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Minireview

MINIREVIEW

Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms

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One sentence summary: This review provides an important input of knowledge on the relevance of biofilm formation in bovine mastitis infections and their role in antimicrobial resistance.
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ABSTRACT

Bovine mastitis (BM) is a disease with high incidence worldwide and one of the most relevant bovine pathologies and the most costly to the dairy industry. BM is an inflammation of the udder and represents one of the most difficult veterinary diseases to control. Biofilm formation is considered a selective advantage for pathogens causing mastitis, facilitating bacterial persistence in the udder. In fact, recently some authors drew attention to the biofilm formation ability presented by several mastitis causing pathogens and to its possible relation with recurrent mastitis infections and with the increased resistance to antimicrobial agents and host immune defence system. Actually, up to now, several researchers reported the potential role of cells in this mode of growth in the previous facts mentioned. As a consequence of the presence of biofilms, the infection here focused is more difficult to treat and eradicate, making this problem a more relevant pressing issue. Thus, we believe that a deeper knowledge of these structures in mastitis can help to determine the best control strategy to be used in veterinary practice in order to reduce losses in the dairy industry and to ensure milk safety and quality. The aim of this paper was to review the existing research and consequently to provide an overview of the role of biofilms in BM infections.

Keywords: antimicrobial resistance; biofilms; bovine mastitis; causative agents

INTRODUCTION

Bovine mastitis (BM) is responsible for major economic losses on dairy farms worldwide, caused by the decrease in milk production, increase in health care costs and increase in culling and death rates (Melchior, Vaarkamp and Fink-Gremmels 2006). Moreover, mastitis poses a threat to human health since it may be responsible for zoonoses and for food toxin infections (Blum *et al.* 2008; Fernandes *et al.* 2011). Staphylococci are the bacteria most commonly isolated from BM (Leitner *et al.* 2011) and *Staphylococcus aureus* is a common cause of this disease (Oliveira *et al.* 2007). However, coagulase-negative staphylococci (CNS) have become the most common BM isolate in many countries and are now predominant over *S. aureus* in most countries and could therefore be described as emerging mastitis pathogens (Tremblay *et al.* 2013). Apart from staphylococci, Coliforms, Enterococci and Streptococci are also frequently isolated from cows with mastitis (Smulski *et al.* 2011). For example, in England and Wales, *Streptococcus uberis* was the most commonly isolated from clinical and subclinical cases of BM (Bradley *et al.* 2007). Depending on their primary reservoir and mode of transmission, mastitis may

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be classified as 'contagious' or 'environmental'. The main contagious microorganisms are *S. aureus* and *Streptococcus* species, being their main source the mammary gland of infected cows. On the other hand, the primary source of environmental mastitis pathogens is the habitat of the cow, and *Streptococcus* species and Gram-negative bacteria (*Escherichia coli* and *Klebsiella*) are examples of microorganisms included in this group (Bogni *et al.* 2011).

Recurrent infections are often attributable to biofilms (Vasudevan *et al.* 2003). Biofilm formation is accompanied by significant genetic and subsequent physiological changes in the microorganisms resulting, inter alia, in a loss of sensitivity to virtually all classes of antibiotics (Melchior, Vaarkamp and Fink-Gremmels 2006). This fact can be a worrying problem since 40% of mastitis cases arise from recurrence of preceding BM infections after failed therapy (Hillerton and Kliem 2002).

Overall, the main objective of this paper was to summarize the general knowledge and research concerning biofilms and mastitis. Therefore, this review will provide an important input of knowledge on the relevance of biofilm formation in BM infections and their role in antimicrobial resistance.

BIOFILMS

In natural, industrial and medical environment, bacteria are able to attach to surfaces and grow in biofilm communities. In fact, this is the predominant mode of growth of bacteria in natural environment (Kaur *et al.* 2009). Biofilms are widely defined as a cluster of cells enclosed in a self-produced matrix. When growing in this mode of life, microorganisms become more tolerant to opsonophagocytosis and conventional antibiotics (Stewart and Costerton 2001), being 100–1000 times less susceptible to antibiotics than their planktonic counterparts (Donlan, 2000). This bacterial tolerance/persistence is responsible for the chronicity of a disease (Burki, Frey and Pilo 2015). Moreover, biofilm formation can also be harmful to host tissues since they can promote the phagocyte release of lysosomal enzymes, reactive oxygen and nitrogen species (McAuliffe *et al.* 2006; Hermeyer *et al.* 2011). Morente *et al.* (2013) drew attention to the role of biofilms in the development and transfer of resistance promoted by microbial interactions happening within biofilm. Biofilm formation can be a strain trait or can be genetically linked to traits, representing a selective advantage in pathogenesis of mastitis (Fox, Zadoks and Gaskins 2005).

