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Control of Bovine Mastitis: Old and Recent Therapeutic Approaches

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Abstract Mastitis is defined as the inflammatory response resulting of the infection of the udder tissue and it is reported in numerous species, namely in domestic dairy animals. This pathology is the most frequent disease of dairy cattle and can be potentially fatal. Mastitis is an economically important pathology associated with reduced milk production, changes in milk composition and quality, being considered one of the most costly to dairy industry. Therefore, the majority of research in the field has focused on control of bovine mastitis and many efforts are being made for the development of new and effective anti-mastitis drugs. Antibiotic treatment is an established component of mastitis control programs; however, the continuous search for new therapeutic alternatives, effective in the control and treatment of bovine mastitis, is urgent. This review will provide an overview of some conventional and emerging approaches in the management of bovine mastitis' infections.

Introduction

Bovine mastitis is the most costly pathology concerning dairy industry. The economic damages resulting of these infections are related with direct and indirect losses. The direct losses include the costs of treatment, discarded milk, herdsman's time, fatalities, and the costs associated with repeated cases of mastitis. The indirect ones include the decreased milk production, decreased milk quality, increased culling, loss of premiums, pre-term drying-off, animal welfare aspects, and other associated health problems [35]. Numerous pathogens are responsible for mastitis with the majority of infections caused by the staphylococci, streptococci, and enterobacteria. There are two types of mastitis, clinical and subclinical infections, and depending on the primary reservoir and mode of transmission this pathology can be contagious or environmental. The main contagious microorganisms are *Staphylococcus aureus* and *Streptococcus* agalactiae, 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. *Streptococcus uberis*, *Escherichia coli*, and *Klebsiella* spp. are examples of microorganisms included in this group [8]. Due to the predominance of infectious mastitis and to the importance of the use of antimicrobial agents in the treatment and control of this pathology, this microbial infection continues to deserve the attention of several researchers and then continues to be a subject of investigation by several groups. Therefore, this review will discuss and focus on old and novel anti-mastitis strategies that have recently been explored.

Antibiotics

As referred, in the dairy industry, mastitis infections are the main cause of the usage of antibiotics. Nowadays, antibiotic therapy (such as natural or synthetic penicillins) is the main strategy for mastitis treatment. However, beyond the costs of its use, the emergence of resistance and the non-responsiveness of cattle to antibiotics has become a critical issue. The overuse and misuse of antibiotics in bovine mastitis may represent a serious problem related with the emergence of resistance and the entrance of resistant

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bacteria in food chain [49]. Taking this into account, antibiotic administration is strictly controlled by veterinarians in the Nordic countries [46]. In addition, bacteria, which are able to reside intracellularly, within the mammary gland, and to form abscesses, are more difficult to combat due to the restriction of their contact with antibiotics. This is the case of *S. aureus* where the percentage of cure using currently approved antibiotics (e.g., pirlimycin) is approximately 10–30 %. Bannerman and Wall [3] concluded that therapies available for the treatment of mastitis caused by *S. aureus* remain suboptimal and therefore inefficacious. Moreover, the use of antibiotics has other disadvantages, as for example the presence of their residues in the milk which is used for human consumption and represents a concern to consumers. Therefore, it is urgent for the search of new antimicrobial therapies, namely non-antibiotics alternatives in order to minimize their use in foodborne animals. Moreover, biofilm formation has been seen as one of the main factors contributing to antibiotic resistance and recurrent mastitis infections [33]. Atulya et al. studied the effect of milk composition on biofilm formation of some of the major mastitis pathogens, and described that pH was one of the factors that affected biofilm formation presenting a negative correlation. Once current therapy is based on the use of acidic antibiotics, the authors suggested the administration of pH enhancing agents with the objective of reduce biofilm formation and consequently long-lasting infections [2]. All these limitations presented by the antibiotics urged the search for new antimicrobial agents effective against mastitis pathogens. Some of these alternatives are listed below.

