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Review

Subclinical Mastitis of Buffaloes in Asia: Prevalence, Pathogenesis, Risk Factors, Antimicrobial resistance, and Current Treatment Strategies

Abstract

Subclinical mastitis (SCM) remains a significant challenge in buffalo farming across Asia, impacting both animal welfare and economic productivity. In this review, we assessed the status, pathogenesis, risk factors, antimicrobial resistance (AMR) and therapeutic measures associated with SCM in buffalo populations. This study revealed a pooled prevalence of SCM in Asia at 41.51% (2513/6054; 95% CI: 40.26-42.76) with considerable variation observed across different regions. Notably, Turkey exhibited the highest pooled prevalence at 61.1% (95% CI: 43.46-76.86), while Nepal reported the lowest pooled prevalence at 23.7% (95% CI: 19.15-28.82). *Staphylococcus* spp. emerged as the most common mastitogen with the California Mastitis Test (CMT) identified as the primary diagnostic method. Risk factors for SCM exhibited variability among studies, reflecting the diverse husbandry practices and environmental conditions across Asian buffalo farming regions. Furthermore, AMR poses a significant concern, with beta-lactam antibiotics (penicillin, ampicillin, oxacillin) commonly found to be resistant in many studies. Herbal therapy derived from both animal and plant sources, along with immunotherapy, emerged as effective strategies for controlling and preventing SCM and clinical mastitis in buffalo. Importantly, these approaches offer promising solutions for combating AMR while promoting sustainable mastitis management practices in Asian buffalo farming. The review emphasizes the need to understand the prevalence, causes, and management of SCM in buffaloes across Asia, calling for targeted interventions and further research to tackle this widespread issue.

Keywords: Subclinical mastitis, Buffalo, Prevalence, Risk Factor, Therapeutic measures, Antimicrobial resistance

INTRODUCTION

Buffalo (*Bubalus bubalis*) is renowned as the ebony treasure of South Asia. Buffalo farming in Asian nations has seen a substantial and rapid increase, playing a role in approximately 13% of the total global milk production over the last fifty years [1]. But a notable barrier to buffalo milk production is mastitis, impacting milk quantity, quality, and safety, resulting in substantial economic losses, heightened antibiotic usage, and compromised animal well-being [2, 3]. It stands as a highly widespread, expensive, and intricate ailment within the dairy sector, causing financial setbacks due to diminished milk output, wasted milk, premature culling, veterinary expenses, and labor expenditures [4]. As previously reported, each buffalo incurred an average yearly economic deficit of 70 USD as a result of mastitis, wherein 55% of the loss was attributed to mastitis intervention and 16% to reduced milk yield [5]. Hence it is depicted that, mastitis not only impacts the health and well-being of the buffaloes directly but also imposes financial losses on dairy farmers due to treatment expenses and reduced milk production.

The milk from buffaloes in their lactating phase acts as an ideal medium for the multiplication of different pathogenic, opportunistic, and spoilage microorganisms and has the capacity to exert an influence on the pathophysiology of mastitis. Based on the dynamics between the host and pathogens, mastitis can appear as either clinical mastitis (CM) or subclinical mastitis (SCM) [3, 6]. Among these, SCM is the prevailing occurrence across all dairy animals and results in more significant financial losses [2]. It is documented to be 15 to 40 times more dominant than the clinical form of mastitis [7]. Clinical mastitis is easily identifiable but detecting SCM is challenging due to its lack of noticeable symptoms [8]. The complexity of SCM etiology is intertwined with factors such as microbial virulence, load, and treatment, encompassing micro environmental conditions, host attributes, milking methods, potential vectors, immunity, and nutritional wellbeing [9]. Major mastitogens encompass "contagious" or "environmental" types, with significant contagious pathogens being *Staphylococcus aureus* and *Streptococcus agalactiae*, and predominant environmental pathogens including *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus uberis*; meanwhile, *Streptococcus dysgalactiae* can function as both an environmental and contagious pathogen [10-12]. Additionally, it is notable that injuries to teats or the udder, whether caused by physical, chemical, or thermal factors, can also result in cases of SCM. Animals affected by SCM can serve as a potential reservoir of infection for other members within the herd. Dairy animals in tropical climates encounter a higher incidence of SCM due to the conducive environmental conditions that promote the growth of pathogenic microorganisms responsible for causing SCM [13, 14]. Moreover, SCM accounts for around two-thirds of the overall economic losses in total milk production [13, 15]. As a result, regular implementation of on-site tests can be highly beneficial in promptly identifying and treating SCM. Dry cow therapy using antibiotics is a recommended approach for treating CM or SCM resulting from bacterial infections. While imprudent use of antibiotics may contribute to the development of antibiotic resistance, ultimately affecting the effectiveness of treatment [16].

There is a notable scarcity of research on antimicrobial resistance (AMR) in SCM among buffalo globally, particularly in Asia. However, numerous studies have identified varying degrees of AMR in pathogens responsible for SCM in dairy cows. These studies reveal that major mastitis-causing agents exhibit resistance to multiple antibiotics [17]. Furthermore, SCM also contributes to the dissemination of antimicrobial-resistant bacteria within dairy herds and infected animals can release resistant pathogens into their environment, contaminating milking equipment, bedding, and other surfaces, potentially leading to the infection of other cows within the herd [18, 19]. Consequently, the antimicrobial-resistant SCM cases results more challenging to treat effectively and the bacteria may not respond to standard antibiotic treatments, leading to prolonged infections, reduced milk production, and economic losses for the dairy farmer [20, 21]. AMR leads to more than 30,000 deaths annually in the EU and 700,000 worldwide; with projections indicating it could result in millions of fatalities. In the EU, the economic impact of AMR is significant, with healthcare and productivity losses estimated at EUR 1.5 billion each year [20]. Hence, timely identification of SCM becomes imperative in order to effectively address this challenge [22]. Various tests, both in field settings and laboratories, can be employed to identify instances of SCM. Embracing progressive and endorsed approaches for managing SCM can serve as a guiding principle to enhance milk production, its quality, and the well-being of dairy livestock. But there is a lack of substantial information concerning SCM of buffaloes in Asia. Despite the economic significance of SCM, there is a dearth of comprehensive studies on this highly prevalent disease in Asian countries.

Considering the facts, the treatment of sub-clinical mastitis considering its prevalence, pathogenesis, risk factors, and AMR has emerged as a significant challenge in recent times. Thus, an updated review on bubaline sub clinical mastitis has been conducted on Asian countries focusing on its prevalence, pathogenesis, risk factors, AMR, and potential therapeutic strategies. The ample scientific data available could help advance future research in investigating, developing, and manufacturing new pharmaceutical formulations that are more potent in combating resistant pathogens.

METHODOLOGY

This comprehensive review is the advancement of an extensive exploration of SCM, drawing insights from a diverse array of articles sourced from renowned academic research database like Scopus, PubMed, Google Scholar, Web of Science, SpringerLink, ScienceDirect, JSTOR, PubMed Central (PMC) and others. Our search strategy employed a multitude of keywords, including 'Mastitis and Buffalo,' 'Sub Clinical Mastitis and Buffalo,' 'Bubaline SCM and Asia,' 'SCM and mastitogen', 'SCM and Diagnosis,' 'SCM and Treatment,' 'Micro-environmental Conditions and SCM,' 'Host Attributes', 'Immunity,' 'Nutritional Wellbeing,' 'SCM and Genetics,' 'SCM and Dry Period,' 'Energy Balance,' 'Bacteriophages,' 'Phyto-additives,' 'Vaccination,' and 'Economics,' among others. This review encompasses articles published in peer-reviewed journals from the year 2000 to 2024, focusing on the objectives of this study. The bibliographic analysis conducted using "VOSviewer software" focused on research articles related to mastitis in buffalo. The articles were selected on PubMed database based on keyword searches including "mastitis," "buffalo," "prevalence," "treatment," "mechanism," "risk factors," and "pathogens." Using the linlog modularity method, these articles were clustered into groups where the cluster groups were identified on different colors in Fig. 1. The analysis revealed a total of 1,043 links among the articles, with cumulative link strength of 3,477, highlighting the interconnectedness and depth of research in this area (Fig. 1).

Status, Etiology, and AMR of Bubaline SCM in Asia

Despite the fact that buffaloes exhibit enhanced sphincter strength and possess a broader protective epithelial layer within the teat canal as compared to dairy cows, it remains evident that the teat canal continues to serve as the primary channel for the entry of infectious microorganisms [23]. Numerous research investigations have consistently demonstrated that the incidence of SCM tends to exceed that of CM in water buffaloes [24]. The collective prevalence rate of SCM globally stands at 42%, with a notable prevalence in North America when analyzed by continent, and Uganda showing a higher prevalence among individual countries. When scrutinized by methodology, SCM rates were particularly elevated in somatic cell counts worldwide [1]. Research conducted in various Asian nations has also consistently demonstrated an ascending trend in the prevalence of SCM among buffalo populations over the past quarter-century [25].

A comprehensive analysis of 20 studies (Table 1 and Fig. 2) meeting the inclusion criteria revealed a pooled prevalence rate of SCM of buffaloes in Asia at 41.5% (2513 out of 6054; 95% CI: 40.26-42.76). Among these, Turkey exhibited the highest prevalence at 61.11% (95% CI: 43.46-76.86), while Nepal reported the lowest prevalence at 23.7% (95% CI: 19.15-28.82). India conducted most studies (6), with a pooled prevalence of 32.64% (687 out of 2105), followed by Pakistan and Bangladesh with rates of 49.1% (95% CI: 46.70-51.46) and 48.3% (95% CI: 45.69-50.89), respectively, as illustrated in Fig. 2 depicting the distribution across the region.

