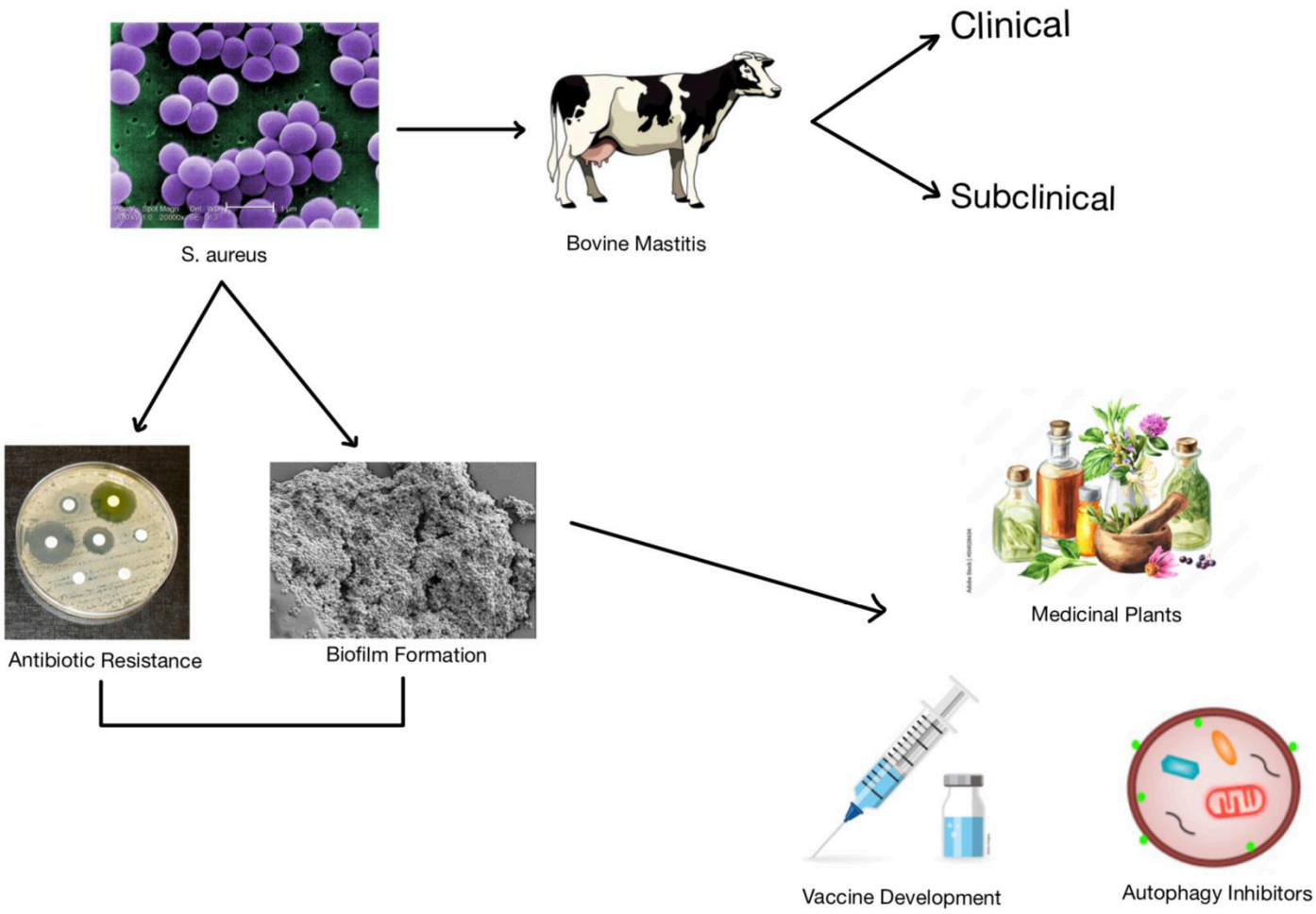
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Staphylococcus aureus Effects on Bovine Mastitis: A Review



Graphical Abstract: *Staphylococcus aureus* is the most common cause of subclinical and clinical bovine mastitis, an infection negatively affecting the dairy industry. The antibiotic resistance and biofilm-forming capabilities of *S. aureus* allow for the bacteria's proliferation and make treatment difficult. Possible treatment methods include vaccines, autophagy inducers, and medicinal herbs (1–7).

