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Bovine Mastitis: Part I

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Abstract

Bovine mastitis is one of the most important bacterial diseases of dairy cattle throughout the world. Mastitis is responsible for great economic losses to the dairy producer and to the milk processing industry resulting from reduced milk production, alterations in milk composition, discarded milk, increased replacement costs, extra labor, treatment costs, and veterinary services. Economic losses due to bovine mastitis are estimated to be $2 billion in the United States, $400 million in Canada (Canadian Bovine Mastitis and Milk Quality Research Network-CBMQRN) and $130 million in Australia per year. Many factors can influence the development of mastitis; however, inflammation of the mammary gland is usually a consequence of adhesion, invasion, and colonization of the mammary gland by one or more mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli*.

Keywords: mastitis, bovine, *Staphylococcus*, *Streptococcus*

1. Introduction

Bovine mastitis is one of the most important bacterial diseases of dairy cattle throughout the world. Mastitis is responsible for major economic losses to the dairy producer and milk processing industry resulting from reduced milk production, alterations in milk composition, discarded milk, increased replacement costs, extra labor, treatment costs, and veterinary services [1]. Annual economic losses due to bovine mastitis are estimated to be $2 billion in the United States [2], $400 million in Canada (Canadian Bovine Mastitis and Milk Quality Research Network-CBMQRN), and $130 million in Australia [3]. Many factors including host, pathogen, and environmental factors influence the development of mastitis; however, inflammation of the mammary gland is usually a consequence of adhesion, invasion, and colonization of the mammary gland by one or more contagious (*Staphylococcus aureus*, *Streptococcoccus agalactiae*, *Corynebacterium bovis*, *Mycoplasmsa bovis*, etc.) or environmental (coliform bacteria, environmental *Streptococcus* spp. and some coagulase negative *Staphylococcus* spp., many other minor pathogens) mastitis pathogens.

2. Etiology of mastitis

Over 135 various microorganisms have been identified from bovine mastitis. The most common bovine mastitis pathogens are classified as contagious and environmental mastitis pathogens [4]. This classification depends upon their distribution in their natural habitat and mode of transmission from their natural habitat to the mammary glands of dairy cows [5]. It is important to mention that all

pathogens lists as environmental or contagious may not be strictly environmental or strictly contagious; some of them may transmit both ways. Environmental mastitis pathogens exist in the cow's environment, and they can cause infection at any time. Environmental mastitis pathogens are difficult to control because they are in the environment of dairy cows and can transmit to the mammary glands at any time, whereas contagious mastitis pathogens exist in the infected udder or on the teat skin and transmit from infected to non-infected udder during milking by milker's hand or milking machine liners. Environmental mastitis pathogens include a wide range of organisms, including coliform bacteria (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp), environmental Streptococcus spp. (*Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus equi*, *Streptococcus zooepidemicus*, *Streptococcus equinus*, *Streptococcus canis*, *Streptococcus parauberis*, and others), *Trueperella pyogenes,* which was previously called *Arcanobacterium pyogenes* or *Corynebacterium pyogenes* and environmental coagulase-negative *Staphylococcus* species (CNS) (*S. chromogenes, S. simulans, S. epidermidis, S. xylosus, S. haemolyticus, S. warneri, S. sciuri, S. lugdunensis, S. caprae, S. saccharolyticus*, and others) [4, 6–9] and others such as *Pseudomonas*, *Proteus*, *Serratia*, *Aerococcus*, *Listeria*, Yeast and *Prototheca* that are increasingly found as mastitis-causing pathogens on some farms [10, 11].

Contagious mastitis pathogens primarily exist in the infected mammary glands or on the cow's teat skin and transmit from infected to non-infected mammary glands during milking by milker's hand or milking machine liners. *Mycoplasma* spp. may spread from cow to cow through aerosol transmission and invade the udder subsequent to bacteremia. The most frequent contagious mastitis pathogens are coagulase-positive *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis,* and *Corynebacterium bovis* [11, 12]. The prevalence of mastitis caused by these different mastitis pathogens varies depending on herd management practices, geographical location, and other environmental conditions [13]. These different causative agents of mastitis have a multitude of virulence factors that make treatment and prevention of mastitis difficult.

2.1 Environmental mastitis pathogens

It is important to mention that all environmental mastitis pathogens may not be strictly environmental, and some of them may transmit both ways (contagious and environmental). However, the vast majority of these organisms are in the environment of dairy cows, and they transmit from these environmental sources to the udder of a cow at any time of the lactation cycle.

