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# Trends In Therapeutic and Prevention Strategies for Management of Bovine Mastitis: An Overview

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## Abstract

Mastitis is one of the most economically significant diseases for the dairy industry for backyard farmers in developing countries and high producing herds worldwide. Two of the major factors impeding reduction in the incidence of this disease is [a] the lack of availability of an effective vaccine capable of protecting against multiple etiological agents and [b] propensity of some of the etiological agents to develop persistent antibiotic resistance in biofilms. This is further complicated by the continuing revolving shift in the predominant etiological agents of mastitis, depending upon a multitude of factors such as variability in hygienic practices on farms, easy access leading to overuse of appropriate or inappropriate antibiotics at suboptimal concentrations, particularly in developing countries, and lack of compliance with the recommended treatment schedules. Regardless, *Staphylococcus aureus and Streptococcus uberis* followed by *Escherichia coli*, *Streptococcus agalactiae* has become the predominant etiological agents of bovine mastitis followed *Streptococcus agalactiae, Streptococcus dysagalactiae, Klebsiella pneumonia* and the newly emerging *Mycoplasma bovis*. Current approaches being pursued to reduce the negative economic impact of this disease are through early diagnosis of infection, immediate treatment with an antibiotic found to either inhibit or kill the pathogen(s) *in vitro* using planktonic cultures and the use of the currently marketed vaccines regardless of their demonstrated effectiveness. Given the limitations of breeding programs, including genetic selection to improve resistance against infectious diseases including mastitis, it is imperative to have the availability of an effective broad-spectrum, preferably cross-protective, vaccine capable of protecting against bovine mastitis for reduction in the incidence of bovine mastitis, as well as interrupting the potential cross-species transmission to humans. This overview highlights the major etiological agents, factors affecting susceptibility to mastitis, and the current status of antibiotic-based therapies and prototype vaccine candidates or commercially available vaccines against bovine mastitis as potential preventative strategies.

## Introduction

Major advances in the fields of animal breeding, animal nutrition and husbandry practices have played a significant role in increasing the global milk yield over the last 2 decades [1] meeting the overwhelming demand for milk and milk products. There have been continuous changes in the predominance of etiological of mastitis [2], greater understanding of the host responses to intra-mammary infections [3,4] and treatment regimens leading to adoption of various control and prevention measures. Regardless, the problem of mastitis continues to pose the greatest challenge to the dairy industry worldwide.

Mastitis is one of the most economically devastating diseases of dairy cattle particularly for the back yard farmers in developing world (Table 1), with different levels of economic losses reported by different countries [5-19].

More than $130 million is lost by the Australian dairy industry ($A200/cow/year) every year due to poor udder health resulting in reduced milk production that is mainly associated with mastitis [18]. A herd without an effective mastitis control programme may witness morbidity as high as 40% with infection, on an average of two quarters of the mammary gland [18]. Of the various clinical manifestations, subclinical mastitis is economically the most important due to its long term effects on milk yields [20-22]. Huge economic losses are also incurred due to unmarketable milk or milk-products contaminated with antibiotic residues originating from treatment in the developing nations as well as from the use of antibiotics as growth promoters particularly in dairy feedlots in the developed world. The prolonged use of antibiotics in the treatment of mastitis has led to the additional problem of emergence antibiotic resistant strains, hence the constant concern about the resistant strains entering the food chain [23-25]. Many organisms associated with mastitis also have zoonotic importance and can cause diseases like brucellosis, tuberculosis, leptospirosis, Q-fever etc. [26].