Biofilm formation begins with the initial adhesion and subsequent agglomeration into multicellular structures. During the development of the biofilm, adhesive forces are required for the colonization of surfaces and the intercellular interaction. Disruptive forces plays a preponderant role in the formation of fluid-filled channels, crucial for oxygen, metabolites and nutrient delivery to all biofilm cells, giving to the biofilm its typical 3D structure. On the other hand, disruptive forces are also important for the detachment of cells, limiting biofilm expansion and promoting the dissemination of the infection (O'Toole, Kaplan and Kolter. 2000). Biofilm formation is a dynamic process, rendering possible the shedding of planktonic cells that present rapid multiplication and are able to occupy other surfaces. This process of high significance can promote the colonization of microbial pathogen in other infection sites, and consequently the formation of new biofilms, leading to the systemic dissemination of infection. This process of detachment lead to a change in gene expression, towards toxin and adhesion molecules and a fast multiplication being frequently linked to reappearance of clinical signs of infection. In veterinary medicine, biofilms seem to be related with several diseases including mastitis and this is supported by the change of antimicrobial susceptibility (Fernandes *et al.* 2011; Atulya *et al.* 2014). As mentioned before, the presence of biofilms in mammary glands results in a decreased effectivity of antibiotherapy. This makes persistent infections hard to treat and impair the eradication of the infection, resulting in a great prejudice to the dairy industry. Furthermore, we know that nowadays biofilm formation can be induced by subinhibitory concentrations of several antimicrobial agents (Melchior, Vaarkamp and Fink-Gremmels 2006). As example, Costa *et al.* (2012) shown that subinhibitory concentrations of enrofloxacin, usually used to treat clinical mastitis, promoted the biofilm formation of mastitis *E. coli* isolates. Therefore, the previous researchers attributed this mechanism of resistance to the recurrence of the disease, promoted by the persistence of the microorganism in question in the mammary gland mediated by biofilm formation. Some researchers tried to simulate the *in vivo* environment and studied the effect of the milk in biofilm formation *in vitro*. Biofilm formation was influenced by milk composition as showed by Atulya *et al.* (2014) that tested six major milk components. Dipotassium hydrogen phosphate concentration and pH negatively affected the biofilm formation of *S. aureus* (MTCC 1430) and *Pseudomonas aeruginosa* (NCIM 5029), respectively. The pH decrease favours biofilm formation and that causes a serious problem since current therapy for mastitis is based in the administration of acidic antibiotics. The authors suggested the use of pH enhancing agents in combination with antibiotic therapy in order to avoid long-lasting infections (Atulya *et al.* 2014).

Baselga *et al.* (1993) proved that ruminant mastitis isolates were able to produce slime *in vivo* when an experimental infection of mammary gland was followed in sheep and that the *in vivo* biofilm formation capacity of *S. aureus* is a major virulence factor determinant to their pathogenicity in mastitis infections (Vasudevan *et al.* 2003). *In vivo* studies of biofilm formation by BM pathogens is a challenge and although supported by *in vitro* studies, no *in vivo* study can directly support the hypothesis that biofilm formation is responsible for the less efficiency of antimicrobial therapy and by the persistence of intramammary infections (IMI) (Melchior, Vaarkamp and Fink-Gremmels 2006; Veh *et al.* 2015).

Several questions have emerged because of the presence of biofilm in the mammary glands. First, several studies have been done *in vitro* but these ones do not guarantee the same results *in vivo* when treating BM (Pyörälä 2009). Moreover, standardized antimicrobial susceptibility tests are usually done with planktonic cells without taking into consideration the general opinion that biofilms are more tolerant to antimicrobial agents (Nadell, Xavier and Foster 2009). Therefore, the current practices used nowadays may fail to prevent the dissemination of virulent strains. Additionally, sometimes the effect of determined control strategy is strain dependent whereby it should be focused on specific strains causing infection in the herds. Sometimes the method of detection of mastitis based on the count of somatic cells can also represent a potential risk of dissemination of the disease since cows with low SCC can be a potential reservoir of bacteria able to form biofilms permitting the persistence of the pathogens in the farm (Cucarella *et al.* 2004). Therefore, biofilms represent a mechanism of microorganism survival in the mammary gland and contribute to the persistence of pathogens on the farm. Thus, it is crucial to understand the role of biofilms and other virulence factors that are involved in the ability of the majority of the mastitis' pathogens to colonize and

Table 1 . Main mastitis causing pathogens and their biofilm formation ability.