2.1.1 Streptococcus uberis mastitis

Streptococcus uberis is one of the environmental mastitis pathogens that accounts for a significant proportion of subclinical and clinical mastitis in lactating and non-lactating cows and heifers [14]. This organism is commonly found in the bedding material, which facilitates infection of mammary glands at any time [15]. Some report also indicated the possibility of contagious transmission of *Streptococcus uberis* [16].

S. uberis has various mechanisms of virulence that increases the chances of this organism establishing infection. These include a capsule, which evades phagocytosis, adherence to, and invasion into mammary epithelial cells [17, 18]. *S. uberis* adheres to epithelial cells using different mechanisms, including the formation of pedestals [19] and bridge formation through *Streptococcus uberis* adhesion molecule (SUAM) and lactoferrin [20–22]. This attachment is specific and mediated through a bridge formation between *Streptococcus uberis* adhesion molecule (SUAM) [23, 24]

on *S. uberis* surface and lactoferrin, which is in the mammary secretion and has a receptor on the mammary epithelial surface [20, 22]. This interaction creates a molecular bridge that enhances *S. uberis* adherence to and internalization into mammary epithelial cells most likely via caveolae-dependent endocytosis and potentially allows *S. uberis* to evade host defense mechanisms [22, 24]. These factors increase the pathogenicity of *S. uberis* to cause mastitis. The *sua* gene is conserved among strains of *S. uberis* isolated from geographically diverse areas [9, 13], and a *sua* deletion mutant of *S. uberis* is defective in adherence to and internalization into mammary epithelial cells [14].

2.1.2 Coagulase-negative Staphylococcus species (CNS)

More recently, coagulase-negative *Staphylococcus* species (CNS) such as *S. chromogenes, S. simulans, S. xylosus, S. haemolyticus, S. hyicus*, and *S. epidermidis* are increasingly isolated from bovine milk [7, 25–27] with *S. chromogenes* being the most increasingly diagnosed species as a cause of subclinical mastitis. *Staphylococcus chromogenes* [28] and other CNS [4, 8] have been shown to cause subclinical infections in dairy cows that reduce the prevalence of contagious mastitis pathogens.

Staphylococcus chromogenes is most commonly isolated from mammary secretions rather than from the environment itself [8, 29]. *S. chromogenes* consistently isolated from the cow's udder and teat skin [30], and some studies showed that it causes long-lasting, persistent subclinical infections [26]. The CNS causes high somatic cell counts in milk on some dairy farms [29, 31]. Woodward et al. [32] evaluated the normal teat skin flora and found that 25% of the isolates exhibited the ability to prevent the growth of some mastitis pathogens. An *in vitro* study conducted on *S. chromogenes* showed that this organism could inhibit the growth of major mastitis-causing pathogens such as *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* [28]. In a study conducted on conventional and organic Canadian dairy farms, CNS were found in 20% of the clinical samples [33]. Recently, mastitis caused by CNS increasingly became more problematic in dairy herds [30, 34–36]. However, mastitis caused by CNS is less severe compared to mastitis caused by *Staphylococcus aureus* [26].

2.1.3 Coliform mastitis

Coliform bacteria such as *Escherichia*, *Klebsiella*, and *Enterobacter* are a common cause of mastitis in dairy cows [37]. The most common species, isolated in more than 80% of cases of coliform mastitis, is *Escherichia coli* [38, 39]. *E. coli* usually infects the mammary glands during the dry period and progresses to inflammation and clinical mastitis during the early lactation with local and sometimes severe systemic clinical manifestations. Some reports indicated that the severity of *E. coli* mastitis is mainly determined by cow factors rather than by virulence factors of *E. coli* [40]. However, recent molecular and genetic studies showed that the pathogenicity of *E. coli* is entirely dependent on the FecA protein that enables *E. coli* to actively uptake iron from ferric-citrate in the mammary gland [41]. The severity of the clinical mastitis and peak *E. coli* counts in mammary secretions are positively correlated. Intramammary infection with *E. coli* induced expression and release of pro-inflammatory cytokines [42, 43]. Recently, it has been shown with mouse mastitis models that IL-17A and Th17 cells are instrumental in the defense against *E. coli* intramammary infection [44, 45]. However, the role of IL-17 in bovine *E. coli* mastitis is not well defined. The result of recent vaccine efficacy study against *E. coli* mastitis suggested that cell-mediated immune response has more protective effect than humoral response [46]. However, the cytokine signaling pathways that lead to efficient bacterial clearance are not clearly defined.