Bacteriophages

Bacteriophage therapy can be a possible alternative to antibiotics in the fight against mastitis infections. Bacteriophages are virus able to infect and kill bacteria [11]. Phages were demonstrated to be determinants as new antimicrobial agents for veterinary applications. Phage K is an anti-staphylococcus phage, with lytic and antimicrobial action and has been used as prophylactic measure in infections caused by *S. aureus*. However, to be used as therapeutic strategy for mastitis it must be active into the bovine mammary gland and when in contact with raw milk. This constitutes a disadvantage of phage K that was inhibited by natural milk and udder secretions [31]. Gill et al. [20] also tested the efficacy of phage K against *S. aureus*. Once again, phage therapy presented several limitations such as degradation/inactivation of phage by milk and its components (whey proteins) and by the immune system. Although the results were presented by the previous studies, some researchers defend that bacteriophage therapy might be a valid alternative weapon against mastitis' pathogens. However, they also defend that more investigations need to be done namely, in terms of pharmaco-kinetics and pharmaco-dynamics, as well as studies concerning the administration of phages into intramammary tissues, aiming to "exploit phages to their full potential" [4]. Kwiatek et al. isolated a new virulent phage (MSA6) from a cow with mastitis, which presented a wide lytic spectrum against staphylococcal strains of bovine origin. This polyvalent nature of MSA6 is crucial for their potential use as universal anti-staphylococcal agent. Moreover, taking into consideration several features presented by MSA6, this phage is similar to others formerly used with success in bacteriophage therapy [27]. Everything indicates its possible use in several staphylococci infections including bovine mastitis. Dias et al. [15] isolated that bacteriophages able to infect *S. aureus* from mastitis-positive cows. These phages presented several features critical to their use as phage therapy, namely wide range of host, high lytic activity, and thermostability. In addition, Fenton et al. [18] tested bacteriophage-derived peptidase; $CHAP_K$ against *S. aureus* isolated from mastitis-infected cows. This study demonstrated the potential use of $CHAP_K$ as prophylactic and therapeutic measures of mastitis infections. $CHAP_K$ was effective against biofilms either by preventing biofilm formation or by disrupting established biofilms of staphylococci strains associated with bovine mastitis. Basdew and Laing [5] tested some phages sensitivity to several simulated stresses. Sabp-P1, Sabp-P2, and Sabp-P3 were the less sensitive to the stresses tested in vitro and therefore demonstrated to be the favorites to future in vivo experiments and to future use as therapeutic strategy to treat mastitis-infected cows. Although still with some limitations, in general, bacteriophage therapy seems to be a serious candidate to antibiotic alternatives to be used in the future control and treatment of bovine mastitis.

Vaccination

During decades an effective vaccine for the prevention of bovine mastitis was searched. However, the "miraculous" vaccine remains elusive. Some vaccines were developed against *S. aureus* mastitis but all presented limited efficacy. The improper immunization schedules, ineffective adjuvant formulation, and the limited range of protection are some of the causes of their ineffectiveness. This last point is crucial for the formulation of vaccines. It is important to find a vaccine capable of protecting against a wide range of strains since multiple strains can be present within a herd and within an individual cow [3]. Due to its endemic nature, the large number of microorganisms that can cause the disease, and the ubiquity of these pathogens, bovine mastitis cannot be completely eradicated. Therefore, different

strategies/vaccines are necessary in different countries in order to respond to the specific requirement of an individual country or segment of the dairy industry. Nowadays, vaccination became an important area in mastitis control strategies and the implementation of an effective control protocol is becoming urgent [4]. Some vaccines for *S. aureus* were tested but their effect depends of several factors such as the type of vaccine used, the age of the cow, and environmental conditions [23, 34]. As example, bacterins made from killed bacteria were not effective in the prevention of new infections of *S. aureus*. When using a toxoid as bacterin adjuvant, a higher protective effect was observed. DNA and recombinant protein vaccines were developed in alternative to conventional bacterin and as antibiotic therapy and proved to be active in protecting mammary glands against *S. aureus* infections [41, 43, 51]. Seegers et al. [39] demonstrated that when *E. coli* vaccination was used in the case of environmental mastitis a higher annual benefit per cow was achieved in comparison with a non-vaccine approach. Moreover, already in the 90s, DeGraves and Fetrow [14] also drew attention to the possible reduction in the losses per cow after immunization. *E. coli* J5 is one existing vaccine for coliform mastitis and is composed of whole antigens derived from J5 mutant strain of *E. coli*. This vaccine showed some efficacy and proved to be able to reduce the number and severity of coliform mastitis by 70–80 % [12, 50]. There are no vaccines available to prevent mastitis caused by *K. pneumoniae* [44]. The mechanisms inherent to the spontaneous cure that occurs in mild cases of subclinical mastitis can be exploited to the production of new vaccines [36, 37] and therefore the research in this area continues.