In the context of Bangladesh (Table 1), a recent study showed a significant upsurge in the overall prevalence of SCM. The findings were quite striking, with SCM rates standing at 27.9% (out of 3491 quarters) when evaluated at the quarter-level and even higher at 51.5% (out of 880 buffaloes) when evaluated at the buffalo-level [26]. From another study in Bangladesh, *S. aureus* emerged as the predominant causative agent of SCM among buffalo cows, accounting for 37.4% of cases. Following closely behind were *E. coli* (7.6%), *S. agalactiae* (6.2%), *Klebsiella* spp. (4.5%), coagulase-negative Staphylococci (CNS) (4.1%), *S. uberis* (3.8%), *S. dysagalactiae* (3.1%), *Bacillus* spp. (2.4%), and *Enterobacter spp.* (1.4%) [27]. Another recent study conducted in Bangladesh established that non-aureus staphylococci (NAS) were the most prevalent pathogens, comprising 24.7% of identified cases[28]. Most of the bacteria were resistant to penicillin, ampicillin, doxycycline, tetracycline, chloramphenicol, and ciprofloxacin [6, 24, 27].

In India (Table 1), the prevalence of SCM in water buffaloes was 20.4%. Among this cohort, 7.8% exhibited latent mastitis, 9.8% had specific mastitis, and 2.8% displayed symptoms of nonspecific mastitis [29]. Subsequent investigations conducted in India in recent years have demonstrated a worrisome escalation in SCM prevalence, with rates reaching 20.4% in 2015, surging to 33.8% in 2018, and reaching a substantial 68.3% in 2019 [29-31]. In another recent study in India, it was observed that the prevalence of SCM stood at 11.3% when assessed at the quarter level and was slightly higher at 22.2% when examined at the animal level [32]. This study observed 51.9% prevalence for *Staphylococcus* spp. Following this, *Streptococcus* spp. was identified in 33.33% of the samples, while *E. coli* was detected in 14.81% of them. Subsequently, the highest degree of sensitivity was exhibited towards gentamicin and enrofloxacin. This was followed by ceftriaxone, moxifloxacin, cefoperazone, and tetracycline. Conversely, the bacterial isolates displayed the lowest sensitivity when exposed to amoxicillin in conjunction with clavulanic acid [32]. Furthermore, a separate study reported a pooled prevalence of 45% for SCM in India [1]. In line with these findings, a very recent study indicated that the overall prevalence of SCM, irrespective of the species, was recorded at 28.14% [33]. Moreover, another recently conducted study in India has unveiled significant findings, indicating that a substantial portion of cases involved infections attributed to *Staphylococcus* (accounting for 83%) and *Streptococcus* (amounting to 76%). Following these, cases of mixed infections involving both bacteria were also noted. In sharp contrast, infections associated with *E. coli* and Diplococci were observed in a mere 7% and 3% of the cases, respectively [34].

Studies in Pakistan (Table 1) have shown the prevalence of 15.2% in 2011, 38.8% in 2018 and 22.9% and 67.3% in 2019, 57% in 2021 [35-38]. In addition, *Staphylococcus* spp. (34%) were the most predominant bacterial isolates from mastitic milk, followed by *E. coli* (19.4%), *Streptococcus* spp. (9%), and *Klebsiella* spp. (8%) [38]. Most of the bacteria were susceptible to gentamicin (92%) and enrofloxacin (88%) [38].

In Nepal (Table 1), initial SCM prevalence in buffalo herds was 78.0% [39]. Nevertheless, subsequent studies reported a prevalence of 30% for SCM in buffaloes in 2021 which is sharply increased to 70% in 2022 [7, 40]. At the quarter level, CNS accounted for 46.3% of SCM cases, while at the individual animal level, both *S. aureus* and CNS contributed to SCM at 36.1% [37]. Both isolates displayed significant susceptibility to amikacin, ceftriaxone, and gentamicin. In contrast, CNS showed higher resistance to ciprofloxacin and gentamicin, while *S. aureus* exhibited increased resistance to enrofloxacin and tetracycline [40].

In Egypt (Table 1), the prevalence of SCM was identified 44%, with *S. aureus* being the most common (31%) mastitis-causing agent, and antimicrobial susceptibility testing indicating that 32.2% of buffaloes affected by staphylococcal SCM showed resistance to cefoxitin, classifying them as methicillin-resistant *S. aureus* (MRSA) [41]. In another research study, SCM was found to have a prevalence of 42.7% [39]. The main pathogens identified were *S. aureus*, with MRSA being particularly prevalent. Results from antibiotic sensitivity tests indicated that ciprofloxacin and linezolid showed 100% sensitivity, levofloxacin exhibited 85% sensitivity, while amikacin and trimethoprim + sulfamethoxazole had shown 80% sensitivity. Tylosin, gentamicin, and oxytetracycline displayed sensitivity of 60%, 60%, and 40%, respectively, against MRSA which was detected in buffalo milk [42].

In Philippines (Table 1), the prevalence of SCM was 42.8%, utilizing the California Mastitis Test, with dams aged less than 3 years having 76% probability, while those aged 3 years had an 82% probability of experiencing SCM [2]. Additionally, in another study, 39 isolates of *S. aureus* were detected, constituting a prevalence of 41.94% (39 out of 93) [43]. Among these 39 identified *S. aureus* isolates, only 24 (61.54%) exhibited resistance to cefoxitinand penicillin while highly susceptible to clindamycin (66.67%), trimethoprim+sulfamethoxazole (95.83%), tetracycline (83.30%), rifampicin (79.17%), ciprofloxacin (95.83%) and gentamycin (87.50%).

In Turkey, a study on subclinical mastitis (SCM) in Anatolian water buffalo (Table 1) found that only two out of 22 coagulase-negative staphylococci (CoNS) strains were resistant to at least two antibiotics [43]. In another study, most strains (81.8%) exhibited vancomycin resistance, while 68.2% were resistant to oxacillin. Multi-drug resistance was relatively low, occurring in only 13.6% of the strains [44].

Risk factors affecting SCM in Buffalo

The etiology of SCM in buffaloes in Asia is a complex and multi-factorial issue influenced by various factors, including the type of the involved bacteria, host factors, herd, and environmental factors (Fig. 3). Understanding the underlying causes and contributing risk factors is crucial for the effective prevention and management of SCM in buffalo herds. These risk factors include:

Pathogen associated factors

SCM in buffalo in Asia can be caused by a variety of pathogens (Fig. 3), including bacteria, fungi, and occasionally, viruses. The prevalence and specific pathogens involved can vary across different regions of Asia.

Bacterial cause

S. aureus and CoNS are frequently identified as common culprits in SCM cases among buffalo in Asia. These bacteria possess the capability to persist within the udder and initiate chronic infections that are notably challenging to treat [6, 45]. Presently, they are recognized as significant pathogens responsible primarily for SCM [46]. *E. coli* is a frequently encountered environmental pathogen that can result in SCM in buffalo. These infections are typically linked to inadequate hygiene and suboptimal management practices [25]. Certain *Mycoplasma* species, including *Mycoplasma bovis* and *Mycoplasma mycoides,* have been documented as responsible agents for SCM in buffalo populations in some Asian countries [15]. Streptococcal species such as *Streptococcus agalactiae, S. dysgalactiae,* and *Streptococcus uberis* are additional contributors to instances of SCM in buffalo populations. *Streptococcus uberis*, in particular, tends to induce a chronic subclinical form of mastitis, although it can also lead to mild to moderate CM [15, 45]. *Corynebacterium* species, such as *Corynebacterium bovis*, have been identified in cases of SCM in buffalo [15].

Non-Bacterial cause

Fungal agents specifically yeast species like *Candida* spp., on occasion, have the potential to trigger SCM in buffalo [47]. Furthermore, SCM has been triggered following a combined intramammary and intranasal administration of bovine herpesvirus 4 to lactating cows, with bovine leukemia virus being identified in mammary tissue of cows experiencing SCM [48].

Animal level risk factors

There are some animal level risk factors (Fig. 3) which are closely related to the occurrence of SCM

Species and breed

Buffaloes exhibit a higher prevalence of SCM compared to cattle, particularly in high-yield crossbred buffalo such as Murrah, which are at an elevated risk of developing SCM [26]. Teats with a funnel shape were linked to a greater occurrence of SCM compared to those with a cylindrical shape. It was previously reported that cylindrical teats in Murrah buffalo are the most prevalent and exhibit a higher incidence of SCM [49] . Conversely, in some other studies, mastitis is found to be more prevalent in cattle than in buffaloes since buffaloes possess tighter sphincter muscles in comparison to cattle. Additionally, crossbred and exotic cattle exhibit a higher susceptibility compared to zebu cattle [50. 51],

Age and Parity

Mastitis cases tend to be more frequent in older animals with higher parity, while in buffaloes, a significant proportion of mastitis cases are observed in those that are in their third or fourth parity [27, 52]. In addition, it was determined that 90.32% of she-buffaloes aged between 9 to 11 years, 77.27% in the age group of 12 to 14 years, 65.78% among those aged 6 to 8 years, and 41.37% in the age range of 3 to 5 years tested positive for SCM [30]. To corroborate the previous point, a recent study indicated that somatic cell count (SCC), a marker for SCM, was notably influenced by factors such as buffalo breed, age, parity, and the time of the year [53].

The stage of lactation and the condition of the udder and teats

Dairy animals, especially those in the first two months of milking after giving birth, are highly vulnerable to mastitis [51]. During the peri-parturient period, increased pressure on the teat canal may lead to leakages, providing an opportunity for pathogens to invade the udder. Studies indicate that certain udder and teat characteristics, such as pendulous udder shape, flat or inverted teat ends, and longer/thicker teats, are associated with a higher risk of intra-mammary infection and elevated milk somatic cell counts [54].