2.2 Contagious mastitis pathogens

2.2.1 Coagulase-positive Staphylococcus aureus

Coagulase-positive *Staphylococcus aureus* is one of the most common contagious mastitis pathogens in dairy cows, with an estimated incidence rate of 43–74% [47, 48]. *Staphylococcus aureus* is grouped under the family *Staphylococcaceae* and genus *Staphylococcus*. It is a gram-positive, catalase and coagulase-positive, non-spore forming, oxidase negative, non-motile, cluster-forming, and facultative anaerobe [49]. The coagulase test is not an absolute test for the confirmation of the diagnosis of *S. aureus* from the cases of bovine mastitis, but more than 95% of all coagulase-positive staphylococci from bovine mastitis belong to *S. aureus* [50]. Other coagulase-positive species include *S. aureus* subsp. *anaerobius* causes lesion in sheep; *S. pseudintermedius* causes pyoderma, pustular dermatitis, pyometra, otitis externa, and other infections in dogs and cats; *S. schleiferi* subsp. *coagulans* causes otitis externa (inflammation of the external ear canal) in dogs; *S. hyicus* is coagulase variable (some strains are positive and some others are negative), species that causes mastitis in dairy cows, exudative epidermitis (greasy pig disease) in pigs; and *S. delphini* causes purulent cutaneous lesions in dolphins.

S. aureus can infect many host species, including humans. In humans, *S. aureus* causes a wide variety of illnesses ranging from mild skin infection to a life-threatening systemic infection. It has been reported that certain strains of *S. aureus* with specific tissue tropism can be adapted to infect specific tissue such as the mammary gland [51]. Furthermore, a study by McMillan [52] showed distinct lineages of *S. aureus* in bovine, ovine, and caprine species. *S. aureus* strains can be host specific, meaning that they are found more commonly in a specific species [51]. Some studies showed that *S. aureus* that causes mastitis belong to certain dominant clones, which are frequently responsible for clinical and subclinical mastitis in a herd at certain geographic areas, indicating that the control measures may need to be directed against specific clones in a given area [53–55]. However, because *S. aureus* is such a big problem in human health, cross-infection has been an important research topic. Several studies have reported cases of cross-infection in several different species [56–58]. In the dairy industry, there have been reports of human origin methicillin-resistant *S. aureus* infecting bovine mammary glands [59, 60]. These studies add to the unease that strains can gain new mutations or virulence factors and adapt to cross the interspecies boundary relatively rapidly [61].

Although the incidence of *S. aureus* mastitis can be reduced with hygienic milking practices and a good management system, it is still a major problem for dairy farms, with a prevalence of 66% among farms tested in the United States [62]. The prevalence of *S. aureus* mastitis varies from farm to farm because of variation in hygienic milking practices and overall farm management differences on the application of control measures for contagious mastitis pathogens. Good hygiene in the milking parlor can significantly reduce the occurrence of new *S. aureus* mastitis in the herd, but it does not remove existing cases within a herd [63]. Neave et al. concluded that it is nearly impractical to keep all udder quarters of dairy cows free of all pathogens at all times. Since this early observation by Neave et al. [63], many studies have confirmed that management practices can reduce new cases of intramammary infection (IMI) [9, 64] but cannot eliminate existing infections. In the United States, the prevalence of clinical and subclinical *S. aureus* mastitis ranged from 10 to 45% [65] and 15 to 75%, respectively.

2.2.1.1 Virulence factors of S. aureus

Staphylococcus aureus has many virulence factors that can be grouped broadly into two major classes. These include (1) secretory factors which are surface localized structural components that serve as virulence factors and (2) secretory virulence factors which are produced by bacteria cells and secreted out of cells and act on different targets in the host body. Both non-secretory and secretory virulence factors together help this pathogen to evade the host's defenses and colonize mammary glands.

