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Emerging Strategies to Better Control Bovine Mastitis: A Perspective for Detection, Diagnosis and Control of Mastitis Pathogens

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Abstract

Bovine mastitis, an inflammation of the mammary gland, is a major economic burden globally. It is mainly caused by bacterial pathogens and manifests in two forms: clinical mastitis, with obvious clinical signs, and subclinical mastitis, characterized by an elevated milk somatic cell count (SCC) (with subclinical mastitis being more common in most dairy herds). The SCC, often transformed into somatic cell score (SCS), is a key indicator of udder health and widely used in genetic evaluations. Mastitis occurrence and severity are influenced by many factors, including pathogen type, the cow's immune response, environmental conditions, and genetic predisposition. This manuscript offers an overview of bovine mastitis, focusing on recent developments in diagnostic techniques that address infectious agents and non-infectious contributors, aiming to improve control strategies. Mastitis-causing bacteria are categorized into contagious, environmental, and opportunistic bacteria. Contagious bacteria primarily spread during milking, whereas environmental pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus uberis* originate from the cow's surroundings. *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma bovis* are recognized as major contagious pathogens. Opportunistic bacteria, e.g., *non-aureus staphylococci*, commonly colonize the teat skin. Effective control relies on stringent hygiene during milking and in the cows' environment, milking equipment maintenance, teat disinfection, appropriate treatment of clinical mastitis and intramammary infections at drying off, and vaccination. Diagnostic approaches have progressed from traditional microscopic examination and SCC testing to rapid cow-side assays, various biosensors, nucleic acid amplification, and genomic analyses. Intrinsic and extrinsic factors significantly influence susceptibility, with older cows and those in early lactation at highest risk. In summary, bovine mastitis is a complex, multifactorial disease that requires an integrated approach combining hygiene, vaccination, genetic improvement, and advanced diagnostics. Addressing both host-related and environmental factors through sustainable herd management is critical to reducing mastitis prevalence, boosting dairy productivity, and enhancing animal welfare worldwide.

Keyword(s): Dairy cattle, Mastitis, Non-pathogens, Pathogens, Strategies

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Introduction

Mastitis, inflammation of the mammary gland, is a common and costly disease affecting dairy cows worldwide. It is typically caused by bacterial infections; *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Klebsiella* spp., and non-*aureus* staphylococci are the most common major udder pathogens (Zadoks and Fitzpatrick, 2009; Al-Harbi et al., 2021). The disease can be classified into two categories based on the degree of inflammation: clinical mastitis (CM) and subclinical mastitis (SCM). Clinical mastitis is the most severe form, where visible abnormalities such as a red and swollen udder and fever are present. Subclinical mastitis is relatively more common and a milder form of the disease, where there are no visible clinical signs, but there is an increase in somatic cells in the milk (Harmon, 1994; Dohoo et al., 2011; Santman-Berends et al., 2012). Somatic cell count (SCC) is commonly used in genetic evaluation for udder health, as it is an indirect method of diagnosing SCM and is closely associated with udder health (Schukken et al., 2003). For monitoring udder health and conducting genetic evaluations, SCC is often log-transformed into somatic cell score (SCS) to fit a normal distribution (Nani et al., 2015; Fonseca et al., 2025).

Mastitis is a complex disease that involves multiple factors, including the type of pathogen, host immunity, environmental and genetic factors affecting incidence and severity (De Vliegher et al., 2012; Germon et al., 2025). Environmental factors have an important role in protecting dairy cattle against intramammary infection (IMI) and mastitis. Proper housing, bedding, and nutrition are crucial in maintaining healthy immune systems, which, in turn, reduce the risk of IMI. Furthermore, the importance of genetic factors in determining mastitis resistance in dairy cattle, and the potential for genetic improvement through selective breeding, is highlighted. In recent years, genomic selection has emerged as a powerful tool to accelerate genetic improvement and provide more accurate estimates of an animal's breeding value for mastitis resistance (Meuwissen et al., 2001; Zhang et al., 2022; Zavadilová et al., 2022).

Moreover, recent advances in "-omics" technologies have made it possible to conduct large-scale genetic studies and identify potential candidate regions and genes regulating mastitis (Asselstine et al., 2019; Kim et al., 2021; Naserkheil et al., 2022). The aim of this manuscript is to provide an overview of mastitis, highlighting advances in diagnostic methods for detecting and identifying both mastitis pathogens and non-pathogenic factors, in order to improve control of bovine mastitis.