Furthermore, teat deformities or injuries, whether caused by chemical injury (ergot, trichothecenes), physical trauma, or heat injury, significantly increase the risk of infection. Uneven milk flow and asymmetrical udder quarters, especially in cases of less pointed and more flattened teats, are associated with higher mastitis rates [55]. Additionally, another study indicates that rear udder quarters are particularly susceptible to mastitis [56].

Energy Balance

Various studies have demonstrated that dairy animals experiencing under or over body condition are more prone to udder health issues [57]. It's crucial to maintain proper body condition scores (BCS) through methods like regular exercise and feeding management. Managing BCS during the calving period significantly improves productivity and reduces udder health problems [58]. Animals with over BCS can be fed high-fiber, low-concentrate diets, while those with under BCS may benefit from dense feeds, although excessive concentrate levels should be avoided to prevent acidosis [59]. Adding 2-5% supplemental fat to lactating animal diet can enhance energy balance and improve production status. Nevertheless, it is crucial to ensure that the ratio of n6 to n3 polyunsaturated fatty acids falls within the range of 3.9 to 5.9 to maintain normal immune functions in lactating animals during the transition period [60].

Dry period length

Studies have shown varying dry period lengths (from 0 to 70 days), but recent study suggests an optimal duration of 8 weeks [61]. This period allows time for nutrient replenishment and prepares the animal's body from a nonproductive to a productive stage [62]. Providing a dry period significantly shorter than optimal can lead to negative energy balance and increase the risk of SCM, especially in young milch animals [62].

Herd and Environmental risk factors

SCM is also related to some environmental risk factors as shown in Fig. 3.

Climate Change

Warm and humid weather promote the proliferation of harmful bacteria [63], and the prevalence of mastitis is notably affected by relative humidity and bedding materials [64]. Research suggests that mastitis cases are highest during rainy (16.28%) and summer (75.9%) seasons, whereas winters (8.75%) see fewer instances [65, 66]. The variation in resistance to specific climatic conditions among lactating animals may be due to differences in their anatomical structure. Adequate ventilation in animal shelters can help to control humidity and temperature [67].

Bedding materials

Studies highlight bedding materials as a significant source of SCM [63]. Select bedding materials that stay dry and clean for extended periods to prevent mastitis and provide comfort to farm animals [68]. Recycled manure solids (RMS) have shown potential but require careful hygiene maintenance and a dry matter content of over 34% [69]. Conversely other materials like rubber mats, concrete floors, and paddy straws have drawbacks. Rubber mats could be expensive investments, while concrete floors may become slippery for animals in wet conditions. Paddy straws are highly susceptible to moisture absorption, which can significantly increase microbial growth. Sand is considered ideal, with a 25 cm layer requiring regular replacement for controlling mastitis [70]. Consistently replacing the top layer of bedding materials and timely raking can aid in maintaining dry and hygienic bedding for animals to move and rest comfortably. This practice may enhance animal comfort levels and contribute to SCM control [71].

Oxidative stress levels

Subclinical mastitis (SCM) leads to oxidative stress and the release of NO-derived free radicals in milk, causing milk loss, reduced antioxidant capacity and vitamin C levels [72]. Vitamin C supplementation in dairy animal diets is recommended to counteract these effects [73]. Prolonged SCM infection negatively affects milk quality and quantity, with increased NO-derived metabolites linked to SCM in milk-producing animals [74].

Milking techniques, milking machine maintenance, and hygiene management.

Milking management is crucial for preventing SCM in dairy animals. Both hand and machine milking methods must be hygienic to avoid SCM [54]. The underhand milking method should be avoided due to its potential for causing teat tissue injuries. Strict hygiene during machine milking is essential to prevent SCM, as unhygienic conditions can promote pathogen growth [54, 75]. Automation in milking practices, along with strict hygiene maintenance, can help control SCM. Hygienic practices such as washing hands, udders, utensils, and milking equipment are recommended. Iodine-based teat dips or sprays are advised for preventing SCM. Cleaning milking machines two to three times daily is crucial, and indirect parts should also be cleaned to prevent bacterial contamination. Chlorine-based disinfectants are effective for dairy utensils, while acid-based disinfectants can remove alkaline deposits in machines. Manual cleaning may suffice for small farms, while larger operations may require machine washing systems [76].

Pathogenesis

Microorganisms from the environment and contagious sources infiltrate the udder through the teat cistern. Inside the alveolus, these invaders multiply and are confronted by neutrophils (white blood cells), causing harm to the milk-producing epithelial cells of the cow's udder. To proliferate significantly, pathogens must multiply after entering through the teats' cistern opening. To do so, they must overcome the host animal's mammary system's immune defense barrier [77]. Typically, sphincter muscles serve to tightly seal the cistern canal, thus preventing the entry of pathogens. The inner lining of the cistern canal is composed of keratin, a protein that contains a waxy substance produced by the outer layer of epithelial tissues. Keratin possesses some antimicrobial properties due to the release of long-chain fatty acids, although its effectiveness in this regard is somewhat limited [78-80]. Furthermore, it's important to note that the teat canal can remain open for up to 2 hours after milking, as it takes about 2 hours for the sphincter muscles to tighten again around the teat canal. During the animal's approaching parturition, there's an increased intramammary pressure followed by teat canal dilation, creating a crucial window for pathogens to invade the host animal's mammary system [81]. Once these pathogens breach the animal's immunity barrier, they multiply and produce toxins. These toxins influence the accumulation of leucocytes and epithelial cells, releasing chemo-attractants. Consequently, various neutrophils are deployed to the infection site. These neutrophils contain bactericidal substances that destroy bacteria and some epithelial cells, leading to reduced milk yield and quality [82]. This process triggers the release of enzymes like N-acetyl-beta-D-glucosaminidase (NAGase) and lactate-dehydrogenase (LDH). The remaining neutrophils are either eliminated through apoptosis or ingested by macrophages (Fig. 4). The damaged epithelial cells and dead neutrophils are released into the milk, resulting in a high somatic cell count (SCC). In advanced cases, alveoli can be severely damaged, allowing various ions to influx into the milk, thereby increasing its pH, which can indicate the presence of mastitis [83-85].

Diagnosis of SCM

Diagnosis of SCM in buffaloes involves a combination of traditional methods and advanced molecular techniques [86]. Traditional methods such as Somatic Cell Count (SCC) analysis, California Mastitis Test (CMT), and Surf Field Mastitis Test (SFMT) are cost-effective and accessible, offering simplicity and affordability for on-farm use. These tests detect elevated SCC levels indicative of an immune response to infection, aiding in early detection even in the absence of visible symptoms. However, they lack numerical SCC values and may yield false positives or negatives, limiting result accuracy. On the other hand, advanced molecular techniques like Polymerase Chain Reaction (PCR), Real-time Quantitative PCR (RT-qPCR), Reverse Transcription PCR (RT-PCR), Loop-mediated isothermal amplification (LAMP), microarray-based assays, and Next-generation sequencing (NGS) provide high sensitivity and specificity in identifying mastitis pathogens at the molecular level [87]. PCR and its variants amplify specific DNA sequences of bacteria and viruses present in milk samples, while LAMP amplifies DNA under isothermal conditions, offering high sensitivity and suitability for on-site testing. Microarray-based assays enable simultaneous detection of multiple pathogens, while NGS technologies provide comprehensive insights into microbial composition and genetic diversity. These advanced techniques facilitate accurate diagnosis, enabling targeted treatment strategies and enhanced mastitis management in dairy herds. In addition to these tests described, there are more on-site tests used for diagnosing SCM depicted in Fig. 5.

Economic Impact

SCM leads to production losses that are three times greater than those caused by CM, making it accountable for a substantial portion of economic losses, comprising 60-70% of the overall losses attributed to mastitis infections [88]. Normally, milk with a somatic cell count (SCC) of around 100,000 is considered healthy. However, if the SCC exceeds 200,000, it is classified as a SCM case [89]. This issue may not be immediately apparent, but it gradually erodes the economic viability of dairy production and eventually results in a decline in financial returns [90]. Various estimates suggest that each lactating cow experiences a milk loss ranging from 100 to 500 kg due to SCM and the poor quality of milk often leads to discarding, further escalating the losses [91]. To illustrate, approximately 80% of the economic losses within the dairy industry are attributed to SCM in India [9]. Studies have shown that SCM can be significantly more prevalent, which leads to substantial economic losses, amounting to approximately INR 4151 Crores, which is nearly 560 million USD [92]. In a recent study, it was reported that the estimated loss of approximately $147 per cow annually is incurred in dairy farming due to mastitis and this loss is mainly associated with reduced milk production and the need to cull animals with prolonged infections [93]. Another study reveals those financial losses due to mastitis stem from various factors: milk production losses (31%), expenses on veterinary services and drugs (24%), discarded milk (18%), laboratory fees, and additional labor for the farmer (4.0%), as well as premature culling or death of dairy animals (23%). Additionally, each infected animal experiences a reduction in their lactation period by approximately 57 days, resulting in a decrease in milk output by 375 kg per lactation [94].

Advanced treatment of SCM

Biosecurity

Biosecurity measures play a key role in preventing the introduction and spread of pathogens in farms, thereby minimizing the risk of disease transmission and reducing antibiotic usage. Efforts are underway to develop and implement biosecurity tools like the BIOCHECK CATTLE® protocol, which assesses biosecurity levels on dairy farms across different regions. However, studies indicate that many cattle farmers do not fully implement adequate biosecurity measures due to practical and financial constraints [20]. Therefore, while biosecurity is increasingly recognized as a preventive measure, further education of farmers on proper implementation could improve mastitis management strategies, potentially reducing the need for antibiotics in disease prevention and treatment.