2.3 Non-secretory factors

Some of surface localized structural components that serve as virulence factors include membrane-bound proteins, which include collagen-binding protein, fibrinogen-binding protein, elastin-binding protein, penicillin-binding protein, and lipoteichoic acid. Similarly, cell wall-bound factors such as peptidoglycan, lipoteichoic acid, teichoic acid, protein A, β-Lactamase, and proteases serve as non-secretory virulence factors. Other cell surface-associated virulence factors include exopolysaccharides, which comprises capsule, slime, and biofilm. Overall, *S. aureus* has over 24 surface proteins and 13 secreted proteins that are involved in immune evasion [66] and about 15–26 proteins for biofilm formation [67, 68].

Surface proteins, such as staphylococcal protein A (SpA), clumping factors A and B (ClfA and ClfB) [69–71], fibrinogen-binding proteins [72], iron-regulated surface determinants (IsdA, IsdB, and IsdH) [69, 73], fibronectin-binding proteins A and B [74], biofilm associated protein (BAP) and exopolysaccharides (capsule, slime, and biofilms) [75–79], play roles in *S. aureus* adhesion to and invasion into host cells [80]. The BAP expression enhances biofilm production and the BAP gene is only found in *S. aureus* strain from bovine origin [81–83]. Evaluation of BAP gene of *S. aureus* from bovine and human isolates using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) showed that bovine and human isolates are not closely related [84]. Thus, some host-specific evolutionary factors may have been developed between both strain types.

Biofilms are considered an important virulence factor in the pathogenesis of bovine *S. aureus* mastitis [77, 78]. Slime, an extracellular polysaccharide layer, acts as a barrier against phagocytosis and antimicrobials. It also helps with adhesion to a surface [85]. If a biofilm forms in a mammary gland, it will protect those bacteria from antimicrobials and the host's immune system [77, 78]. In addition, once the biofilm matures and the immune attack has subsided, the biofilm can break open and allow reinfection of the mammary gland [86]. There are many contributors to biofilm production, such as polysaccharide intercellular adhesin (PIA) also known as poly-N-acetyl-β (1-6)-glucosamine (PNAG), MSCRAMMS, teichoic acids, and extracellular DNA (eDNA) [75, 76] that are known to help these bacteria cells to hold onto a surface [87]. Various proteins encoded by intercellular adhesion loci such as icaA, icaB, icaC, and icaD are involved in PIA production which in turn result in biofilm formation [75, 76]. Vasudevan et al. [88] evaluated the correlation of slime production and presence of the intercellular adhesion (*ica*) genes with biofilm production. These authors [88] found that all tested isolates were positive for *icaA* and *icaD* genes, and most tested isolates produce slime, but not all slime positives produced biofilms *in vitro*. Similarly, a study in Poland found that all isolates were positive for *icaA* and *icaD* [80] genes. While adhesion is promoted with biofilm production, the *bap* gene prevents the invasion of host cells [83]. Despite the presence of the *ica* gene strongly support biofilm production, the presence of

the *ica* gene is not mandatory for biofilm production since *S. aureus* lacking *ica* gene can still produce biofilm through other microbial surface components recognizing adhesive matrix molecules (MSCRAM) and secreted proteins [89, 90].

2.4 Secretory factors

Some of the known secretory virulence factors are toxins which include staphylococcal enterotoxins, non-enteric exfoliative toxins, toxic shock syndrome toxin 1, leucocidin, and hemolysins (alpha, beta, delta, and gamma) [91, 92]. Similarly, enzymes such as coagulase, staphylokinase, DNAase, phosphatase, lipase, phospholipase, and hyaluronidase serve as virulence factors of *S. aureus* [93].