Detection and Diagnosis of Mastitis Pathogens

1. Historical Background and Key Pathogens in Bovine Mastitis

Bacterial causes of mastitis were first identified in the late 1800s, as mentioned by Plastridge (1958). One of the early mastitis investigators, Murphy (1947), described the progression of mastitis as a 3-phase process: invasion, infection, and inflammation. Furthermore, two pathogens, namely *Strep. agalactiae* and *Staph. aureus*, were identified as the most important contagious pathogens (Murphy, 1947; Plastridge, 1958; Vakkamäki et al., 2017; Keane, 2019). Currently, > 200 bacterial species are known to cause IMI. Pathogens have been identified as a significant risk factor associated with the incidence of bovine mastitis, and this factor is considered in most mastitis control and treatment programs (Klaas and Zadoks, 2018). Because measures for prevention and control of mastitis differ considerably among udder pathogens, is it very important to determine which bacterial species cause IMIs in a particular herd. Therefore, aseptic sampling of CM and SCM cases needs to be a routine practice.

2. Classification and Control of Contagious Mastitis Pathogens

Bovine mastitis is caused by a range of Gram-positive and Gram-negative bacteria, classified into three types: contagious, environmental, and opportunistic (Schukken et al., 2011; Haxhiaj et al., 2022). Contagious mastitis is transmitted among cows, especially during milking, and is caused by pathogens like *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. These pathogens colonize and grow in the teat canal and establish particularly SCM, leading to an increase in SCC (Kibebew, 2017). An elevated SCC, comprised of leukocytes, macrophages, and epithelial cells, is a strong indicator of the presence of IMI (Sharma et al., 2011). The prevalence of contagious mastitis has considerably decreased in the last decades, particularly in well-managed dairy herds, with most of these herds now having a low bulk tank SCC (< 150,000 cells/mL), are free of *Strep. agalactiae* and have a low incidence of *Staph. aureus* IMI. Effective ways to prevent and control contagious IMIs include minimizing contact between infected and uninfected cows during milking, ensuring proper maintenance of milking equipment, good miking technique, post-milking teat disinfection, culling of affected cows, adequate treatment of clinical mastitis, and targeted dry cow therapy (DCT) (Smith and Hogan, 1993).

3. Environmental and Opportunistic Pathogens: Sources, Risks, and Prevention

Environmental pathogens, which differ from contagious pathogens, are present in the bedding and housing. Examples of pathogens in this class include *E. coli*, *Strep. uberis*, and *Klebsiella* spp, whereas

Streptococcus dysgalactiae can have either an environmental or contagious mode of transmission. Opportunistic pathogens such as most non-*aureus* staphylococci species, e.g., *Staphylococcus simulans* and *Staphylococcus chromogenes*, are common residents of teat skin (Bradley, 2002; De Buck et al., 2021; Moroni et al., 2018). When a cow's natural immunity is suppressed, environmental and opportunistic pathogens can enter the teat during milking, leading to IMI which will then often cause SCM and CM (Bradley, 2002). Prevention and control of these pathogenic infections is more difficult than control of contagious pathogens. Important elements in the control of environmental mastitis are vaccination, maintaining hygiene of the udder and milking process to protect healthy cows from infected cows, reducing exposure of teat ends to pathogens, as well as boosting the immune system by providing trace elements, essential amino acids, and vitamins through good nutrition (Smith and Hogan, 1993).

4. Prevalence, Challenges in Treatment, and Role of Bacterial Biofilms in Mastitis

The prevalence of SCM varies considerably among countries depending on bulk tank SCC levels; however, it is in general higher than the incidence of CM (15-30% during lactation in many countries) (Krishnamoorthy et al., 2021). Treating various forms of mastitis has proven challenging due to the ability of many bacteria to produce biofilms as a defense against the host's immune response and adaptation to the host environment (Oliveira et al., 2011). Biofilms, or structured communities of bacterial cells, consist of clusters of cells that are enclosed in a self-produced matrix containing exopolysaccharides, teichoic acids, proteins, extracellular DNA, and enzymes. These communities adhere to biotic or abiotic surfaces. Formation of these communities begins with bacterial attachment to an abiotic surface and can be facilitated by hydrophobic or electrostatic interactions. Subsequently, cell wall-associated adhesins such as flagella, fimbriae, and pili can drive the process (Melchior et al., 2006; Gomes et al., 2016).

Additionally, antimicrobial resistance of udder pathogens has become an important problem, particularly in low- and middle-income countries where restrictive use of antimicrobials is not applied.