Antibiotic therapy and nano therapy

Antibiotics are extensively used in intensive livestock production, accounting for over 50% of global antimicrobial use in veterinary medicine [95]. By 2030, antimicrobial use in food-producing animals is projected to increase significantly, with estimates ranging from 11.5% to 67% [96, 97]. In dairy production (cattle an buffalo), mastitis is the primary reason for antibiotic use, mainly administered via intramammary preparations or systemic applications. Common antibiotics for mastitis include penicillins, sulfonamides, ampicillin, cloxacillin, and aminoglycosides. Additionally, symptomatic and supportive therapies help reduce inflammation, improve antibiotic efficacy, and accelerate recovery and milk production [11, 95]. However, controlling mastitis requires the cautious administration of antibiotics, often given preventively during the dry period. When treating CM, antibiotics should be selected based on the results of culture and sensitivity tests [16], considering the diverse antibiotic susceptibility patterns observed in mastitis-causing pathogens. Resistance issues highlight the importance of careful antibiotic selection to avoid ineffective treatments [98]. However, antibiotic usage can lead to residue concerns in milk and poses a risk of antibiotic resistance. Alternative strategies, such as non-steroidal anti-inflammatory drugs, are explored for supportive therapy [99]. For challenging pathogens like *S.aureus,* traditional antibiotic therapies have limited effectiveness due to biofilm formation and unique host adaptations, driving the search for innovative solutions like vaccines and novel peptides [16]. Effective mastitis management also requires continuous monitoring of antibiotic resistance, awareness campaigns, and legal frameworks promoting judicious antibiotic use [100,101]. In some cases, extended antibiotic treatments or combination therapies are explored to improve cure rates, but the overall efficacy varies based on factors such as pathogen type and udder environment. For challenging pathogens like *Prototheca* spp. no effective therapies exist, leading to investigations into alternative disinfectants like guanidine [102]. Additionally, nano therapy has garnered a significant attention for delivering antimicrobial agents as drug delivery vehicle in the treatment of SCM. Different types of nanoparticles, such as liposomes, nanogels, polymeric nanoparticles, inorganic nanoparticles, and solid lipid nanoparticles, have demonstrated potential in managing SCM caused by bacteria like *S. aureus* [103]. Studies have demonstrated their effectiveness against multi-drug resistant strains, enhancing antibacterial activity and reducing dosing intervals for antibiotics. Specifically, chitosan nanoparticles [104] and metal nanoparticles like silver, copper, and zinc oxide [105] have exhibited antibacterial properties without harming mammary glands. Plant-derived nanoparticles, such as silver-nanoparticle-decorated quercetin and curcuminnano-formulations, have also displayed anti-biofilm activity against multi-drug resistant bacteria causing SCM [106]. Combining chitosan with antibiotics like cloxacillin has proven effective in inhibiting biofilm formation, improving clearance, and reducing intracellular bacteria viability, offering a novel, contamination-free method for mastitis prevention, particularly against multi-drug resistant strains [107]. However, further research is needed to validate these findings *in vivo*.

Probiotics

Probiotics are indeed live microorganisms which are administered in adequate amounts to provide health benefits to the host [108]. These benefits are achieved through various mechanisms, including the modulation of the gut microbiota, enhancement of the gut barrier function and mucosal integrity, modulation of the immune system, and inhibition of pathogens [102,103]. Lactic acid bacteria, a type of probiotic, have become popular in the treatment and prevention of mastitis, an inflammatory condition common in dairy animals. Strains of *Lactobacillus* have strong immunomodulatory properties and can protect against mastitis when used as feed supplements, teat dips, or through intramammary inoculations [109]. These bacteria form protective biofilms in the udder, inhibiting the growth of mastitis-causing pathogens. Studies have identified specific strains like *Lactobacillus brevis* 1595, *L. brevis* 1597, *L. plantarum* 1610, *Lactobacillus lactis subsp. lactis*, and *Lactobacillus perolens* among the total of 165 isolates obtained from sampling of the teat canal exhibited**,** high colonization capacities in bovine mammary epithelial cells, preventing invasion by pathogenic bacteria [110]. The two strains of lactic acid bacteria, *L. lactis subsp. lactis* CRL 1655 and *L. perolens* CRL 1724, isolated from bovine milk, demonstrated inhibitory activity against bovine mastitis pathogens. This effect was achieved through co-aggregation and adherence to the epithelial cells of the bovine teat canal during in vitro evaluation [111]. Incorporating lactic acid bacteria into animal feed is considered effective in preventing bovine mastitis. These bacteria adhere to mammary gland surfaces, hindering pathogenic bacteria's invasion. *Lactobacillus* strains eg. *L. casei* BL23 also modulate the innate immune response, reducing pro-inflammatory cytokine expression in infected mammary epithelial cells and inhibiting bacterial adhesion and internalization [112]. Intramammary infusion of lactic acid bacteria (*L. lactis subsp. lactis* CRL 1655 and *L. perolens* CRL 1724) induces pro-inflammatory activity, promoting neutrophil influx into milk during lactation and drying-off periods [113]. Probiotic-based teat disinfectants have proven superior to commercial disinfectants, reducing mastitis-associated bacteria by altering the teat apex microbiota and preventing colonization by pathogens. Although probiotics hold potential, using them alone or in conjunction with repeated milk-out is not advised for the management of CM in lactating dairy animals. Moreover, microbial extracts derived from actinomycetes have exhibited antimicrobial properties against a range of bacteria responsible for mastitis, indicating their potential as viable options for mastitis treatment [114]. These extracts effectively hinder bacterial growth and are utilized for the development of efficient mastitis treatments.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are potent antimicrobial agents that play a crucial role in the innate immune system [115]. These peptides are widely available in nature and can provide antimicrobial activity against different organisms including bacteria, viruses, fungi, and parasites. They function through various mechanisms, such as disrupting bacterial membranes-leading to cell lysis and death or by modulating the immune system [109]. AMPs are known to exhibit broad-spectrum activity against various bacteria, including drug-resistant strains, and can work synergistically with conventional antibiotics. AMPs like beta defensins and cathelicidins are crucial in the innate immunity of vertebrates. Beta defensins act as the first line of defense against intramammary infections (IMIs) in dairy animals [116], while cathelicidins, released by infiltrating neutrophils during mastitis, have broad-spectrum antimicrobial activity [117]. Bovine cathelicidins, such as BMAP-27 and BMAP-28, show potential for mastitis treatment [118]. Bacteriocins, another type of AMP, are synthesized and secreted by various bacteria and are considered alternatives to antibiotics. Examples like Nisin and Bovicin HC5 have demonstrated antimicrobial efficacy against mastitis-causing bacteria [119]. However, bacterial ability to develop resistance against these compounds through different mechanisms, including protease production, surface charge modification, efflux pump activation, and potential toxicity and low bioavailability poses significant challenges in widespread use of AMPs as antimicrobial agents [106, 110-120].

Immunotherapy and native secretory factors

Immunotherapy provides an alternative approach to mastitis treatment, utilizing immunological techniques. It was previously reported that, microbeads carrying specific antibodies and an enhancer of phagocytosis, known as Y-complex, were used to treat mastitic cows infected with *E. coli*, *S. dysgalactiae*, or coagulase-negative staphylococci (CNS) [121] This treatment was as effective as antibiotics and superior to non-steroidal anti-inflammatory drugs (NSAIDs) in eliminating bacteria. Meloxicam, an NSAID, when used alongside antibiotics, improved cow fertility [122]. Additionally, interleukin-2 (IL-2) injected into the skin region after calving enhanced milk markers (SCC, serum amyloid A, lactoferin, NAGase) related to immune responses [123]. Immuno-stimulants such as *Saccharomyces cerevisae* yeast extracts and egg yolk immunoglobulins (IgYs) have demonstrated potential. The infusion of yeast extract into the mammary gland increased immune cell activity, reducing the risk of new infections. Specific egg yolk immunoglobiulin IgYs exhibited inhibitory and phagocytic activity against *E. coli* and *S. aureus* isolated from mastitic animals in vitro, suggesting their potentials for therapeutic treatment against mastitis in dairy cows [124]. Furthermore, antibodies targeting *S. uberis* adhesion molecule (SUAM) provided better protection against *S. uberis* infection in dairy cows, reducing clinical symptomsand bacterial counts, indicating improved clearance of pathogens and reduced intramammary infections [125]. A summary of the use and functioning of immunotherapy in treating mastitis is depicted in Fig. 6. In addition, a native secretory factor is also a naturally occurring substance released by cells or tissues into the bloodstream or surrounding environment, playing vital roles in biological processes such as cell signaling and immune response modulation. These factors encompass a range of molecules including hormones, cytokines, growth factors, and enzymes [126]. Lactoferrin (Lf), a natural whey protein derived from mammary glands and classified as a native secretory factor, exhibits notable antibacterial and anti-inflammatory properties (Fig. 6). It enhances penicillin's inhibitory activity against bacteria, especially in penicillin-resistant strains [127], by blocking beta-lactamase activity [128]. Bovine lactoferricin gene *(LFcinB)* transfected into mammary cells increases *LFcinB* secretion, exhibiting strong antibacterial activity against *S. aureus* and *E. coli* [129]. Phospholipases A2 reduce inflammation and improve cell viability in vitro, and a single PLA2G1B application in chronic *S. dysgalactiae* cases clears inflammation and bacteria [130]. Homeopathy shows limited effectiveness compared to antibiotics, with suboptimal bacteriological and cytological cure rates [131]. In CM of dairy lactating cows, non-antimicrobial treatments like homeopathy are not recommended.