2.4.1 Hemolysins

S. aureus isolates from bovine mastitis produce alpha (α), beta (β), gamma (γ), and delta (δ) hemolysins that cause hemolysis of red blood cells of the host [94] and all are antigenically distinct. α-hemolysin is a pore-forming toxin that binds to a disintegrin and metalloproteinase domain-containing protein-10 (ADAM10) receptor resulting in pore formation and cellular necrosis [95, 96]. It is also known to increase the inflammatory response and decrease macrophage function [97]. α-hemolysin damages the plasma membrane of the epithelial cell resulting in leakages of low-molecular-weight molecules from the cytosol and death of the cell [98]. It is produced by 20–50% of strains from bovine IMI [99]. A study reported that the α-hemolysin might be required for a cell to cell interaction during biofilm formation [100]. β-hemolysin hydrolyzes the sphingomyelin present in the plasma membrane resulting in increased permeability with progressive loss of cell surface charge [101]. It is produced by 75–100% of *S. aureus* strains from bovine IMI [99]. α-hemolysin expression requires specific growth conditions *in vitro* because its growth is inhibited by agar [102]. α-hemolysin producing strains cause complete hemolysis of sheep red blood cells, whereas β-hemolysin producing strains cause partial hemolysis within 24 h of incubation at 37°C [103]. Partial hemolysis caused by β-hemolysin becomes completely lysed after further storage at 4–15°C, which is also expressed as hot-cold lysis [104]. β-hemolysin producing strains are the most frequent isolates from animals [105]. δ-hemolysin causes complete hemolysis of red blood cells of wide range of species including human, rabbit, sheep, horse, rat, guinea pig, and some fish erythrocytes. δ-hemolysin migrates more slowly through agar than the α-hemolysin so the effect takes longer time to express. Double (α- and β-) hemolysin producing strains caused complete hemolysis in the middle with partial hemolysis on the peripheral area around each colony [105]. γ-hemolysin is produced by almost every strain of *S. aureus*, but γ-hemolysin is not identifiable on blood agar plates, due to the inhibitory effect of agar on toxin activity [106].

2.4.2 Enterotoxins Enterotoxins

These toxins are heat stable and can resist pasteurization. *S. aureus* produces staphylococcal enterotoxins A, B, C, D, E, G, H, I, and J–Q as well as toxic shock syndrome toxin 1 (tsst-1) [105, 107, 108]. Enterotoxins can get into the food chain through the consumption of contaminated food and cause food poisoning [109]. Staphylococcal enterotoxins tend to contaminate dairy products and cause foodborne illness [110, 111]. Staphylococcal enterotoxins G to Q (SEG–SEQ) are prevalent among *S. aureus* isolates from cases of bovine mastitis and are also implicated in the pathogenesis of mastitis. Some of these toxins are known to function as

superantigens that cause increased immunological reactivity in the host [110]. Some studies showed that about 20% of *S. aureus* isolates from IMI produce toxic shock syndrome toxin-1 [109, 112]. Toxic shock syndrome toxin causes toxic shock syndrome and can be fatal [113]. Besides the superantigenic effect of enterotoxins, their role in the pathogenesis of mastitis is unknown. It may be specific to each strain or area based on selective pressures in the habitat [114]. Enterotoxin prevalence seems to vary between geographical regions. The strains producing enterotoxin C have been isolated relatively frequently from cases of bovine mastitis [108, 115, 116].

Enterotoxins are believed to have a role in the development of mastitis since *S. aureus* isolates from cases of mastitis had a high prevalence of enterotoxins than isolates from milk of cows without mastitis [117, 118]; however, staphylococcal enterotoxins expressions are controlled by several regulatory elements [119] that respond to a variety of different micro-environmental stimuli and the exact mechanisms by which enterotoxins contribute to the development of mastitis are not clearly known and yet to be determined.

In addition to specific virulence factors, *Staphylococcus aureus* also possesses different mechanisms or traits such as biofilm formation, adhesion to and invasion into mammary epithelial cells, and formation of small colony variant (SCV) that enable this pathogen to resist host defense mechanisms. The ability of *S. aureus* to invade mammary epithelial cells during mastitis plays a significant role in the pathogenesis of *S. aureus*. Internalized bacteria can hide from the host's immune system inside the host cell and continue to multiply inside the host cell [120]. There may be many mechanisms that *S. aureus* uses to invade into host cells, and each mechanism can be strain dependent. *S. aureus* strains have a fibronectin-binding protein that can link to the fibronectin on the mammary epithelial cell surface. Fibronectin binding protein is thought to be a common way for the bacteria cells to invade bovine mammary epithelial cells. Fibronectin-binding protein-deficient strains cannot invade host cells [121]. The presence of a capsule prevents adherence to epithelial cells [122, 123].

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 [124]. Adhesion is dependent on surface proteins called adhesins, which help the bacterium to recognize and attach to host cells. Staphylococci 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 [124]. 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 [125, 126].

Adhesion to and invasion into epithelial cells [124], intracellular survival in macrophages [127], and epithelial cells allow them to avoid detection by the host immune system and resist treatment with antibiotics [120]. Due to its poor response to treatments, *S. aureus* infections often become chronic with a low cure rate [128]. Treatment of *Staphylococcus aureus* mastitis with cloxacillin cured only 25% of the clinical cases and 40% of subclinical cases in the study by Tyler and Baggot [129]. *Staphylococcus aureus* also has a known ability to form biofilms [77, 78, 86] and acquire antimicrobial-resistance genes via horizontal resistance gene transfer, which enables this bacterium to develop antimicrobial resistance [130, 131].

