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Chapter

# *Staphylococcus aureus* and Dairy Udder

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## Abstract

*Staphylococcus aureus* is a major causative agent of intra-mammary infections in dairy animals with potential virulence of surface components, toxins, and extracellular enzymes. About 74% quarter prevalence of *S. aureus* in bovine udder with overall prevalence exceeding 61% in dairy animals. About 17 different serotypes of dairy originated *S. aureus* have been reported with 24 virulence coding genes for leukocidins (lukED/lukM), pyrogenic toxin super antigen (PTSAg), haemolysins (hla-hlg), toxic-shock syndrome toxin (tst), enterotoxins (sea-seo, seu), exfoliative toxins (eta, etb), and genes for methicillin (mecA) and penicillin (blaZ) resistance. Attainment of refuge inside the macrophages and neutrophils is a major cause of *S. aureus* mastitis persistence. Mammary prebiotics and probiotics are recently being used as alternatives to antibiotic for the prevention of mastitis. Literature showed anti- staphylococcus vaccines with different results depending upon types of immunization, route of administration and adjuvant used. Studies has shown that herd specific as well as commercial *S. aureus* vaccines reduce new infections in dairy animals. Experiments are still in progress for the use of vaccines against *S. aureus* mastitis with optimal efficacy and reliability. Perhaps, there might be bright future because of highly satisfactory trial results of mastitis vaccines in the lab animals.

**Keywords:** *S. aureus*, dairy udder, transmission, pathogenesis, economic impacts, treatment, prevention

## 1. Introduction

*Staphylococcus aureus* is a symbiotic and opportunistic microorganism that can colonize various sites of different animals and humans. This bacteria can cause serious infections in humans and animals [1]. In animals, bovine mastitis, commonly caused by various bacteria, is one of the most devastating disease in dairy farming worldwide. Of these bacteria, *Staphylococcus aureus* is the leading pathogen causing the most dangerous mastitis in cattle and the most difficult dairy product in most countries. *Staphylococcus aureus* has emerged as superbug of dairy udder, compromising animal health and economy [2]. Its virulence is due to its

ability of producing wide array of virulence factors that enhances its attachment, colonization, longer persistence and escaping the immune response. Such resistant strains are distinguished by systemic heterogenicity, genetic variety, interactions between complex community and the extracellular matrix of macromolecular substances [3].

*Staphylococcus aureus* has a variety of strains, most notably multi-drug resistance and biofilm formation. The latter has received a lot of attention due to its ability to minimize the effects of antibiotics, colonize the mucous membrane of the epithelium, last longer, avoid immune reactions, and contribute to etiology [4]. Methicillin resistant *S. aureus* strains have been designated as emerging pathogen in livestock and dairy animals. Hospital acquired MRSA and community associated MRSA are limited to humans only, but livestock occupational personals may have infections with human originated MRSA [5].

The successful mastitis therapy depends on various factors such as accurate diagnosis, elimination of causative agent, stage of disease, severity of the infection, selection of the drugs, route of drugs administration along with other supportive treatments [6, 7] and some other factors regarding mastitis causing organisms. However, irrespective of the appropriate use of antibiotic, the mastitis may not be treated successfully [8]. The treatment failure mainly occurs due to insufficient contact of antimicrobials and disease-causing microorganisms in the udder. Mastitis can incur economic losses in both ways either directly and indirectly [9]. The direct losses include veterinary expenditure, labor costs, reduced production, poor quality milk and discarded milk. Whereas, the indirect losses are not obvious to the producers and are termed as "hidden losses" which include increased risk of other diseases, poor fertility rate, increased culling rate and sometime mortality. So, total cost can be much more than the direct losses [10–12]. This chapter addresses the following aspects such as transmission, pathogenesis, strains spectrum, economic impact, emerging treatment and prevention strategies to control *S. aureus* dairy udder infection.

