Causes, Symptoms, Prevention and Treatment of Mastitis (Mast) in Dairy Cows

Samuel Jawahar B. and Princess Rajendran A.

1Department of Zoology, St. Johns College, Palayamkottai, Tirunelveli, Tamil Nadu, India
2Department of Zoology, Rani Anna Government College for Women, Tirunelveli, Tamil Nadu, India

*Corresponding Author

Received: 20th November, 2021; Accepted: 18th December, 2021; Published online: 31st December, 2021

https://doi.org/10.33745/ijzi.2021.v07i02.095

Abstract: A drop in milk output and a drop in milk quality result in financial hardship. Farmers should discard milk from cows with clinical instances of mastitis (M AST) and cows receiving anti-infective therapy according to the withdrawal period to allow the antimicrobials to exit the cow's system. M AST also alters the structure and composition of milk, resulting in lower cheddar output and a shorter period between uses of the resultant dairy product. Due to the additional time necessary to treat M AST animals, medical and veterinary expenditures will increase, as will labour costs and dairy productivity. Veterinary government support is a matter for concern, notwithstanding the financial problems, because research reveals that M AST can be unpleasant and can cause discomfort in cattle. As a result, cattle with proven clinical M AST or active subclinical M AST have a greater treatment risk. Certainly, udder medical issues are frequently referring to as one of the top three reasons for dairy cow separation. Another reason in dairy groups is low milk production, which might be linked to M AST. Harmful M AST, a severe form of the disease that causes significant aggravation and septicemia, can potentially cause cow death. The widespread use of antibiotics (ABX) raised worries about the growth of ABX-resistant bacteria, prompting the dairy industry to restrict ABX use. As a result, alternative treatments for the prevention and treatment of bovine M AST have been investigated, notably natural compounds derived from plants and animals. This study examines bovine M AST in terms of risk factors, management, and therapies, as well as developing therapeutic options for the treatment of bovine M AST.

Keywords: M AST, Nanoparticles, Herbal medicines, Antibiotics, Mastitis


Introduction

Among bovine illnesses, M AST has a significant impact on animal welfare as well as the financial system (Harmon, 1994; Jones and Bailey, 2009; Cha et al., 2011; Contreras and Rodriguez, 2011; Bardhan, 2013; Sinha et al., 2014; Abebe et al., 2016; Izquierdo et al., 2017; Aghamohammadi et al., 2018; Das et al., 2018; Cheng et al., 2019). The inflammation of the mammary glands/udder in cows is known as bovine M AST. The disease is caused largely by bacterial infections and is classified into several types depending on epidemiology, including infectious and...
environmental MAST (Garcia, 2004). The former is caused by infectious microorganisms (MOs) such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp., which are spread from an inflammatory cow to a healthy cow during milking via bacterial reservoirs such as hands, towels, and/or the milking equipment.

Environmental MAST, on the other hand, is caused by MOs that occur often outside of the milking parlour, i.e., the causative MOs come from the cow’s surroundings, which include bedding material, dirt, dung, faeces, and stagnant water (McInerne *et al.*, 1992; Garcia, 2004). Furthermore, it has a negative influence on both the composition and the value of milk (Halasa *et al.*, 2007; Kalinska *et al.*, 2017; Cobirka *et al.*, 2020). Environmental MAST is greatly influenced by control methods (Garcia, 2004), necessitating the use of more advanced technological and organic equipment, as well as suitable encouragement and rewards. Farmers and field veterinarians must work in accordance with legal guidelines when using antimicrobials that are mandated (Klaas and Zadoks, 2018).

Massive advancements in managing MAST were accomplished throughout the last century; nevertheless, changing population dynamics, herd form, and more stringent processing requirements have rendered MAST a complicated illness that continues to be a major headache for the dairy industry. As a result, extensive research within the neighbourhood is also required (Ruegg, 2017). MAST is a major financial problem in farm animals and buffalo in India (Das *et al.*, 2018), Canada (Aghamohammadi *et al.*, 2018), Germany (Hamann, 2001), The United Kingdom (Bennett *et al.*, 1999), The Netherlands (Hogeveen *et al.*, 2011), and The United States of America (Hadrich *et al.*, 2018).

Bovine MAST is associated with a daily loss of 1 to 2 litters of milk in the first weeks after commencement, and a total loss of 100 to 552 kg during the lactation, depending on the parity and time of incidence. MAST also has a long-term influence on milk output, since cows will no longer be able to reclaim their peak milk yield during the last portion of the lactation (Rajala-Schultz *et al.*, 1999). Despite various improved control methods in farm animals and buffalo raising inside the dairy sector, MAST remains a terrifying disease and one of the most common financial issues faced by farmers and dairy owners. India is at the top of the milk-producing countries (farm animals and buffalo milk combined). MAST causes a monetary loss of Rs. 575 million a year in India, and it reduces milk consumption by 21% (Bardhan, 2013). Furthermore, the consumption of MAST-affected milk may be harmful to individuals since antibiotic resistant bacteria may be transferred through contaminated unpasteurized milk; hence, it is a major public health problem (Oliver and Murinda, 2012).

Furthermore, health hazards connected with increased microbial resistance and ABX residues in milk have increased consumer demand for natural products, as they believe that foods produced through conventional agricultural systems are healthier and safer to consume (Ruegg, 2009). Due of zoonotic risks, MAST milk cannot be consumed and therefore cannot be sold, resulting in significant financial losses. Infected udder lowers the price of animals on the market and places a financial strain on the owner due to treatment costs (Gonzalez and Wilson, 2003; Seegers *et al.*, 2003). Although the link between MAST and pathogenic MOs was established in 1887, the most common infections were identified most effectively during the 1940s.

The discovery of the multi-factorial aetiology of bovine MAST in the 1960s cleared the way for more MAST research (Singh and Singh, 1994; Noguda *et al.*, 2018) such as molecular epidemiology of the causative pathogens; comparative strategies for identifying the pathogens on a subspecies level; virulence gene arrays; whole genome sequencing; and investigations of *in vitro* antibiotic susceptibility pattern. As days goes, ABX (penicillin) medicine
grew more widely available until 1945; nevertheless, it lost its effectiveness against all infections that cause MAST. Control methods should aim to reduce the pre-calving time in heifers in order to reduce the risk of MAST in later phases (Naqvi et al., 2018).

Subclinical MAST and IMIs in heifers at some time during calving are often caused by significant pathogens, such as coagulase-negative Staphylococci, which are the most common cause of heifer MAST. Many factors impact IMIs in early lactation, including the type of the disease, pathogen virulence, calving time, infection/cure duration, host immunity, pregnancy status, and managerial methods, as well as chance linked to season and herd location. A short-term prepartum ABX treatment is an effective way to manage heifer MAST, but it's almost ever recommended owing to the long-term negative effects on udder health and milk production, lowering farmers' earnings (De Vliegher et al., 2012). MAST detection is the most important need of the dairy industry for simple milk production, not only for financial reasons and public health concerns, but also for animal welfare reasons. Early, rapid, and accurate diagnosis is desired for MAST prevention or early identification for control or therapeutic purposes.