Abstract:

Bovine mastitis is an extremely prevalent and pressing issue in the dairy industry. The two types of mastitis include clinical and subclinical. The most common cause of this infection is *Staphylococcus aureus*, which has developed antibiotic resistance and biofilm-forming capabilities. There are a wide variety of lineages and strains of *S. aureus*, each of which cause different symptoms and effects on the cow. *S. aureus* induces autophagy, and replicates in autophagosomes, allowing for its survival. Bovine mastitis is very difficult to treat, but strategies including vaccines, autophagy inducers, and medicinal herbs are currently being tested.

1. Introduction

1.1. Bovine Mastitis

Mastitis is an infection characterized by inflammation of the breast tissue and can affect almost all domestic mammals (8). Bovine mastitis particularly refers to mastitis in cows and is one of the most prevalent and critical diseases affecting the dairy industry today. Mastitis can be caused by a variety of non-infectious and infectious factors, both environmental and pathogenic (8). The infectious causes of mastitis include yeast and fungi, viruses, and bacteria (8). The most common infectious pathogen causing bovine mastitis is the bacteria *Staphylococcus aureus* (8). There are two types of mastitis, clinical and subclinical (8–10). Clinical mastitis causes abnormalities in the composition of milk produced by the cow, including flakes, clots, or watery secretions (8). Clinical mastitis also causes inflammation of the udder, swelling, pain, increased body temperature, reduction in milk yield, and even death of the cow (8,11,12). In contrast, subclinical mastitis does not cause any outward symptoms for the cow and is therefore more difficult to diagnose (11). Subclinical mastitis is primarily characterized by a decrease in somatic cell count such as white blood cells (12) and siginficantly decreased milk production (8,11). Subclinical mastitis is more common, prevails for longer periods of time in cows, and spreads quickly through herds (8), often through the milking process (13), when infected milk touches the milkers' hands or milking equipment before touching a healthy cow (14,15). Bovine mastitis causes immense financial

loss and economic burden on the dairy industry, due to the expenses towards trying to treat the disease as well as the loss of revenue from decreased milk production, and the disease has negative effects on the environment (11). In addition, cows infected with *S. aureus* produce milk contaminated with enterotoxins (toxins produced by *S. aureus* that cause harm to the digestive system), causing the milk to be dangerous and unfit for human consumption (16).

1.2. S. aureus

S. aureus is one of the most common and well-known bacterial pathogens (17). It is a gram-positive bacteria with a spherical shape and a cluster formation (18). *S. aureus* is an opportunistic pathogen, meaning it is often commensal, or healthy, in the body, until the immune system is compromised, after which the bacteria becomes pathogenic, or capable of causing disease (19). The bacteria is present in 20-30% of humans at all times in its commensal form (19,20). *S. aureus* can cause many different levels of infection, including acute, or short-term; recurrent, or repeating again and again; and chronic, or persisting long-term (19). *S. aureus* infections were once able to be treated with antibiotic penicillin, but many strains of *S. aureus* have developed resistance due to their production of penicillinase, an enzyme which breaks down penicillin and interferes with its effectiveness (19,20). Antibiotics called β-lactam were then used to treat *S. aureus*, but this treatment soon led to resistance to these drugs through the development an encoding gene for β-lactamase, an enzyme which breaks down β-lactam and once again renders the antibiotic ineffective (19). Methicillin-resistant *S. aureus* (MRSA) is now widespread (19), and this inability to be eradicated with antibiotics has led to increased death rates in those infected with *S. aureus* (17).