Pathogen	Mastitis type	Biofilm formation	Reference
S. aureus	Subclinical mastitis	+	Darwish and Asfour (2013); Fabres-Klein *et al.* (2015)
CNS	Subclinical mastitis	+	Darwish and Asfour (2013)
E. coli	Clinical mastitis	+	Costa *et al.* (2014); Milanov *et al.* (2015)
E. faecalis	Clinical mastitis	+	Elhadidy and Zahran (2014)
S. uberis	Clinical mastitis	+	Crowley *et al.* (2011); Kromker *et al.* (2014)
S. dysgalactiae	Clinical mastitis	+	Olson *et al.* (2002)
S. agalactiae	Mastitis	+	Rosini and Margarit (2015)

+: biofilm-forming bacteria.

maintain infections. Nowadays, it is already accepted that biofilm production is an important virulence mechanism of pathogens causing BM (Table 1). As example, and taking into account this notion, enzymes involved in biofilm production and components of biofilm such as extracellular polymeric substance and other matrix constituents could be potential new targets for new anti-mastitis drugs used as prophylactic and therapeutic strategies in the treatment of that disease.

Staphylococcus species

Several authors showed that staphylococci causing BM have the ability to produce biofilm, which is an important factor that contributes for unusual problems with eradication of infection and recurrent infections of mammary glands (Darwish and Asfour 2013; Raza *et al.* 2013; Seixas *et al.* 2014). Intracellular survival of staphylococci into mammary epithelial cells was considered one of the main mechanisms of pathogenicity (Garzoni and Kelley 2009). Bardiau *et al.* (2014) studied the possible correlation of capsular profile, biofilm formation and intracellular survival, on *S. aureus* BM isolates and concluded to exist a correlation between these three features responsible for long-lasting infections.

Most studies concerning biofilms and BM are researches focused on staphylococci species namely *S. aureus*, that despite of the large-scale control programmes continues to be one of the main pathogens involved in ruminant mastitis worldwide namely subclinical, clinical, recurrent and chronic mastitis (Bogni *et al.* 2011; Raza *et al.* 2013). *Staphylococcus aureus* is part of the commensal flora of several mammalian species. However, when subjected to a combination of endogenous and exogenous factors they can become pathogenic and a source of mastitis (Melchior, Fink-Gremmels and Gaastra 2006; Melchior, Vaarkamp and Fink-Gremmels 2006). This pathogen is responsible for between 5% and 70% of cows and 90% of herds affected worldwide with BM (Zecconi and Scali 2013). *Staphylococcus aureus* produces glycocalyx, an extracellular polysaccharide layer surrounding cells and whose function is to promote bacterial adhesion onto the mammary epithelial cells and protect bacteria from opsonization and phagocytosis (Arslan and Özkardes 2007). *Staphylococcus aureus* mastitis infections are usually difficult to treat and are prone to resurgence (Le Maréchal *et al.* 2011). Oliveira *et al.* (2011) demonstrated that *S. aureus* cells are able to invade mammary epithelial cells where they can persist for long periods. They also showed no relation between biofilm formation and invasiveness of epithelial cells since some strains tested were good biofilm producers and presented a low level of invasive ability and the most invasive strain was biofilm negative. Several genes are involved in biofilm formation by staphylococci from BM such as loci *ica* (intercellular adhesion), *bap* (biofilm-associated protein), *agr* (accessory gene regulator) and *sar* (staphylococcal accessory regulator).

However, the expression of all of them is not necessary, suggesting that other mechanisms can be determinant in biofilm growth (Cucarella *et al.* 2004; Planchon *et al.* 2006).

Therefore, the possible role of biofilms in chronic infections drew attention of several researchers in the characterization of genes implicated in biofilm formation (Tormo *et al.* 2005). Vasudevan *et al.* (2003) concluded that *ica* operon is crucial and a virulence factor in the pathogenesis of mastitis, evidencing that biofilm formation can be a possible reason to the therapy resistance observed in these strains. Moreover, the ability of biofilm formation within animals can modify the efficacy of antimicrobial agents and this can have implications in the treatment of the infection caused.