Traditional techniques are clearly inexpensive, simple, surprisingly available, and subject relevant, but they are typically non-unique. Superior inspections are expensive, requiring technical expertise as well as complex infrastructure and equipment, yet they are typically accurate and unique for various MAST types (Swarup et al., 1989; Malik et al., 2016; Hussein et al., 2018; Chakraborty et al., 2019). Blanket dry cow treatment, targeted culling, and well-defined biosecurity standards are all effective ways to control and prevent the return of virulent Streptococcus agalactiae and Staphylococcus aureus strains (Kefee, 2012). Furthermore, a combination of antibiotic treatment and culling of non-responsive cows resulted in a decrease in transmission rate and IMMS (Halasa, 2012).

For the treatment of MAST, a variety of traditional and advanced therapeutic methods are available, including ABX, vaccination, nanoparticle (NPs)-based therapy, natural therapy, and bacteriocins (Gomes and Henriques, 2016). Various retailers contribute to the reduction of udder infections, including MAST in cows, as well as to the improvement of milk quality (Skowron et al., 2019). ABX treatment and immunisation are the most often utilised methods for MAST treatment. Extensive and uncontrolled use of ABX for treatment, along with the formation and persistence of biofilm-related ABX resistance in MAST, has resulted in a decreased response to antibiotic treatment (Park et al., 2012; Babra et al., 2013). Although immunisation is ineffective against bovine MAST because so many MOs are involved in its formation, Staphylococcus aureus, Streptococcus uberis, and Escherichia coli have been identified as the most important targets for vaccine development (Wilson et al., 2009; Bradley et al., 2015; Collado et al., 2016; Cote-Gravel and Malouin, 2019; Ashraf and Imran, 2020).

Due to the obvious ABX's and vaccines' flaws, a slew of other treatment options has arisen to fill in the gaps. Few superior healing mechanisms or methods that might be promising for the prevention of MAST include NPs production and bacteriocins [antimicrobial peptides (AMP)] (Castelani et al., 2019; Godoy-Santos et al., 2019). The present evaluation discusses a variety of aspects of MAST/IMIs, with a focus on the disease's genesis, rapid incidence, diagnosis, control, and improvements in treatment and the development of innovative medicines for combating this important disease that affects bovine populations and dairy herds (Fig. 1).

MAST causing MOs:

The majority of infections that cause clinical bovine MAST are found in the environment. Contagious agents, on the other hand, are mostly associated with subclinical illnesses (Martin et al., 1997; Cheong et al., 2008; Ryan and Adley, 2010;
Fig. 1: MAST Influencing Factors.

Ranjan et al., 2011; Abebe et al., 2016; Mekonnen et al., 2017; Klaas and Zadoks, 2018; Dufour et al., 2019; Ngu Ngwa et al., 2020). MAST is a multi-etiologic illness caused by bacteria, with clinical, subclinical, contagious, and environmental MAST being the most common. The bacteria most frequently involved are Yeast, Nocardia, Yersinia ruckeri, Staphylococcus intermedius, Staphylococcus aureus, Vibrio fluvialis, Sphingomonas paucimobilis, Streptococcus agalactiae, Streptococcus pyogenes, Kyctococcus sedentarius, Peptococcus indolicus, Trueperella pyogenes, Mycobacterium bovis, Bacillus cereus, Escherichia coli, klebsiella pneumonia, Streptococcus canis, Aeromonas hydrophila caviae, Arcanobacterium pyogenes, Klebsiella oxytoca, Staphylococcus hyicus, Enterobacter aerogenes, Staphylococcus chromogenes, Serratia liquefaciens, Staphylococcus hyicus and Pasteurella spp. (Williamson and Di Menna, 2007; Chen et al., 2012; Verjan Garcia et al., 2015; Bi et al., 2016; Levison et al., 2016; Carvalho-Castro et al., 2017; Vakkamäki et al., 2017; Pașca et al., 2017; Abdalhamed et al., 2018; Shinozuka et al., 2018; Zhang et al., 2018; Dufour et al., 2019; Tarazona-Manrique et al., 2019; Ngu Ngwa et al., 2020).

Staphylococcus aureus, Streptococcus dysgalactiae and Streptococcus agalactiae are among the infectious pathogens. The most common organisms are Staphylococcus aureus, whereas the most common environmental pathogens are members of the Enterobacteriaceae family, including Escherichia coli and Streptococcus uberis (PeterssonWolfe et al., 2010; Dufour et al., 2019). In clinical MAST, Agalactiae is the most frequent Gram-positive bacteria, followed by Staphylococcus aureus, with Proteus spp., Brucella spp., Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella spp., Staphylococcusintermedius, and Escherichia coli being the most identified Gram-negative bacteria (Kefee, 2012; Cortinhas et al., 2016; Dalanezi et al., 2020). Because Streptococcus agalactiae and Staphylococcus aureus are disseminated mostly by contact, herd biosecurity is an essential preventative strategy for reducing and eliminating reservoirs (Fig. 2).
The majority of microbial infections can induce both clinical and subclinical inflammation. *Trueperella pyogenes*, on the other hand, is responsible for only clinical inflammation (Malinowski et al., 2006). *Staphylococcus aureus*, enteric bacterium species, and *Escherichia coli* are the most common causes of milk loss in mother cows. Infections by *Streptococci* spp., *Trueperella pyogenes*, *Staphylococcus aureus*, enteric bacteria spp., and *Escherichia coli* cause significant losses in older cows (Grohn et al., 2004). *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* are the most prevalent infections that cause inflammation, but *Eubacteria bovis* is less frequently involved (Wernicki et al., 2014; Vakkamaki et al., 2017). Coagulase-negative *Staphylococci* and their function in causing inflammation should also be properly considered (Krukowski et al., 2001). Wilson et al. (1997) found that *Streptococcus agalactiae*, along with other pathogens such as *Prototheca* sp., *Streptococci* spp., and *Trueperella pyogenes*, is linked to the majority of instances of inflammation. Coliforms, CAMP-negative *Streptococci* spp., *Trueperella pyogenes*, *Streptococcus agalactiae*, fungi, and *Prototheca* spp. cause inflammation in its most severe form when infections are present (Wilson et al., 1997; Bronislaw Malinowski et al., 2006).

Richard Steele and McDougall (2014) discovered *Eubacterium* spp. (40 per cent) and *Staphylococcus aureus* (32 per cent) as the most prevalent isolates in cases of sub-clinical inflammation in New Island. *Prototheca* spp. are infective algae and opportunistic infections that induce inflammation in farm herds and have the potential to cause animal illness (Alves et al., 2017). *Staphylococcus aureus* is the most frequent MOs associated with inflammation (McParland et al., 2019). Magro et al. (2018) reported penicillin-resistant *Staphylococcus aureus* (CC22-MRSA-IV) as IMMS MOs. MRSA was identified as an epidemic UK-EMRSA-15 grouping in CC22 after genotyping with DNA microarrays.
B-lactam and macrolide resistance genes were found in these isolated isolates. Milkers and farm cows provided isolates, suggesting that animal illness may be reversed. In thirteen of the total samples obtained from farm cows, routine sampling and analysis of milk revealed the presence of inflammation-causing bacteria. The most frequent pathogens discovered among the isolated pathogens were *Staphylococcus aureus*, *Streptococci* spp., *Trueperella pyogenes*, and *Corynebacterium bovis* (Cvetnic et al., 2016).