The mode of transmission from infected mammary glands or colonized udder skin to healthy mammary glands is through contact during milking procedures with milker's hand, towel, and milking machine [58]. *S. aureus* usually causes subclinical or chronic infections and is difficult to clear with antibiotic treatment [132].

2.4.3 Streptococcus agalactiae

The most important virulence factor of *S. agalactiae* is the capsular polysaccharide [133], which protects this bacterium from being engulfed by macrophages and subsequently phagocytosed [133]. Another virulence factor of *S. agalactiae* is the Rib protein, which confers resistance to proteases. Emaneini et al. [133] found that the Rib encoding gene (*rib*) was detected in 89% of the isolates from bovine origin. *Streptococcus agalactiae* causes persistent infections that are usually difficult to clear without antibiotic treatment [134]. Though *Streptococcus agalactiae* is highly contagious, it has good response to treatment with antibiotics, which makes it possible to eliminate from herds with current mastitis control measures [129]. Since the adoption of hygienic milking practices, the incidence of mastitis caused by *S. agalactiae* has dramatically decreased and is now rarely observed in dairy herds [135].

2.4.4 Mycoplasma mastitis

Mastitis caused by *Mycoplasma* spp. is a growing concern in the United States. It is believed that this organism has been underreported due to the difficulty of isolation by culture method [136]. The incidence of *Mycoplasma* mastitis varies across the globe, with a 3.2% prevalence rate in the United States that may increase to 14.4% in larger herd size of greater than 500 cows [47, 48, 62, 137]. A risk factor for *Mycoplasma* mastitis increase with herd size, and most of the *Mycoplasma* mastitis cases are subclinical infections with outbreaks linked to asymptomatic carriers [138]. Pathogenesis of most *Mycoplasma* spp. infection is characterized by adherence to and internalization into host cells resulting in colonization of the host with immune modulation without causing severe disease [138]. *Mycoplasma* species lack a cell wall, thus not sensitive to beta-lactam antibiotics, but showed sensitivity to non-beta-lactam antibiotics [139].

3. Routes of entry of mastitis pathogens to the udder

In general, it is believed that mastitis pathogens gain entrance to the udder through teat opening into the teat canal and from the teat canal into the intramammary area during the reverse flow of milk due to vacuum pressure fluctuation of the milking machine [9]. However, the detailed mechanism of mastitis pathogen colonization of the mammary gland may vary among species of bacteria and the virulence factors associated with particular strain in each species. An example of this is in some cases; it has been shown that *E. coli* can penetrate the teat canal without the reverse flow of milk [9]. Some of the major mastitis pathogens, such as *E. coli* [140], *Staphylococcus aureus*, and *Streptococcus uberis* [20–22] can adhere to and subsequently invade into the mammary epithelial cells. This adherence and subsequent invasion into mammary epithelial cells allow them to persist in the intracellular area as well as to escape the host immune defenses attack and action of antimicrobial drugs [120, 140–144]. Dogan et al. [145] compared *E. coli* strains known to cause chronic infections with strains known to cause acute infections and found that chronic strains were more invasive to the epithelial cells, leading to the difficulty in clearance and persistent infection compared to acute strains. *S. aureus* enters the mammary gland through the teat opening and subsequently multiply in the mammary gland where they may form biofilms, attach to, and internalize into the mammary epithelial cells causing inflammation of mammary glands characterized by swelling, degeneration of epithelial cells, and epithelial erosions and ulcers [146, 147].

4. Clinical manifestation of mastitis

Depending on clinical signs, mastitis can also be divided into clinical and subclinical mastitis. Clinical mastitis is characterized by visible inflammatory changes (abnormalities) in the mammary gland tissue such as redness, swelling, pain, increased heart, and abnormal changes in milk color (watery, bloody, and blood tinged) and consistency (clots or flakes) [9]. Clinical mastitis can be acute, peracute, subacute, or chronic. Acute mastitis is a very rapid inflammatory response characterized by systemic clinical signs which include fever, anorexia, shock, as well as local inflammatory changes in the mammary gland and milk. Peracute mastitis is manifested by a rapid onset of severe inflammation, pain, and systemic symptoms that resulted in a severely sick cow within a short period of time. Subacute mastitis is the most frequently seen form of clinical mastitis characterized by few local signs of mild inflammation in the udder and visible changes in milk such as small clots. Chronic mastitis is a long-term recurring, persistent case of mastitis that may show few symptoms of mastitis between repeated occasional flare-ups of the disease where signs are visible and can continue over periods of several months. Chronic mastitis often leads to irreversible damage to the udder from the repeated occurrences of the inflammation, and often these cows are culled.