## 2. Transmission of *Staphylococcus aureus* in udder infections

The main reservoirs of *Staphylococcus aureus* are infected mammary glands, ducts, and papillary lesions. However, this bacteria also found on the skin, nose and teat passages. The bacteria spread to uninfected areas through the lining of the teat cups, milker's hands, towels and fruit flies. *Staphylococcus aureus* does not persist on healthy teat skin, but tends to colonize damaged skin and teat lesions. The body reproduces the infected lesion, increasing the likelihood of teat colonization and subsequent udder infection. Heifers infected during calf pregnancy are an important reservoir that can be passed on to uninfected *Staphylococcus aureus* herds. There has been much controversy over the route of infection with *Staphylococcus aureus* in early prenatal heifers, but it is possible that the cause is a calf that was fed on a mother infected with *Staphylococcus aureus*. Data is limited, but if you have a problem with *Staphylococcus aureus* on your farm, you should definitely consider choosing scrapes carefully (such as cryo-sterilization). Obviously, a good treatment plan for mastitis will take into account the absence of this disease in heifers [13].

## 3. Pathogenesis of *S. aureus* in udder infection

Bacterial pathogens can recognize, respond and adapt to the harsh environmental conditions that prevail in mammalian hosts during infection. Despite the

host's immune response and antibacterial treatment, it helps to invade, calm, and survive within the host [14]. *Staphylococcus aureus* produces a variety of enzymes, including coagulase, which can coagulate plasma, converting plasma fibrinogen to fibrin, coat bacterial cells, and inhibit nutrition. Hyaluronidase (also called diffusion factor) can break down the hyaluronic acid present in tissues and support the spread of *Staphylococcus aureus* in the host. It also produces DNase (deoxyribonuclease), an enzyme that breaks down DNA. Lipase, which breaks down lipids, and staphylokinase, which breaks down fibrin. It is also known that *Staphylococcus aureus* produces β-lactamase, esterase, elastase and phospholipase for drug resistance, and these enzymes promote colony formation and pathogenicity. Other toxic factors of *Staphylococcus aureus* include leukocidin (which can cause cytolytic destruction of phagocytic cells in some animals) and toxic shock syndrome toxin (TSST). The latter can cause an overproduction of lymphokine, which can lead to tissue damage [15]. Depending on the strain, *Staphylococcus aureus* can release some toxins that are major virulence factors. These toxins act on cell membranes containing superantigens, exfoliating toxins, and some two-component toxins such as alpha toxins, beta toxins, gamma toxins, delta toxins and Panton Valentine's toxins and leukocidin (PVL). It can be divided into three categories, for example toxins [16]. Protein A, which plays an important role in strategies for evading immunity, is immobilized on the staphylococcus-peptide-glycan-pentaglysin bridge using transpeptidase sortase (**Figure 1**). Protein A is able to bind to fragments of the crystal region (Fc) of IgG antibodies (γ-immunoglobulins). This phenomenon is due to the fact that Protein A binds to an IgG antibody produced against the target microorganism and reacts with the corresponding antigen usually present in the patient sample to perform an aggregation test in which a visible aggregation reaction can be observed. The *Staphylococcus aureus* strain is known to produce pigments such as staphyloxanthin and gold carotenoid pigments. These pigment acts primarily as a toxic factor, acting as a bacterial antioxidant and helping microorganisms escape the host's immune system and kill reactive oxygen species used by the pathogen [18]. The toxins produced by *Staphylococcus aureus* destroy the cell membranes and tissues that directly produce milk. White blood cells (leukocytes) are attracted to the area of inflammation and try to fight the infection. First, bacteria damage the tissues lining the teat and mammary gland within 1/4 of a second, eventually leading to scar tissue formation. The bacteria