**Clinical significance of MAST:**

MAST can be classified as infectious or environmental MAST, and they are caused by a wide range of infections. In addition, MAST can be categorised as either clinical or subclinical in nature (Garcia, 2004; Abebe et al., 2016). Any increase in the amount of moisture and pollutants in the barn's environment will increase the load of pathogens in the environment. One study found a 74.7 per cent prevalence of MAST at the herd level and 62.6 per cent prevalence at the cow level. In terms of subclinical and clinical MAST, the former appears to be responsible for the majority of instances (59.2%) compared to the latter (3.4%) (Garcia, 2004; Abebe et al., 2016).

Clinical MAST can be identified largely via visual signs such as redness in the afflicted half or entire breast, warmth, swelling, discomfort upon biting, milk clots, discoloration, and a change in milk quality. Udder inflammation is the most common symptom. Environmental infections that include coliforms are the most common causes of clinical MAST. Four hundredth of the 20,000 clinical MAST cases in The Netherlands were caused by *Streptococcus uberis* and *Streptococcus dysgalactiae*, 30% by *Staphylococcus aureus*, and half-hour by *Escherichia coli* (Steeneveld et al., 2011).

During some conditions, the cow's mammary may exhibit shrivelled status as well as inflammatory resistance (Fig. 3). Such circumstances include long-term antibiotic medication to the mother, a greater frequency of mammalian fungal infection caused by mineral-vitamin and inhibitor insufficiency, nutritional imbalance, and bad environmental conditions such as weather fluctuations (Wawron et al., 2010). Kumar et al. (2010) investigated the incidence and cost of clinical MAST.

In comparison to clinical MAST, there are no clinically evident symptoms in subclinical MAST, however, a change in milk composition might be an indication. As a result, it is identified and verified by a laboratory analysis of milk or an animal-side test such as the Coliform MAST Test, which is followed by a laboratory isolation of the cause. In a healthy cow, the Somatic Cell Count (SCC) in the milk should be less than 200,000 per millilitre. White blood cells (WBCs) infiltrate neutrophils and macrophages into mammalian tissue as a result of inflammation (Akers and Nickerson, 2011). *Streptococcus agalactiae* is typically found within the mammalian mammary gland, where it produces long-term infections with greater SCC (Kefee, 2012).

The immune response of the host to pathogenic microorganisms that damage the udder causes MAST (Gurjar et al., 2012). The bacteria balance in a healthy udder is typically favourable. The microbiota of the IMMS is a complicated colony comprising various MOs (Rainard, 2017; Andrews et al., 2019). In a healthy udder, the commensal mammary microbiota plays a crucial role in immunological homeostasis (Derakhshani et al., 2018). As a result, a change in the udder microbiota's diversity may have an impact on MAST. The normal microbiome of the udder is an important factor to investigate when diagnosing MAST because healthy quarters include some bacteria. In udders, bacteria such as *Ruminococcus*, *Oscillospira*, *Roseburia*, *Dorea*, *Prevotella*, *Bacteroides*, *Paludibacter*, and *Bifidobacterium* are prevalent. MAST is caused by any udder or teat assault or congenital anomaly, such as a teat fistula, a teat spider, a leaky teat, or udder sores that expose the udder to external MOs or cause milk retention (Rambabu et al., 2011).
Severe inflammation was seen in MAST-affected tissue, as well as significant reductions in the alveolar epithelium and lumen, as well as an increase in stromal connective tissue and leucocytosis (Nickerson *et al.*, 1995). External infections are exposed to the udder, or the udder’s internal defences are weakened. The presence of *Staphylococcus* spp. or *Escherichia coli*, as well as disturbance of the normal microbiome, are characteristics of the clinical form of MAST.

According to scientists, MAST develops and spreads as a result of pathogen-induced alterations in the normal microbiota or antibiotic therapy that lasts too long (Falentin *et al.*, 2016). According to a comprehensive molecular epidemiological study, the majority of dairy cattle in the United States had more than 10 coagulase-negative *Staphylococcus* spp., which were identified at different stages of lactation (Wuytack *et al.*, 2020). MAST is the consequence of numerous factors interacting at the host level, and it is a complex and damaging effect. Pathogens, their development patterns in the udder parenchyma, signalling pathways that cause clinical symptoms, and other molecular processes mediated by pathogen-associated molecular patterns are all implicated. Initiating udder inflammation induced by microbial infections, as well as a range of environmental factors, the host’s pattern recognition receptors, including as Toll-like receptors, NOD-like receptors, and RIG-like receptors, make this feasible. As a result, a well-coordinated approach to diagnosis and management of this life-threatening disease is essential (Bhattarai *et al.*, 2018).

**MAST Classification:**

Paying care to the cows’ health always pays off. When diagnosing MAST, it is essential to understand how bad the problem is. The MAST grading system (Fig. 4) is a great tool for evaluating the severity of MAST infection. Because a dairy employs a large number of people, it is important that the milkers, who are often the first to notice MAST, understand its seriousness. The chart is used to develop a standardised method and to incorporate training for all dairy employees. Using the chart, milkers may determine the exact measures to take. They know to take a sample for testing in a mild or moderate case, and they know to notify the herdsman immediately away in a severe case. If a farm can culture (on-farm or with their veterinarian) and have results within 24 h, they may be able to...
segregate mild and moderate cases to the hospital pen and postpone treatment until they have more information (Pyörälä and Pyörälä, 1998; Ruegg et al., 2009; Zigo et al., 2021).

**Economic significance:**

In addition to the cost of medication and other expenses, both clinical and subclinical MAST result in milk loss since it must be discarded (Halasa et al., 2007; Kumar et al., 2010). Subclinical MAST causes three times as many productivity losses as clinical MAST, accounting for 60-70 per cent of all MAST-related economic expenditures due to infections (De Vliegher et al., 2012; Sinha et al., 2014). Sinha et al. (2014) investigated the prevalence and economics of subclinical bovine MAST in India’s Central Region using dairy cows. Milk value loss accounted for about 49% of MAST losses, while veterinary expenses accounted for 37%. The cost of treating an animal includes both the cost of medicine (31%) and the cost of services (5.5 per cent). Due to their high production potential, which was hindered during the MAST period, cross-bred cattle losses were much higher.

**Frequently used MAST diagnostic tests:**

Modern diagnoses are quantitative, highly specific, and sensitive, whereas traditional MAST diagnostic procedures are frequently qualitative, with lesser specificity and sensitivity (Godden et al., 2017; Hussein et al., 2018; Chakraborty et al., 2019). At the species and subspecies levels, advanced molecular techniques based on phenotyping and genotyping procedures enable rapid and specific identification methods for MAST-causing infections (Gurjar et al., 2012). To choose the optimal antibiotic for medicinal purposes and to select the best processing technique for dairy products in particular, it is important to identify the type of bacteria. For this reason, identification (automated) methods such as VITEK identification cards are available, which provide consistent bacterial identification findings (Harjanti et al., 2018).

According to Kandeel et al. (2018), even if there is no overt clinical MAST, all dairy calves brought to any veterinary facility should be treated as if they have IMMS infection. Chakraborty et al. (2019) have reviewed many advancements in diagnostics appropriate for early and accurate MAST diagnosis, including phenotyping (general kind of testing) and genotyping (specialised type of testing). The
former comprises physico-biochemical, non-specific cultural, and proteomics testing, while the latter includes a particular culture, polymerase chain reaction (PCR) and its many variants (e.g., qRT-PCR) (Behera et al., 2018), loop-mediated isothermal amplification (LAMP), and lateral flow assays (Sheet et al., 2016; Barreiro et al., 2017; Griffioen et al., 2020).