Subclinical mastitis is the inflammation of the mammary gland that does not create visible changes in the milk or the udder. Subclinical mastitis is an infection of mammary gland characterized by non-visible inflammatory changes such as a high somatic cell count coupled with shedding of causative bacteria through milk [9]. During this inflammatory process, the milk samples showed a rapid increase of somatic cells, characterized by increased number of neutrophils in the secretion [146, 148]. Despite increased recruitment of somatic cells into infected mammary glands, evidenced by an increased number of neutrophils, infection usually does not clear but became subclinical. Intramammary infections during early lactation may become acute clinical mastitis characterized by gangrene development due congestion and thrombosis (blockage) of blood supply to the tissue but most new infection during late lactation or dry period become acute or chronic mastitis [149, 150].

The increase in somatic cell count during subclinical infections leads to a decrease in useful components in the milk, such as lactose and casein [151]. Lactose is the sugar found in milk, and casein is one of the major proteins in milk and decreases in these two components affect the quality and quantity of milk yield [9]. During mastitis, there is an increase in lipase and plasmin, which have a detrimental effect on the quantity and quality of milk due to the breakdown of milk fat and casein [9]. Subclinical infections can reduce milk production by 10–12% when just one-quarter is infected [152]. These subclinical infections cause some of the greatest unseen economic [20] losses because of their detrimental impact on production and milk quality without showing visible signs of infection [152].

5. Risk factors for mastitis

There are host-, pathogen-, and environmental-related risk factors that predispose dairy cows to mastitis. The host risk factors include age (parity), stage of lactation, somatic cell count, breed, the anatomy of the mammary glands/morphology of udder and teat (diameter of teat canal and conformation of the udder), and immune competence (immunity) [153] (**Figure 1**). The environmental risk factors include the proper functioning status of milking machine, udder trauma, sanitation, climate, nutrition, management, season, and housing condition [154]
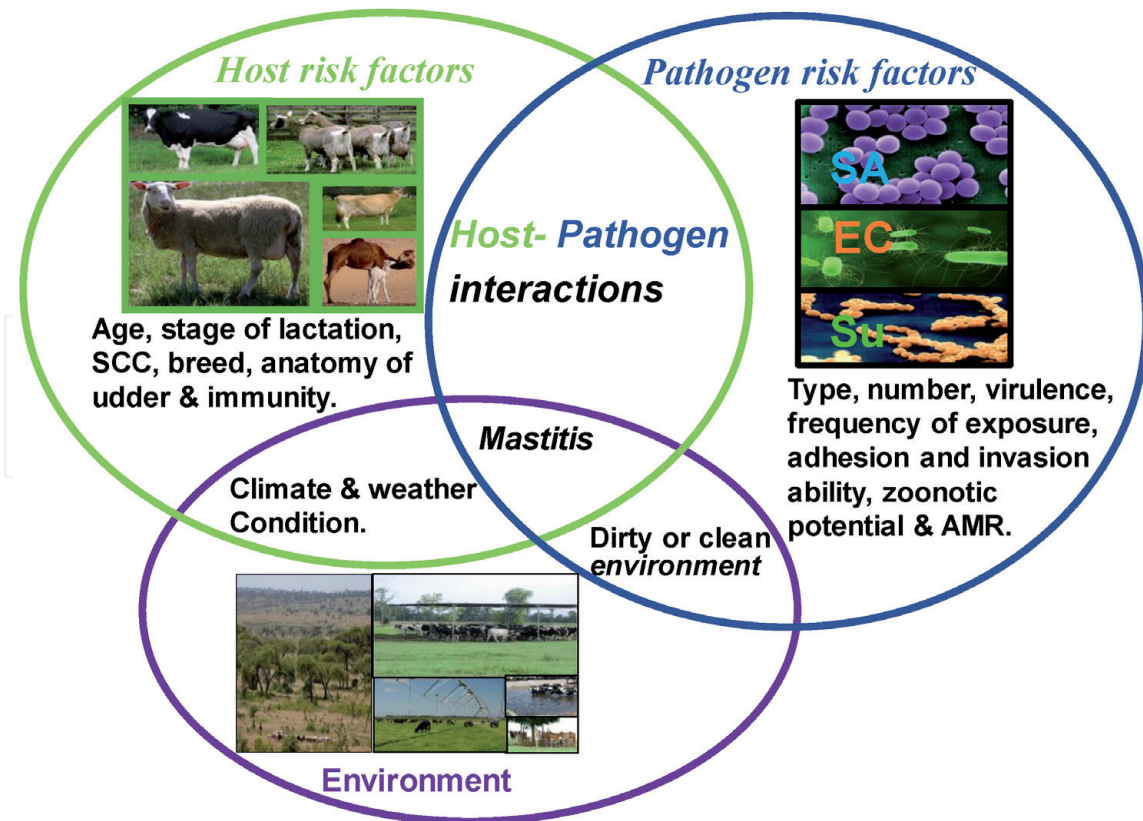
Figure 1.
Risk factors for mastitis. SA, Staphylococcus aureus; EC, Escherichia coli; SU, Streptococcus uberis; SCC, somatic cell count; AMR, antimicrobial resistance.