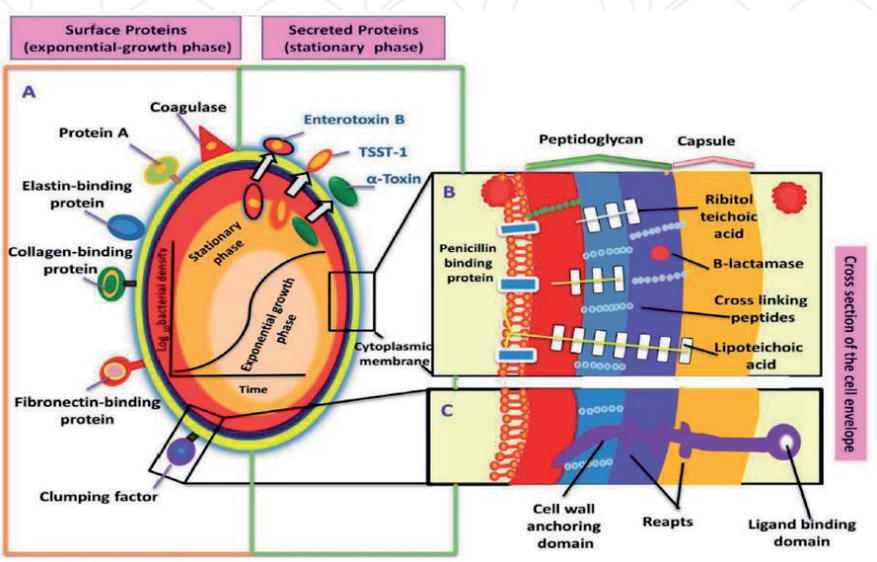



**Figure 1.**
*Various virulence factors of S. aureus [17].*

then migrate into the duct system, forming deep-rooted infectious pockets in the lactating (alveolar) cells. The second is the formation of abscesses that prevent their spread, thus avoiding detection by the immune system. Abscesses prevent antibiotics from entering bacteria. This is the main reason for poor response to treatment. However, bacteria can also escape the lethal effects of some antibiotics by hiding in neutrophils (white blood cells) and other host cells preventing exposure to antibiotics. When the white blood cells die (usually within a day or two), the bacteria are released and the infection continues [19].

During infection, the destruction of alveolar and tubular cells reduces the lactation yield. These damaged cells can attach to leukocytes and block the mammary canal that drains the alveolar region, resulting in additional scar tissue, blockage of the canal, and decreased lactation. The teat canal can be opened later, but this usually results in the release of *Staphylococcus aureus* to other areas of the udder. The spread of *Staphylococcus aureus* in the glands leads to the formation of additional abscesses, which can become very large and appear as lumps in the udder (**Figures 2** and **3**). Most cases of *S. aureus* mastitis are asymptomatic, but chronic cows typically have high SCC, abnormal udder tissue, and recurrence of clinical mastitis. Clinically infected areas are usually swollen, milk has visible clots (large clots). Acute infections with *Staphylococcus aureus* usually develops late in lactation. Clinical symptoms such as udder swelling and hardness, milk appearance change) do not appear until the start of the next stage. It is difficult to cure an infection well, because the drug cannot penetrate all foci of infection, and bacteria can avoid contact with antibiotics in the white blood cells. Many strains of *Staphylococcus aureus* have acquired antibiotic resistance (the ability to produce enzymes that