A popular diagnostic biomarker for evaluating bovine MAST is haptoglobin (acute phase protein) (Kalmus et al., 2013). A label-free chemiluminescence bioassay based on magnetite nanoparticles was demonstrated in a study for the early, sensitive, and rapid detection of haptoglobin at clinically relevant levels in milk, resulting in quantitative detection of haptoglobin with a detection limit of 0.89 pg/ml (Nirala et al., 2020). The oxidative state of animals with mammary gland inflammation in cows has been studied (Kleczkowski et al., 2017). The synthesis of many inflammatory mediators, as well as reactive oxygen species, is involved in MAST-related inflammation (Turk et al., 2017). Increased levels of inflammatory and oxidative mediators have been related to MAST. The levels of interleukins in the blood, tumour necrosis factor (TNF), acid glycoprotein (alpha-1 AG), and haptoglobin have all altered considerably (Kleczkowski et al., 2017).

Differential protein expression in subclinical and clinical MAST was previously investigated using the cow serum proteome. In both preclinical and clinical situations, the comparative analysis assisted in assessing the systemic inflammatory and oxidative stress response. The findings of the study show that vitronectin, an inflammatory protein, is overexpressed in both subclinical and clinical MAST. As a result, vitronectin is an important mediator in the progression of MAST and can be utilised as a biomarker to diagnose MAST in its early stages (Turk et al., 2012). In clinical and subclinical MAST, serum paraoxonase-1 activity was measured to assess systemic inflammatory and oxidative stress responses (Kovacic et al., 2019).

Paraoxonase-1 activity was significantly lower in rats with clinical and subclinical MAST than in the control group, according to the findings of Kovacic et al. (2019). We may deduce that subclinical MAST-induced oxidative stress and inflammation significantly decreased paraoxonase-1 activity in the blood and milk of infected cows (Nedic et al., 2019). As a consequence, the activity of paraoxonase-1 might be utilised as a biomarker to detect MAST in the early stages. The virulence of the causative agent determines the pathogen-invading mammary glandular response. Furthermore, the causative agent’s infectivity is influenced by the microbial environment as well as the host (relative). As a result, MAST epidemiology differs depending on infections, host factors, and environment (Klaas and Zadoks, 2018). Despite the fact that the origins and epidemiology of this specific inflammatory illness of the mammary gland in food animals are well established, the difficulty of properly diagnosing the ailment remains a significant challenge (Sordillo, 2011).

Conducting research based on molecular epidemiology can thoroughly characterise the routes of transmission, sources, and prognosis of numerous diseases that cause MAST in cattle. Understanding how viruses evolve to evade host defences might aid our understanding of the host adaptation process (Zadoks et al., 2011). Pathogen persistence may also be assessed more efficiently by identifying the allelic profiles of virulence or house-keeping genes, and molecular epidemiology research can help with this. In this respect, it's important to note that the development of the multilocus sequence typing (MLST) technique has aided these molecular epidemiology studies (Sordillo 2011; Shibata et al., 2014). The Internet of Things (IOT) has recently been used to identify MAST and foot and mouth disease (FMD) using Neural Networks and smart sensors, which may help in a better way for a significant reduction of both diseases (Vyas et al., 2019). This can benefit the Agriculture and Dairy Industries in a number of ways by reducing the poor quality of milk.
supplied by cows and, as a result, lowering dairy processing expenses.

**MAST treatment:**

Understanding the pathophysiology of infection, devising innovative sensitive tests for early screening, implementing suitable management practises to reduce the risk of transmission, and avoiding uninfected animals are all necessary components of an effective MAST control programme. Antibiotic residue in milk and antimicrobial resistance must be addressed by a control programme that includes the strategic use of antimicrobials (Ruegg et al., 2017). The underlying cause of udder infection must be identified before pharmaceutical therapy may begin.

Teat and udder affections including teat fistulas, leaking teats, teat spiders, and udder sores require immediate attention. Because these affections break the protective barrier and expose the teat canal or udder to external MOs, early treatment is necessary. According to one study, the number of cattle teat surface surgical affections, as well as the number of teat surgical affections in buffaloes, was significantly higher than the number of teat surgical affections in other areas (Misk et al., 2018). Treatment was not necessary for all teat and udder issues. A total of 24 per cent of the 282 cow and buffalo cases went untreated, while 73.8 per cent were treated using medicinal and surgical treatments (Misk et al., 2018).

During milking season, which is a high-risk period for new IMIs, the majority of prophylactic measures are centred (Keefe, 2012). Disinfecting the teat before and after milking, as well as full milk-out, can improve the health and hygiene of dairy cows (Keefe, 2012; Yu et al., 2017). Heifers in early lactation are more likely than cows to acquire clinical and subclinical MAST due to their management and physiological state. Improved prepartum management measures in the areas of environmental and animal hygiene, such as the use of teat sealants and antiseptics, vector control, separation of heifers from older cows, and reduced MAST milk feeding to calves, are recommended to minimise heifer MAST (McDougall et al., 2009). Although prepartum heifer treatment resulted in a much higher cure rate, minimal milk loss, and a lower risk of antibiotic residues, lower SCC and high milk production are not always achieved in all herds (Borm et al., 2006). Furthermore, throughout the first 200 days of the first lactation, IMMS treatment showed no influence on preparturient heifer reproductive performance (Borm et al., 2006).

Lactational therapies in preclinical IMIs of dairy cows led in decreased transmission rates of *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli*, as well as fewer flareups and lower IMI-related expenditures, according to the study. However, *Staphylococcus aureus* transmission was not stopped. To enhance udder health, dairy cows require lactational treatment, which must be preceded by management measures (van den Borne et al., 2010). Antibiotic therapy, identifying the causal agent, parity, stage of breastfeeding, history of previous SCC, clinical MAST, and other systemic diseases all have a role in clinical MAST treatment (Steeneveld et al., 2011).

Due to restrictions imposed by the organic certification process, such as no use of antimicrobials or hormones, use of organic feeds, and stress-free husbandry practises, organic farmers in the United States treat clinical MAST cases with a variety of alternative therapies, including homoeopathy, botanicals, vitamin supplements, and whey-based products (Ruegg, 2009). The recommended cow-specific treatment for clinical MAST was not demonstrated to be economically viable in a study done in the Netherlands (Steeneveld et al., 2011). On the other hand, herd-specific therapies such as cow-specific medication and culling strategies against preclinical and clinical IMIs may be highly cost-effective in the management of MAST (Gussmann
et al., 2019). MAST treatment comprises both preventive and therapeutic treatments, the most frequent of which is antibiotic medication. Recent MAST treatments, on the other hand, have included the use of natural medicines such as zeolites and propolis, which might be used instead of antibiotics (Benic et al., 2018).

**Treatment with ABX:**

ABX is widely used as a MAST preventive method during the dry season. Antimicrobial therapy of dry cows is allowed as a preventative technique among livestock animals. ABX should be chosen for the treatment of clinical MAST based on the patient’s medical history, pathogenesis, antibiotic sensitivity profile, and, most importantly, proposed therapeutic principles. Antibiotic resistance has been found in pathogens isolated from MAST milk across a wide spectrum of ABX.

