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Role of nanotechnology in mastitis treatment for dairy cows: a mini review

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Abstract

Mastitis is among the most popular ailments in dairy cow that results in the greatest financial losses for dairy farms. Different strains of bacteria, fungus, and algae are responsible for causing mastitis. Mostly this disease is typically caused by bacteria that grow in the udder and lymph nodes, causing damage to the udder parenchyma. Antibiotics are the primary method of treatment for mastitis. However, long-term use of antibiotics has caused pathogens that cause mastitis to become more resistant to antibiotics. As a result, various strategies for eliminating pathogenic bacteria that cause mastitis are being researched. Researchers have looked for alternative therapeutic modalities to antibiotics for the treatment of mastitis. These efforts are helped by the innovative invention of nanotechnology for mastitis therapy and management. As a result, nanotechnology may become the primary type of mastitis treatment in the near future. *In vitro-In vivo* research of alternative remedy for mastitis have indicated that many microorganisms responsible for this disease can be successfully inhibited and destroyed. The current review article discusses different nanoparticles for mastitis treatment in dairy cow herds.

1. Introduction

Animals with mastitis suffer from an inflammatory reaction to the udder tissue in their mammary glands, which can result from physical trauma or infection caused by microorganisms. This is the most common disease that leads to dairy companies money losses because of lower output and poor milk quality (Cheng and Han 2020). On dairy farms, 60-70% of antimicrobials used treat and prevent mastitis (Stevens et al. 2016). As a result of bovine mastitis, the entire failure cost per cow is estimated at $147 per year, with culling and milk production losses representing 11 to 18% of gross margin per cow (Hogeveen et al. 2019). Around 70% of total losses are caused by mammary tissue injury (Sharma et al. 2018). Different breeds of cattle are reared in different places, and different lactation stages, parity, ages, geographic locations, weather conditions, farm management system, and previous episodes could contribute to the differences in the prevalence and distribution of mastitis (Krishnamoorthy et al. 2021).

The Bovine mastitis can be categorised in three groups based on the inflammation degree: clinical, sub-clinical, and chronic. The clinical mastitis in dairy cows is easily diagnosed and is identified by visual abnormalities like red and swollen udder and fever. There are flakes and clots that appear in watery cow milk (Argaw 2016). On the other hand, mastitis in the subclinical stage is unobservable and characterised by a sudden increase in milk somatic cell counts with no visible abnormalities in milk or udders. Chronic mastitis occurs in irregular intervals over several months, with an inflammatory process lasting several months (Pal et al. 2019). Most scientists consider that subclinical mastitis has a greater economic impact when compared to clinical mastitis. Chiefly, the overall prevalence for subclinical mastitis was reported to be 59.43% (Singh et al. 2016) with its quarter level incidence of 34.78% (Bhat et al. 2016). The most frequent pathogens responsible for causing mastitis are bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Enterococcus*, *Staphylococcus epidermidis*, and *Pseudomonas spp.*, as well as fungus such as *Cryptococcus* or *Candida spp.* (Kalinska et al. 2018).

The mainstay of mastitis treatment and prevention is antibiotics, but their effectiveness is decreasing as bacteria become more resistant. It is believed that the issue has been caused by the excessive use of antibiotics in treating milch animals, resulted in the emergence of drug-resistant bacterial strains (Pascu et al. 2022). Many scientists have observed that there is increasing antibiotic resistance of primary mastitis pathogens. Henceforth, in order to treat mastitis, scientists have had to come up with new solutions. Nanomaterial have been employed in human health for both diagnosis and treatment, but their usage in animal health is still comparatively new. However, nanotechnology is a rapidly expanding science that allows for the creation of new materials at the Nano scale level (1 to 100 nm). It has the incredible potential to change the agri-food industry by presenting cutting-edge treatments for common and expensive diseases like bovine mastitis (Neculai-Valeanu et al. 2021). In order to limit pathogen resistance to drugs, many studies are currently being conducted to investigate alternative options to antibiotics in the treatment as well as prevention of animal diseases. The review paper's objective is to describe the current state of knowledge regarding the use of nanotechnology in the treatment and prevention of mastitis in dairy cow.