Cucarella *et al.* (2004) demonstrated that *Staphylococcus* strains expressing Bap protein showed a high ability to infect and persist in the mammary gland and were more tolerant to antibiotic treatment. Szweda *et al.* (2012) studied phenotypically and genotypically several *S. aureus* isolates of BM in Poland relatively to their ability to form biofilms. Most isolates (57.6%) were classified as biofilm producers. Fox, Zadoks and Gaskins (2005) determined that *S. aureus* milk isolates produce biofilm with higher frequency than isolates from extramammary sites. As previously mentioned, the detachment of biofilms can be one explanation to the occurrence of chronic problems at the herds level. The *agr* expression seems to contribute to the detachment of biofilms and consequent colonization of new sites, increasing the ability of *S. aureus* to spread through a herd (Boyen *et al.* 2009).

CNS are among the most common agents of subclinical mastitis (Schwarz *et al.* 2010). Half of IMI caused by these pathogens are able to persist in the udder for long periods (Taponen *et al.* 2007). Several papers stated that milk of a healthy udder is believed to be sterile (Lyer, Anand and Dang. 2010; Caldwell 2012; Verdier-Metz *et al.* 2012). However, CNS are part of the normal teat skin flora and can colonize the teat canal and therefore they are designated as skin flora opportunists (De Vliegher *et al.* 2004, Janus 2009; Philip, Radhakrishnan and Mathew 2012). Simojoki *et al.* (2012) reported that CNS isolates from IMI rarely produced matrix and that no relation was observed between biofilm production ability and persistence/severity of mastitis. Slime production was thus considered non-crucial to persistence of IMI caused by CNS. No correlation was observed between phenotypic biofilm formation ability or slime production and evasion and adhesion ability of CNS (Oliveira *et al.* 2011). Although slime production is rarely observed, it occurs more frequently in persistent infections when compared with spontaneously cured infections and in *Staphylococcus epidermidis* comparatively to other

CNS. This fact may be a possible explanation to the common prevalence of *S. epidermidis* in some herds. The *eno* gene (encoding laminin binding protein) is the only microbial surface components recognizing adhesive matrix molecules gene expressed by CNS isolated in mastitis being involved in bacterial adhesion to host tissues (Simojoki *et al.* 2012).

In conclusion, the transition to a chronic mastitis infection is potentially mediated by the emergence of biofilms in the udder tissue and this type of cell growth is a possible explanation to the frequent therapeutic failures (Dubravka *et al.* 2010).

Escherichia coli

Escherichia coli is another of the common infectious agents of BM being considered one of the major agents worldwide (Blum *et al.* 2015, Kempf, Loux and Germon 2015). In England and Wales, *E. coli* was one of the major mastitis pathogens most commonly isolated from clinical cases (Bradley *et al.* 2007).

Escherichia coli is a pathogen able to infect the mammary gland by entering the udder via the teat canal (Lipman *et al.* 1995).

Although the improvement in managing and housing of dairy cattle, mastitis caused by this bacterium continue to be a problem in several countries. *Escherichia coli* presents several virulence factors/mechanisms such as toxins, adhesins, invasins, capsule production, the ability to resist serum complement and iron scavenging. To be able to promote disease, *E. coli* needs the successful combination of virulence factors, being these ones required to overcome the host's selection pressure and to colonize, multiply and survive in the udder (Kaper, Nataro and Mobley 2004). Clinical *E. coli* mastitis can range from mild with only local signs to severe disease with systemic clinical signs. In severe cases, *E. coli* can cause acute tissue damage and complete loss of milk production and even the death of the infected animal. According to Burvenich *et al.* (2003), the severity of mastitis promoted by this microorganism is related with factors other than strains characteristics, namely host factors. Generally, intramammary *E. coli* infections are spontaneously eradicated by host defences. However, this is not the case of recurrent infections that are more difficult to treat. Recurrent coliform mastitis infections can be caused by the persistence of the microorganism within the mammary gland (Bradley and Green 2001), being possibly the biofilm production responsible for this persistence. Accordingly, Fernandes *et al.* (2011) demonstrated that all their *E. coli* mastitis' isolates were able to form biofilm although at different levels. Any *E. coli* pathotype even non-pathogenic strains can cause mastitis (Shpigel *et al.* 2008). More recently, Suojala *et al.* (2011) showed that mastitis-causing *E. coli* are typical commensals. Blum and Leitner (2013) evidenced that the most common virulence factors exhibited by this bacterium causative agent were lpfA (long polar fimbriae), iss (increased serum resistance) and astA (enteroaggregative *E. coli* heat-stable enterotoxin 1). However, none of them was specific to mastitis *E. coli* pathogens since none characterized the majority of the strains studied. lpfA was reported as having a potential role in the improvement of virulence in the mammary gland by promoting epithelial adhesion (Dogan *et al.* 2012). *Iss* was repeatedly found in *E. coli* BM isolates. Shpigel *et al.* (2008) proposed that it can exist as an association of unknown factors with pathogenicity of mastitis *E. coli* persistent bovine IMI strains.