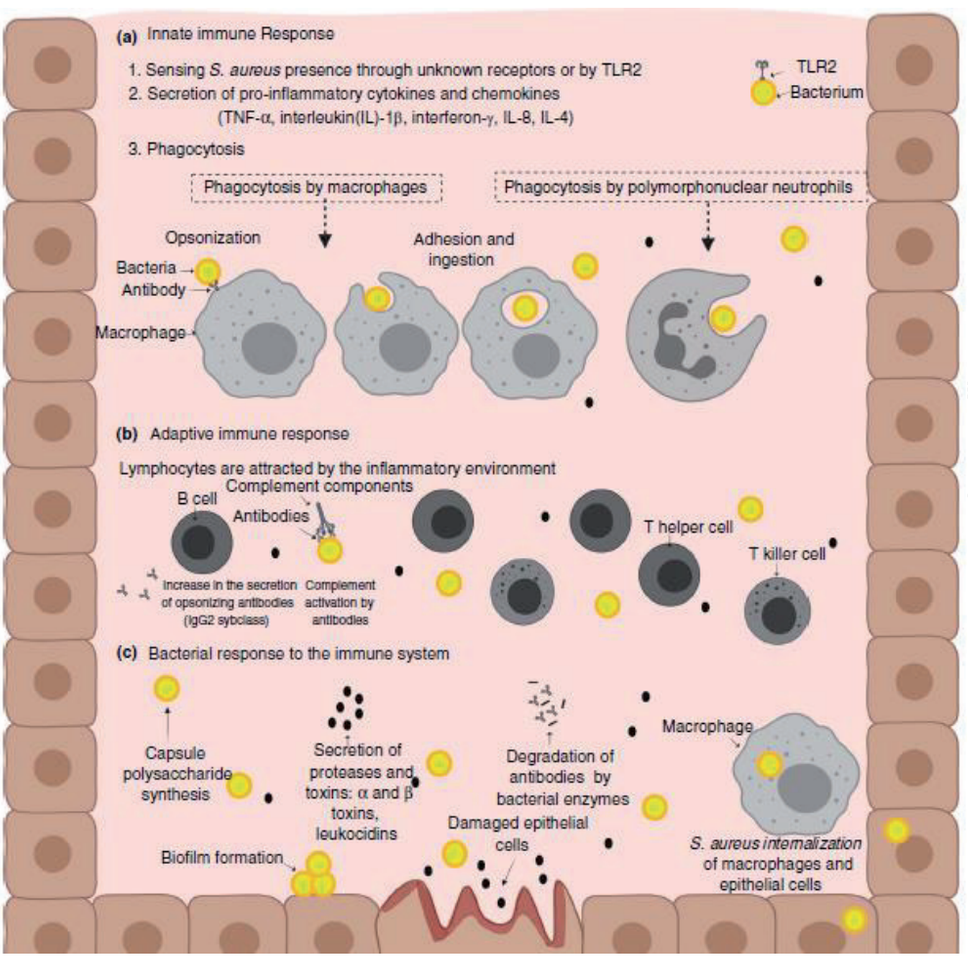



**Figure 2.**
*Immune response to* S. aureus *and vice versa inside the mammary gland [20].*

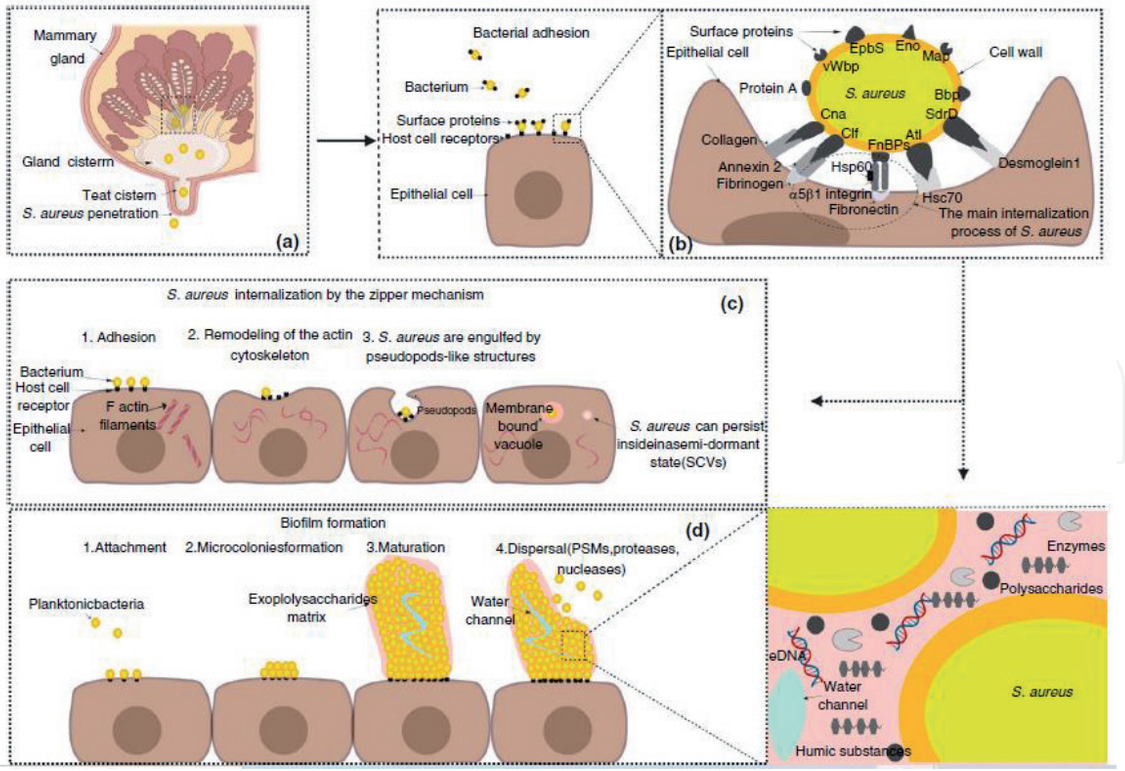



**Figure 3.**
*Intracellular invasion of* S. aureus *inside mammary gland [20].*

inactivate penicillin and other antibiotics), making treatment impossible. The development of antibiotic resistance during treatment with certain β-lactam antibiotics (such as penicillin) is another reason for treatment failure [20].

## 4. *Staphylococcus aureus* strains spectrum

*Staphylococcus aureus* is a major causative agent of intramammary infections in dairy animals with potential virulence of surface components (adhesins, capsular polysaccharides, protein A), toxins, extracellular enzymes and coagulase [21]. About 74% quarter prevalence of *S. aureus* in bovine udder [22] with overall prevalence exceeding 61% in dairy animals [4]. A wide array of genotypic variations has been observed with great genetic diversity in the isolates of bovine as well as caprine origin. Some of the variants are common throughout the globe as ruminant specific *S. aureus* while others are geographic related in the literature [23]. Inflammatory respondent metabolic pathways (BoLA-DRA, GLYCAM1, FCER1G, B2M, CD74, NFKBIA and SDS), milk constituent associated (CSN2 and CSN3) and immunity related (B2M and CD74) are also specific strains of *S. aureus* of dairy mastitis [24, 25].

*Staphylococcus aureus* genotyping is mostly done by pulsed field gel electrophoresis(PFGE), multi locus sequence typing (MLST), polymerase chain reaction (PCR), *S. aureus* protein A (spa) typing, agar typing and typing on the basis of virulence and resistance coding genes [23, 26–31]. Thirty-nine electrophoretic types of *S. aureus* with diverse MLST