Bacteriophage therapy and bacteriophage endolysins

Bacteriophages, or phages, are viruses that specifically infect bacteria. They can be used therapeutically to target and destroy pathogenic bacteria without harming the host's cells or beneficial microbiota [132]. Treating bacteria that form biofilms is challenging due to their resistance to conventional antibiotics. Various bacteriophages have been studied for their effectiveness against mastitis-causing bacteria like *S. aureus, S agalactiae, S uberis, Klebsiella pneumonia*, *Klebsiella oxytoca*, and *E. coli* [20, 133]. While promising, these evaluations have been primarily *in vitro*, requiring further *in vivo* studies to confirm their efficacy in clinical cases. One approach involves phage cocktails, which have shown superiority over individual phages in mouse models of mastitis [134]. However, phages can induce specific immune responses, potentially affecting their therapeutic success [135]. Thermostable phages, resistant to high temperatures, have been identified, and the lytic effectiveness of a bacteriophage mixture comprising three phages, STA1.ST29, EB1.ST11, and EB1.ST27, was assessed against *S. aureus* isolates. The marked reduction in *S. aureus* bacterial density highlighted the therapeutic potential of bacteriophage therapy [136]. Despite these advancements, further research is essential to validate the *in vivo* efficacy of bacteriophage therapy for managing bovine mastitis. Additionally, bacteriophage-derived endolysins have proven effective against Gram-positive pathogens by breaking down the peptidoglycan layer of bacterial cell walls, facilitating phage release during the lytic cycle [137]. A novel bacteriophage-derived peptidase, CHAPK, demonstrated efficacy in disrupting biofilm-forming staphylococci, making it a potential candidate for preventing *S. aureus* colonization on udder skin when included in teat-dip solutions [138] Other anti-staphylococcal peptidoglycan hydrolases like lysostaphin, LasA, ALE-1, broth lysate, CsCl, LytM, AtlA, AtlE, LysK, SAL-1, MV-L, ClyS, and LysH5 have also been identified for controlling and treating staphylococcal infections [139].

Herbal therapy (Plant and Animal derived compounds, Essential oils)

Herbal therapy is a promising approach for mastitis treatment due to its lack of adverse effects. Ethno-veterinary medicine, focusing on herbal remedies offers alternatives to manage bovine mastitis, demonstrating antibacterial, anti-inflammatory, and immune-modulatory properties (Table 2) [140-167]. Chinese herbs, such as *Diploclisiaglaucescens* and *Curcuma longa*, exhibit analgesic and anti-inflammatory effects comparable to standard medications [168]. Various administration methods, including topical, oral, and intramammary routes, are utilized. Herbal therapies such as plant extracts, like moringa, possess anti-inflammatory and antioxidant properties, aiding in udder inflammation and oxidative stress reduction. It also inhibited the expression of pro inflammatory cytokines (TNF-α, IL-1β, and IL-6), cyclooxygenase-2 expression, downregulated NF-$\kappa\beta$ as well as upregulated heme- oxygenase-1 and NADPH [169]. In southern Brazil, plants like *Achilleamillefolium* and *Baccharistrimera* are used orally and topically for their anti-inflammatory and immunomodulatory effects. *Oxytropisglabra* inhibits biofilm formation in bacteria (*S. epidermidis*) associated with mastitis [170]. Integrating herbal extracts with conventional treatments improves mastitis management. Some herbal preparations, like PHYTO-MASTVR, containing FDA-recognized safe ingredients, show potential for mastitis treatment, although effectiveness may vary [171]. Animal-derived compounds like bee venom, lactic acid bacteria from honeybees, and propolis possess anti-inflammatory properties and demonstrate antibacterial activity against major mastitis-causing pathogens in laboratory settings. They also exhibit inhibitory effects on *S. aureus* and *E. coli* [141-143]. Essential oils derived from *Allium sativum*, *Cinnamon cassia*, lemongrass, and *M. verticillata* exhibit inhibitory properties against various species of *Staphylococcus* and *E. coli,* while also enhancing the phagocytic activities of immune cells. Furthermore, they demonstrate immunomodulatory effects and inhibit *Streptococcus uberis* strains [156-162]. A depiction of the role and mechanism of action of herbal therapy in treating mastitis is provided in Fig. 7.

Stem Cell Therapy

Bovine mammary stem cells play a crucial role in maintaining udder health and can be utilized to treat mastitis-induced structural/cytological defects [172]. Mesenchymal stem cells derived from fetal bone marrow and adipose tissue exhibit antibacterial activity, enhancing bacterial clearance by promoting innate immune responses and anti-microbial peptide expression which is mediated by b-defensin 4 A and NK-lysine 1 activity [173]. Human mesenchymal stem cells show broad-spectrum antimicrobial activity, mediated by the enzyme indoleamine 2,3-dioxygenase (IDO), while murine stem cells lack this activity, indicating species-specific differences [174]. Allogeneic adipose tissue mesenchymal stem cell therapy reduced bacterial count in mastitis-affected cows without adverse effects [175]. Intramammary inoculation with allogeneic ATMSCs (2.5 × 10^7) lowered the bacterial count in the milk of cows with CM compared to untreated cows. Bovine mammary stem cell therapy can be applied to regenerate mammary tissues by either repairing or replacing damaged tissue. These stem cells have the capacity to differentiate into epithelial, myoepithelial, and/or cuboidal/columnar cells of the udder tissue. Utilizing bovine mammary stem cells helps mitigate the risk of rejection and potential side effects. Given that mammary stem cells are essential for the growth, renewal, and turnover of mammary epithelial cells, they can be employed for tissue repair and enhancing milk production [170]. Additional research on the isolation and characterization of mammary stem cells is crucial for gaining a deeper understanding of normal epithelial cell development in mammary tissue [172]. Despite various established and emerging treatment techniques, mastitis remains a challenge due to its diverse causes and clinical manifestations. Farmer's knowledge and skills in mastitis management are crucial, as highlighted in a survey completed by Swedish dairy farmers. Overall, while stem cell therapy holds promise, further advancements and research are necessary before its widespread application in mastitis treatment [175].

CONCLUSIONS AND FUTURE PERSPECTIVES

Subclinical mastitis (SCM in buffaloes throughout Asia poses a significant challenge, affecting both animal health and dairy productivity. The review underscores the high prevalence of SCM in buffalo populations across Asia, with Turkey showing the highest rates and Nepal the lowest. *Staphylococcus* spp. is identified as the leading causative agent, with the California Mastitis Test (CMT) being the main diagnostic method. Various factors contribute to the occurrence of SCM in buffaloes, including breed, age, parity, lactation stage, udder and teat condition, dry period duration, bedding materials, and oxidative stress levels. Effective management practices addressing these factors are crucial for reducing the impact of SCM. Moreover, conventional diagnostic techniques for mastitis, though economical, often lack sensitivity and specificity. Advanced diagnostic tools provide rapid results and improved sensitivity but still fall short in specificity and economic viability due to the need for technical expertise and advanced equipment. Once mastitis is diagnosed, the main challenge is to treat it effectively to avoid economic burdens. Various therapeutic strategies, including antibiotics, vaccines, anti-inflammatory drugs, and homeopathic treatments, have been evaluated but none have proven universally effective due to varying pathogen responses. Antibiotics have been the primary treatment, but the rise of bacterial resistance, largely due to irrational use, necessitates alternative treatments. Promising advanced therapies such as bacteriophages and their endolysins, immunotherapy, herbal therapy, and nanoparticle technology require further research. Effective mastitis management demands the simultaneous development of accurate diagnostic techniques and targeted treatments, ensuring early diagnosis and specific therapy to control and treat mastitis effectively.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Figure legends

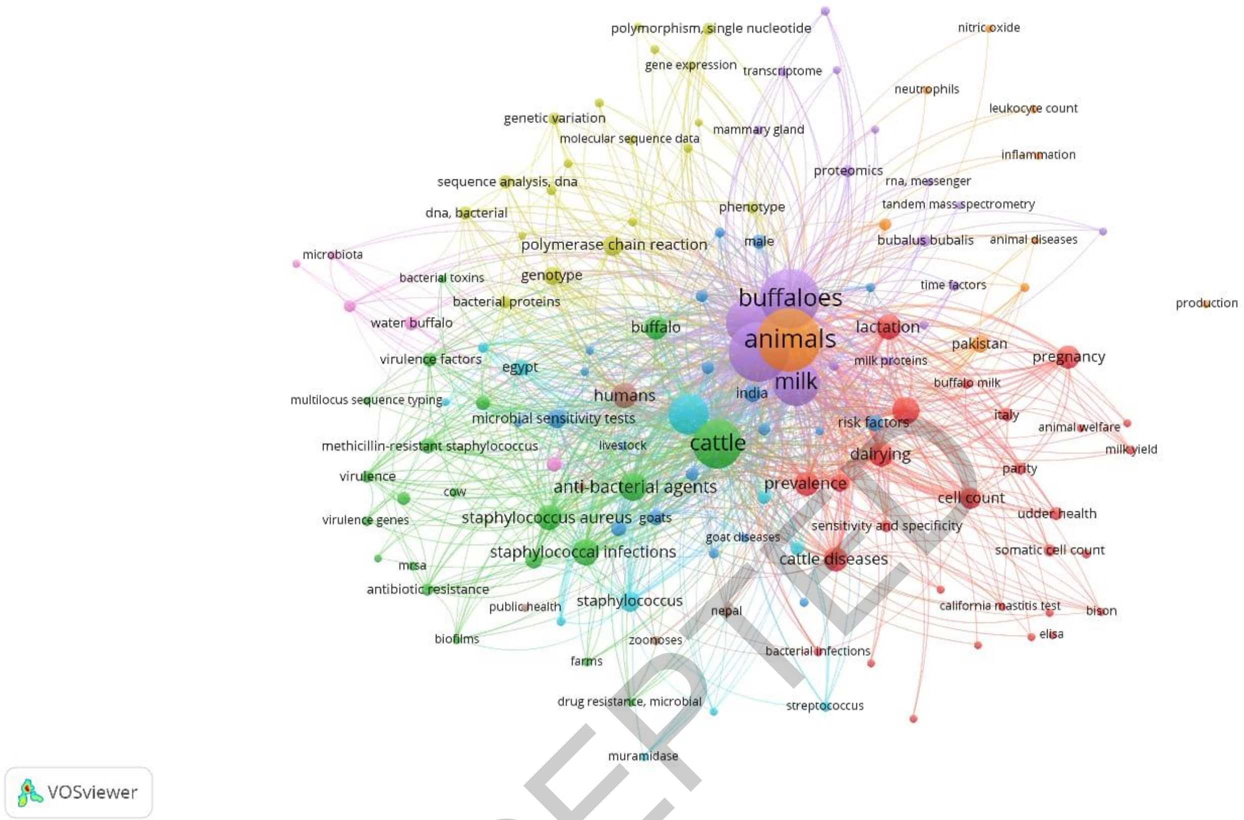

Figure 1. Bibliometric analysis of the associated research mainly focused on Buffalo and mastitis. The analysis was conducted using VOSviewer.exe. The different color indicates cluster of similar research regarding mastitis in buffaloes.

