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Review on Biofilm Forming Microbials in Cases of Bovine Mastitis and its Impact on Treatment

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Abstract

Biofilms are communities of microorganisms that are attached to a surface and play a significant role in the persistence of bacterial infections in both animals and humans. Biofilm bacteria are highly resistant to antimicrobial agents and host immune responses resulting in chronic infection of bovine mastitis. Biofilm formation is an important virulence factor that may result in recurrent or persistent udder infections and treatment failure through increased resistance to antibiotics and protection against host defenses. There is paucity of information regarding biofilm bacteria in bovine mastitis of veterinary importance and their role in disease pathogenesis. Bovine mastitis is an inflammation of the udder and represents one of the most difficult dairy diseases to control. Biofilm formation is considered a selective advantage for pathogens causing mastitis, facilitating bacterial persistence in the udder. Recently biofilm need attention due to its possible relation with mastitis causing pathogens and with the increased resistance to antimicrobial agents and host immune defense system. As a consequence of the presence of biofilms, the mastitis infection 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 microbial biofilms in bovine mastitis infections in Ethiopia.

Keywords: Antimicrobial resistance • Biofilm • Bovine Mastitis

Abbreviations: BM: Bovine Mastitis; CNS: Coagulase Negative Staphylococci; CRA: Congo Red Agar; EPS: Extracellular Polymeric Substance; MSCRAMMs: Microbial Surface Components Recognizing Adhesive Matrix; PBS: Phosphate Buffered Saline; QS Quorum Sensing; TM: Tube Method

Introduction

Biofilm is as an assemblage of microbial cells that is surrounded by a matrix of Extra-Polymeric Substance (EPS) secreted by those cells. The formation of a biofilm is a developmental process in which a quorum sensing signal molecule, an auto inducer, functions to induce the secretion of EPS and leads to the formation of a characteristic three dimensional biofilm architecture and also exhibit an altered phenotype with respect to growth rate and gene transcription [1]. The transition of a single cell into groups of cells necessitates development of intercommunication to coordinate growth and maximize efficiency. The final discrete stage of biofilms leads to cell separation, and the separated cells shut down the genes encoding EPSs and up regulate the genes encoding chemotactic proteins or flagella required by planktonic bacteria [2].

Biofilm formation is a multistage process that starts with microbial adhesion with a subsequent production and accumulation of an extracellular matrix composed by one or more polymeric substances such as proteins, polysaccharides, humic substances, extracellular DNA and sometimes other molecules such as those involved in cell-to-cell communication [3]. The cells of bacteria in a biofilm have a complex polymorphic organization with a certain cytoarchitectonics. The multilayer topography affects the metabolism and physiological activity of cells. Within the biofilm, changes can occur that include the reaction of general stress, the stop of key metabolic processes and the induction of protective mechanisms. Reduced metabolism of microorganisms in a biofilm leads to the emergence of antibiotic resistance, since antibacterial drugs are most effective against metabolically active cells [4].

The ability of bacteria to produce a biofilm is considered an important virulent property in pathogenesis of mastitis. In the majority of cases, intramammary infections are treated with antibiotic agents. Biofilms protect microorganisms against these antibiotic agents, phagocytosis and sanitizers [5]. The ability of

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bacteria to produce biofilms leads to difficulties with pathogen elimination which can give rise to persistent infections [6]. In vitro studies suggest that there are many more pathogens such as Staphylococcus aureus, Escherichia coli, Streptococcus uberis, and Streptococcus dysgalactiae and coagulase negative staphylococci, possessing the ability to cause biofilm-related mastitis. Literature reports that the cure rates for Staphylococcus aureus infected udders are lower (27%) in comparison to cure rates of Streptococcus uberis (64-81%) or Coagulase Negative Staphylococci (CNS) mastitis (80-90%). Bacteria in a biofilm are more resistant to antibiotics than planktonic bacteria. It has been estimated that biofilm cells can be up to 1000 times more resistant to antibiotics than planktonic cells. The ability of bacteria to produce biofilms leads to difficulties with pathogen elimination, which can give rise to persistent infections.