5. Advancements in Mastitis Detection: From Traditional Tests to Modern Diagnostic Technologies

In the last century, there has been emergence of many methods and tests for detecting mastitis to ensure milk product quality and public safety. Direct microscopic examination of milk for bacteria, enumeration of milk leukocytes, bacteriological culture, and detection of various abnormal milk constituents has facilitated identifying and managing affected cows and producing high-quality dairy products (Shaw, 1937; Ramuada et al., 2024). Leukocyte counting tests were developed and widely used to detect SCM as a herd management tool by applying a threshold of 500,000 cells/mL (Plastridge, 1958; Lipkens et al., 2019). The California Mastitis Test (CMT) and the Wisconsin Mastitis Test (WMT) were introduced as rapid and inexpensive methods for detecting and managing SCM (Schalm, 1957; Postle, 1965). Automated cell counters such as the Fossomatic (Gonzalo et al., 2003) and the 'portable' De Laval Cell Counter (Gonzalo et al., 2006) were developed to estimate SCC as a faster and reliable method for enumerating somatic cells in milk, indicating an IMI with a sensitivity and specificity of 80%, and providing an overview of udder health (Paape et al., 1965; Dohoo and Leslie, 1991). Differential SCC (Halasa and Kirkeby, 2020) and in-line methods to estimate SCC in automated milking systems (Damm et al., 2017) have recently become available.

The United States and the European Union initially set maximum bulk tank SCC limits at 750,000 and 500,000 cells/mL, respectively. However, in 1992, the EU decreased the cut-off to 400,000 cells/mL. In general, it was possible to adapt to this change (European Commission-Joint Research Centre, 2020), and average bulk tank SCC in these countries decreased to 200,000 – 250,000 cells/mL. Monthly SCC testing of individual cows by Dairy Herd Improvement Associations, which defines mastitis exclusively based on culture of milk samples, was accepted as a very useful mastitis management tool in 1994 (Harmon, 1994; Tommasoni et al., 2023). Development of the somatic cell score (SCS), a logarithmic transformation of SCC, enabled researchers to quantify the linear relationship between SCM and reduced milk production, with each 1-unit increase in SCS resulting in a production loss (Ali and Shook, 1980; Wiggans and Shook, 1987).

Researchers are constantly striving to develop diagnostic methods that are better, faster, and more cost-effective. Diagnostic techniques for mastitis are currently classified into two main categories: laboratory techniques and cow-side tests (summarized in Table 2). These methods generally include immunoassays, hand-held biosensors, nucleic acid tests, loop-mediated isothermal amplification (LAMP), infrared thermography (IRT), and enzymatic assays, as well as advancements in genomics, proteomics, metabolomics, transcriptome analysis, nano-assembly, and micro-modeling of compact or mobile tools (Viguier et al., 2009; Wu et al., 2015; Zhao et al., 2015; Bosward et al., 2016; Kosciuczuk et al., 2017; Ashraf and Imran, 2018). These innovations have much potential to facilitate mastitis detection. In addition, automatic digital diagnostic tools such as the Afimilk mastitis detector, DeLaval cell counter, and Draminski mastitis detector have been developed (Godden et al., 2017; Cho et al., 2024).

Table 1. Details regarding some of the most important agents causing intramammary infections (IMI) in cattle (Haxhiaj et al., 2022; Duarte et al., 2015; Adkins et al., 2017; Cheng and Han, 2020; Lynch and Helbig, 2021; De Buck et al., 2021; Lücken et al., 2022; Idamokoro, 2022; Liu et al., 2022).