2. *S. aureus* Strains

A bacterial lineage refers to a sequenced representative of a single bacterial genus (21). There are multiple lineages and over one hundred strains of *S. aureus* (22). While many studies regarding the effect of *S. aureus* on bovine mammary epithelial cells choose only one single strain to use while testing their results, these findings might not be entirely accurate due to the differing properties and immune responses

of each strain (23). Being able to distinguish between different lineages and strains and their properties will help determine how the disease is diagnosed and the most effective treatment in each case of the disease (23). Two of the major bacterial lineages associated with mastitis include CC97 and CC151, each of which are comprised of their own strains (23). CC97 strains produce a much stronger immune response compared to the CC151 strains, which display a lesser and delayed immunogenicity (23). The production of cytokines, which are proteins produced by and affecting the immune system (24), and neutrophils, which are white blood cells that help fight infection (25), can also be affected by the bacterial lineages (23). CC97 strains stimulate cytokine production and recruit a greater number of neutrophils than C151 strains (23). The CC97 strains typically cause subclinical mastitis, while CC151 strains cause acute clinical mastitis (23).

The specific strains MOK023 of lineage CC97 and MOK124 of lineage CC151 each cause similar symptoms in cows with mastitis, but to varying degrees of extremity (26). MOK023 typically causes subclincial mastitis, while MOK124 typically causes clinical mastitis (26). In comparison with MOK023, MOK124 results in a more significantly reduced milk yield, higher blood and milk neutrophil concentration, higher concentration of anti-*S. aureus* antibodies, and increased inflammation and udder damage levels (9,26). These differing properties demonstrate the need to perform studies on a wide range of *S. aureus* strains in the future to ensure that we are learning as much as possible about the effects that *S. aureus* has on bovine mastitis.

3. Autophagy

Autophagy is a cellular process that can provide a method for eliminating pathogenic organisms such as *S. aureus* (27). Autophagy is a mechanism in cells that allows for cell components that are no longer needed or are not working properly to be degraded and recycled in an orderly manner (27,28). This process, shown in Figure 1, occurs in all eukaryotic cells and is a highly conserved mechanism which is vital for cell survival (27). Autophagy is prompted to begin when a cell experiences starvation or nutrient deprivation, leading to the formation of a double-membraned structure called a phagophore

(28). This phagophore elongates to form a closed autophagosome encapsulating the cytoplasm and organelles to be broken down (28). The autophagosome is then transported to a lysosome, an organelle responsible for breaking down and digesting cellular material, and the lysosome and autophagosome fuse together (28). This forms an autolysosome, which allows the lysosome to release enzymes into the autophagosome and begin the degradation process (28). This is the most important step which allows autophagy to create biological changes (29). The cellular materials are then degraded into macromolecules such as amino acids, recycled and ready to be reused by the cell (28). The process is regulated by autophagy-related genes (ATGs), which form a ubiquitin-like system, serving to regulate and stabilize target proteins (27,28).