There are various methods to detect biofilm production but Congo Red Agar (CRA) and Tube Method (TM) are routinely used in clinical laboratory for determining ability of bacteria to form biofilm because of their specificity, low operational cost and availability of materials. Bacteria in biofilm behave differently from planktonic bacteria, especially in terms of their response to antibiotic treatment. Biofilm-associated bacteria are highly resistant to antibiotics. The complicated structure of biofilm with extracellular polymeric matrix could prevent antibiotics from reaching the bacteria. Bacteria in biofilm could also adopt a slow growing or starved state due to the altered microenvironment such as depletion of nutrition and accumulation of waste. The changed physiological state of bacteria could make them more resistant to antibiotics, which target more active cell processes. Therefore the objectives of this paper is to review the existing research and consequently to provide an overview of the role of biofilms in BM infections and its implications on antibiotic treatments and its control.

Literature review

Biofilms in bovine mastitis

Biofilms are a defense mechanism for prokaryotes and some eukaryotes (algae) as the conditions at that time were too harsh for their survival. Biofilms protect prokaryotic cells by providing them with homeostasis, encouraging the development of complex interactions between the cells in the biofilm. Aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of Extracellular Polymeric Substances (EPSs) adhere to each other and to cell surface. A biofilm is a system that can be adapted internally to environmental conditions by its inhabitants. The self-produced matrix of extracellular polymeric substances, which is also referred to as slime, is a polymeric conglomeration generally composed of extracellular biopolymers in various structural forms.

A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of Extracellular Polymeric Substances (EPSs). The cells within the biofilm produce the EPS components which are typically a polymeric conglomeration of extracellular polysaccharides, proteins, lipids and eDNA. Because they have three-dimensional structure and represent a community lifestyle for microorganisms they have been metaphorically described as "cities for microbes .

Biofilm forming bacteria in bovine mastitis

Mastitis in dairy cow herds is a serious problem for milk producers because it leads to decreased milk production, high costs for medical treatment and an increased culling and death rate. The ability of bacteria to produce a biofilm is considered an important virulent property in pathogenesis of mastitis. In the majority of cases, intramammary infections are treated with antibiotic agents.

Biofilms protect microbial agents, phagocytosis and sanitizers. The ability of bacteria to produce biofilms leads to difficulties with pathogen elimination which can give rise to persistent infections. Resistance to antimicrobial agents results from the retardation of antibiotic diffusion through the biofilm matrix, an increased rate of mutation, the production of enzymes that degrade antibiotics, the presence of dormant bacterial cells with low metabolic activity and increased doubling times in the inner layers of biofilms.

As indicated in Table 1 there are many more pathogens such as Staphylococcus aureus, Escherichia coli, Streptococcus uberis, Streptococcus dysgalactiae and coagulase negative staphylococci, possessing the ability to cause biofilm-related mastitis as conformed by vitro tests. Literature reports that the cure rates for Staphylococcus aureas infected udders are lower (27%) in comparison to cure rates of Streptococcus uberis (64-81%) or Coagulase Negative Staphylococci (CNS) mastitis (80-90%) (Table 1).