2. Risk factors of mastitis

The incidence of bovine mastitis is known to be significantly influenced by a numerous risk factors, as well as pathogen-host and environmental factors (Fig. 1). The most frequent cause of bovine mastitis is thought to be bacterial intra-mammary infection. Several species of bacteria have been identified, which potentially causes the bovine mastitis. There are two types of bacterial infections- contagious and environmental based on the source of infection (Lakew et al. 2019). Bovine mastitis transmitted from cow to cow, particularly during milking, is referred to as contagious mastitis. The udder and teat skin of cows harbor infectious pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus*, alongside some uncommon species such as *Mycoplama bovis* (Kibebew 2017). Environmental pathogens, unlike contagious pathogens, live in herd's housing and bedding rather than that of cow's skin and udder. Therefore, these are best illustrated as opportunistic pathogens i.e., continuously looking for opportunity to infect (Nitz et al. 2021). Environmental mastitis has been associated with a wide variety of bacteria species, including *Streptococcus spp.* (such as *Strep. uberis*), coliforms species (such as *E. coli, Klebsiella spp., Enterobacter spp.*), *Pseudomonas spp.*, etc (Williamson et al. 2022). Mastitis susceptibility and resistance are affected by genetic factors and dairy cow breeding. Purebred or crossbred high-yielding cattle, particularly Holstein-Friesian cattle, appear to be genetically more susceptible to mastitis than medium-yielding breeds (Shaheen et al. 2016). The udder's structure also influences susceptibility to infection. Subclinical mastitis is more common in cattle that have large teats or udder with pendulous shapes, as well as in animal that have blind quarters after calving (Persson et al. 2014). Age is another factor that affects infections. Because of the or permanently/ partially-open or wider teat canal brought on by frequent milking, older cows are more prone to infections (Kibebew et al. 2017). During transition period or periparturient period, dairy cows are more likely to contract mastitis (Drackley et al. 1999). The three weeks after and before parturition is known as the 'transition period' or 'peripartum period'.

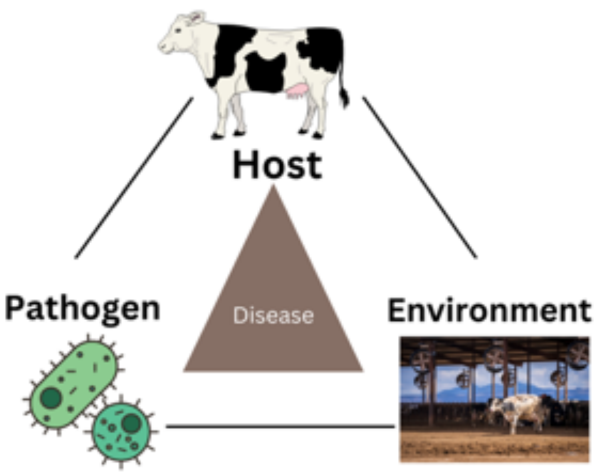

Fig.1 *Risk factors of mastitis*

3. What is nanotechnology?

Nanotechnology is among the most promising technologies of the future and its inevitable and rapid technological advancements have even further increased its applicability and reliability with reduced cost. Nanotechnology is utilised in numerous industries, including agriculture, food, and pharmaceutics. It is used to create nanoparticles with distinctive physical, optical, and mechanical properties from their bulk counterparts. Nanotechnology is being used in medicine for medication delivery, diagnostics, and disease treatment (Beyene and Ghosh 2021). Current area in which nanoparticle technology is being developed as a delivery method for drugs and antimicrobial agents is the application of nanoparticle technology to antibiotics (Gomes and Henriques 2016). Mastitis has already been treated with different types of nanoparticles showing positive consequences (Kalinska et al. 2019; Orellano et al. 2019). By increasing the uptake of active compounds by phagocytes, nanoparticle formulations will improve their antibacterial activity. These drugs are efficient and effective against numerous multi-drug-resistant bacteria, which possess a plausible threat to the society (Castelani et al. 2019).

4. Use of nanotechnology in the diagnosis

Novel nanoscale material manipulation techniques have revolutionised nearly all medical applications, including the detection, treatment, and development of vaccines. The fast development of nanotechnology allows for the creation of sophisticated diagnostic tests for the early and accurate detection of mastitis. One of these main diagnostic tests involves the use of nano-biosensors, a unique class of analytical methods for the detection of mastitis. Biosensor is a device that combines bioreceptors unique to the antigen or chemical being investigated with a physical nano-transducer (Driskell and Tripp 2009). These sensors use electric impulses to detect the presence of certain biological molecules. There are numerous types of biosensors based on the nanoparticle employed, the kind of transductor, system for signaling/ recognition, and the nature of the target molecule (Martins et al. 2019).