On the other hand, Blum and Leitner (2013) and Suojala *et al.* (2011) concluded that mastitis *E. coli* strains do not have known specific markers of virulence and therefore until now none virulence factor was associated with their pathogenicity, being this pathotype still uncharacterized. Additionally, Fernandes *et al.* (2011) also shown that the pathogenicity of BM *E. coli* isolates is not mediated by a single and specific virulence factor but classified this bacterium as 'opportunistic pathogen with different virulence factors'. They reported several combinations of different virulence factors in the *E. coli* isolates. The *fimH* gene, responsible for type 1 fimbriae expression and which has an important role on biofilm formation, was observed in all the isolates tested. This is in agreement with previous studies that proved the existence of these adhesins both in commensal and pathogenic *E. coli* isolates (Fernandes *et al.* 2011).

Taking this into account and despite the numerous attempts, it was impossible to group these isolates into pathotypes able to cause mastitis. As general conclusion these researchers abolish the idea of the existence of a specific mammary pathogenic *E. coli*.

No association was also found between virulence factors, phylogeny groups and antimicrobial resistance traits and the severity of mastitis caused by this microorganism (Suojala *et al.* 2011). Several authors reported that a strain selection during infection can take place in the mammary gland (Blum *et al.* 2008, Blum and Leitner 2013).

Enterococcus species

Enterococcus faecalis represents a major mastitis causing pathogen. This Gram-positive bacterium is generally present in organic bedding material being opportunistic invaders of mammary glands. Once again persistent and recurrent infections provoked by this pathogen are caused by its ability to form biofilms (Elhadidy and Zahran 2014). Elhadidy and Zahran (2014) proved that a correlation exists between biofilm formation and adherence/invasion to mammary gland epithelium. This ability contributes for recurrent infections and pathogenesis of *E. faecalis* mastitis. In addition to this competence, *E. faecalis* has an innate resistance to several antimicrobial agents presenting therefore an enhanced resistance to these compounds. Both contribute to make this microorganism an important reservoir for dissemination of resistance genes, becoming *E. faecalis* biofilm-related infections a therapeutic challenge and very difficult to treat (Elhadidy and Elsayyad 2013). Little information is available about the genetic basis of biofilm formation by *E. faecalis*. However, some authors highlighted the possible role of Esp, a specific cell surface protein of *E. faecalis* in biofilm formation. This protein, whose function is still unknown, has high sequence similarity with Bap protein that was previously reported to be involved in biofilm formation (Cucarella *et al.* 2001). Elhadidy and Elsayyad (2013) tried to establish a relationship between *esp* gene and biofilm formation. However, no association was observed between them, but a significant ability of biofilm formation was demonstrated by *E. faecalis* isolates from BM.

Streptococcus species

Streptococcus uberis is also an etiologic agent of BM. In the United Kingdom, this pathogen is responsible for about 33% of BM cases causing persistent infections and being resistant to antimicrobials (Pullinger *et al.* 2007). An evidence of this fact was the result obtained by Milne *et al.* (2005) where chronic infection by *S. uberis* was unresponsive to therapeutic treatments. Once again, biofilm growth seems to have an important role in *S. uberis* mastitis. Varhimo *et al.* (2011) were the pioneers in demonstrating that several *S. uberis* strains isolated from mastitis were able to