genotyping had been reported, most of them were showed genetic heterogenicity and classified to one of the eight clonal complexes, suggestive of multiclonal nature of the *S. aureus* isolates from single dairy herd [32]. Clonal 8 complex (i.e., USA300), a lineage known for human infections, has also been isolated from bovine mastitis, suggestive of recent host shift and new adoptive genotypic strain of bovine mastitis [27].

PFGE typing of *S. aureus* from dairy origin showed that PFGE type A was significantly related to teat skin while PFGE type Q was more exclusive to milk and exhibit marked biofilm potential [33]. Overall, PFGE clusters of isolates showed same endotoxin coding genes with indistinguishable banding patterns. Phylogenetic studies based on MLST sequencing classified these clusters into clonal complexes with similar staphylococcal endotoxin genetic profiles [23]. Genotyping of dairy originated *S. aureus* showed five clonal types (PFGE A consisting on sequence type 747 [ST747] and spa type t359; PFGE B with spa type ST750 and t1180; PFGE C with spa type t605 and ST126; PFGE D with spa type t127 and ST751; PFGE F with spa type t002 and ST5). About 63% isolates harbor major clone A but negative for Panton-Valentine leukocidin and exfoliative toxin D genes [26]. Another reported PFGE typing of dairy originated *S. aureus* revealed 16 PFGE types (from A – P), with M, I and O as most frequent but not significantly variant strains in the field, respectively. PCR typing based on endotoxin genes presence showed that 11.7, 1.8, 2.7, 0.9 and 7.2% isolates carried seb, seb and sec, sec, see, and tsst-1, respectively with zero prevalence of sea and sej genes. PFGE types M and O showed clustering behavior with β-hemolysin and least prevalence of endotoxin coding genes [34].