## Etiology of Mastitis

Mastitis (Mast: breast, it is: inflammation) is an inflammatory

| Rank | Country | Milk production (milliontons)* | Losses due to mastitis | | | |
|------|---------|-------------------------------|------------------------|---|---|---|
| | | | Annual (US$ equivalent, million) | Per cow per year (US$ equivalent) | Year of estimate | Reference number |
| 1. | India | 107.03 | 1150-1200 | | 2001 | [5] |
| | | | 320 | | 1994 | [6] |
| 2. | United States of America | 85.88 | 1700-1800 | 185 | 1996 | [7] |
| 3. | China | 38.51 | | 160 | 1991 | [8] |
| 4. | Pakistan | 33.61 | NA | NA | | |
| 5. | Russian Federation | 32.33 | NA | NA | | |
| 6. | Brazil | 30.00 | | 95-142 | 2005 | [9] |
| 7. | Germany | 29.20 | | 285 | 2001 | [10] |
| 8. | France | 22.65 | | 102.42 | 2006 | [11] |
| 9. | New Zealand | 15.67 | | 67.70 | 1993 | [12] |
| | | | 141.70 | 28.73 | 2006 | [13] |
| 10. | United Kingdom | 13.24 | NA | 191.26 | 1999 | [14] |
| | | | | 75-131 | 1993 | [15] |
| 11. | Poland | 12.45 | NA | | | |
| 12. | Turkey | 11.61 | NA | | | |
| 13. | Netherlands | 11.47 | | 22-257 | 2011 | [16] |
| | | | | 72.56 | 1990 | [17] |
| 14. | Ukraine | 11.36 | NA | | | |
| 15. | Mexico | 10.55 | NA | | | |
| 16. | Italy | 10.70 | NA | | | |
| 17. | Argentina | 10.37 | NA | | | |
| 18. | Australia | 9.39 | 130 | 200 | 2011 | [18] |
| 19. | Canada | 8.21 | | 19.6 | 1996 | [19] |
| 20. | Japan | 7.91 | NA | | | |

NA=Not available

*Combined output from cows and buffaloes as of 2009

(FAO: http://faostat.fao.org/site/339/default.aspx).

**Table 1**: Economic losses due to mastitis in different countries.

reaction of the udder, which may result due to microbial, thermal or physical causes. The predominant causal organisms are cell-walled pathogens, although mycoplasma, yeast and algae have also been reported to cause mastitis [27-29]. Interestingly, 137 species and subspecies of potential pathogens can be associated with infection of the mammary gland [30]. However, mastitis in dairy herds is generally of two types: environmental mastitis and contagious mastitis.

Environmental mastitis is caused by potential pathogens found generally in the digestive tract [referred to as "coliforms"] of cattle or their surroundings such as faeces, soil, bedding material and manure [31]. These microorganisms generally proliferate substantially in bedding [approximately 1,000,000 or more cells per gram of bedding]. This increases the probability of infection of mammary glands leading to clinical mastitis [32]. There is a positive correlation between the number of coliforms present in the bedding material and the bacterial load on the teat ends as well as the occurrence rates of clinical mastitis [33]. Coliforms--particularly *Escherichia coli*, *Enterobacteraerogenes*, *Klebsiella pneumonia* and *Serratiamarcescens*, and a *Streptococcussp*, *Streptococcusuberis* are the chief organisms found to cause environmental mastitis. Environmental mastitis has previously constituted less than 10% of total mastitis cases, but more recently there has been an increase in the incidence of environmental mastitis [32,34-38] particularly associated with *Suberis* infection. This pathogen is most often associated with chronic mastitis, which does not respond to antibiotic treatment [31].

Contagious mastitis is caused by bacterial pathogens that thrive on the udder skin and lesions of teat. They cannot survive for long periods in the environment and generally are transmitted from one cow to another by the milking machine, the hands of milkers, milk-contaminated fomites or the sponge used while milking [39,40]. The pathogens mainly associated with contagious mastitis are *Staphylococcus aureus* and *Streptococcus agalactiae* [41-43]. Although *Streptococcus dysgalactiae* is considered as an environmental pathogen, there is evidence of its transmission from cow to cow as a contagious pathogen causing mastitis [41]. Mycoplasma species also cause contagious mastitis. *Mycoplasma bovis* is the predominant species sometimes leading to severe problems like sudden onset, rapid transmission and reduction in milk yield and lack of response to treatment [39,44]. However, the most recognised pathogen in the majority of clinical and subclinical mastitis cases in most countries is *Staphylococcus aureus* [45-49]. These bacteria are of immense importance, causing over 25% of intra-mammary infections and adversely affecting the quality of milk in a large number of clinical cases [50,51]. They are also considered the emerging pathogens causing bovine mastitis since they are the most commonly isolated bacterial pathogens [49,52].