S.N	Pathogen	Mastitis type	Biofilm formation	References
1	*S. aureus*	Subclinical mastitis	+	19, 20
2	CNS	Subclinical mastitis	+	19, 6
3	*E. Coli*	Clinical mastitis	+	21
4	*E. faecalis*	Clinical mastitis	+	22
5	*S. uberis*	Clinical mastitis	+	23
6	*S. dysgalactiae*	Clinical mastitis	+	24
7	*S. agalactiae*	Mastitis	+	25

+: **Biofilm-forming bacteria**

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

Biofilm formation and pathogenic mechanisms in bovine mastitis

Biofilm formation in bovine mastitis: Bacterial biofilms are organized by microbial aggregates that live in extracellular polymeric matrices that are irreversibly attached to the surface of an object, living body or tissue and are difficult to remove unless deterred and inhibited quickly. The biofilms consist of 5–35% cell volume and the extracellular matrix is compound of 97% water, 2% protein and polysaccharide and 1% DNA and RNA. Biofilm formation is a dynamic cyclic process in four stages involving bacterial attachment and cohesion, micro colony formation, biofilm maturation and bacterial dispersion. Planktonic bacteria reach the attachment surface through Brownian motion, hydrodynamic motion and active swimming motion. They use the interaction of their own pili, adhesions carried on flagella, electrostatic action, hydrophobicity and other physical and chemical actions to provide adhesion force between the attachment surfaces.

When adhesion is stable, the QS system, a communication system among bacteria, is activated. Through the QS system, chemical signal molecules are transmitted to regulate bacterial proliferation and division, as well as bacterial density. The formation of Extracellular Polymeric Substances (EPSs) occurs at the stage of biofilm attachment to the surface which provides strength for the interaction of bacteria in biofilms. EPSs are key matrix components adhering to substrates and biofilms required to maintain structure. EPSs act as a bridge to attach negatively charged bacteria to positively charged surfaces. The transition of a single cell into groups of cells necessitates development of intercommunication to coordinate growth and maximize efficiency. The final discrete stage of biofilms leads to cell separation and the separated cells shut down the genes encoding EPSs and up regulate the genes encoding chemotactic proteins or flagella required by planktonic bacteria.

According to 31 and 34 the development of a biofilm, depicted as a five-stage process.

Stage 1. Initial attachment: Colonizing bacteria anchor to a surface through basic adhesion techniques.

Stage 2. Irreversible attachment: After the cells aggregate they form micro colonies and excrete EPS or "slime" to form an irreversible attachment that can weather shear forces and maintain a steadfast grip on the surface.

Stage 3. Maturation I: The biofilm is fully formed. As it matures the biofilm becomes a multi-layered cluster.

Stage 4. Maturation II: The biofilm continues to grow and become three-dimensional. As the biofilm matures it is able to provide protection against the host immune system anti-microbial, disinfectants and antibiotics.

Stage 5. Dispersion: The detachment of the microbial cells is a consequence of certain microbial enzymes that tear down the extra polymeric matrix resulting in the dispersal of the microbial cells and enables the microbes to colonize new surfaces.

The ability to colonize and invade the bovine mammary epithelium helps bacteria evade the immune response and infect persistently. The bacteria exhibiting greater motility also have greater virulence. The extracellular polymer matrix of biofilm-forming bacteria makes them more resistant to various chemical substances with antimicrobial activity produced by cells, as well as protecting them from the innate immune system. Adhesion is the first step in the formation of biofilm or the invasion of host cells which protects the bacteria from the host immune system and facilitates chronic infection. Adhesion is dependent on surface proteins called adhesions which help the bacterium to recognize and attach to host cells. Bacteria are coated with a wide variety of surface proteins that help them to adhere to host cells and extracellular matrix components. Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) of the host are the most common surface proteins that are involved in adhesion . The ability to bind to host tissue or the host's cell surface is a pivotal part of the bacteria's pathogenicity because adhesion is typically the first step in the invasion and biofilm formation.

The fermentation by microorganisms in infected mammary gland makes that concentrations of lactose and glucose achieve lower levels than normal. As bacteria breaks down the lactose into galactose and glucose in raw milk and this last is the main carbohydrate used by this bacteria, lower levels of glucose could provide a stressful environment to the bacteria which could result in increased production of biofilms during specific phases of the disease.