Pathogen	Abbreviation	Classification of mastitis pathogens	Gram	Form of mastitis[1]	Ways to prevent the infection	Effect on udder tissue	Biofilm producer
Staphylococcus aureus	*Staph. aureus*	Contagious	Positive	CM and SCM	• Stringent milking hygiene and technique • Maintenance of milking machine • Post-milking teat disinfection • Effective monitoring and culling strategies • Prudent antimicrobial use guided by susceptibility testing • Continued research into vaccination and alternative control measures	Irreversible damage	Yes
Streptococcus agalactiae	*Strep. agalactiae*	Contagious	Positive	SCM	• Strict milking hygiene and technique • Maintenance of milking machine • Post-milking teat disinfection • Regular monitoring, prompt treatment, and culling of infected animals • Biosecurity measures • Maintaining overall herd health to break transmission and reduce infection impact	Epithelial cell damage, edema, and neutrophil infiltration, progressing to fibrosis and atrophy of mammary tissue.	Yes
Mycoplasma spp	*Mycoplasma spp.*	Contagious	-	CM, SCM or chronic mastitis	• Meticulous milking hygiene • Early detection and culling of infected animals • Rigorous biosecurity, and overall herd health management due to the pathogen's high contagion • Immune evasion and limited treatment options	Damages secretory tissues, and induces gland and lymphatic nodule fibrosis and abscesses	Yes
Escherichia coli	*E. coli*	Environmental	Negative	CM and transient	• Focus on hygiene and environmental management • Vaccination to boost specific immunity • Selective antimicrobial use and particulary treatment of symptoms (CM) • Good nutrition and calf management practices to strengthen overall herd health and resistance to infection	Irreversible tissue damage in mammary gland, complete loss of milk production, and sometimes death	Yes
Streptococcus uberis	*Strep. uberis*	Environmental	Positive	CM and SCM	• Focus on rigorous hygiene practices • Proper environmental management to minimize exposure (indoors and outdoors) • Careful milking protocols • Prompt treatment of infections • Supporting cow immunity through nutrition and management	Changes in mammary gland tissue: 1. Slight swelling; less pliable udder. 2. Moderate swelling; firm udder, the udder became red and hot, causing discomfort. 3. Severely swollen; the udder is very hard, red, and hot	Yes
Streptococcus dysgalactiae	*Strep. dysgalactiae*	Environmental and Contagious	Positive	CM and SCM	• Requires integrated hygiene practices • Environment cleanliness • Targeted treatment with susceptibility testing • Farm-specific control programs considering both contagious and environmental infection routes • Stringent milking hygiene and technique • Maintenance of milking machine • Post-milking teat disinfection	Infected mammary glands and teat injuries	Yes
Klebsiella spp	*Klebsiella* spp	Environmental	Negative	CM and SCM	• Environmental and milking hygiene • Early detection and isolation of infected animals • Prudent use of antimicrobials, guided by sensitivity • Management of feed and water contamination	Severe and long-lasting duration of intramammary infection; often accompanied by a considerable decrease in milk production	Yes
Staphylococcus simulans	*S. simulans*	Opportunistic	Positive	CM and SCM	• Milking hygiene • Environmental management and monitoring • Targeted antimicrobial treatment • Herd health practices	Destroys udder tissue	Yes
Staphylococcus chromogens	*S. chromogens*	Opportunistic	Negative	CM and SCM	• Focuse on comprehensive hygiene • Diligent monitoring and treatment guided by susceptibility • Environmental control and improving diagnostic precision to reduce impact	Destroys udder tissue	Yes

[1] CM = Clinical mastitis, and SCM = Subclinical mastitis.

Some automated milking systems (i.e., "milking robots") also have SCC counters, enabling real-time observation of milk quality, particularly somatic cells and early identification of potential udder health problems (Nogalski et al., 2011; Simões Filho et al., 2020).

Impacts of Factors Other Than Pathogens on Bovine Mastitis

To effectively address bovine mastitis, it is essential to understand its non-pathogenic factors that can be classified into two categories: intrinsic (host-related) and extrinsic (environment-related) factors. These factors include species, age, parity, lactation period, duration of the condition, housing, and common handling practices (Tekle and Berihe, 2016).

1. Host Factors

The incidence of IMI is influenced by various host factors. One such factor is age, with older cattle being more susceptible to infections due to frequent milking and increased permeability of mammary epithelium (Król et al., 2013). Udder anatomy (i.e., large, funnel-shaped teats and deepness of the udder) is another factor that can affect susceptibility to infection (Persson Waller et al., 2014). Additionally, dairy cattle are at a higher risk of acquiring mastitis during calving and the first month of lactation, due to IMIs acquired during the dry period (Drackley, 1999; De Visscher et al., 2016). Nutritional stress and the immune system are also important factors during lactation. Cattle have a high demand for energy and nutrients to synthesize colostrum and milk, especially in the first month of lactation, which inevitably results in negative energy balance, increasing susceptibility to infections and causing immunosuppression at cellular and humoral levels, especially at the beginning of lactation (Shaheen et al., 2016). Adequate vitamin E, selenium, and zinc can help prevent udder infection, and boost the immune system by improving neutrophil function (Bayril et al., 2015; Khan et al., 2024). Much current research is done on developing pathogen-specific Gram-negative and Gram-positive vaccines and advancing immunology to more rapidly clear IMIs and prevent progression of CM, reducing economic and welfare losses associated with these infections (Piepers et al., 2017). However, these vaccines generally only work if other preventive practices are implemented that reduce the number of pathogens that a cow encounters, e.g., hygiene during milking and the cow's environment.

Table 2. Current mastitis diagnostic approaches.