In a study conducted in the Zenica area of Bosnia and Herzegovina, the highest antimicrobial resistance was observed against benzyl penicillin (C₁₆H₁₈N₂O₅S) (56.3%) and oxytetracycline (OxyA, OxyB, OxyC, and OxyP) (46.2%). (Burovic, 2020). Antimicrobial susceptibility of *Trueperella pyogenes* infections in domestic animals was studied in vitro. ABX such as florfenicol (C₁₂H₁₄C₂FNO₄S), ceftazidime (C₂₃H₂₇N₅O₆S₂), cephalexin (C₁₆H₁₇N₃O₅S), and ceftiofur (C₁₉H₁₉N₃O₇S₃) were shown to be effective (Ribeiro et al., 2015). Antibiotic selection for MAST treatment should be based on culture and sensitivity data rather than empirical therapy, given the emergence of ABX resistance (Tiwari et al., 2013). Another important drawback of ABX treatment is its proclivity for producing ABX residues in milk, which may be detrimental to the health of consumers (Oliver and Murinda, 2012; Gomes and Henriques, 2016). These ABX residues have been shown to be persistent for long periods of time and can have harmful effects on users as well as cause resistance (Kurjogi et al., 2019). The use of ABX in cows, such as oxytetracycline (C₂₂H₂₄N₂O₉), amoxicillin (C₁₆H₁₉N₃O₅S), and ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid), resulted in antibiotic residue in both raw and boiled milk at different time intervals, according to the report (Anika et al., 2019).

Despite the fact that ABX are frequently used to treat MAST regardless of the severity of the disease, most cases of non-severe clinical MAST will not benefit from such uncontrolled usage. To cope with such situations, alternative approaches should be employed (Ruegg, 2017). Targeted ABX therapy aimed against particular organisms is one of the current proposed approaches for treating clinical MAST caused by Gram-positive agents. Such treatment techniques provide the remaining cases adequate time to recover on their own (Ruegg, 2017). Clinical cure rates are improved with ABX therapy that uses several administration channels, such as systemic and IMMS. This might be due to higher antimicrobial concentrations in milk and mammary tissues (Lima et al., 2018).

In clinical circumstances, ABX selection based on culture and sensitivity may not guarantee 100% efficacy. This is owing to discrepancies in ABX susceptibility data in vitro and the failure of such sensitive medicines in clinical settings. In *in vitro*, MAST generated by *Staphylococcus aureus* is sensitive to a variety of ABX, but owing to Staphylococci’s unique biology, as well as their adaptability to the bovine host environment, the development of microabscesses, and the creation of biofilms, many ABX therapies become clinically ineffective (Rainard et al., 2018). To ensure the optimal and appropriate use of ABX in suspected MAST cases in bovine species, careful and cautious interpretation of laboratory data is required to prevent antibiotic treatment of Staphylococci without considering clinical relevance (Wald et al., 2019). The ABX susceptibility spectrum has a lot of variation in it. This can make the outcome of ABX treatment even more complicated. Novel compounds must be researched as soon as feasible for MAST prevention and therapy. For the prevention of new IMIs in dairy cattle, Lago et al. (2016) investigated post-milking barrier teat
disinfectants based on glycolic acid (C\textsubscript{2}H\textsubscript{4}O\textsubscript{3}) and iodine (I). Glycolic acid (C\textsubscript{2}H\textsubscript{4}O\textsubscript{3})-based disinfectants were shown to be non-inferior to iodine (I)-based disinfectants in terms of safety and efficacy as post-milking teat disinfectants. They decreased the incidence of new IMI (NIMI) by about 17\%, but had no effect on SCC or teat conditioning. Martins et al. (2017) studied disinfectants with high free iodine (I) and barrier quality in dairy cows to see if they might prevent new IMMS and clinical MAST from emerging spontaneously.

Teat disinfectants with barrier properties and higher free iodine content were able to reduce the risk of clinical MAST when provided after milking; however, the influence on new infections was only seen at weekly intervals. Barrier after milking teat disinfectant (BAR)-treated animals had a 46 per cent lower incidence of clinical MAST than non-barrier post-milking teat disinfectant-treated animals (NBAR). The risks of NIMI were lowered by 54 per cent and 37 per cent, respectively, using NBAR disinfection.

Several studies looking at the antibiotic sensitivity of bacteria isolated from bovine MAST in vitro showed varying degrees of antibiotic resistance across isolates from throughout the world (Leon-Galvian et al., 2015; Shah et al., 2019). Penicillin, clindamycin, and cefotaxime resistance was found in bovine MAST isolates from Mexico (Leon-Galvian et al., 2015). All Escherichia coli isolates from clinical MAST cow milk were resistant to cloxacillin in a study conducted in southern Taiwan, although some isolates were resistant to tetracycline, neomycin, gentamycin, ampicillin, ceftriaxone, cefotaxime, and ceftazidime (Shah et al., 2019).

In India and Thailand, methicillin resistance genes were discovered in Staphylococcus aureus isolates from bovine MAST patients (Shah et al., 2019). Several resistant microbial isolates have also been identified from clinical cases of cow MAST in India. Fourth-generation cephalosporins were shown to be somewhat superior than the conventional cloxacillin and ampicillin combination in the treatment of asymptomatic Streptococcus agalactiae MAST (Rossi et al., 2019). Although oxytetracycline might be used as a first-line treatment in cattle with acute Escherichia coli MAST, its effectiveness is unknown (Shinozuka et al., 2019).

In mild to moderate Escherichia coli MAST, ABX should be avoided; however, in severe cases, ABX such as fluoroquinolones and cephalosporins given parenterally are recommended to minimise the risk of bacteremia (Suojala et al., 2013). The efficacy of ABX in the treatment of MAST is affected by all changes in the susceptibility spectrum, as well as the establishment of resistance. In Indian crossbred cows, ceftizoxime has been demonstrated to be effective in treating acute staphylococcal MAST (Buragohain et al., 2019). Changes in the susceptibility spectrum will affect how MOs respond to antimicrobial medicines. Although extensive use of ABX for MAST prophylaxis may result in antibiotic resistance and residues in milk, the benefits of ABX much exceed the disadvantages stated above (Oliver and Murinda, 2012). Steele and McDougall (2014) looked at how protracted penethamate hydriodide (PH) therapy affected the bacteriological cure per cent and SCC in dairy cows. Following PH treatment, the proportion of bacteriological cures increased, the percentage of glands infected after therapy decreased, and SCC decreased.

As a result, the pH aids in the treatment of intramural infections in preclinical MAST; however, older cows and those infected with Staphylococcus aureus, particularly those resistant to penicillin, have a lower impact. Fuenzalida and Ruegg (2019) observed that IMMS ceftiofur had no effect on culling rate, milk yield or production, or SCC when used to treat non-severe culture-negative clinical MAST cases. Klebsiella pneumoniae MAST was worsened by intraamammial (IMM) ceftiofur, resulting in chronic IMMS and poor clinical outcomes. IMM
ceftiofur is not considered necessary in the occurrence of *Escherichia coli* MAST (Fuenzalida and Ruegg, 2019). However, there is presently no viable alternative to ABX.