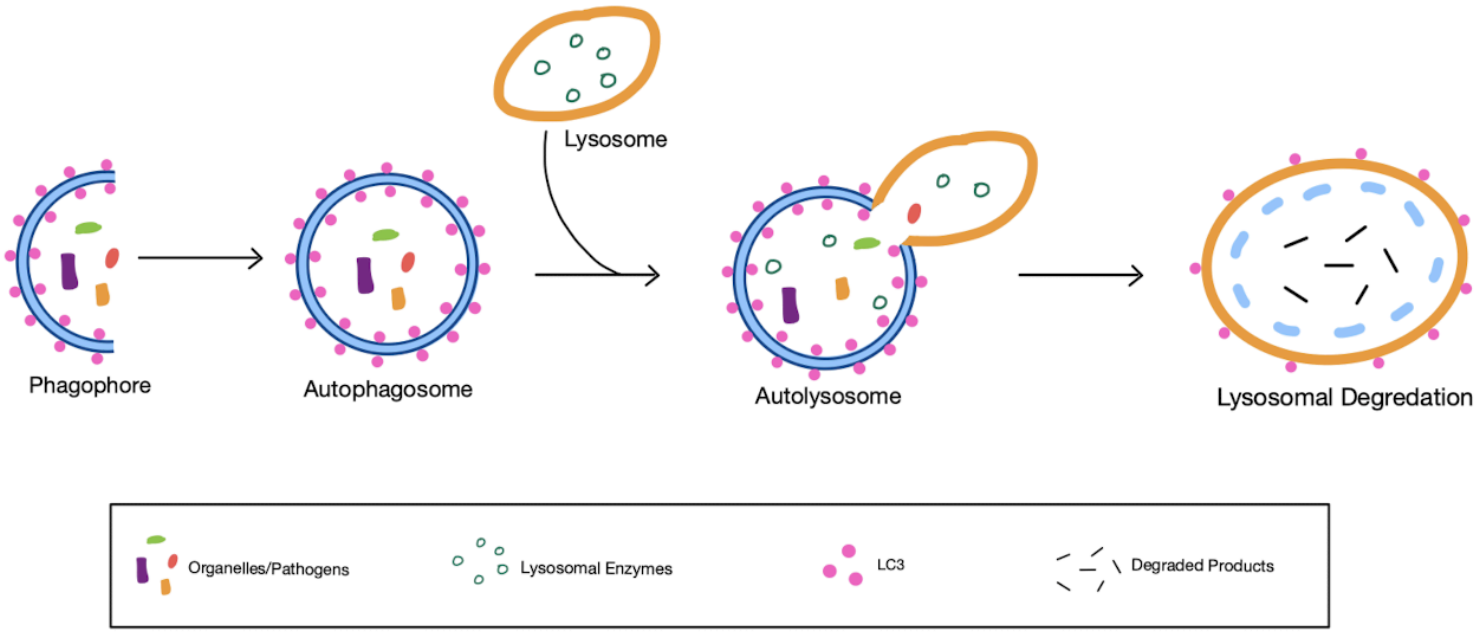


Figure 1. Process of autophagosome formation and degradation. The phagophore elongates around cytoplasm, organelles, and pathogens to form an autophagosome, which fuses with a lysosome containing enzymes for degradation.

Typically, the autophagosome has the ability to engulf a pathogen, which would then proceed to the degradation process to eliminate the pathogen (27–29). However, some bacteria are able to survive by interfering with the autophagic process and avoid degradation (27). During autophagy, a specific complex of ATGs conjugates to another key protein of autophagy, microtubule-associated protein 1 light

chain 3 (LC3) (27). LC3-II is a form of LC3 found in the inner and outer membranes of the autophagosomes and remains attached to the membrane throughout the process of autophagy (27). Nucleoporin p62 is a third key protein involved – it is an autophagy receptor protein that takes ubiquitinated proteins and forms a complex with LC3-II, and then gets degraded in the autolysosome (27). Studies have shown that increased expression of LC3-II and p62 in bovine mammary epithelial cells (BMEC) after treatment with *S. aureus* indicates that autophagy was induced by *S. aureus*, but was not allowed to proceed through complete autophagic flux to lead to the degradation phase (27,29). Not only is *S. aureus* able to avoid degradation during autophagy, but studies have also shown that *S. aureus* is able to use autophagy to its benefit by using it as a means to replicate intracellularly (28). However, this has only shown to be true in some strains of *S. aureus*, particularly those containing a certain gene regulator known as the *agr* system (28). Additional studies have shown that *S. aureus* works to diminish the storage of macromolecules, such as glucose and amino acids, in its host, in order to induce autophagy through starvation and subsequently use the autophagic process to replicate as well as obtain the broken-down glutamine for its metabolic needs (27,28). Although this particular study was performed using human cells, it is likely that the same process occurs in bovine mammary epithelial cells, although future research would be necessary to ensure that this is the case.

4. Biofilm-Forming Capabilities

S. aureus has the ability to form biofilms, which are matrices of microbial cells produced by the bacteria which protect it from antibacterial agents and the immune responses from the host (13,15,30). The process of biofilm formation, shown in Figure 2, begins with planktonic (free-floating) bacteria attaching to a surface within the body or to another bacteria (31). This initial attachment is not permanent, and the bacteria still have a chance to detach (31). Next, the bacteria become irreversibly attached and begin multiplying to form microcolonies (32). The cells form a mature 3-D structure – a tower-like shape with three layers (one for regulating, one for protection, and one with cells that are ready to escape the biofilm) (32). The final stage of biofilm formation is dispersion, which can occur as a result

of numerous factors including lack of nutrients or unfit environmental conditions (32). The biofilm ruptures and the microbes are dispersed, followed by a repetition of the cycle from the beginning (32).