Staphylococcal protein A types t084, t304 and t688 from subclinical mastitis showed divergent virulence and heterogenicity traits while a novel spa-type t18546 was also reported from dairy udder ailments [35]. Prevalent clonal types of *S. aureus* from bovine udder exhibited generic alterations of epigenetic modulators to surpass immune response of host. The study reported 35,878 transcripts of these strains which differ 23% from reference genomic cluster. Expressive nature of 20,756 transcripts were observed with more than 1 fragment per kilobase of transcript per million mapped fragments and 25.95% of multi-exonic genes alternatively spliced. Alternative Splicing (AS) events for more than 100 immunogenic genes were noted with 379 alternate AS events coding for transcription and splicing proteins. Spa typing of ovine originated *S. aureus* showed 14 diversified clones, most prominent of which were t1773, t967 and t1534 as 62.32, 5.79 and 5.79% respectively. Three novel spa types were also identified with repeats successions (07–23–12-34-12-12-23-03-12-23), (04–31–17-24-25-17-17) and (04–31–17-24-17-17) [36].

Screening of *S. aureus* for endotoxins (SE) showed that >90% isolates were positive for SE genes while 70.1% with exaggerative response. All isolates were positive for biofilm encoding genes (icaA/D, clf/B, can, fnbA). A total of 7 spa types (1 novel spa type t17182), 5 STs, 14 SmaI-pulso-types and 3agr types (no agrII) were reported. PFGE cluster II-CC1-ST1-t127-agr III was the most prevalent clone (56.3%). Isolates of agr III (PFGE Cluster I/II-CC1-ST1-t127/2279) exhibited higher number of virulence genes than other agr types. The MSSA-ST398-t1456-agr I clone showed higher antibiotic resistance, weak biofilm expression and lower level of virulence genes expression [37]. Another study reported agr-I strain harboring penicillin resistance genes while agr-III strains were devoid of that pattern. Antimicrobial resistance encoding genes (tet (L), tet (K), erm (B) and bla (Z)) were frequent in these strains [36]. Thus, the data narrated agr-I and II as different subspecies of dairy originated *S. aureus* [38]. Disruption of the ica operon in a bap-positive *S. aureus* strain showed no alteration in biofilm expression, indicating Bap gene compensatory mechanism for deficit PIA/PNAG product (a biofilm matrix polysaccharide) [39]. 17 different pulsotypes of dairy originated *S. aureus* have been reported with 24 virulence coding genes for leukocidins (lukED/lukM), pyrogenic toxin superantigen (PTSAg), haemolysins (hla-hlg), toxic-shock syndrome toxin (tst), enterotoxins (sea-seo, seu), exfoliative toxins (eta, etb), and genes for methicillin (mecA) and penicillin (blaZ) resistance. PTSAg-encoding genes and plasmid encoded sei, sed and blaZ genes were frequent in persistent intramammary ailments [40].

*Staphylococcus aureus* classification based on *mec*A gene is narrated as methicillin susceptible (MSSA) and methicillin resistant (MRSA) strains. Studies reported 84% MSSA prevalence with MRSA isolation rate up to 4%. Spa typing of the isolates showed frequent presence of t034 and t529 in MSSA while t121 was noted in MRSA strains. Both types of isolates were positive for endotoxin B, C, D, and E. MLST and PFGE typing of isolates revealed composite genotype profile of ST 5-PFGE USA100-unknown spa type which is of hospital origin and ST 8-PFGE USA300-spa type t121 genotype, commonly designated as community-associated MRSA clone [28]. Another study reported 77.8% MRSA from goat mastitis as strong biofilm producers. Spa typing revealed 44% t127, 33.3% t2049 and 22.2% t7947 type among total MRSA isolates [41].

## 5. Economic impacts due to *S. aureus* udder infection

Economic impacts of the mastitis are of great financial importance. Mastitis negatively impacts numerous aspects of cow and herd management. Mastitis can incur economic losses in both ways either directly and indirectly [9]. The direct costs include veterinary expenditure, labor costs, reduced production, poor quality milk and discarded milk. Whereas, the indirect losses are not obvious to the producers and are termed as "hidden costs" which include increased risk of other diseases, poor fertility rate, increased culling rate and sometime mortality. So, total cost can be much more than the direct losses [10–12].

A 15–20% of total cow population of the countries having major share in the milk production is affected by mastitis each year. Production losses per effected quarter are estimated 30% of productivity loss whereas, 15% production is lost during entire lactation/cow. The mastitis rate in the heifer can be up to 97% and *S. aureus* has major significance in imparting the huge economic losses. *Staphylococcus aureus* effects animals of various stage and parity e.g. nulliparous, primiparous, primigravid and multiparous [42, 43].