Tilmicosin intraamammary infusion during drying might be a viable option for avoiding NIMI, which is caused mostly by environmental streptococci and coagulase-negative *Staphylococcus* (Dingwell et al., 2002). An IMMS infusion of ceftiofur protects against *Streptococcus uberis* infection, according to an experimental study. When compared to the 2-day treatment option, the 8-day extended therapy had a greater impact (Oliver et al., 2004). There were no significant variations in MAST cure rates between two ABX, tylosin base and penethamate hydriodide; 79.8% vs. 82.0% of cows treated, respectively (McDougall et al., 2007). Several variables, including microbe type, udder environment, and milking technique (machine/hand), impact the efficacy rate of medications in MAST.

Non-steroidal anti-inflammatory medicines resulted in fewer SCC, reduced milk output losses, improved clinical outcomes, and lower culling rates as compared to antimicrobial therapy alone (McDougall et al., 2009). Nonsteroidal anti-inflammatory drugs (NSAIDs) have been found to be effective in the treatment of *Escherichia coli* MAST and are currently recommended for use as supportive therapy in the treatment of clinical MAST (Suojala et al., 2013). Due to its unique pathophysiology, contagiousness, environmental persistence, skin or mucosal colonisation, and poor response to existing treatment drugs, *Staphylococcus aureus* is one of the few etiological organisms responsible for clinical MAST that can give the clinician/veterinarian a headache (Rainard et al., 2018).

Therapeutic interventions for *Streptococcus agalactiae* can be successful rapidly, but they are usually useless against *Staphylococcus aureus* (Kefee, 2012). Increased biofilm formation among methicillin-resistant *S. aureus* strains has been found to enhance pathogenicity (Shah et al., 2019).

*Staphylococcus aureus* is the most difficult agent to eliminate from herds because of all of these features. Antibiotic therapy in *Staphylococcus aureus*-induced MAST is no longer effective, most likely due to overuse (Park et al., 2012) or the development and persistence of biofilm-associated antibiotic resistance in *Staphylococcus aureus*-induced MAST (Babra et al., 2013). That might reflect the continuous attempt to develop vaccines against MAST induced by *Staphylococcus aureus*, which have yet to prove successful (Cote-Gravel and Malouin 2019). According to a recent study, the NZ2114 derivative peptide H18R (H2) can be used to treat *Staphylococcus aureus*-induced MAST in a safe and promising way (Wang et al., 2019).

According to one study, nasal immunisation against *Staphylococcus aureus* associated MAST in bovines results in an increase in anti-*Staphylococcus aureus* specific IgA antibodies in milk and a negative correlation between anti-*Staphylococcus aureus* specific IgA antibodies and *Staphylococcus aureus* counts in the treated udder, suggesting that nasal vaccines could be used to treat *Staphylococcus aureus* infections. To successfully incorporate antibiotic usage in MAST reduction, diagnostic effort should be enhanced so that unnecessary antibiotic use may be minimised (Kromker and Leimbach, 2017).

Prudent antibiotic use should be carried out with sufficient knowledge, a scientific foundation for minimising antibiotic use, and a legal requirement for caution, taking into account the cost of MAST therapy and the potential benefits (Doehring and Sundrum, 2019). Antimicrobial therapy of cows with newly acquired subclinical MAST (RASCM) should be conducted only in severe cases, given the current emphasis on the limited and cautious use of antimicrobials in animal husbandry treatment methods (van den Borne et al., 2019). Antibiotic resistance of major bacterial infections that cause MAST in cows must be continuously monitored, as well as harmonisation of techniques and interpretations.
(Chehabi et al., 2019). When compared to a preparation containing tetracycline, neomycin, bacitracin, and prednisolone, an intraammary preparation of ceftiofur hydrochloride showed no significant difference in overall clinical cure, bacteriological cure, or new infection when used to treat non-severe clinical MAST in dairy cows (Cortinhas et al., 2016).

Pirlimycin was used as an extended therapy for Staphylococcus aureus intra-mammary infections in heifers by Skoulikas et al. (2018). The treated group had a considerably higher cure rate (64.8 per cent) than the control group (34.1 per cent). As a result, they predict that treating heifers with the extended treatment method right after calving will result in higher cure rates for Staphylococcus aureus IMMS infections. Extending the length of IMMS therapy may help prevent clinical failures, but it has no effect on cure proportion, somatic cell count, or the incidence of new infections.

McDougall et al. (2019) found that IMMS treatment with a combination of amoxicillin, clavulanic acid, and prednisolone administered over short (3 times at 12 h intervals) and long (5 times at 12h intervals) periods significantly reduced clinical failures while having no effect on cure proportion, SCC, or new infection rate. Because there are no effective therapies for MAST caused by Prototheca spp, it is exceedingly difficult to treat (Alves et al., 2017). Previously, the in vitro algicidal efficacy of guanidine on Prototheca zopfii genotype 2 strains isolated from clinical and subclinical bovine MAST was studied. At low concentrations, guanidine possesses algicidal action and can be used as an alternate disinfectant, antiseptic for cleaning, chemical dry treatment of bovine teats, or even as an IMMS therapeutic agent (Alves et al., 2017).

The resistance of biofilm-producing bacteria to conventional ABX makes treatment challenging. Alternative types of therapy must be utilised in such situations if the etiological culprit is to be successfully eliminated. Bacteriophages are bacteria-infecting and destroying viruses (Tiwari et al., 2014). They have the capacity to target and destroy specific bacteria, as well as the ability to grow quickly, making them a viable competitor in the fight against dangerous bacteria (Carson et al., 2010). The capacity of several bacteriophages to eradicate pathogenic microorganisms associated to MAST has been found and studied (Amiri Fahliyani et al., 2018; Varela-Ortiz et al., 2018). There have been a number of potential candidates for bacteriophage therapy of Staphylococcus aureus (Varela-Ortiz et al., 2018), Klebsiella oxytoca (Amiri Fahliyani et al., 2018), and Escherichia coli (Porter et al., 2016). Because all of the evaluations were based on in vitro research, further in vivo studies are needed to show efficacy in clinical settings. Although bacteriophages are effective against bacteria, the majority of them are unstable in the environment and must be stored and managed in certain ways.

The isolated bacteriophages demonstrated thermostability and significant lytic capability, according to Amiri Fahliyani et al. (2018), making them good options against Staphylococcus aureus antibiotic resistant strains. All of the thermostable phages maintained a high titer even after a 5-min incubation at 100 C (Amiri Fahliyani et al., 2018). The use of a phage cocktail rather than a single virus is another development in bacteriophage treatment. A phage cocktail was shown to be more successful than each of the individual phages used alone in treating Staphylococcus aureus-induced MAST in a mouse model (Geng et al., 2020).

Mice treated with phage cocktail had the highest IMMS phage titer when compared to other groups, and its efficacy was comparable to that of the antibiotic ceftiofur sodium. Phages trigger phage-specific humoral responses and memory, thereby jeopardising therapeutic efficacy (Krut and Bekeredian-Ding, 2018). STA1, ST29, EB1.ST11, and EB1.ST27 were used to assess the lytic efficacy of a bacteriophage combination that included three phages, STA1.ST29, EB1.ST11, and EB1.ST27, against Staphylococcus aureus isolates.

1054
The significant reduction in *Staphylococcus aureus* germ density showed that bacteriophage therapy could have therapeutic potential, which has to be confirmed *in vivo* (Titze et al., 2020). More study is needed to show that bacteriophage therapy is effective in treating cow MAST *in vivo*.