5. Nanotechnology in mastitis treatment

The veterinary, pharmaceutical, and medicine industries are increasingly utilising nanoparticles because of their properties. Nanotechnology is one of the world's most quickly advancing scientific area. A unique physical and chemical characteristic of nanomaterials, as well as their vast surface area in comparison to their volume, make them useful for managing infections, such as those that cause mastitis in dairy cow (Algharib et al. 2020). According to the literature, the most commonly used nanoparticles in the eradication of harmful bacteria are silver, copper, and gold nanoparticles (Wernicki et al. 2014; Lange et al. 2021; Neculai-Valeanu et al. 2021).

Silver nanoparticles rupture by denaturing proteins of the cell membranes, resulting in creation of microenvironment that is saturated with silver ions, hindering replication of DNA, generating reactive oxygen species and also expressing respiratory chain enzymes and proteins which are responsible for their bactericidal and fungicidal effects (Xu et al. 2020). Copper nanoparticles can be toxic to pathogens because they produce reactive oxygen compounds and peroxides which destroy the DNA chain, lipids, and proteins (Angele-Martinez et al. 2017). Gold nanoparticles act differently from natural gold in that they alter membrane potential and the rate at which pathogen cells synthesises adenosine triphosphate, ultimately inhibiting metabolism in pathogenic bacteria (Mikhailova 2021). It is mainly the cell wall structure of pathogenic microorganisms that determines the effectiveness of nanoparticle therapy. It has been found that copper nanoparticles are more toxic to Gram-positive bacteria with thicker cell walls, while silver and gold nanoparticles are more toxic to Gram-negative bacteria (Sanchez-Lopez et al. 2020).

5.1 Silver nanoparticles

According to Ali et al. (2021), silver nanoparticles improve the effects of traditional antibiotics against *Staphylococcus aureus,* the causative agent of bovine mastitis. Yu et al. (2018) reported the quercetin nanoparticles a composite material obtained by combination of silver nanoparticles and the plant-derived drug component namely quercetin, exhibited stronger anti-biofilm and antibacterial properties against strain of multi-drug resistant *Escherichia coli*, isolated from a dairy cow suffering with mastitis. Gram negative bacteria are more sensitive to nanoparticles of silver; possessing antimicrobial properties (Dakal et al. 2016). Yuan et al. (2017) also found that the minimum inhibitory concentrations for silver nanoparticles for *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains isolated from mastitis-infected goat milk were 1 and 2g/ml, respectively. A study by Dehkordi et al. (2011) exhibited that at low doses silver nanoparticles killed *Escherichia coli* strains, but were less toxic to *Staphylococcus aureus*. The results of this study depicted that mean time of silver nanoparticle antimicrobial action against *Staphylococcus aureus* is 7 min. The gel formulation containing nano-silver effectively inhibited by *Streptococcus typhi*, *Streptococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Candida yeast-like fungi in a study by Jain et al. (2009). The lowest minimum inhibitory concentration observed for *Escherichia coli* (1.56 g/ml) caused 50% of strains to die, while the highest concentration was observed for *Streptococcus aureus* (6.25 g/ml). In a previous study, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria were found to be most sensitive to silver nanoparticles, while *Streptococcus aureus* bacteria were revealed to be least sensitive. The bacterial strains treated with nanoparticles showed decreased glutathione expression, lower superoxide dismutase, and catalase expression, but increased glutathione S-transferase expression.