In Holland, the financial losses resulted due to clinical mastitis by Staphylococci were €293/Cow. Whereas, it was €277/cow in every clinical case of mastitis due to staphylococci in first three months after post calving and €168/cow onward for the rest of the lactation. In USA dairy, the annual losses incurred by the mastitis were estimated around $2billions, while $400 M in Canada and $130 M in Australia excluding the antibiotic residue in human diet, expense to preserve the nutritive quality of milk and to prevent milk degradation [12, 44, 45]. There is variation in cost of each component between the herds, partially due to the performance of herd and partially due to difference in preferences of the farmers when the mastitis is detected. Mastitis can impart economic losses to the farmers in following ways.

### 5.1 Low milk yield

The loss of yield depends on certain factors of great importance like severity of mastitis, nature of causative agent, stage of lactation at the time of mastitis. Losses are severe in primiparous cows due to clinical mastitis caused by *Staph. aureus*, *E. coli* with *Klebsiella*. However, the maximum loss of production in multiparous is caused by *Streptococcus* spp., *Staphylococcus aureus* and others pathogens [46, 47]. The loss of production is higher in multiparous cows than primiparous. Clinical mastitis occurring before peak production stage/yield causes more extensive loss as compared to rest of the lactation and loss of milk yield is persistent throughout the lactation [12, 48]. According to a study, this yield loss for multiparous could be 300-400 kg (4–6% of lactation) while 200-300 kg in primiparous

animals. The magnitude of yield loss in 30% cases of clinical mastitis reached up to 950-1050 kg per lactation. Whereas, in subclinical mastitis the losses incorporated are 80 kg/lactation (1.3%) and 120 kg/lactation (1.7%) in primiparous and multiparous respectively [49, 50].

### 5.2 Altered milk composition

The mastitis milk is low in fats and casein due to reduction in the synthetic capacity of secretary tissues of the udder parenchyma. The reduction in the fats up to 3-22 kg (1.5–7.5%) and casein protein contents up to 0 to 15 kg (0 to 8.5%) of the milk and higher SCC incurs the penalty to the producers in premium payment [51, 52].

### 5.3 Veterinary and medicinal cost

The veterinarian cost for treatment fee, travel and labor charges. On an average a handsome expenditure of $444 in clinical mastitis case are charged. This include (128$) directly in term of diagnostic (10$), medicinal expenditure ($36), discarded milk ($25), Veterinary charges ($41), extra labor ($4) while death losses ($32). The indirect costs are ($316) which include ($125) through future production losses and ($182) for culling and replacement and whereas reproductive losses are ($9). Therefore, to take an accurate decision to control the mastitis depends on understanding of economic impact of mastitis [43, 53]. Mastitis is among the main reason in cattle for the use of antibiotics and this exposure of animal to antibiotics is main reason behind the development of antibiotic resistant bacteria and antimicrobial residues in milk which are of a great public health concern [4, 7].

### 5.4 Discard of milk

Milk of the cow is discarded after mastitis diagnosis or while cow is being treated with antibiotics due to presence of antibiotic residues in milk during withdrawal period. The length of the withdrawal period depends on the drugs used and production system (*i.e*. conventional or organic). This discarded milk cost higher per unit than the milk not produced due to feed costs inclusions [53, 54].

### 5.5 Extra labor

Clinical mastitis requires extra labor for veterinary visits and medicine administration. Milking order is also changed in the clinical mastitis giving rise to less efficient milking and increasing the labors cost because hours of extra time required to manage the mastitis case [12, 53].

### 5.6 Subsequent disorders

The probability of subsequent clinical mastitis increases in cows once infected with mastitis. As the affected udder act as reservoir for the pathogen, the affected cows increase the spreading of mastitis in the herd. Cows having experienced one case of clinical mastitis often develop a subsequent case of clinical mastitis later in lactation. Mastitis is associated with increased risk of lameness, ketosis, displaced abomasum (LDA/RDA), and paresis and fertility problems. The economic cost of various disorders and fertility problems arise after mastitis and it is also included in the total cost of mastitis [55–57].