(**Figure 1**). The pathogen risk factors include type (bacteria, fungi, yeast, and algae), number (large number and small number), virulence (highly, moderate, or less virulent), frequency of exposure (dirty farm floor, dirty milking machine, and dirty teat drying towels frequently expose to pathogen; clean floor, clean milking machine, and clean teat drying towels less exposure to pathogens), ability to resist flushing out of the glands by milk (ability to adhere or attach to and invade or internalize into mammary epithelial cells), zoonotic (transmit from cow to human or vice versa) potential, and resistance to antimicrobials [4] (**Figure 1**). The warm, humid, and moist climate favors the growth of bacteria and increases the chances of intramammary infection (IMI) and mastitis development [154]. The incidence of mastitis varies from farm to farm due to the combined effects of these different factors that increase the risk of disease development.

Dairy cows are highly susceptible to IMI during the early dry period due to increased colonization of teat skin with bacteria. Bacterial colonization of teat increases during the early dry period because of an absence of hygienic milking practices including pre-milking washing and drying of teats [155], as well as pre- and post-milking teat dipping in antiseptic solutions [156, 157] that are known to reduce teat end colonization and infection. An udder infected during the early dry period usually manifests clinical mastitis during the transition period because of increased production of parturition inducing immunosuppressive hormones [158, 159], negative energy balance [160], and physical stress during calving [161].

6. Role of mastitis on public health

Mastitis is increasingly becoming a public health concern due to the ability of the causative bacterial pathogens and/or their products, such as enterotoxins, to

enter the food supply and cause foodborne diseases [109, 162], especially through the consumption of raw milk [29] and undercooked meat of culled dairy cows due to chronic mastitis that are usually sold to the slaughter (abattoir) for meat consumption. The Center for Disease Control (CDC) estimated that roughly 48 million people in the United States a year become sick from foodborne diseases [163]. Foodborne pathogens have been detected in bulk tank milk in multiple studies [164–167]. These authors found that the number of foodborne pathogens detected in bulk tank milk vary with location, management practices, hygiene, and number of animals on the farm [165]. Similarly, a study on bulk tank milk from east Tennessee and southwest Virginia by Rohrbach et al. [168] showed that 32.5% of the samples analyzed contained one or more foodborne pathogens. Even dairy producers who used proper hygienic milking practices, pre- and post-milking teat disinfectant and antibiotic dry cow therapy, had foodborne pathogens in their bulk tank milk [164]. The isolation of these foodborne pathogens from bulk tank milk samples across the United States demonstrate the threat that mastitis pathogens and zoonotic mastitis causing pathogens create on public health if raw milk is consumed or if these pathogens make it through processing.

7. Conclusions

Bovine mastitis is the most important multifactorial disease of dairy cattle throughout the world. Mastitis is responsible for huge economic losses to the dairy producers and milk processing industry due to reduced milk production, alterations in milk composition, discarded milk, increased replacement costs, extra labor, treatment costs, and veterinary services. Many factors including pathogen, host, and environment can influence the development of mastitis. Mastitis, the inflammation of the mammary gland is usually a consequence of adhesion, invasion, and colonization of the mammary gland by one or more mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli*.

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