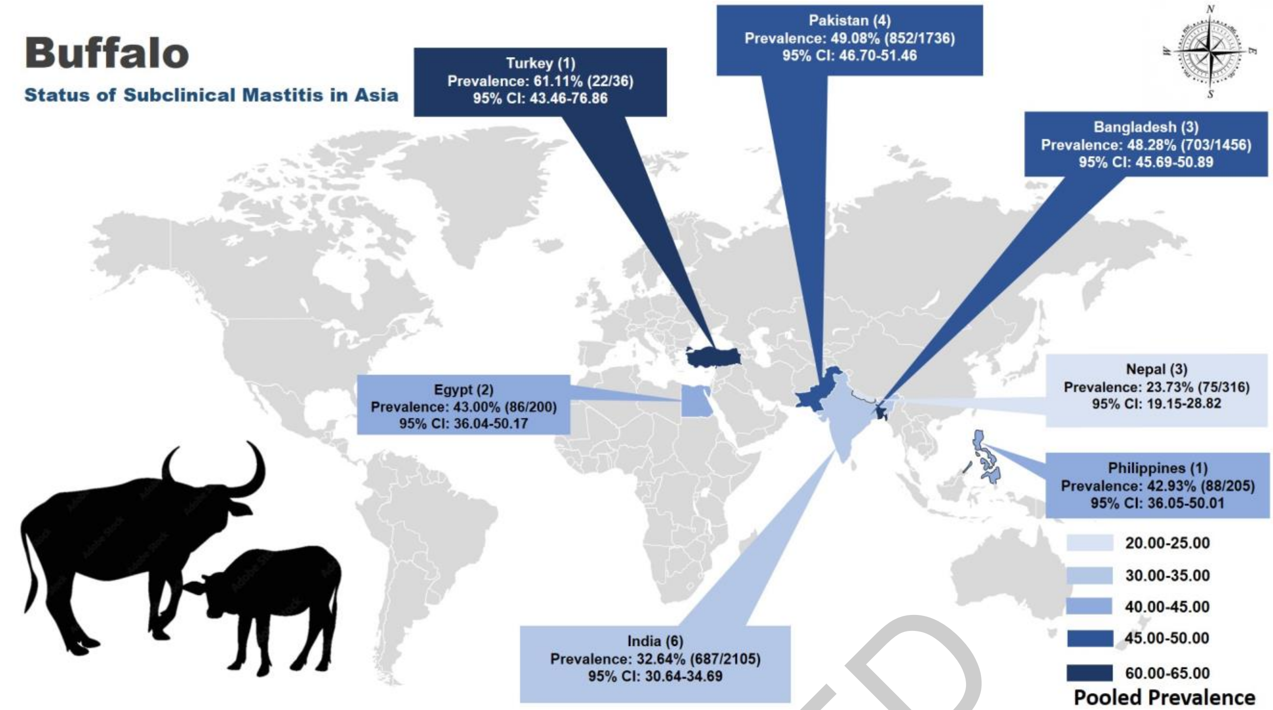

Figure 2. Current status of subclinical mastitis (SCM) around the Asian continent. The choropleth map indicates the pooled prevalence of SCM in buffaloes in Asian cuntries. Light to deep color spectrum indicates low to high prevalence.

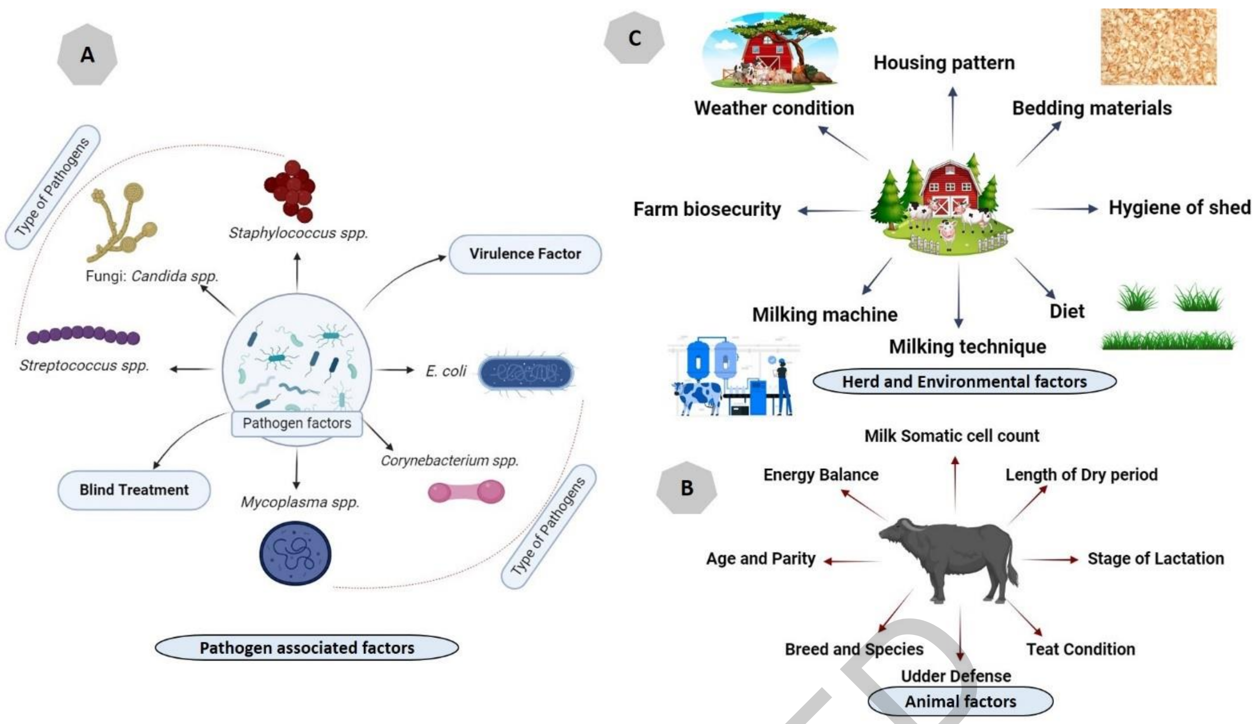

Figure 3. Risk factors associated with occurrence of sub-clinical and clinical mastitis in buffalo. Fig. 3A shows pathogen associated factor, Fig. 3B indicates animal related factors and Fig. 3C indicates herd and environmental associated factors related with mastitis in buffaloes.

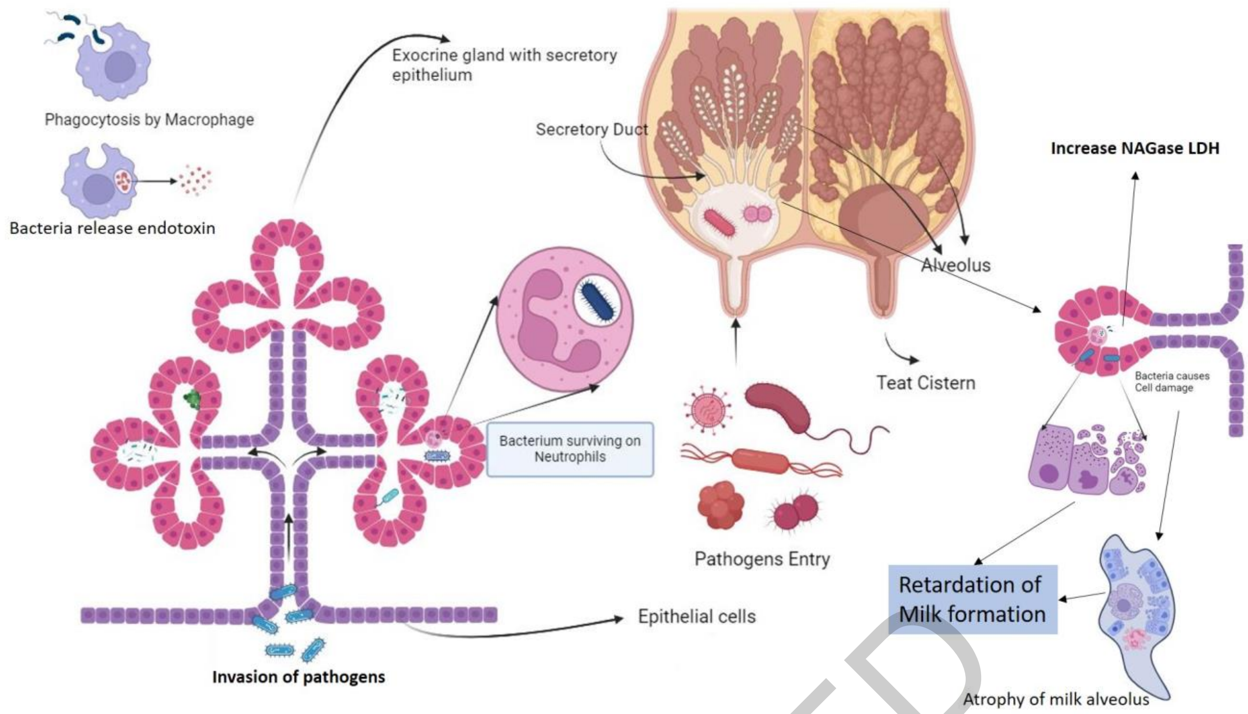

Figure 4. The immune response to pathogens that cause mastitis and pathogenesis leading to the development of subclinical and clinical mastitis. The figure indicates pathogen entry and recognition, activation of innate immune response, involvement of neutrophil and macrophage finally development of mastitis.