produce biofilm and that milk components are strong inducers of biofilm formation. This is in accordance with Almeida *et al.* (2003) that previously reported the importance of milk and protein milk in biofilm growth namely in the adherence and internalization of this pathogen to mammalian cells. Crowley *et al.* (2011) proved that biofilm formation was more pronounced in clinical isolates of *S. uberis* than in strains isolated from healthy cows, with the former evidencing a greater biofilm biomass. Moreover, they showed that glutamine transport, adherence interactions and sugar metabolism are important processes in early biofilm growth, while at the transcriptional level, an increase in mRNA levels of lactoferrin binding protein (*lbp*) was apparent. *Streptococcus uberis* biofilm formation is predominantly mediated by proteins. When adding milk to strains originally classified as non-biofilm producers, most of the strains presented a significant increase in biofilm production. Alpha-casein and b-casein and their degradation by the extracellular proteolytic activity of *S. uberis* are determinants to biofilm formation, being crucial for the achievement of a maximal production. Therefore, the biofilm formation promoted by host protein and extracellular proteolytic activity is possibly involved in the persistence of IMI resulting from the presence of this bacterium in the mammary gland. On the other hand, metallo and cysteine proteases inhibit biofilm formation in *S. uberis* (Varhimo *et al.* 2011).

Streptococcus dysgalactiae is also frequent among the causative agents of clinical and subclinical BM. Several virulence genes were frequently detected in bovine isolates namely genes encoding streptolysin S, glyceraldehyde-3-phosphate dehydrogenase, the plasminogen-binding M-like protein PAM and the collagen-like protein SclB. Moreover, several virulence determinants were found in this bacterium including alpha-2-macroglobulin, plasminogen, albumin, fibrinogen, fibronectin, vitronectin and collagen whose role is the interaction with plasma or extracellular matrix proteins of the host; and alpha-2-macroglobulin-, immunoglobulin G- or immunoglobulin A-binding protein Mig, the alpha 2-macroglobulin- or immunoglobulin G-binding protein Mag and a fibrinogen-binding M-like protein with an important role in mastitis. These genes are responsible for the increased virulence of *S. dysgalactiae* subsp. *dysgalactiae* from mastitis, possibly contributing to the dissemination to different tissues of the host, taking advantages of new niches and consequently to the propagation of the infection that can become persistent (Rato *et al.* 2011). Moreover, their biofilm-producing ability (Olson *et al.* 2002) can make them highly difficult to eradicate.

This pathogen is an important microorganism to take into consideration since it can cause invasive diseases in humans and the subspecies *dysgalactiae* is nowadays regarded as an emerging zoonotic pathogen (Rato *et al.* 2011).

Streptococcus agalactiae is a Gram-positive bacteria and a major contagious pathogen causing bovine subclinical mastitis (Rosini and Margarit 2015). Although this bacterium has a short life span in the environment, it can survive indefinitely within the mammary gland as an obligate pathogen of the udder (Kefee 1997; Rosini and Margarit 2015). *Streptococcus agalactiae* biofilm formation has been demonstrated in several *in vitro* studies and seems to be tightly controlled by environmental conditions (Rosini and Margarit 2015). The capsular polysaccharide is the main virulence factor of this bacterium. Among the most frequently virulence genes found in bovine isolates are *bca* (encoding C^{a} protein-induction of protective immunity), *scpB* (fibronectin-binding protein) (Duarte *et al.* 2005) and *lmb* (laminin-binding protein), responsible for binding and invasion into host epithelial cells; *hylB* gene encoding hyaluronidases, which are involved in cleaving hyaluronic acid, a component of the extracellular matrix in many tissues and then assisting invasion into the mammary gland (Bogni *et al.* 2011).

Overall, *Streptococcus* spp. are able to grow in biofilms, facilitating its persistence under environmental stress conditions, presenting an innate resistance to the majority of therapeutic drugs.

CONCLUSIONS

The economic losses on the dairy industry involving BM causative agents, the rapid emergence and exhibition of multidrug resistance as well as their great tendency to cause persistent, chronic and recurrent infections, make this disease a continuous challenge and a subject of investigation by several research groups justifying the continued attention in this area. Independently from the origin of the infection, biofilms have been shown to be important in pathogenicity and therefore may play a role in the biology of recurrent infections, antimicrobial agents/host immune defence system resistance, being consequently more difficult to control/eradicate the disease. The role of biofilms in mastitis infections is crucial to determine and study the best control strategies to be used in veterinary practice in order to reduce losses in the dairy industry and to ensure milk safety and quality.

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