Diagnostic Approach	Test	Testing Methods	References
Laboratory techniques	Somatic Cell Count (SCC)	• Automated system ▪ ADAM™-SCC2 is an automated somatic cell counter integrated with fluorescence optic and image analysis software. ▪ The NucleoCounter® SCC-100™ somatic cell counter is a high-quality image cytometer, ideal for milk. • Manually by preparing and staining slides and then viewing with a microscope • Most laboratories use flow cytometry or combine flow cytometry and fluorescence	Gunasekera et al., 2003; Dufour et al., 2011
	Bacterial Culturing (BC)	• The standard plate count (SPC) with a healthy target of < 5,000 colony-forming units (CFUs)/mL is a common method.	Murphy et al., 2016
	Polymerase Chain Reaction (PCR)	• Culture plate identification of bacteria is considered the gold standard to identify bacteria involved in CM and SCM • Electrospray ionization mass spectrometry ESI-MS. • Other molecular techniques such as real-time quantitative PCR (qPCR), next-generation sequencing (NGS), and loop-mediated isothermal amplification (LAMP) methods. • Matrix-assisted laser desorption/ionization time-of-flight (MALDI–TOF) mass spectrometry	Graber et al., 2007; Bexiga et al., 2011; Perreten et al., 2013; Cameron et al., 2017; Li et al., 2017; Anis et al., 2018
Cow-side tests	California Mastitis Test (CMT)	• Plastic paddle with four cups (to collect milk from each quarter). • Mix equal volumes of milk and anionic surfactant and visually assess to estimate relative SCC.	Schalm, 1957; Sargeant et al., 2001
	Somaticell®	• Could be a replacement for CMT. Also, the test is a correction of the Wisconsin Mastitis Test. • Estimates concentration of somatic cells in milk by a chemical reaction.	Thompson and Postle, 1964; Rodrigues et al., 2009; Ferronatto et al., 2018
	Electrical Conductivity (EC)	• Automatic milking systems with sensors that measure EC to detect mastitis. High EC is due to increased sodium and chloride concentrations and decreased lactose and potassium.	Py, 2003; Khatun et al., 2017
On-Farm Culture	-	• Biplates and triplates are major on-farm testing methods to detect bacteria in milk • Enzyme-linked immunosorbent assays (ELISA) methods for diagnosing *Staph. aureus, E. coli, Strep. dysgalactiae,* and *Strep. agalactiae* IMI. • Various on-farm techniques to monitor udder health include measuring enzymes, pH indicators, strip plates, or portable SCC measurements. • Emerging innovations with biotechnology and nanotechnology.	Royster et al., 2014; Kandeel et al., 2019

2. Environmental Factors

Livestock health and welfare are influenced by various factors, including environmental conditions and herd management. Factors such as high humidity and temperature, solar radiation, and climate variables affect the incidence of IMI (Hammami et al., 2013). To alleviate thermal stress, environmental modifications can be done, such as misting or sprinklers along with fans to promote evaporative cooling (Collier et al., 2006). Housing cows in a free stall with a misting system also reduced incidence of CM (e.g., Keister et al., 2002). Maintaining a clean, comfortable environment will also reduce proliferation of mastitis pathogens and decrease mastitis occurrence (Zeinhom et al., 2016). Jingar et al. (2014) reported that hot and humid climates and seasons adversely affected the incidence of mastitis in most cow breeds and Murrah buffaloes. Preventing mastitis requires attention to improving nutrition, housing, environment, and milking techniques, including maintenance of robotic milking systems. Other critical management practices include proper identification and treatment of cows with CM during lactation, segregation of cows with a contagious IMI, culling persistently infected animals, targeted dry cow antimicrobial therapy, application of internal teat sealants at drying off, establishing udder health goals, record-keeping, and regular monitoring of udder health status. These practices contribute to sustainable strategic management in preventing mastitis (Pyörälä, 2002; Kerro Dego et al., 2020; Stanek et al., 2024).

Conclusions

Bovine mastitis is a complex and economically impactful disease shaped by a variety of pathogenic and non-pathogenic factors. The range of causative agents, including contagious, environmental, and opportunistic bacteria, highlights the need for focused prevention and control measures such as rigorous hygiene practices, vaccination programs, and selective breeding to enhance genetic resistance. Progress in diagnostic methods, from conventional somatic cell counting to advanced genomic and biosensor technologies, provides valuable tools for earlier and more precise detection, leading to better mastitis management. Moreover, managing intrinsic host factors like age, immune function, and nutrition, along with external environmental influences such as housing conditions and climate, have crucial roles in reducing susceptibility and promoting udder health. Having a comprehensive, multifaceted strategy that integrates these elements through sustainable herd management is vital for reducing incidence and prevalence of mastitis, boosting dairy productivity, and ensuring animal welfare on a global scale.

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