In addition to *Staphylococcus sp*, *Corynebacterium sp* constitute some of the emerging pathogens causing bovine mastitis. *Corynebacterium bovis* is frequently isolated from milk in many dairy farms and causes moderate inflammation of the mammary gland [53,54]. These infections result in a slight increase in bulk tank somatic cell counts, changes in the composition of milk, sudden reduction in milk production and clinical mastitis [39]. Four species of non-lipophilic *Corynebacteria* found to cause clinical and sub-clinical mastitis are *Camycolatum, C ulcerans, C pseudotuberculosis, and C minutissimum* [53]. Other species

of *Corynbacterium* isolated from cases of clinical mastitis in sheep are *C mastiditis* and *C camporealensis* [55,56].

Recent studies have revealed that coagulase negative staphylococci (CoNS) isolated from teat skin, teat canal, and vagina as well as from the coat and the nostril comprises a major interest area of mastitis causal organisms [57,58]. Mastitis in heifers at calving is mainly caused by CoNS. More than 50 species and subspecies are included in this group [52]. *Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus saprophyticus, Staphylococcus hyicus, Staphylococcus warneri, Staphylococcus chromogenes, Staphylococcus scrimi*and *Staphylococcus xylosus* are the commonly encountered species of CoNS in bovine mastitis [59]. The various species of CoNS isolated from bovine mastitis cases show varied pathogenicity, antimicrobial susceptibility and virulence factors [60,61].

Mastitis caused by fungi and yeast is uncommon. The fungi commonly associated with mastitis are *Candidasp, Trichosporonsp, Saccharomyces sp* and *Aspergillussp* [62]. A very rare case of mastitis caused by yeast like fungus, *Geotrichumcandidum* has also been reported [62]. Though the incidence of mycotic mastitis is very low, serious problems may arise when it occurs in enzootic form [63].

## Factors Affecting the Susceptibility to Mastitis

The large number of predisposing factors that contribute to the emergence of mastitis in dairy cattle may be physiological, genetic, pathological or environmental [64] described below:

### Age of the cow

It has been demonstrated that occurrence of mastitis in infected quarters increases with age incows [39,65-68], being the highest at 7 years of age [69]. This may be due to an increased cellular response to intramammary infection or due to permanent udder tissue damage resulting from the primary infection. Efficient innate host defence mechanisms of the younger animals are one possibility that makes them less susceptible to infection [70]. However, at least one study conducted using 4133 cattle including both cross-bred and non-descriptive breeds revealed the highest risk of occurrence of mastitis to be between the ages of 4-6 years, followed by the age group between 2-4 years, with the least occurrence noted between 6-8 years of age [71]. Interestingly it was noted in this study that the crossbred animals were 2.55 times more susceptible to mastitis than the non-descriptive ones.

### Inherited features of the bovine

Various genetic traits may also have a considerable impact upon the susceptibility of the animal to mastitis. These genetic traits include the natural resistance, teat shape and conformation, positioning of udders, relative distance between teats, milk yield and fat content of milk. High milk yielders with higher than average fat content are reported to be more susceptible to mastitis [72-74]. The conformation of the udder and shape of the teat are inherited characteristics that may also affect susceptibility to mastitis. Cows with elongated teats are more vulnerable to mastitis infection than cows with inverted teat ends [75,76]. Broad udders, lower hind-quarters and teats placed widely help the infectious agent and should be selected against it [77]. Another important predisposing factor for mastitis is super numerous teats, which provide additional reservoirs for potential pathogens leading to manifestation of mastitis.