Biofilm in milking equipment of dairy cows: Bacterial adhesion to surfaces and the formation of biofilms in dairy processing equipment are the main source of contamination of dairy products and transmissions of the disease. Biofilm-forming bacteria can colonize the surfaces of equipment in the dairy industry, may adversely affect the safety and quality of the milk and its products. Biofilm-forming bacteria are known to be a major source of both spoilage and pathogenic micro flora in the dairy industry. Therefore, bacteria that form biofilms may adversely affect the safety and quality of milk and its products. The main source of contamination of dairy products is often associated with the formation of biofilms on the surfaces of milk transport pipes, milking containers, milking machines and accessories in the dairy industries.

Despite numerous efforts to combat biofilm formation, there is still no effective technological means to thoroughly solve the biofilm problem in the dairy industry. Here, we introduced peptide-based coating in order to modify the physical properties of the stainless steel surface by affecting its availability for bacterial adhesion. We found that the coated surface displays a notable decrease in the ability of bacterial cells to attach. This peptide, DOPA-Phe (4F)-Phe (4F)-OMe, consists of 3, 4-dihydroxy-L-phenylalanine (L-DOPA), which can adhere to various surfaces and two amino acids of phenylalanine with fluorinated residues, which direct their self-assembly onto a surface and alter the surface properties, thus preventing the attachment of the proteins and bacteria. In the dairy industry, CIP (cleaning in place) systems generally involve the sequential use of caustic (sodium hydroxide) and acid (nitric acid) wash steps, and chemicals originally selected for their ability to remove organic (proteins and fat) and inorganic (calcium phosphate and other minerals) fouling layers. To enhance cleaning

effectiveness, caustic detergents and caustic additives have been developed, which contain surfactants, emulsifying agents, chelating compounds, and complexing agents.

Pathogenic mechanisms of biofilm forming bacteria in bovine mastitis: Diseases caused by bacterial biofilms are associated with the formation of biofilms on the surface of diseased tissues and the continuous release of free bacteria, causing persistent infection and chronic inflammation. The structural characteristics and properties of bacterial biofilms make them less susceptible than planktonic bacteria to antibiotics and the body's immune system. Under simulated physiological conditions, leukocytes can be attached to mature biofilms and produce cytokines. Neutrophils accumulated on biofilms, even though they still have phagocytic effects, cannot migrate from the contact points, which may lead to the release of oxidants to damage the host immune defense mechanism. Necrotic neutrophils can also act as a biological matrix that promotes the formation of bacterial biofilm. These factors will increase the body's inflammatory response.

Biofilm formation is a dynamic process, rendering possible the shedding of planktonic cells and is 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. Biofilm formation and Intracellular survival in mammary epithelial cells are the main mechanisms of pathogenicity. Various virulence genes expression (Table 2) is one of the factors responsible for pathogenicity by enhancing adherence to specific surface and neighboring cells and extracellular matrix (Table 3).

S/N	Types of bacteria	% of biofilm formed	Research area	Diagnostic methods	Reference
1	*S. aureus*	74	Brazil	Molecular method	26
2		40	Brazil	CRA	27
3		41.66	Algeria	Molecular method	28
4	CNS (*S.Epidermidis*)	5	Brazil	CRA	26
5	*E. Coli*	66.7	Ethiopia (hawassa)	Laser scanning microscope	28
6	*E. Coli*	55	Brazil	Laser scanning microscope	28
7	*Enterococcus*	60	Ethiopia	Laser scanning microscope	
8	*Klebsella*	46.7	Ethiopia	Laser scanning microscope	

Table 2. Prevalence of biofilm forming bacteria from bovine mastitis.