5.2 Copper nanoparticles

According to Kalinska et al. (2019), copper nanoparticle could be used to treat bovine mastitis. The findings indicate that commercially available nanoparticles were of high quality and had no toxic effect on mammary gland tissue. Copper nanoparticles also influenced or reduced pathogen viability. It has been demonstrated that using copper oxide nanoparticles can lower levels of harmful microorganisms such methicillin-resistant *Escherichia coli* and *Staphylococcus aureus*. The combination of copper oxide and silver nanoparticles revealed the full elimination of harmful germs (Ren et al. 2009). According to Kruk et al. (2015) studies on monodisperse copper particles revealed that copper nanoparticles had strong antibacterial and antifungal impacts against the Gram-positive bacteria, involving *Staphylococcus aureus* methicillin-resistant strains, comparable to nano-silver, as well as fungicidal activity against *Candida spp*. An extract from *Rheedia brasiliensis* fruit as well as its new copper metal complex was evaluated for its antibacterial potential against *Staphylococcus* strains isolated from bovine mastitis (De Barros et al. 2017). The antibacterial properties of such compounds suggest that they could be used to treat mastitis. Lange et al. (2021) stated that copper nanoparticles prevent the proliferation of pathogen and destroy their membranes such as *Streptococcus agalactiae* and many more. It is possible to enhance the primary antibacterial effect of copper nanoparticles by surface functionalizing them with additives to make them very effective as antimicrobials. Copper nanoparticles have a significant surface-to-volume ratio, making them ideal as antimicrobials (Ingle et al. 2014).

5.3 Gold nanoparticles

Umadevi et al. (2011) found that gold nanoparticles were more useful against Gram-negative bacteria, while Gram-positive bacteria required much larger particles in order to achieve their bactericidal effect. Furthermore, gold nanoparticles inhibited the growth of mastitis causing fungal pathogens (Wani and Ahmad 2013). According to Shamaila et al. (2016), the minimum inhibitory concentration for *E. coli* and *Staphylococcus aureus* were 2.93 g/ml and 3.92 g/ml, respectively, for gold nanoparticles with a size range of 7 to 34 nm. Omara (2017) examined *in-vitro* the effects of gold nanoparticles and honey on vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) that were isolated from cow milk of mastitis udder. He found that at a concentration of 2.56 g/ml, gold nanoparticles with a 30 nm diameter inhibited MRSA and VRSA growth, whereas a combination of nano-gold with honey from citrus fruit had a minimum inhibitory concentration that was approximately 50% lower.

In addition, nanoparticles exhibit their greatest reactivity in a new environment that does not contain other structure or substances, like proteins, bacteria or lipids. In several papers, nanoparticle synthesis and properties have been studied (Wang et al. 2011; Pramanik et al. 2012; Zain et al. 2014; Santhoshkumar et al. 2019). The properties of metal nanoparticles still require further investigation if metal nanoparticles are to be used as mastitis prevention agents in dairy cattle.

6. Role of mastitis disease in public health

There are a number of serious problems associated with clinical and subclinical mastitis, such as causing damage to mammary tissues and reducing milk production, in addition to causing financial loss due to animal health issue; they are also largely responsible for lowering the nutritional value of milk, which is a serious threat to human health (Cobirka et al. 2020). Mastitis affects milk's physical, chemical, bacterial, and organoleptic qualities. Milk lactose and solid non-fat decrease in milk when somatic cell counts rise because of mastitis (Rathaur et al. 2020). However, the magnitude of this shift depends on the source. Mastitis also reduces protein and calcium levels in milk, and injured epithelial cells release peculiar enzymes, which can be used as biomarkers to diagnose the condition (Hussain et al. 2012). Antibiotic residues in milk can induce allergic responses when ingested, however, this can be prevented by discarding milk during antibiotic therapy. Bacterial resistance is constantly evolving as a result of antibiotic usage, and its transmission to people cannot be disregarded (Ashraf and Imran 2020). Humans can develop antimicrobial resistance from mastitic milk obtained from dairy animals treated with antibiotics. Antibiotics must be controlled excessively in order to ensure the safety and quality of milk (Shamila-Syuhada et al. 2016).

7. Conclusions and future prospective

Animal welfare and economic losses are caused by mastitis, which deteriorates milk quality, reduces production performance, increases culling rates, and treatment costs. Both clinical and subclinical forms of the disease can be caused by a variety of microbial organisms. Currently, mastitis control programs still use antibiotics as part of their treatment plan. The effectiveness of antibiotics is not always satisfactory when combined with other therapies. As a result, looking for new therapeutic options is essential. As an alternative to existing therapy products, nanomaterials have great potential for development of new drug formulas. In addition to the diversity of excipients, these new types of formulas are characterised by their particular characteristics, including relative abundance, biocompatibility, biodegradability, non-irritability, innocuity, and low cost. Nanoparticles may serve as treatment agents in bovine mastitis due to their potential uptake by phagocytes. In the future, advanced treatment solutions, such as nanoparticle technology, may provide a solution to the current problem.

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