### Stage of lactation

The incidence of mastitis is reported to be higher immediately after parturition, early lactation and during the dry period, especially the first 2-3 weeks [69,78-80] due probably to increased oxidative stress and reduced antioxidant defence mechanisms during early lactation [81]. An increase in somatic cell numbers or count (SCC) which are mainly neutrophils, is observed immediately after parturition, which remains high for a few weeks irrespective of the presence or absence of infection [82,83]. This increased SCC is the cow's natural first line of defence to prepare for the onset of the new lactation. Relatively recent studies have revealed that cows in late lactation always show a higher than average SCC than that seen at other stages of the lactation period [84], potentially representing increased subclinical infection, leading to a fall in milk production.

### Mammary regression

There are significant functional changes in the udder during the early and late lactation and dry period, which affect the cow's susceptibility to infections. Lactating cows under stress show premature mammary regression. Such a condition compromises udder's natural defence mechanisms [85,86] leading to invasion of the teat canals by potential pathogens. The same condition prevails during the healing process of lesions because the resistance to causal agents remains less effective.

### Milking machine

Extraneous factors such as the milking habits of farmers and faulty milking machines favour the pathogens to gain access to mammary gland and proliferate, potentially leading to mastitis [87]. In farms where machines are employed for milking it is important to maintain physiologically optimal pressure [50 kPa for most machines], because pressures in excess of this may lead to injury in the teat [88]. Fluctuations in the pressure due to inadequate vacuum reserve must be avoided to prevent occurrence of mastitis. Proper installation as well as the correct maintenance of milking machines is important to avoid an inadequate vacuum level, teat and tissue damage and incomplete milking [89]. The vacuum level created by the vacuum pump is another important factor for complete and high quality milking. Experiments have shown that a teat subjected to a vacuum level of 10.5-12.5 inches at the time of peak milk flow results in rapid, complete and high quality milk yield, and the teat suffers minimum physical pressure [90]. Two-chambered teat cups are found to be better than single chambered teat cups in regard to achieving complete milking as well as fewer incidences of teat injuries [91]. However, there is a report of increased risk of both contagious and environmental mastitis causing pathogen due to machine induced changes, which widen the orifice of the teat canal in cows [92].

### Nutrition

The quality and plan of nutrition appears to be an important factor that influences clinical manifestation of mastitis in heifers and cows [93] although no relationship between the incidence of mastitis and either high energy or high protein feed in cows has been reported [94,95].

Vitamin E is one of the important supplements in dairy feed to boost the immune response of cows [96] as it has been reported to enhance the neutrophil function as well as the phagocytic properties of neutrophils after parturition [97]. Vitamin E is often combined with selenium, which acts as an anti-oxidant by preventing oxidative stress [98,99]. A number of investigations have demonstrated that neutrophils of selenium fed cows are more effective at killing mastitis causing microorganisms than those not supplemented with selenium [100-102].

Beta-carotene and Vitamin A have also been found to be effective

in preventing the occurrence of mastitis, most probably due to their antioxidant and immune-enhancing properties and contribution mucosal surface integrity of the mammary gland respectively [103,104]. Zinc and copper are also important nutritional elements that contribute mammary gland health by promoting cellular repair, wound healing and reduction in SCC [103,105,106] aided by increases in metallothionein synthesis with antioxidant potential. Various studies have shown that feed supplemented with copper and fed to heifers reduces the severity of subclinical mastitis as well as clinical mastitis induced by *Escherichia coli* [107,108].

## Weather and climate

The incidence of mastitis is greatly influenced by the weather conditions and prevailing climatic conditions. Heat, humidity, cold and draught are the important predisposing factors [109-114]. A higher incidence of mastitis has been reported to occur particularly during summer rainy months [113-117]. As heat and humidity increases, so does the bacterial multiplication as well as the load of pathogens in the environment [118]. Conversely, an alternative study has reported a higher incidence of coliform mastitis during the cold months of the year when the temperature was reported to be less than 21°C [119,120].