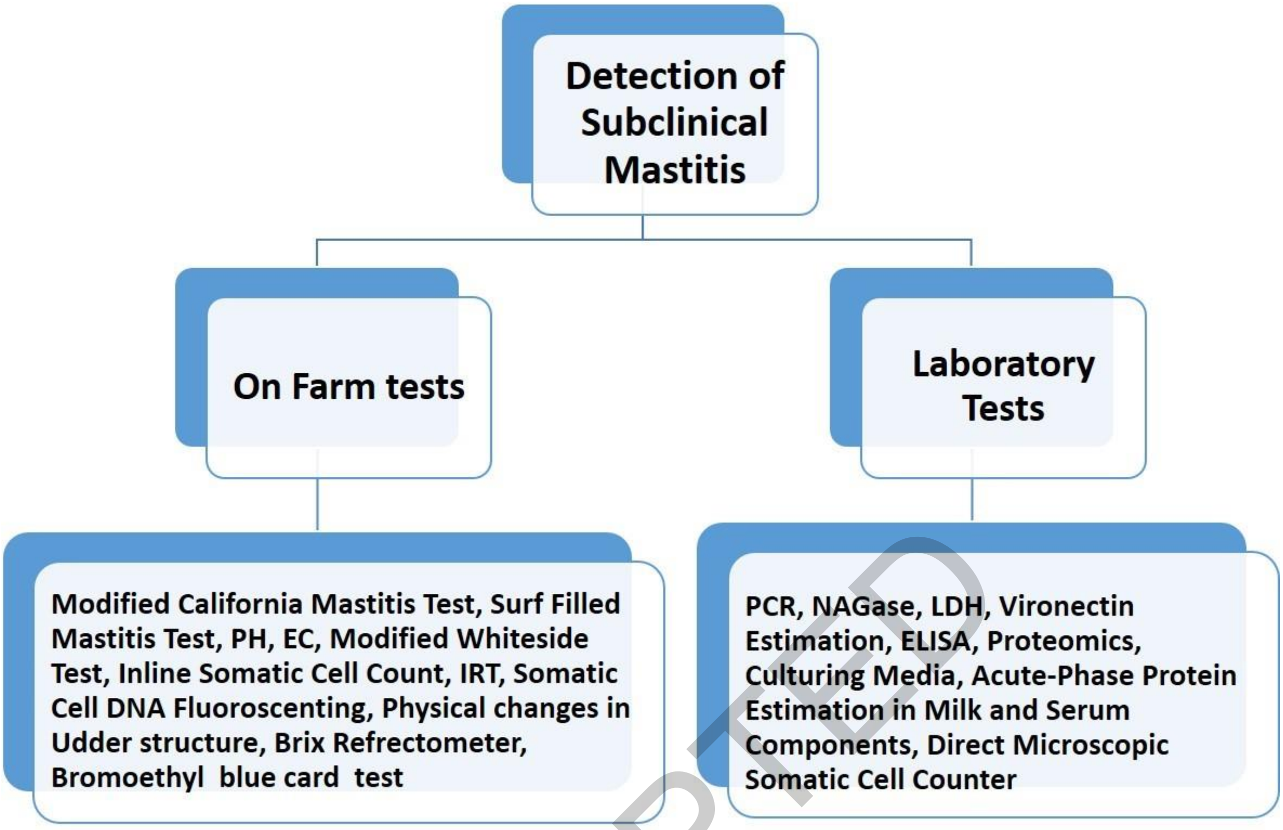

Figure 5. Different diagnostic tests used for the detection of Subclinical Mastitis.

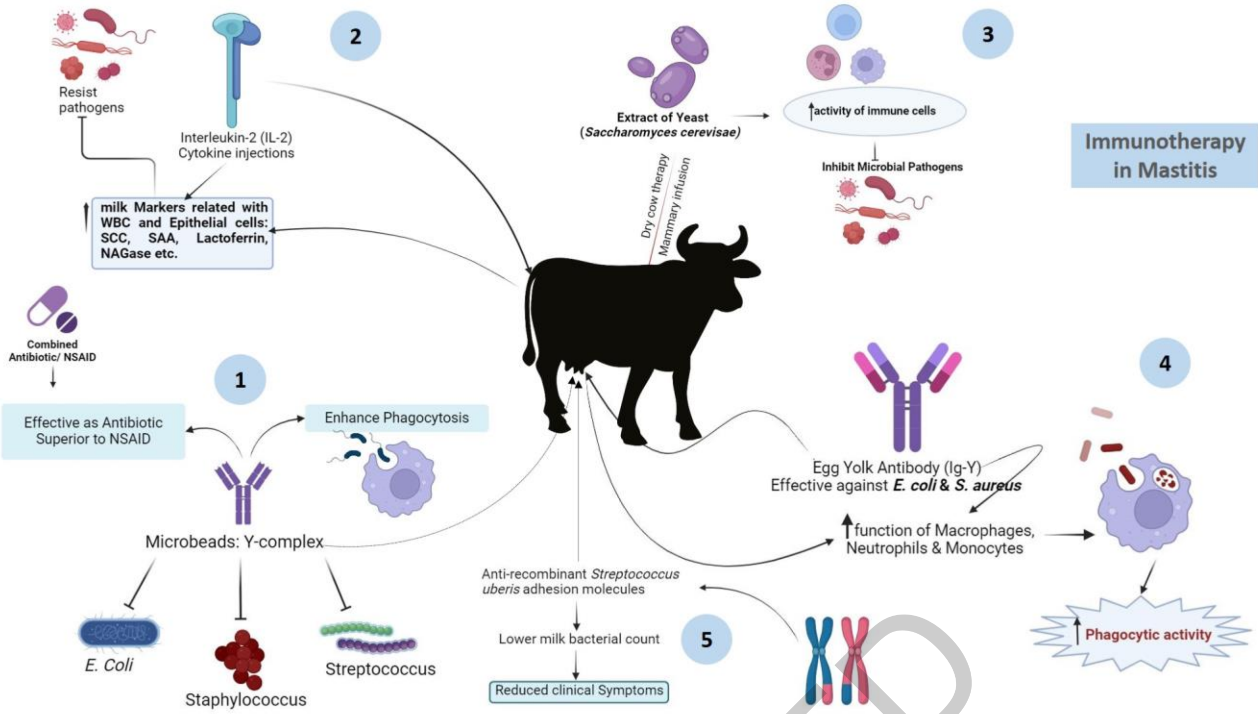

Figure 6. Immuno-therapeutic approaches for the treatment of sub-clinical and clinical mastitis. Method 1: Microbeats and formation of Y-complex, Method 2: Injecting Interleukin-2 (IL-2) and Cytokine, Method 3: Mammary infusion of *Saccharomyces cerevisae*, Method 4: Egg yolk antibody (Ig-Y) therapy, Method 5: Using anti-recombinant *Streptococcus uberis* adhesion molecules.

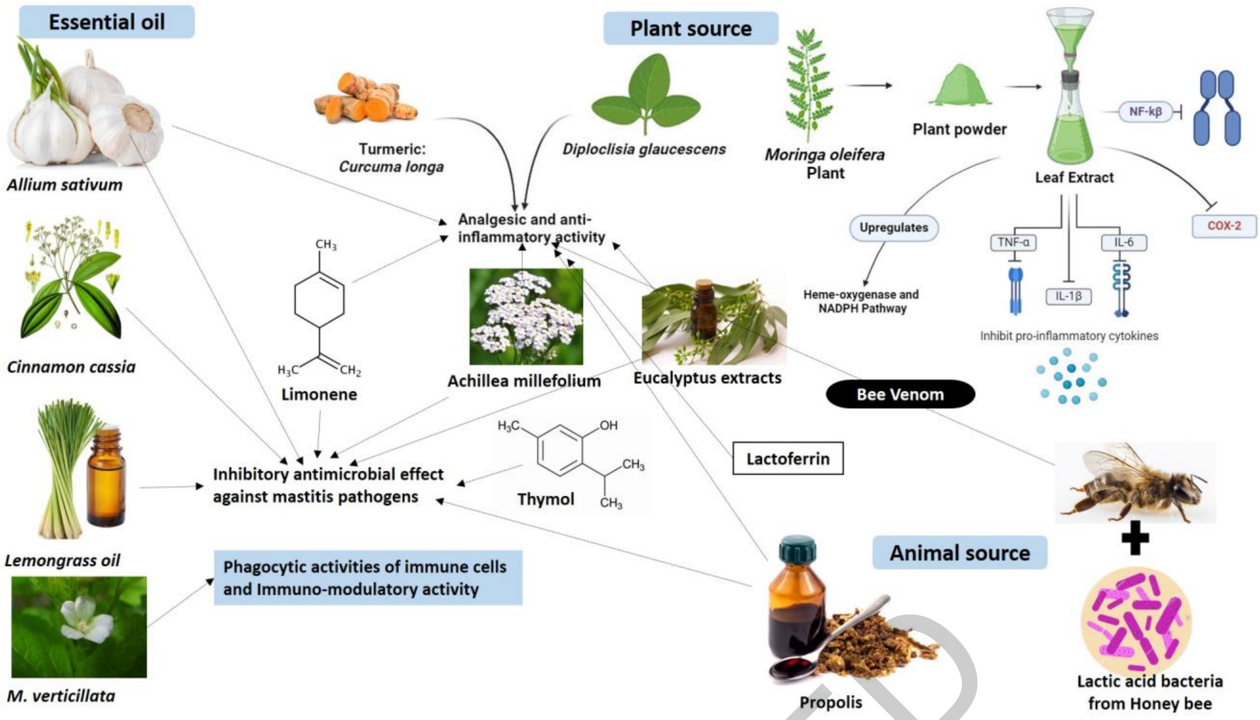

Figure 7. A promising approach for the use of herbal and animal derived medicines in the treatment of mastitis. Antibacterial, analgesic and anti-inflammatory properties of medicinal herbs.Plant sources (*Moringa oleifera*, *Diploclisia glaucescens*, *Curcuma longa*, *Achillea millefolium*), Plant extract (Limonene, Thymol, Lactoferrin, Eucalyptus extract), animal source (Lactic acid bacteria from honey bee, Propolis etc.), Essential oils from (*Allium sativum, Cinnamon cassia, Lemongrass oil*, *M. verticillata*).

