treatment of bovine mastitis in Brazil, and it is suggested that these drugs are responsible for triggering a selective pressure on dairy farms. In fact, 80% of the isolates in this study showed resistance to gentamicin. In addition, all

Table 2. Antimicrobial resistance profile e Multiple Antimicrobial Resistance (MAR) Index of 15 isolates of *S. aureus*.

Sample	Antimicrobial resistance pattern	Antimicrobial resistance profile	IRMA
ST4	CFE, OXA, PEN, GEN, NEO, DOX, TET, CLO, VAN, AMC, SUT, CTF	1	1.0
ST6	CFE, OXA, PEN, GEN, NEO, DOX, TET, CLO, VAN, AMC, SUT, CTF	1	1.0
ST11	CFE, OXA, PEN, GEN, NEO, DOX, TET, CLO, AMC, SUT	2	0.83
ST15	CFE, OXA, PEN, GEN, NEO, DOX, TET, CLO, AMC, SUT	2	0.83
ST7	CFE, OXA, PEN, GEN, TET, CLO, VAN, AMC, SUT, CTF	3	0.83
ST3	CFE, OXA, PEN, DOX, TET, CLO, VAN, AMC, SUT, CTF	4	0.83
ST12	CFE, OXA, PEN, GEN, DOX, TET, CLO, VAN, AMC, SUT	5	0.83
ST1	CFE, OXA, PEN, GEN, DOX, TET, CLO, AMC, SUT, CTF	6	0.83
ST8	CFE, OXA, PEN, GEN, DOX, TET, CLO, AMC, SUT	7	0.75
ST9	CFE, OXA, PEN, GEN, DOX, TET, CLO, AMC, SUT	7	0.75
ST14	CFE, OXA, PEN, GEN, DOX, TET, CLO, AMC, SUT	7	0.75
ST5	CFE, OXA, PEN, GEN, CLO, AMC, SUT, CTF	8	0.66
ST10	CFE, PEN, GEN, NEO, TET, CLO, AMC, SUT	9	0.66
ST13	CFE, OXA, PEN, TET, CLO, AMC, SUT, CTF	10	0.66
ST2	CFE, OXA, PEN, DOX, TET, CLO	11	0.5

isolates from dairy farms in the Northeast of Brazil, reported in the work of SILVEIRA-FILHO et al. (2014), were resistant to gentamicin and bacitracin, which denotes the high rate of *S. aureus* resistant to gentamicin on dairy farms in different regions of Brazil. FREITAS et al. (2018), when identifying *S. aureus* in dairy properties of Rio Grande do Sul, found that 96.7% of the isolates were resistant to tetracycline, gentamicin (86.7%), and neomycin (96.7%).

Ceftiofur, a third generation cephalosporin, and the only one approved for farm animals (SATO et al., 2014), was the drug with the lowest resistance (33.34%) in this study. This group of antimicrobials prevents the synthesis of the cell wall, responsible for the functions of protection, support, and maintenance of the bacterial morphology (AULETTA et al., 2016).

For PÉREZ-RODRÍGUEZ & MERCANOGLU TABAN (2019) the excessive and indiscriminate use of antimicrobials for therapy and prophylaxis of bacterial infections in production animals are promoting the development of multi-resistant pathogens to commonly used drugs. Microbial resistance is the ability inherited from microorganisms to multiply in the presence of antibiotics, regardless of the duration of treatment (LEVIN-REISMAN et al., 2019).

All *S. aureus* isolates formed biofilms at different temperatures and degrees of adherence. Only one sample (ST13) did not form biofilms at 3°C. Regarding virulence factors, 86.7% of the strains formed capsules, 60% showed the presence of protease, 26.7% expressed the factor α-hemolysin and 13.3% β-hemolysin (Table 3).

It is important to highlight the detection of biofilm formation at 3°C, since this temperature is considered safe for the transport and conservation of milk on the farm. According to IN 77 (BRAZIL, 2018), in the case of a direct expansion refrigeration tank, the temperature for milk storage must be equal to or below 4°C, for a maximum time of 3 hours, regardless of its capacity, with the performance and efficiency characteristics according to specific technical regulations. The temperature of cooled raw milk in transport and reception should not exceed 7°C, exceptionally allowing its reception at up to 9°C.

Of the 15 isolates, 40% were classified as strongly adherent, i.e., strongly biofilm forming. GUIMARÃES et al. (2012), when studying the production of biofilm by Staphylococcus, obtained different results from those observed in our study, because 13/30 (43.3%) of the strains analyzed did not form biofilms and only 8/30 (26.7%) were strongly biofilm formers.

OLIVEIRA et al. (2006), working with mastitis isolates, found 18.7% of the *S. aureus* strains producing biofilms, while NOEL et al. (2016), who studied a biofilm formation in *Staphylococcus* spp. also from bovine mastitis, but in the Rio de Janeiro state, detected biofilms in 74.4% of the isolates. STEPANOVIC et al. (2000) emphasized that the plate adherence test is one of the most used methods to quantify biofilms produced by *Staphylococcus* spp.