## Strategies for Therapy and Prevention of Mastitis

### Antibiotics

Antibiotics ranging from narrow to broad spectrum have been used extensively over the past 40 years in the control of bovine mastitis [42,83]. However, because of the emerging antibiotic resistance believed to be probably due to their overuse [121-128] and the induction of prolonged persistent antibiotic resistance in biofilms by many mastitis-causing pathogens, as demonstrated recently for *S aureus* isolated from cases of bovine mastitis [129], effectiveness of antibiotic therapy has been compromised. As such the control of bovine mastitis has become one of the most challenging problems on dairy farms today. Cows suffering from mastitis are culled due to high SCCs and repeated occurrence of clinical mastitis. Although culling and selective antibiotic therapy have been found to cause a reduction in the manifestation of clinical mastitis [130], dairy farmers are often reluctant to cull affected the devastating financial impact on backyard farmers due to losses in milk production as is often the case in developing countries.

Intramammary infections have been traditionally treated with systemic or intramammary antibiotic therapy [131,132]. Despite scheduling treatment regimens to prolong the availability of appropriate antibiotics for an extended period of time in the infected area [131,133], the cure rates of mastitis particularly for *S aureus* infections have been reported to vary from 0% to 80% [131,133-135]. This is presumably due to the induction and persistence of biofilm-associated antibiotic resistance [129] depending upon the intensity of infection as reflected by SCC counts and management practices [136-139]. Milking animals with a SCC of less than 1 million showed greatest success of antibiotic therapy while those with a higher count responded poorly [134,140]. This is notwithstanding the fact that some antibiotics used for the treatment of mastitis such as penicillin, oxytetracycline, lincomycin and neomycin may affect the phagocytic properties of polymorphonuclear leukocytes (PMN) by altering the oxidative burst property of PMN [141-143] leading to a recurrence of intramammary infections.

### Bacteriophage therapy for mastitis associated infections

Given the problems associated with antibiotic therapy of mastitis, development of alternative treatment strategies for management of clinical and sub-clinical mastitis are warranted. One such alternative treatment is bacteriophage therapy, which uses pathogen specific bacteriophages in the treatment of a bacterial infection. Recent interest in phage therapy in veterinary medicine was sparked by some early success in the treatment of *E. coli* infections in animal models including a chicken model for respiratory infections [144], a mouse model for meningitis [145] and a calf model for diarrhoea [146,147]. However, the few studies that have been carried out using bacteriophages to treat mastitis caused by *S aureus* infection have yielded variable results. While intramammary infusion of bacteriophage into *S aureus* infected quarters of lactating dairy cattle did not show significant protection [148,149]. Kwiatek et al. [150] isolated and characterised a bacteriophage from the milk of cows suffering from mastitis with broad-spectrum activity against methicillin- resistant *S aureus* (MRSA). It is suggested that additional research is required to explore the therapeutic potential of bacteriophages to treat clinical and subclinical mastitis associated bacterial infections.

### Mastitis vaccines

It is beyond the scope of this overview to describe detailed experimental approaches undertaken for the development of vaccines against bovine mastitis caused by the major bacterial pathogens thus far. In this overview, a brief description of the vaccines currently being formulated with the hope of reducing the incidence mastitis on-farm or backyard farming, and promising prototype vaccine candidates of the mastitis-associated pathogens, is presented. The use of vaccination particularly with autogenous killed whole cell vaccines to control infectious diseases on-farm in dairy cattle is common, and vaccination against mastitis pathogens is no exception. Several efforts have been made to develop a vaccine against mastitis, but few have claimed satisfactory outcomes [151-158], neither in the field nor on backyard farms. It is clear that a single vaccine will not prevent mastitis caused by the plethora of pathogens and their different mechanisms of pathogenesis [159].

**Vaccines against *Staphylococcus aureus*:** Numerous attempts to develop a vaccine against *Staphylococcus aureus* using varied approaches have been made [47]. These include whole organism vaccines [154], DNA vaccine encoding clumping factor A [156], live attenuated (*aroA*) *S aureus* [160], capsular polysaccharide (CPS)-protein conjugate vaccines [161,152] and recombinant *S aureus* mutated enterotoxin type C [162].