Bacterial species	Virulence genes	Purpose of the gene in biofilm	Reference
S. aureas	Ica	Intracellular adhesions	47
	Bap	Biofilm associated protein, promote adhesions, increase invasion into mammary gland epithelial cells.	
	Agr	Accessary gene regulator, Detachment of biofilm and colonization of new sites	
CNS	Eno	Encode laminin binding protein, involve in bacterial adhesions to host tissue	46
E.coli	LpfA	Encodes long polar fimbriae,	48
	Curli gene	Encodes curli fibers, proteinaceous extracellular fibers are involved in surface and cell–cell contacts that promote community behavior and host colonization.	
	Fim H	Responsible for type 1 fimbriae which is important in biofilm formation	
E.faecalis	Esp	Specific cell surface protein of *E.faecalis* in biofilm	20

S.uberis	Lbp	Host gene induce biofilm formation in *S.uberis*	50
S.dysag	Bca	Encoding protein induction of protective immunity	51
	ScpB	Fibronectin binding protein	
	Lmb	Laminar binding protein used for invasion into host epithelium	
	HylB	Encoding hyaluronidase for cleaving hyaluronic acid used invade host tissue	

Table 3. Several virulence genes involved in biofilm forming microbial in pathogenesis of bovine mastitis.

Biofilm and antibiotic resistance in bovine mastitis

Bacteria in a biofilm are more resistant to antibiotics than planktonic bacteria. It has been estimated that biofilm cells can be up to 1000 times more resistant to antibiotics than planktonic cells. It is highly probable that multiple factors work together to protect biofilm cells from antibiotic treatment.

The *Exopolysaccharide* matrix (EPS) prevents the penetration of antibiotic inside the biofilm. Charged polysaccharides and DNA of the matrix can trap several kinds of antibiotics. Due to slow growth rate there is limited availability of oxygen and nutrients inside biofilms, so biofilm cells, especially those in the deep layers.

These features make biofilm bacteria insensitive to antibiotic that target dividing cells. For example, the targets of β-lactams are dividing cells, so when they are used on *E. coli* biofilms, their bacteriolytic activity is diminished.

In biofilms, there is a small subpopulation of cells called persister cells. Their growth rate is zero or extremely slow. Most of the antibiotics acts at this stage of microbial cell growth or division are not effective against persister cells. Efflux pumps are another important factor, which allow bacteriasl cells to pump intracellular toxins out, including antibiotic drugs. Efflux pumps are also expressed in planktonic cells, but some efflux pump genes are up regulated in biofilm indicating that they contribute to antibiotic resistance.

Plasmid borne antibiotic resistance could also be possible in single-species or multi-species biofilm by horizontal gene transfer. In biofilms, the frequencies of horizontal plasmid transfer are much higher than between planktonic cells. Studies on *S. aureas* biofilms showed that biofilms promote the spread of plasmid-borne antibiotic resistance genes by conjugation/mobilization.

The majority of intramammary infections are treated with antibiotic agents. Biofilms protect microorganisms against these antibiotic agents, phagocytosis. The ability of bacteria to produce biofilms leads to difficulties with pathogen elimination, which can give rise to persistent infections. Resistance to antimicrobial agents results, amongst other factors, from the retardation of antibiotic diffusion through the biofilm matrix, an increased rate of mutation, the production of enzymes that degrade antibiotics, the presence of dormant bacterial cells with low metabolic activity and increased doubling times in the inner layers of biofilms.

Diagnosis of biofilm forming bacteria in bovine mastitis

There are various methods to detect biofilm production but Congo Red Agar (CRA) and Tube Method (TM) are routinely used in clinical laboratory for determining ability of bacteria to form biofilm because of their specificity, low operational cost and availability of materials.

The Congo Red Agar (CRA) culture and micro plate adhesion tests are used to detect biofilm production by bacteria. The former is used to determine the phenotype based on the color of the colonies that grow and the latter is used to quantify components of the biofilm matrix. Quantitation of extracellular proteins and polysaccharides can be used to characterize the composition of the biofilm matrix, as the concentration of proteins and polysaccharides represent two factors that can be used to evaluate the virulence of each strain.