An important characteristic of *S. aureus* is its ability to form biofilms on biological and inert surfaces. In relation to mastitis, the adhesions of *S. aureus* to the epithelium of the mammary gland are considered the first critical point in the pathogeny of this disease, because the biofilm helps in the adherence and colonization of microorganisms in the epithelium of the mammary gland (MELO et al., 2012). Thus, in many clinical cases, the immune response of the host to

Table 3. Biofilm formation and virulence factors of 15 isolates of *S. aureus*.

Sample	Adhesion at 550nm					Virulence factors		
	3°C	9°C	25°C	36°C	42°C	Capsule	Protease	Hemolysin
ST1	WEAK	WEAK	MODERATE	WEAK	MODERATE	+	+	-
ST2	MODERATE	WEAK	MODERATE	STRONG	STRONG	+	+	+β
ST3	WEAK	MODERATE	MODERATE	STRONG	STRONG	+	-	+α
ST4	WEAK	WEAK	MODERATE	WEAK	WEAK	+	+	-
ST5	WEAK	STRONG	WEAK	STRONG	STRONG	+	+	+β
ST6	WEAK	WEAK	WEAK	STRONG	STRONG	+	-	+α
ST7	WEAK	MODERATE	MODERATE	STRONG	STRONG	+	+	-
ST8	WEAK	WEAK	STRONG	STRONG	STRONG	+	-	+α
ST9	WEAK	WEAK	MODERATE	WEAK	MODERATE	+	+	-
ST10	WEAK	WEAK	WEAK	STRONG	STRONG	+	-	+α
ST11	WEAK	WEAK	WEAK	MODERATE	MODERATE	+	+	-
ST12	WEAK	WEAK	WEAK	MODERATE	MODERATE	+	+	-
ST13	-	WEAK	MODERATE	MODERATE	STRONG	-	-	-
ST14	MODERATE	MODERATE	STRONG	STRONG	STRONG	-	-	-
ST15	WEAK	WEAK	MODERATE	MODERATE	MODERATE	+	+	-

persistent infections is ineffective and may result in chronic conditions (ARCHER et al., 2011).

Although the effect of surface roughness on biofilm formation is contradictory in the literature (MARCHAND et al., 2012), the results of biofilm on liners with milk residues from milking and on clean liners, even after detergency and sanitization, highlight the urgency of alternative measures for biofilm dispersion. LATORRE et al. (2010) observed biofilms on liners, mainly associated with scratches on their surface, using scanning electron microscopy.

Regarding virulence factors, *S. aureus* is able to develop several mechanisms to escape from immune responses. To resist phagocytic depuration, this pathogen expresses a polysaccharide capsule, which effectively masks the bacterial surface and the proteins associated with the surface, the opsonins, resulting in a higher virulence and tissue invasion ability from a peripheral focus, prolonging the persistence of the pathogen in the bloodstream of the host (O'RIORDAN & LEE, 2004). In addition, the enhancement of *S. aureus* pathogenesis is determined by the secretion of proteases that cleave specific components of the host immune system or disturb the integrity of the extracellular matrix and intercellular connections, compromising the stability of host tissues and contributing to the spread of infection (KOZIEL & POTEMPA, 2013).

S. aureus is one of the most common human and animal pathogens. Some staphylococcal species produce hemolysins that differ according to the lytic action on erythrocytes. Bovine strains produce mainly beta-hemolysin, while human isolates have the ability to produce alpha-hemolysin (ECONOMOU & GOUSIA, 2015). Beta- and alpha-type strains are also important in the pathogenesis of intramammary infections in dairy cattle due to the induction of pro-inflammatory changes in the cells, inactivating the immune system by their direct cytotoxic effect and by degrading tissues (ZSCHOCK et al., 2005). MARQUES et al. (2013) found only 13.2% (33/250) hemolytic *Staphylococcus* spp. Of these, 48.5% (16/33) presented total hemolysis, 36.4% (12/33) partial hemolysis, and 15.1% (5/33) partial hemolysis. CHIH-WEI; YIU-KAY; YU-TSUENG (2011) defines the production of hemolysins as decisive for the pathogenicity of microorganisms, since by degrading tissues, they allow the invasion and dissemination pf the pathogen, besides escaping the immune response of the host.

Alpha hemolysin is considered by some authors as one of the main pathogenicity factors of this bacterium due to its hemolytic, dermonecrotic, and neurotoxic effects (DINGES; ORWIN; SCHLIEVERT, 2000). LARSEN; AARESTRUP; JENSEN (2002) suggested that strains of Staphylococcus spp. producing partial hemolysis are more virulent to cattle than non-hemolytic strains. Partial hemolysis in Staphylococcus spp. is represented by beta-hemolysin, which is toxic to several cell types and relevant in cases of mastitis since the udder is rich in sphingomyelin (COELHO et al., 2011).

The virulence factors act on the induction and persistence of infections, ensuring the success and survival of the agent. Since the pathogenicity of *S. aureus* depends on these factors, the development of alternatives that promote their inhibition and, consequently, prevent their clinical manifestations (ESCAICH, 2008), becomes a promising strategy.

CONCLUSION

The identification of biofilm forming antimicrobial multiresistant *S. aureus* reveals the importance of prevention and control of intramammary infections in the herds and of the persistence of the etiological agent in the milking facilities, in order to avoid the propagation of this pathogenic microorganism in the milk production chain.

SUPPLIERS

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ACKNOWLEDGEMENTS

To Capes/Prosup/UPF for supporting this study for supporting this study through scholarships.

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