**Bacteriophage endolysins as a unique type of antibacterial agent:**

Another potential therapeutic medication that is effective against Gram-positive bacteria is endolysins generated by bacteriophages. They are the proteins that allow the phage to escape from the bacterial cell by breaking the peptidoglycan layer of the bacterial cell wall during the lytic cycle (Breyne et al., 2017). CHAPK, a novel bacteriophage-derived peptidase identified by Fenton et al. (2013), is an effective biocidal agent capable of rapidly breaking biofilm-forming *Staphylococcus*. Because of CHAPK's *in vitro* efficacy, it can be used in teat-dip solutions to prevent *Staphylococcus aureus* colonisation on udder skin (Fenton et al., 2013). To prevent and cure infections caused by the staphylococcal bacterial group, several new peptidoglycan hydrolases have been identified. Anti-staphylococcal peptidoglycan hydrolases include Lysostaphin, LasA, ALE-1, broth lysate, CsCl, LytM, AtlA, AtlE, LysK, SAL-1, MV-L, ClyS, and LysH5 (Gill et al., 2006; Szweda et al., 2012).

**Herbal medicine:**

Herbal therapy has no harmful side effects, making it a viable treatment choice for MAST. Ethno-veterinary medicine is a branch of veterinary medicine that focuses on treating diseases with herbal remedies (Tiwari et al., 2018). In the treatment of cow MAST, medicinal herbs can be used as an alternative therapy or as an auxiliary medicine. They can be used as an antibacterial, anti-inflammatory, and immunomodulatory treatment for MAST (Mushtaq et al., 2018). The anti-inflammatory and antibacterial characteristics of Chinese herbs have been effectively employed in the treatment of cow MAST (Muluye et al., 2014; Yang et al., 2019). They can also be used instead of ABX and antipyretics, which are often prescribed for MAST therapy (Muluye et al., 2014).

Methanolic extracts of herbal preparations including *Diploclisia glaucescens* leaf and *Curcuma longa* rhizomes in equal amounts produced analgesic and anti-inflammatory effects (Ranjith et al., 2018). The analgesic effectiveness of the herbal extract was found to be comparable to that of ibuprofen and indomethacin (Ranjith et al., 2018). Depending on the formulation, herbal therapy uses a number of administration techniques. Topical administration (Hase et al., 2013), oral administration (Dash et al., 2016), and IMMS administration are the most often used methods (Yang et al., 2019). Antibiotic therapy was shown to be more successful than herbal and homoeopathic complex therapy in a research evaluating the efficacy of homoeopathic complex therapy, herbal therapy (Neem seed extract), and antibiotic therapy for the treatment of preclinical MAST in dairy buffaloes. When it came to treatment costs, herbal therapy was shown to be the most cost-effective (Younus et al., 2018). As a result, it can be utilised as an adjuvant to ABX in the treatment of clinical MAST without causing major cost increases.

Some herbal extracts may have anti-inflammatory and antioxidant qualities that help with udder inflammation and oxidative stress reduction. Moringa extract has been demonstrated to decrease inflammatory mediators and increase antioxidant mechanisms in cow udder epithelial cells. TNF-a (tumour necrosis factor alpha), IL-1b (interleukin 1 beta), and IL-6 (interleukin 6) expression were all repressed, as was cyclooxygenase-2 (COX-2) expression, and nuclear factor-kappaB was downregulated, while heme-oxynase-1, NAD(P)H, and quinone oxidoreductase-1 were upregulated (Cheng et al., 2019). Numerous plant species are used in southern Brazil to prevent and cure cattle MAST due to their anti-inflammatory, immunomodulatory, and antibacterial
characteristics (Avancini et al., 2008; Xu et al., 2015).

Herbal medicine was made from plant stems, leaves, bark, seeds, flowers, bulbs, fruits, and aerial parts. Plant species like Aloe vera, Achillea millefolium, Allium sativum, Mentinga calabura, Alternanthera brasiliana, Nigella sativa, Baccharis trimera, Origanum vulgare, Chenopodium ambrosioides, Cuphea carthagagensis, Ocimum sanctum, Foeniculum vulgare, Acacia nilotica, Phytolacca dioica, Sambucus nigra, Sida rhombifolia, Mentha pulegium, Solanum mauritianum, Tinospora cordifolia, Moringa oleifera, Garcinia indica, Atractylodis macrocephalae Koidz, Withania somnifera, Eugenia caryophyllus, Allium sativum, Agastache foeniculum, Solidago chilensis, Lavandula angustifolia and Althaea officinalis were used orally, among which Alternanthera brasiliana, Populus nigra, Plantago lanceolata, Baccharis trimera, Evernia prunastri, Ocimum basilicum, Crassula multicava and Sambucus nigra were also used as topical agents (Pașca et al., 2017; Sserunkuma et al., 2017; Johri et al., 2018; Mushtaq et al., 2018; Maramulla et al., 2019; Ren et al., 2020; Kovačević et al., 2021).

Ocimum basilicum and Parapiptadenia rigida were the two plant species that were used by IMMS route in bovine MAST (Avancini et al., 2008). Staphylococcus epidermidis is one of the most common causes of medical device-related infections and bovine MAST, in addition to its biofilm-forming abilities. Oxytropis glabra is a Fabaceae plant that is commonly used as a Chinese herbal composition in Western China. According to Mihaylova et al. (2013) Asteraceae, Lamiaceae, and Fabaceae family plants are frequently utilised for MAST pathogen therapy in India. In vitro tests evaluating the effect of Oxytropis glabra decoction on the creation of Staphylococcus epidermidis biofilms discovered putative inhibitory mechanisms that may be explored further in the development of new biofilm-associated infection treatments (Ren et al., 2020).

Leaf extract exhibited strong bio-enhancing and anti-oxidant properties, which can be utilised in conjunction with ABX in a research to examine the efficacy of Ocimum sanctum leaf juice as supportive treatment for the management of chronic staphylococcal MAST (Dash et al., 2016). Rather employing herbal therapy as a stand-alone treatment for clinical MAST, integrating it in the treatment regimen as an adjuvant with other modalities of therapy can yield better results. A 500 mg/ml concentration of Terminalia chebula ethyl acetate extract showed the same antibacterial efficacy as conventional amoxicillin against molecularly defined isolates of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus megaterium (Kher et al., 2019). This finding raises the prospect that herbal extracts might one day replace ABX as the only therapy for clinical MAST (Zhen et al., 2008; Zecconi et al., 2009; McPhee et al., 2011; Wang et al., 2011; Leitner et al., 2013; Pinedo et al., 2013; Almeida et al., 2015; McDougall et al., 2016; Petzl et al., 2018).

NPs applications:

NPs technology is another topic that is rapidly gaining traction as a delivery technique for antibacterial and other drugs (Gomes and Henriques, 2016). Several types of NPs have been tried in the past for MAST therapy, with encouraging results (Castelani et al., 2019; Kalinska et al., 2019; Orellano et al., 2019; Pinheiro Machado et al., 2019). The absorption of active chemicals by phagocytes will be increased by NPs formulations, enhancing their antibacterial effectiveness (Gruet et al., 2001). They have been demonstrated to work against a wide range of multidrug-resistant bacteria that pose a serious threat to civilisation (Yu et al., 2018; Castelani et al., 2019).