Congo Red Agar (CRA) method: Congo Red Agar (CRA) method that is a qualitative assay for detection of biofilm producer microorganism, as a result of color change of colonies inoculated on CRA medium. The CRA medium is constructed by mixing 0.8 g of Congo red and 36 g of sucrose to 37 g/L of Brain Heart Infusion (BHI) agar. After incubation period that was 24 h at 37°C, morphology of colonies that undergone to different colors is differentiated as biofilm producers or not. Black colonies with a dry crystalline consistency indicate biofilm producers, whereas colonies retained pink are non-biofilm producers. A colour change from red to black is seen in response to the EPS slime layer produced by the biofilm forming isolates.

Tube method (TM): Tube Method (TM) that is a qualitative assay for detection of biofilm producer microorganism, as a result of the occurrence of visible film. Isolates are inoculated in polystyrene test tube which contained TSB and incubated at 24 h at 37°C. The sessile isolates of which biofilms formed on the walls of polystyrene test tube are stained with safranine for 1 h, after planktonic cells are discharged by rinsing twice with Phosphate Buffered Saline (PBS). Then, safranine-stained polystyrene test tube is rinsed twice with PBS to discharge stain. After air drying of test tube process, the occurrence of visible film lined the walls, and the bottom of the tube indicates biofilm production.

The molecular diagnosis of biofilm in bovine mastitis: Molecular methods can be used for two purposes in the diagnosis of biofilm. The first is to identify the microorganisms in the biofilm via molecular target genes. Once the microorganism is known, the second purpose is to demonstrate the genes responsible for biofilm formation, adhesion, and slime production. Molecular method may be an amplification-based method with a single target, or a DNA array system that targets

gene under and overexpression in the biofilm. Genes responsible for adhesion vary depending on the species of microorganism. When planning a study of biofilm diagnosis, it is recommended to choose the biofilm genes and check the target gene sequence in a gene bank before designing primers. A molecular study can be performed after separating each microorganism in the biofilm mass or the target genes can be studied after directly isolating biofilm DNA/RNA before dispersal.

Treatment of biofilm associated bovine mastitis

Since bacterial biofilms comprises of mixed population of pathogens rather than a single species, it would be difficult to design a compound effective against a mixed population of bacterial biofilms. The bacterial biofilms are considered to be resilient and are persistent which makes them to withstand the conventional methods of treatment and this in turn has changed the perspective of research in order to develop innovative options of counteracting the biofilm infections that are promising therapy. If the microorganism responsible for a biofilm infection has been isolated, prevention can be achieved by early preemptive antibiotic treatment before the onset of clinical signs or symptoms.

Bacteriophages for treatment of biofilm in bovine mastitis: Phage and phage derived lysin products have recognized potential for narrow spectrum antibacterial therapy. Bacteriophages are used to treat chronic bacterial infections which are often thought to be associated with bacterial biofilms by inducing depolymerization of EPS. The unique selectivity of this method depends on the orientation of bacteriophages. Phage moves through the water channels in biofilms and reach the bacteria in the deepest biofilm layer. Impediments to phage penetration into the biofilms, perhaps creating refuges from phage attack, likely include the biofilm matrix, i.e., EPS. If EPS is not immediately produced by replicating biofilm bacteria, the EPS surrounding recently split bacteria may be more susceptible to penetration by phage virions because of the newer and peripheral EPS being purer, less complex, less dense, or thinner. Phage tails, to the extent that they display smaller diameters than phage virions as a whole, may contribute to nonenzymatic virion translocation into the EPS, perhaps with longer and narrower tails permitting deeper or faster local penetration to biofilm surface-located bacteria.