Nanoparticles

Over the last few years, nanoparticles drew attention of some investigation groups since these structures can be used as delivery vehicles for antimicrobial agents. Several researchers demonstrated that nanoparticles can have potential use in biomedical applications, namely on bovine mastitis infections. As example, silver nanoparticles showed to inhibit *S. aureus* isolated from subclinical mastitis [13] and to be ideal for a highly cost-effective antimicrobial solution. The synergistic effect of silver nanoparticles and antibiotics was also evaluated, and a successful combination was obtained using antibiotics that inhibit protein translation, such as erythromycin, in combination with silver nanoparticles against *S. aureus* [24]. On the other hand, Wang et al. [48] showed the potential of tilmicosin-solid lipid nanoparticles against *S. aureus*. Xuefeng [47] also reported amoxicillin nanoparticles as biologically active against *S. aureus*, *E. coli*, and *S. agalactiae*. Cardozo et al. demonstrated that NO (nitric oxide)-nanoparticles were able to inhibit *S. aureus*, and in general could be used in the treatment and prevention of bovine mastitis. In addition to the low price, safety NO-nanoparticles can be an alternative to overcome the problem of bacterial resistance development [10]. Berni et al. [7] tested violacein nanoparticles against bovine mastitis. Violacein was considered a powerful antibactericidal agent and its nanoparticles were more efficient against *S. aureus* than the free agent. In conclusion, antimicrobial agents encapsulated in nanoparticles and consequently the controlled drug delivery are among the potential strategies to control and prevent bovine mastitis infections.

Cytokines

The emergence of antibiotic resistance has prompted the investigation of the immunotherapeutic use of recombinant cytokines in the treatment of bovine mastitis. Cytokines are small proteins that have an important role in cell signaling. Some cytokines (recombinant bovine cytokines, such as IL-2, IFN-c, and TNF-a) demonstrated to be responsible for stimulating innate and acquired immunity in mammary gland. However, this enhanced immunity was not enough to prevent or treat bovine mastitis. Nevertheless, when combined with antibiotics, there was an additive effect being possible their use as adjuvant mastitis therapy [1].

Natural Compounds

Plant-Derived Antimicrobials

As mentioned before, there is an urgent need for alternatives to antibiotics for controlling bovine mastitis. Plants are promising sources of new biologically active agents with antimicrobial action. Moreover, plant-derived drugs have the advantage of not inducing resistance after prolonged exposure [16, 32]. Some examples of plant-derived natural compounds are diterpenes. Fonseca et al. tested three diterpenes: manool, ent-kaurenoic acid, and ent-copalic acid against several bovine mastitis pathogens. Ent-copalic acid (CA) was the most active against most of the microorganisms tested. Besides its antibacterial potential, CA did not to cause a cytotoxic effect on human fibroblast cell line, so this fact encourages its possible use on bovine mastitis control and treatment [19]. Baskaran et al. [6] also investigated the antimicrobial activity of plant-derived molecules on a wide array of bacterial mastitis pathogens in milk, namely, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *S. aureus*, and *E. coli*. This in vitro study demonstrated that all plant-derived molecules (*trans*-cinnamaldehyde (TC), eugenol, carvacrol, and thymol) were effective against all microorganisms tested being TC the most effective. Since it was

classified as safe by the United States Food and Drug Administration and presented encouraging results against the major bacterial mastitis pathogens, it was suggested by Baskaran et al. [6] as a potential alternative/adjuvant to antibiotics in the prevention and treatment of bovine mastitis. Additionally, ethanolic extracts of propolis (EEP), which are a resinous mixture collected by honeybees from parts of plants, were tested against *S. aureus*. These extracts were biologically active against this mastitis pathogen. However, when tested in cells cultivated in milk, a lower inhibitory effect was observed, concluding the authors that milk component affect the antimicrobial activity of EEP. Nevertheless, given their results and taking into consideration that propolis-resistance appears to be a phenotype not easily selected, the authors suggested that EEP might be a potential antimicrobial agent for mastitis control in vivo [38]. Khayatnouri and Topchi [25] tested the effect of monolaurin, a food grade glycerol monoester of lauric acid found in coconut oil, on *S. aureus* isolates from bovine mastitis. Once again this compound demonstrated antibacterial effect against *S. aureus*. Gopinath et al. [21] tested the effect of *Tabernaemontana divaricata* (L.) extracts on a panel of microorganisms responsible for bovine mastitis. A significant antibacterial effect was observed on all bacteria studied. This preliminary research drew the attention to the possible use of natural compounds from *Tabernaemontana divaricata* (L.) as anti-mastitis drug. In vitro tests conducted by Tolosa et al. [45] demonstrated that crude extracts of two medicinal plants, *Combertum molle* and *Commicarpus pedenculosus*, showed to have a good antimicrobial effect against *S. aureus* isolated from bovine clinical mastitis.