Somato-Staph®/ Lysigin vaccine has been used in the United States of America since the mid-1970s [163,164]. This is a polyvalent whole cell vaccine comprising 5 phage types of lysed cultures. It reduces the clinical severity of bovine mastitis and lowers the SCC in milk [165]. However, it failed to prevent occurrence of new infections [163,166]. "MASTIVAC-1", which is composed of three different field strains, is another vaccine against *S aureus* that initially showed promising results in field trials [154]. However, various field trials of these vaccines against *S aureus* mastitis have shown that, although clinical severity of the disease is reduced, new infections are not prevented from taking hold. A trivalent vaccine composed of *S aureus* serotype 5, 8 and 336 lysates was reported to stimulate the production of IgG1 and IgG2 in serum in heifers and the vaccines formulated with adjuvant such as Freund's incomplete or aluminium hydroxide produced more IgG2 than IgG1 [167]. However, the efficacy of the vaccine against intramammary infections caused by *S aureus* was not evaluated. O'Brien et al. [168] reported that a conjugate vaccine composed of *S aureus* capsular polysaccharide type 5, 8 and 336 combined with poly(DL-lactide-coglycolide) microspheres enhanced phagocytosis and produced high

antibody titre in cows. Nour El-Din et al. [156] reported a high level of antibody response in dairy cattle when vaccinated with DNA vaccine encoding ClfA. Promising results were also reported in a vaccine trial in dairy heifers vaccinated with a virulent mutant RC122 *S aureus* strain [157]. Strong and specific levels of IgG were reported in both milk and blood of the vaccinated heifers. A trial of vaccine composed of extracellular component from *S aureus* in twelve gestating cows resulted in a reduction of multiplication of *S aureus* in the mammary gland [169]. However, there was no difference observed in terms of clinical symptoms in both control and vaccinated animals. In a recent study, a conjugate vaccine composed of ClfA and deacetylated poly-N-β-(1,6)-acetyl-glucosamine (dPNAG) of *S aureus* was reported to be highly immunogenic in a murine bacteraemia model [170]. The same combination of the vaccine was also found to be immunogenic in multiple animal species including goat, rabbit and rhesus monkey in the same laboratory [170].

**Vaccines against coliform bacteria:** Coliforms (*Ecoli, Klebsiella sp.*) are etiological agents of environmental mastitis. Coliform mastitis generally causes clinical mastitis mostly during the peri-parturient period [171,172]. Early investigations used heterogeneous oligosaccharide antigens derived from *Ecoli* to develop a vaccine against coliform mastitis. These vaccines were administered during the non-lactating period with the aim of preventing mastitis in subsequent lactations [173,174]. These vaccines reduced the severity of infection initially, but their effect gradually diminished over time [175]. J-5Bacterin, Mastiguard™ and J Vac® are the three vaccines available in the market against coliform mastitis [164]. A new vaccine, Startvac (Hipra), has recently been made available in the market targeting not only coliforms but also coagulase-negative staphylococci and *S aureus*. J-5Bacterin, also known as the *E coli* J5 vaccine, is composed of the J5 mutant strain of *E coli*. Cows vaccinated with this vaccine showed a significant reduction of clinical mastitis cases under field conditions [176,177]. Only 20% of the vaccinated animals showed clinical infections, although there was no difference in the incidence of new coliform mastitis cases among the vaccinated compared to the non-vaccinated animals [177]. However, no vaccines against mastitis caused by *K pneumoniae* are available in the marketplace.

**Vaccines against Streptococcus uberis:** The high global incidence of clinical mastitis due to *S uberis*, an environmental pathogen, has warranted the development of vaccines to prevent mastitis caused by this specific etiological agent [178]. Repeated immunisation in experimental animals with killed *S uberis* vaccine resulted in a significant reduction in the number of bacteria in milk but failed to reduce the SCC count [179]. Vaccination with bacterin from *S uberis* demonstrated protection against *S uberis* mastitis caused by the homologous strain but failed to protect against a heterologous strain [180]. In another study, plasminogen activator derived from *S uberis* showed promising results in reducing the severity of infection [178,181]. In a recent study, cows vaccinated with a recombinant adhesion molecule of *S uberis* (rSUAM) by the subcutaneous route showed an increased antibody titre in milk and serum, which was found to reduce adherence and internalisation of the organism into the epithelial cells of the mammary gland *in vitro* [182].