Staphylococcus aureus-caused bovine MAST is particularly difficult to treat with conventional therapies due to its efficient pathogenesis, unique facultative intracellular parasitism, biofilm formation, and growing antimicrobial resistance.
As a result, NPs-based therapy techniques such as liposomes, nanogels, polymeric nanoparticles, inorganic NPs, and solid lipid NPs are gaining popularity as viable tools for treating *Staphylococcus aureus* MAST (Algharib et al., 2020). Honey has significant antimicrobial action in vitro against methicillin-resistant and vancomycin-resistant coagulase-positive *Staphylococcus aureus* MAST strains when coupled with gold nanoparticles (AuNPs) (Omara, 2017). When NPs are used in high quantities, they harm numerous organs and cause pathological changes (Elbehiry et al., 2019).

To identify the biological effects of NPs, more study is required (Kalinska et al., 2019). Chitosan nanoparticles (CHS-NPs) (Orellano et al., 2019), propolis NPs, and cationic nisin-lipid nanoparticles (Castelani et al., 2019) have all been found to be beneficial in the treatment of MAST. When coupled with AgNPs, ABX which inhibit protein synthesis have a powerful synergistic effect (Ahmadi et al., 2014). The antibacterial activity of antibiotics like tilmicosin and amoxicillin can be improved via nanoformulation (Yang et al., 2009; Zhu et al., 2018). Tilmicosin solid lipid NPs’ antibacterial efficacy against *Staphylococcus aureus* agar and *Streptococcus agalactiae* was prolonged and enhanced (Zhu et al., 2018). Amoxicillin nanoparticles improved the post-antibiotic impact and lowered the dose interval when used against pathogenic bacteria that cause bovine MAST (Yang et al., 2009). *Staphylococcus aureus* strains collected from MAST patients were found to be toxic to AgNPs and AuNPs. Because *Staphylococcus aureus* strains gain resistance to AuNPs less frequently, AuNPs outperformed AgNPs (Elbehiry et al., 2019). The therapeutic efficacy of an IMMS nanosuspension based on a-linolenic acid (ALA-NS) for the treatment of subclinical MAST was studied.

SREBP-1c (sterol response element-binding protein-1c), NF-jBp65 (nuclear factor kappa-light-chain-enhancer of activated B cells), and UCHL-1 (ubiquitin carboxyl-terminal hydrolase-1) expression, as well as total microbial count and somatic cell count, were all found to be significantly lower after treatment with ALA-NS (Yadav et al., 2020). In the treatment of bovine MAST, CHS-NPs have been demonstrated to have a high therapeutic potential (Orellano et al., 2019). Commercially available AgNPs and CuNPs decreased the in vitro survival of *Staphylococcus aureus* and *Escherichia coli* without causing damage to the mammary gland (Kalinska et al., 2019).

In the IMMS treatment of clinical bovine MAST caused by *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*, sonochemically synthesised capped zinc oxide nanoparticles (ZnONPs) demonstrated greater antibacterial activity than particles synthesised by auto-combustion method, implying its potential for MAST control (Hozyen et al., 2019). In vivo studies are required to validate the antibacterial action of NPs. The ability of the MAST-causing opportunistic bacteria *Escherichia coli* to form biofilm causes antibiotic resistance (Yu et al., 2018). Plant-derived NPs are becoming more popular as a therapy for MAST (Chaitanya Kumar et al., 2013; Yu et al., 2018).

The AgNPs-decorated quercetin NPs made by combining AgNPs with plant-derived quercetin showed substantial anti-bacterial and anti-biofilm activity against MAST-infected dairy calves’ multidrug resistant *Escherichia coli* strains (Yu et al., 2018).

Due to its rapid departure from the body, curcumin, a polyphenol produced from turmeric, has a low oral bioavailability, limiting its anti-inflammatory effect. In a *Staphylococcus aureus*-infected mammary tissue animal model, curcumin nanoformulation can increase oral bioavailability and reduce proinflammatory mediators (Suresh et al., 2018). Similarly, silver nanoparticles produced from aloin, a key component of *Aloe vera*, exhibited significant antibacterial action against *Staphylococcus aureus* in an experimental murine MAST model (Chaitanya Kumar et al., 2013). The
use of NPs in MAST therapy requires more research.

Chitosan and cloxacillin were combined to study their effects on planktonic cultures, bacterial biofilms, and intracellular growth in udder cells (Breser et al., 2018). It was observed that combining chitosan with cloxacillin inhibited the formation of biofilms and improved the clearance of biofilms that had already formed. It also decreased intracellular bacteria viability, which is considered to be linked to increased IL-6 production by affected mammary epithelial cells. As a result, this therapy has the potential to be a one-of-a-kind way to prevent MAST in a safe, effective, and contamination-free way, especially in the face of multidrug-resistant bacteria.

Conclusion

MAST has a negative influence on animal welfare and results in economic and production losses due to decreased milk quality, lower production performance, increased culling rate, treatment costs, and death connected to the acute phase of the illness. Both clinical and subclinical symptoms of the illness can be caused by a variety of microbiological species. Subclinical MAST is more economically important than clinical MAST because it has a higher proclivity for lowering milk quality to the point where it cannot be seen visually but has an influence on overall quality.

For the diagnosis of MAST, there are several well-established and inexpensive conventional diagnostic techniques available, however, they lack sensitivity and specificity. They can not be widely used in the current dairy sector since they can not offer quick results. Improved diagnostic methods for detecting etiological agents in MAST have recently been developed and are simple to use, fast, and sensitive, although they still lack significant specificity. Due to the need for skilled support, sophisticated technology, and infrastructure, such an approach lacks economic viability.

When MAST is discovered, the first concern for the veterinarian or producer is to treat the animals in such a way that the disease does not worsen and becomes an economic burden on the production system. Several therapeutic strategies have been evaluated for efficacy in treating MAST, including ABX, vaccines, bacteriocins, herbal therapy, immunotherapy, and nanoparticle technology, but no single technique has been found to be effective in controlling or treating the disease due to the variable response of etiological agents to therapeutic techniques.

ABX were widely used as the only therapeutic agent in the therapy of MAST until recently, but with the increase of bacterial resistance due to uncontrolled antibiotic use, alternative treatment methods are being researched. A necessity for the twenty-first century is the development of a universal therapeutic agent/technique that can be utilised instead of antibiotic therapy.

This sort of drug/method has the potential to address the growing problem of bacterial resistance. Advanced treatment approaches, such as bacteriocins and NPs technologies, should be further researched in order to discover a solution to the existing problem. Diagnostic techniques and treatment methods should be developed in tandem in order to achieve an accurate early farm level diagnosis, which may then be combined with specialised therapy against the identified microorganism to enable successful MAST management and treatment. Antimicrobial use is a problem in India. Addressing the flaws identified in our study can help minimise AMR, but the mechanism by which this happens (i.e. the change process) can be studied by involving stakeholders in the theory development process.

Interventions addressing AMR from the perspective of animal health should be promoted and organised in a way that allows for monitoring and evaluation. The National AMR Containment Program, the National Action Plan on AMR, and the National Health Policy indicate the government’s commitment to addressing the country’s AMR problem and should be supported.
It is time to start thinking about how to encourage more people to follow the rules. Incentives that encourage actors to change their behaviours and use AMR therapies should also be studied.

**Acknowledgements**

Authors are thankful to ASB Life Cell Research Technologies, Chennai, Tamil Nadu, for the technical support for this research work.

**References**