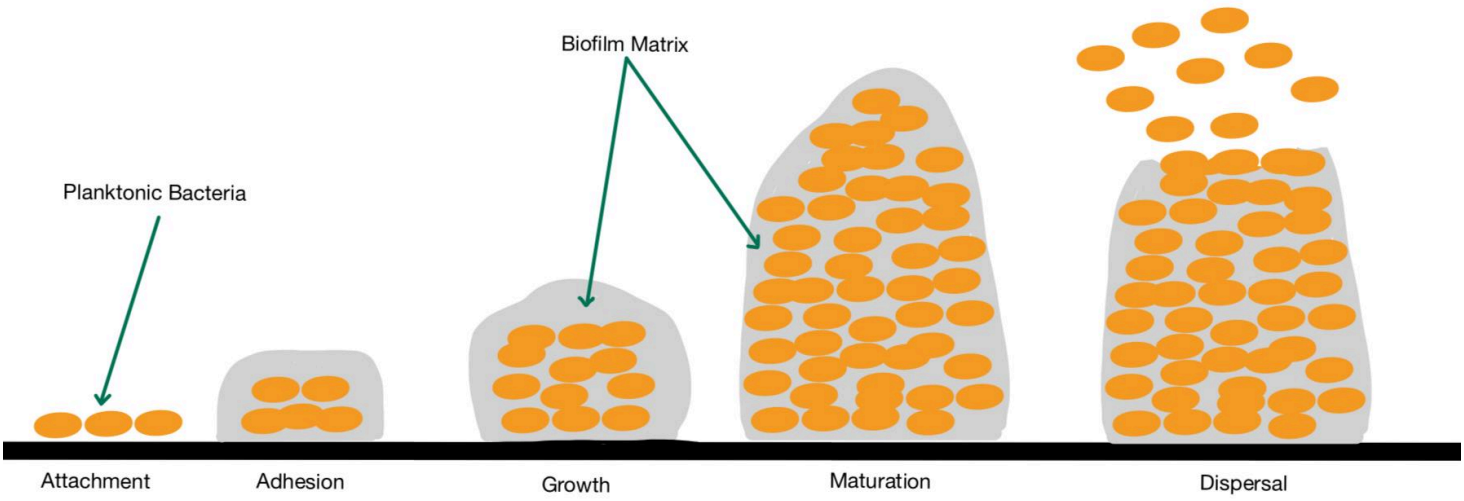


Figure 2. Process of biofilm formation, proceeding from left to right in chronological order.

The biofilm allows the bacteria to have a slower growth rate, which also means that it takes in the antibiotic at a slower rate, such that the antibiotic has less of an effect (33). In addition, antibiotics are hindered and prevented from penetrating the biofilm matrix, protecting the bacteria (33,34). Biofilm formation varies depending on growth conditions and the expression of biofilm-related genes (15). With regards to mastitis, *S. aureus* is able to create biofilms and protect itself from the host's immune response, promoting its growth and survival and causing the disease to persist (13).

5. Treatment Strategies

There are many limitations that make it very difficult to develop effective treatment strategies for cows suffering from mastitis. Most antibiotics are ineffective, due to the antibiotic resistance developed by *S. aureus*, as discussed earlier (19,20), as well as biofilm-forming capabilities (13). Due to the vast number of *S. aureus* strains and the different properties of each, it is challenging to develop a universal

vaccine or treatment that will eradicate all (13,35). Additionally, gene expression is subject to change throughout the progression of infection, even within one single strain of the bacteria (13). The small-colony variant, a phenotypic variant of *S. aureus* formed through a point mutation, is a common cause of mastitis, but is resistant to antibiotics and very persistent because of its slow growth and metabolism, and is able to take over host cells to perpetuate its own survival (13). Although the properties of *S. aureus* present numerous obstacles in the way of finding the optimal treatment for bovine mastitis, researchers have developed some possible solutions which will require significant future research.