Moreover, extracts from twenty medicinal plants were tested by Doss et al. against a wide array of infectious agents responsible for bovine mastitis. These extracts were active against the microorganisms tested [17, 30]. *Centella asiatica* is another plant with a wide array of antimicrobial agents such as triterpenes. Crude extracts of this plant were tested against *S. aureus* isolated from mastitis-infected cows. Ethanol extracts demonstrated to be biologically active against the strains tested. Although the authors suggest more studies namely in vivo, they drew attention of the potential use of these compounds and consequently of this plant as future alternative of antibiotics [42]. Plant antimicrobial peptides (PAP) (γ-thionin and thionin Thi2.1), produced by bovine endothelial cells, were tested on intracellular *S. aureus*. These antimicrobial peptides showed to be effective against this pathogen and therefore seem to be an attractive and potential antimicrobial approach to prevention and treatment of intracellular infections, namely intracellular *S. aureus* bovine mastitis. Additionally to their antimicrobial activity, γ-thionin and thionin Thi2.1, works as immunomodulators [29]. In general, all these studies showed positive results when testing herbal extracts on mastitis-causing pathogens and allowed to suggest the potential use of several plants as a sustainable alternative treatment to replace antibiotics.

Animal-Derived Antimicrobials

The use of immunomodulators, naturally produced by mammals, such as lactoferrin, was appointed as potential non-antibiotic antimicrobial agents for treatment and prevention of bovine mastitis. Lactoferrin is a glycoprotein found in several body secretions such as saliva, tears, bronqueal mucus, and milk. This molecule exhibited an antibacterial effect against some major mastitis-causing pathogens namely *E. coli*, *S. aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *K. pneumonia* [26]. Hafez et al. also isolated lactoferrin from bovine milk whey and tested its antimicrobial potential against *S. aureus*, *E. coli*, *S. agalactiae*, and *P. aeruginosa*. Lactoferrin showed a significant inhibitory effect against all isolates tested being more effective against *E. coli* and less effective against *S. aureus* [22]. In general, their results are in accordance with the work of Kutila et al. [26] and also suggest the possible use of lactoferrin as antimicrobial and immunomodulator agent. β-Lactoglobulin is a protein present in the whey of most mammals. When tested against several mastitis-causing pathogens this protein showed an inhibitory effect on *S. aureus* and *S. uberis*. When tested in combination with lactoferrin an additive effect was observed against *S. aureus*. In addition to the increased combined effect of these two proteins, the fact that they have different effect on different bacteria can enlarge/complement the spectrum of antimicrobial activity. In addition, marine sponges are described as a source of new antimicrobial agents. Extracts of these sessile aquatic animals presented inhibitory activity against several species of coagulase-negative staphylococci (CNS) isolated from bovine mastitis cases. Among the marine sponges tested, *Cinachyrella* sp., *Haliclona* sp., and *Petromica citrine* were active against 61 % of the microorganisms studied, being the extracts of *P. citrine* the most effective against CNS strains [28].

Bacteria and Bacteria-Derived Antimicrobials

Natural compounds produced by bacteria and presenting antimicrobial action are also suggested by several researchers as potential option for alternative antibiotics for the control and treatment of bovine mastitis. *Weisella confusa* (lactic acid bacteria) and its metabolites demonstrated to be active against *S. aureus* and *S. agalactiae* [40]. *S. aureus* is one of the most common etiological agents in mastitis of cows. The success rate of antibiotic treatment is

low due to the ability of *S. aureus* to penetrate the mammary gland tissue and form abscesses. Taking this into consideration, Bouchard et al. [9] tested live *lactobacillus casei* as mammary probiotics. In fact, these bacteria were able to prevent the internalization of *S. aureus* into mammary epithelial cells and therefore are appointed as possible strategy to reduce chronicity and recurrence of *S. aureus* mastitis infections.

Conclusions

Currently, the administration of antibiotics is the most common method of treatment of bovine mastitis. However, this kind of strategy has some disadvantages including low cure rate, increasing occurrence of resistance, and the presence of antibiotics residues in the milk. Therefore, a wide array of alternatives to antibiotics was investigated by several groups of researchers in order to find an effective approach for management of bovine mastitis. Bacteriophages, vaccines, nanoparticles, cytokines, and natural compounds from plants, animals, and bacteria are some examples of valid substitutes to antibiotics. *In vitro* studies showed encouraging results but more tests, namely in vivo, are still critical.

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Compliance with Ethical Standards

Conflicts of interest None.

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