Numerous efforts to develop *S uberis* vaccines to prevent mastitis have not proven successful and no commercial vaccines for prevention of this infection are available in the market. No immune response is induced in the mammary gland even after intramammary infection with *S uberis*, which further complicates the development of a vaccine [183]. Sortase-anchored proteins derived from *S uberis* may

be potential candidates for vaccine as they are important potential virulence antigens contributing to the pathogenesis of bovine mastitis [184]. Recently, Denis et al. [185] reported that cows which developed mastitis after environmental exposure to *S uberis* developed bactericidal antibodies and T cells in blood and milk, resulting in an increased level of interferon-gamma (IFN-γ) that was specific for *in-vitro* killing of *S uberis* [185]. Clearly, research on the development of an effective vaccine against *S uberis* – associated mastitis is highly warranted.

**Vaccines against Streptococcus agalactiae and Streptococcus dysgalactiae:** *Streptococcus agalactiae* is an important pathogen for humans [infants, pregnant women and immune compromised elderly patients] has nine serotypes [159], each having a serologically distinct polysaccharide capsule; a capsular conjugate vaccine, using the capsule of predominant serotypes, has been evaluated in Phase 2 trials with encouraging results. However, little information available on the capsular types of this pathogen which also causes mastitis in the dairy population. Furthermore, no commercially attractive prototype vaccine candidates are available against mastitis caused by *S agalactiae* or *S dysgalactiae* despite many attempts that have been made to develop an effective vaccine against these pathogens. Recently, a recombinant vaccine composed of *S aureus* cluming factor A (ClfA) and surface immunogenic protein (rSip) of *S agalactiae* was shown to increase the serum IgG1 antibody titre in experimental mice immunised by an intramammary route [158]. Not much effort in developing a vaccine against bovine mastitis due to *S dysagalactiae* has been made either. However, the surface proteins GapC and Mig of *Streptococcus dysgalactiae* were reported to be potential protective antigens against bovine mastitis [186].

## Genetic selection of cattle for resistance to clinical mastitis

Breeding production animals for resistance to infectious diseases is not new [187], but generally the breeding of farm animals has been confined to enhancement of production traits such as increased milk production. Such selection, while enhancing milk production, has been reported to increase the incidence of many infectious diseases including bovine mastitis [188,189]. In attempts to overcome this problem, a strategy based on enhancing the overall immune response-- including antibody-mediated immune response (AMIR) and cell-mediated immune response (CMIR)--has been proposed [190]. However, a negative genetic correlation between AMIR and CMIR [191] has been recorded, making a balanced genetic selection more complex and requiring further investigations.

## Ancillary non-specific strategies for prevention of mastitis

**Non-specific immunostimulants against mastitis:** In the absence of the availability of effective commercial vaccines for prevention of mastitis caused by multiple pathogens, attempts are continually being made to evaluate the potential of non-specific immune stimulants for prevention of bovine mastitis [192]. Lysate of *Corynebacterium cutis* has been considered as one of the non-specific immune stimulants against mastitis. There are reports of a reduction in SCC [193] in the milk of dairy cows receiving a subcutaneous injection of lysate of *C cutis* possibly due to the boosting of the immune system of those animals [194]. Intramuscular injection of *C cutis* to pregnant ewes resulted in an increased level of IgG in serum on the 140th day and in colostrum up to 3 days post-parturition [195]. Clearly, further studies on the potential of the non-specific immune stimulants are warranted.