5.1 Vaccine Development

While there is currently a commercially available inactivated *S. aureus* vaccine for bovine mastitis, it is not highly successful due to the genetic diversity of the bacteria (13). In recent studies, researchers have been able to identify genes and proteins of interest that could be potential candidates for developing vaccines or drugs to treat mastitis, including a gene called VraG (13). These genes of interest were found expressed in numerous cows, and were expressed by several strains of *S. aureus* without changing throughout the infection period (13). Because these genes of interest were widespread among herds and across various strains of *S. aureus*, they have the potential to help combat the issue of *S. aureus's* genetic diversity, and should be studied further for vaccine development. In addition, using antibodies to detect proteins involved in *S. aureus* infections can allow new vaccine candidates to be identified (13). Live vaccines, which contain a weakened strain of the bacteria, are typically most effective in treating diseases (13). In the future, using a weakened mutant of *S. aureus*, while taking into consideration the formation of the small-colony variant and creating a small-colony variant mutant that is not able to sustain an infection, could allow for the creation of a vaccine that recognizes a variety of strains of *S. aureus* (13).

5.2 Autophagy Inhibitors

Treating cows with certain autophagy inhibitors is a possible solution to eliminating *S. aureus* from the body (27). Studies have shown that 3-Methyladenine (3-MA), a known autophagy inhibitor,

leads to a significant decrease in autophagosomes formed during autophagy, and a significant decrease in survival of *S. aureus* (27). Because *S. aureus* uses autophagy as a means of survival and replication, inhibiting autophagy will remove *S. aureus's* ability to proliferate through this process and will limit its chance of survival (27). Further studies are needed to ensure that 3-MA's ability to inhibit autophagy is the definitive cause of decreasing *S. aureus* survival, and that the 3-MA is not simply able to kill the bacteria directly. Subsequently, a range of autophagy inhibitors should be tested in the future to determine if certain inhibitors have a stronger impact on *S. aureus* survival and could serve as more effective treatments. A possible limitation of this approach is that these treatments may allow for other bacterial infections to occur (28).

5.3 Herbal Therapy

Medicinal herbs can also be used to treat mastitis. Medicinal plants have properties that can relieve the symptoms of mastitis, including anti-inflammatory, anti-bacterial, and immunomodulatory properties, to help treat the disease (12,36). *Oxytropis glabra* is a plant that has been used to inhibit the biofilm-forming capabilities of *Staphylococcus epidermidis*, another common bacteria (12). Therefore, in the future, further studies in this area can be performed in order to determine whether or not *O. glabra* plants could potentially be used to inhibit *S. aureus* from forming biofilms as well, which would limit the proliferation of the bacteria, and act as another treatment strategy. In addition, mastitis causes oxidative stress, which is a harmful process that can lead to cell damage (37). Plants contain antioxidants, which can be used to prevent the oxidative stress caused by mastitis and can help fight the infection (36). These antioxidants are particularly effective when combined with antibiotics (12). As there are no negative effects stemming from herbal therapy, this is a good strategy to use in combination with other possible treatments as an added remedy (12).

6. Conclusion

Bovine mastitis is an extremely widespread and common disease affecting the dairy industry, spreading quickly through herds and causing chronic infection. Both subclinical and clinical mastitis are

most commonly caused by *S. aureus*, resulting in a variety of symptoms including decreased milk production. This places a large economic burden on dairy farmers and is the most critical issue affecting the industry today. Although it is a very difficult disease to treat due to the antibiotic resistance of *S. aureus* as well as the multitude of strains and its intracellular survival methods, effective treatments are being studied and developed to combat this infection. It is clear that there is a need for extensive future research in developing a treatment for bovine mastitis. Future studies should be done on a wide variety of *S. aureus* strains to ensure that we are gaining as much knowledge as possible about the different responses that each strain has. Research is also necessary to attempt to develop a vaccine that will offer more complete protection against numerous *S. aureus* strains. In addition, more research should be done regarding the effects of autophagy inducers and inhibitors on the survival of *S. aureus.* Studies are also needed to determine the effect of *O. galabra* on biofilm formation inhibition in *S. aureus* as a treatment method.

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