Table 1: Characteristics of the studies showing the prevalence, etiology and risk factors associated with subclinical mastitis in buffalo.

Country	Publication date	Sample size	Prevalence	Prevalent bacteria	Risk factors	Diagnostic tests	Resistant Drugs	Reference
Bangladesh	August, 2021	299 quarter 76 Buffaloes	QL-42.5% AL-81.6%	Non-aureus staphylococci (NAS) (24.7%)	not identified	CMT; MALDI-TOF; SCC	Penicillin	[28]
	March,2023	3491 quarters 880 Buffaloes	QL-27.9% AL-51.5%	Not identified	quarter position, rearing System, teat shape, udder symmetry, number of milkers, season, mortality, quarantine facility	CMT BMSCC (Bulk milk somatic cell count)	Not identified	[26]
	September,2022	500 Buffaloes	QL-37.6%	*S. aureus* (37.4%)	Not identified	Bacterial Culture Biochemical test DNA extraction PCR Antibiotic sensitivity Plasmid extraction	Ampicillin Doxycycline Tetracycline Chloramphenicol Ciprofloxacin	[27]
India	May, 2015	800 quarters 200 Buffaloes	20.4%	*Staphylococcus* spp. (39%)	Education of owner, type of labor, feeding after milking, method of milking, pre- and post-milking milking dipping	Bacteriological exam CMT SCC	Not identified	[29]
	September,2018	1299 Buffaloes	33.76%	*Staphylococcus* spp. (51.16%)	Not identified	Bacteriological culture Antibiotic sensitivity	Streptomycin, penicillin, neomycin low sensitive	[31]
	February,2019	120 Buffaloes	68.33%		Age, breed, stage of lactation			[25]
	March, 2021	81 Buffaloes	QL-11.33% AL-22.22%	*Staphylococcus* spp. (51.85%)	-	CMT Electrical Conductivity Culture Biochemical	Amoxicillin/ Clavulanic acid least sensitive	[32]
	May, 2022	328 Buffaloes	14.63%	Not identified	Not identified	CMT	Not identified	[33]
	April, 2023	77 Buffaloes	77%	*Staphylococcus* spp. (83%)	Not identified	Culture Antibiotic sensitivity	Oxytetracycline	[34]
Pakistan	April, 2018	1036 Buffaloes	QL-16.20% AL-38.8%	-	-	CMT	-	[35]
	January, 2019	196 Buffaloes	67.3%	-	Location, age, breed, BCS, milk yield, lactation stage, number of lactations, udder shape, teat shape, infected quarter, other disease, milk leakage, quarantine, deworming, fly control, type of farm, type of shed, source of drinking water, feed type, udder preparation, teat dipping, milking technique, manure change, bedding change, sharing of feed, sharing	CMT	-	[36]

					of animals.			
	April, 2019	34 Buffaloes	22.9%	*Staphylococcus* spp. (25%)	Age, type of animal, breed, urbanicity, teat washing, bedding area, lactating stage, previous exposure of mastitis	surf field mastitis test culture antibiotic sensitivity	Fosfomycin Kanamycin Oxacillin Penicillin Trimethoprim	[37]
	October, 2021	470 Buffaloes	66%	*Staphylococcus* spp. (34%)	-		Sulfamethoxazole, lincomycin, oxytetracycline, ampicillin, Doxycycline	[38]
Nepal	July, 2020	216 Buffaloes	18%		Use of concrete floor use of dry floor, wipe udder, strip of milk, wash udder with soap, cut nails, wash hands before milking, let animal stands after milking, used stainless steel milk container	CMT		[39]
	January, 2021	50 Buffaloes	30%	*E. coli* (16.5%)	Age, type of animal, breed, stage of parity, milk per day, history of mastitis, teat injury, stage of lactation, other diseases, housing, feeding practice, Udder washing, milking utensils, frequency of barn cleaning	CMT, Culture, Biochemical, Antibiotic sensitivity	Ceftriaxone	[7]
	June, 2022	50 Buffaloes	42.8%	Coagulase Negative Staphylococci (46.33%)	Age, parity, lactation, milk yield, history of mastitis, teat injury	CMT, Culture, Antibiotic sensitivity	Ciprofloxacin, Gentamycin, Enrofloxacin, Tetracycline	[40]
Egypt	May, 2020	50 Buffaloes	44%	*S. aureus* (31%)	-	CMT, Culture, PCR, Antibiotic sensitivity	Penicillin Tetracycline	[41]
	July, 2022	150 Buffaloes	42.7%	MRSA (35.7%)	-	CMT, Culture, PCR, Antibiotic sensitivity	Cefoxitin	[42]
Philippines	March,2012	205 Buffaloes	42.76%		age, lactation length, parity, sex, calving month	CMT		[2]
	December 2020	39 Buffaloes	41.94%		age, parity, stage of lactation, previous history, presence of teat lesion	CMT	Cefoxitin Penicillin	[43]
Turkey	March, 2022	36 Buffaloes	-	CoNS (61.1%)	-	CMT, Culture, PCR, Antibiotic sensitivity	Oxacillin, Vancomycin	[44]

AL: Animal level prevalence, QL: Quarter level prevalence

Table 2: List of important animal-based, plant-derived compounds, and essential oils showing their therapeutic potentials for the prevention and control of mastitis.

Source	Utilized items	Therapeutic potential	References
Animal	Bee Venom	Anti-inflammatory property	[140]
	Lactic Acid Bacteria from honey	Shown potential antibacterial activity against major mastitis pathogens under *in vitro* conditions	[141]
	Lactoferrin	Potential anti-microbial and Anti-inflammatory properties	[142]
	Propolis	Inhibitory effect upon *Staphylococcus aureus, E. coli*; Anti-inflammatory property	[143]
Plant	Baicalin	Inhibitory effect on apoptosis, Antimicrobial action against *E. coli,* Reduces antimicrobial resistance effects, Inhibitory effect upon *Staphylococcus aureus*	[144-146]
	Citral and linalool	Inhibitory effect upon *Staphylococcus aureus* and suppressing effect upon virulence of other microbes	[147]
	Eucalyptus and Juglans extracts	Inhibitory effect upon *Staphylococcus aureus*	[148]
	Limonene	Inhibitory antimicrobial effect upon *Streptococcus uberis,* immuno-modulatory effects, improves phagocytic activities of immune cells	[149]
	Liquidambar leaf extracts	Inhibitory effect upon *Staphylococcus aureus* and *Staphylococci spp.*	[150]
	Poncirus fruit extracts	Inhibitory effect upon, *Clostridium perfringes, E. coli, Haemophilus spp., Pantoea spp.*	[151]
	Terminalia fruit extracts	Inhibitory effect upon *Staphylococcus aureus, Pseudomonas spp., E. coli, Bacillus spp.*	[152]
	Thymol	Inhibitory effect upon *Staphylococcus aureus*	[153]
	Herbal choline obtained from a combination of *Achyrantes aspera, Trachyspermum ammi, Azadirachta indica, Citrullus colocynthis* and *Andrographis paniculata*	Reduced both clinical and subclinical mastits.	[154]
	A combination of *Trachyspermum ammi* L., *Curcuma longa* L., *Cuminum cyminum* L., *Trigonella foenum-graecum* L., *Foeniculum vulgare* Mill., *Anethum graveolens* L, and *Zingiber officinale* Roscoe	Significantly improved neutrophil's function was observed thereby leading to better immunity response in dairy buffaloes	[155]
Essential oils	*Cinnamon cassia*	Inhibitory activities against different species of *Staphylococcus spp* and *E. coli*	[156]
	Copaifera spp.	Inhibitory effect against major bacteria responsible for mastitis	[157]
	Extracts of *Allium sativum*	Inhibitory activities against major mastitis causing bacteria, especially against *Staphylococcus aureus* and *E. coli*	[158,159]
	Geranium, Cinnamon, Cedar, Patchouli, Thyme	Inhibitory activities against *S. aureus, S. epidermidis, P. aeruginosa, E. coli*	[160]
	Lemongrass oil	Anti-microbial activity against major bacteria responsible for mastitis	[161]
	M. verticillata	Improves phagocytic activities of immune cells, Immuno-modulatory and Inhibitory effect upon *Streptococcus uberis* strains	[162]
	Origanum vulgare	Inhibitory effect upon *Staphylococcus aureus, E. coli,* and reduced SCC and white blood cells	[163]
	Punica granatum	Inhibitory activities against *S. aureus, S. saprophyticus*	[164]
	Syzygiumaromaticum and *Cinnamomum Zeylanicum*	Inhibitory effect upon *Staphylococcus aureus* and prevents the formation of biofilm by pathogens	[165]
	Thymus serpyllum and Thymus vulgaris	Inhibitory effect against major microbes responsible for mastitis	[166]
	Valencia orange	Inhibitory effect upon *Staphylococcus aureus* by altering its interplay with mammary cells, Reduced invasion of mammary alveolar cells	[167]