**Lactation therapy:** Treatment of mastitis during lactation with antibiotics is referred to as "lactation therapy", which is used by many

producers to reduce the clinical signs of mastitis and bring back the normal milk production of cows. This therapy has proven useful in reducing the SCC in milk and thereby maintains the quality of milk [196-198]. However, lactation therapy for subclinical mastitis is not suggested as it is not economically viable and shows poor efficacy [198]. Factors such as SCC in milk during treatment, stage of lactation, immune status of the animal, age of the cow and type of pathogen also play an important role in the success or failure of lactation therapy [196,199,200]. New intramammary infections in cows have been shown to respond better to antibiotic therapy than chronic infections [201,202], and young animals show better response to treatment than older animals [203-207]. Spontaneous cure by lactation therapy for clinical as well as subclinical mastitis caused by *S aureus* is very rare [197-208]. Lactation therapy in chronic clinical cases of mastitis caused by *S aureus* has been found equally ineffective [130]. However, an extended period of treatment with antibiotics at therapeutic levels has been reported to yield better cure rates for clinical mastitis caused by *S aureus* [205]. A serious drawback of this therapy is the loss of milk because of antibiotic residues.

**Dry cow therapy:** Dry cow therapy with antibiotics has been suggested as one of the options to control intramammary infections and prevent development of mastitis [209,210]. During the dry period, the cow is at the greatest risk of acquiring new intramammary infections with both gram-positive and gram-negative environmental or contagious pathogens [211-213]. It has been reported that about 61% of new infections are acquired during this period [213]. Treatment during dry period is advantageous because it allows treatment of infections with antibiotics without the need to discard milk from treated quarters. Antibiotics are administered towards the end of lactation [214] and may remain in the udder in concentrations high enough to kill pathogenic bacteria for 20-70 days, depending upon the kind of formulations that are used. The antibiotic has an enhanced penetration due to prolonged exposure and the probability of curing intramammary infections increases markedly, unless resistance to new antibiotics is acquired by the invading pathogen's biofilm formation in the udder [129]. Dry cow therapy has been reported to eliminate almost 100% of mastitis caused by *S agalactiae* [210,215]. However, dry cow therapy is comparatively less successful to prevent *S aureus* mastitis than streptococcal mastitis [209]. The cure rate of dry cow treatment against *S aureus* mastitis was reported as approximately 50% and vaccination against this pathogen during the dry period may enhance the antibiotic efficacy [213]. Dry cow therapy for a period of two weeks showed significant reduction in the number of clinical mastitis cases due to infection with *S dysgalactiae* and *S uberis* [216].

**Teat sealer:** The development of internal and external teat sealants for use during the dry period is a promising progress towards control of mastitis and its aftermath [118,217-221]. External teat sealants such as DryFlex and Delaval also showed potential in reducing new infections of the mammary gland during the dry period [134]. However, lack of persistence is the main drawback of external teat sealers [222]. Bismuth subnitrate as an internal teat sealer used in field conditions was reported to reduce new infections upto tenfold [217,223]. Internal teat sealer used with long acting antibiotics during the dry period showed a 30% and 33% reduction in new intramammary infections and incidence of clinical mastitis, respectively [103]. Bismuth subnitrate combined with cloxacillin as dry cow therapy demonstrated reduction in both clinical and subclinical cases of mastitis [220,221]. There are several studies that have demonstrated the usefulness of OrbeSeal, an internal sealer in reducing new infections of the mammary gland in lactating animals [219,224].

## Conclusions

Bovine mastitis is an economically important disease due to its involvement in the quantity and quality of milk production. The dairy industry all over the world suffers from significant economic losses incurred due to mastitis. Application of hygienic measures during milk collection, using milking machines, lactation and dry cow therapy, teat sealers, dietary supplements and culling are likely to reduce but not control the incidence of both clinical and subclinical mastitis. The effects of mastitis on dairy cattle health and milk production highlight an urgent need to develop effective strategy of prevention and control. The constantly changing predominance of etiological agents in different geographical locations must be considered while adopting and developing mastitis control strategies. Research aimed at developing an effective broad-spectrum universal vaccine capable of providing protection against the predominant environmental and contagious pathogens causing bovine mastitis is highly warranted for reduction of the incidence of bovine mastitis worldwide.

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