Quorum sensing system inhibitors in biofilm of bovine mastitis: New antibiotics and novel treatments for biofilm Studies are ongoing to develop new antibiofilm drugs that will be effective antibiotic therapies against both the biofilm and planktonic cells. New drugs that combine “Quorum-sensing” inhibitors (e.g., antibiofilm substances such as lactonase, patulin, penicillic acid, baicalin hydrate) with antibiotics (e.g., tobramycin, gentamicin, and ciprofloxacin) are promising. There are also ongoing studies about anti-inflammatory treatment approaches for tissue damage surrounding the biofilm infection due to the anti-inflammatory host response, drugs that disperse or degrade the biofilm matrix, and enzyme chelators or biofilm components. It prevents the secretion of protein effectors, destroys QS, inhibits biofilm formation, blocks signaling systems associated with virulence factor gene expression, and attenuates infection by blocking bacterial virulence.

Monoclonal antibodies in treatment biofilm in bovine mastitis: An antibody is an immune protein with high affinity and specificity for foreign invading pathogens, and it is used as an adaptor molecule to recruit various immune cells with effector functions and plays an important role in humoral immunity. Antibodies inhibit bacterial biofilms by interfering with the formation of biofilms or by promoting the disintegration of established biofilms. In many cases, natural antibodies are not sufficient to eliminate mature biofilms, so vaccination with active or passive vaccines is used to enhance the antibody response and is now one of the more aggressive approaches to biofilm infections. The unlimited number of monoclonal antibodies produced by hybridoma technology with a single antigen-binding specificity targets infections caused by biofilms.

Prevention and control biofilm forming bacteria in bovine mastitis

The release of cells from biofilms formed in the alveoli in the mammary gland allows for the contamination of other healthy mammary glands, as well as the contamination of equipment including insufflators and hoses. In light of this, it is essential that all equipment that comes into contact with the animals be properly cleaned and disinfected, particularly the milking machine sets. Control measures mainly rely on detailed screening and inspection of dairy farms, evaluation of welfare plans and the use of prognostic diagnostic tests. These monitoring actions can identify animals at risk and the spread of disease can also be controlled. Prevention can be achieved by early preemptive antibiotic treatment before the onset of clinical signs or symptoms. Major mastitis pathogens have an ability to form a biofilm which makes them more resistant and difficult to control by causing recurrent infections. In-depth knowledge of biofilm formation and control mechanisms could lead to better control.

Discussion

Ideally, preventing biofilm formation would be a more logical option than treating it. The main strategy to prevent biofilm formation is to clean and disinfect regularly before bacteria attach firmly to surfaces. The cleaning process can remove 90% or more of microorganisms associated with the surface, but cannot be relied upon to kill them. Bacteria can redeposit at other locations and given time, water and nutrients can form a biofilm. Therefore, disinfection must be implemented. Also prevention can be achieved by early preemptive antibiotic treatment before the onset of clinical signs or symptoms.

Conclusion and Recommendations

The rapid emergence and exhibition of multidrug resistance as well as their great tendency to cause persistent, chronic and recurrent infections of bovine mastitis make the disease a continuous challenge and a subject of investigation by several research groups justifying the continued attention in in dairy farm. Independently from the origin of the infection, biofilms in dairy industry have been shown to be important in pathogenicity and therefore may play a role in the biology of recurrent udder infections, antimicrobial agents/host immune defense system resistance, being consequently more difficult to control/eradicate the disease from dairy farm. Disinfection and early preemptive antibiotic treatment must be implemented for

prevention before the onset of clinical signs or symptoms. It is known that the much investigation is required to specify the pathogenic mechanisms of biofilm forming bacteria and way to control biofilm. 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.

So based on this the following recommendations are indicated:

- The research gap regarding biofilm forming bacteria in bovine mastitis requires special attention, especially in Ethiopia to divert the problems of biofilm in dairy industry.
- Specific and clear strategies to control the biofilm formation in dairy farm still require further studies.
- Regular monitoring the health of the udder and their sanitation must be enhanced to promote the prevention of the problem of biofilm forming microbials in bovine mastitis.
- Awareness creation is crucial strategy to divert such chronic diseases in dairy farm to promote the productivity of lactating cows.

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