### 5.7 Culling of animal

The risk of culling and mortality rate is increased with clinical mastitis. Similar to milk loss the increased culling also augments the hidden cost. Involuntary culling is associated with replacement costs and is an important component of total mastitis cost. Economic cost also increases as cows recovered after mastitis do not reach their full production potential [9, 10, 53].

## 6. Review of emerging treatment options for *S. aureus* of dairy udder

### 6.1 NSAIDs, plant extracts, and nanoparticles as therapeutic agent

Aqib et al. [58] conducted a study to check the antibacterial of NSAIDs, plant extracts and nanoparticles against mecA positve *S. aureus*. Zinc oxide particles ZnO and Zn (OH) 2 were synthesized by the sorbothermal method and characterized by X-ray diffraction (XRD), calcination and scanning electron microscopy (SEM). Plant extracts were produced by the Soxhlet extraction method. The study showed that 34% (n = 200) of subclinical samples obtained from *Staphylococcus aureus* milk were significantly ($p < 0.05$) associated with suspicious risk factors and pathogens. Antibacterial studies have shown that *Staphylococcus aureus* is 55, 42, 41 and 41% resistant to oxacillin, siroxacin, streptomycin and enoxacin, respectively. Amoxicillin showed higher zone of inhibition increase at 100 mg of *Calotropis procera* extract (31.29%), followed by 1 mg/ml (28.91%) and 10 mg/ml (21.68%) eucalyptus. The combination of amoxicillin with diclofenac, aspirin, ibuprofen and meloxicam up to 500 μg/ml increases the ZOI by 42.85, 37.32, 29.05 and 22.78%, respectively. The Fractional Inhibitory Concentration Index (FICI) shows the synergistic effects of amoxicillin with diclofenac and aspirin, as well as with ibuprofen and meloxicam. Preliminary studies of the combination of micro-particles and amoxicillin in vitro have been found synergistic. In combination with zinc oxide and zinc hydroxide, the ZOI of amoxicillin increases by 26.74% and 14.85%, respectively. NSAIDs, herbal extracts and micro-particles immediately focused on the regulatory resistance of the pathogenic *Staphylococcus aureus* to explore alternative sources of antibacterial agents.

### 6.2 Lysostaphin as an anti-staphylococcal therapeutic agent

Lysostaphin is a potent staphylococcus-degrading enzyme containing a peptidase that can specifically cleave the polyglycine bridge specific to the cell wall of *Staphylococcus aureus*. Lysostaphin activity is measured by its ability to lyse *Staphylococcus aureus* cells. It is influenced by enzyme concentration, pH, temperature, ion and salt concentration. *Staphylococcus aureus* is enveloped in a thick layer of peptide glycans, and lysostaphin destroys the layer of peptide glycans, causing lysis and cell death. Peptide glycans impart strength and rigidity to the cell walls of gram-positive microorganisms, grow and divide, maintain cell shape, and prevent osmotic lysis of *Staphylococcus aureus*. Recombinant lysostaphin (rLYS) is a zinc metal enzyme that hydrolyzes the glycylglycine bond of a peptide glycan to a pentaglycine bridge on the cell wall of *Staphylococcus aureus*. A rodent model was used for the treatment of mastitis. The first study of rLYS in the sand showed that the rate of reduction of udder infection was over 87%, and the activity of dissolving stones in the body had a detrimental effect on the host. Instead, it reveals that it is a traditional antibacterial agent. Efficacy of rLYS in lactating dairy cows with experimentally induced *Staphylococcus aureus* infection. At least one intra-mammary

injection of 100 mg rLYS95 in 60 ml phosphate buffered saline (PBS) cures 95% of udder infection with *Staphylococcus aureus*. The antibacterial activity of rLYS *in vitro* persisted for 72 hours, but *in vivo* most of the infected mammary glands remained in the body for 72 hours after treatment [59].

### 6.3 Endolysin as therapeutic agent

*Staphylococcus aureus* is a serious threat to human and animal health, and there is an urgent need to develop new antibacterial agents to combat this pathogen. The aim of this study was to obtain active recombinant hemolysin from a novel bacteriophage (IME-SA1) and to conduct a clinical study of its effectiveness against bovine mastitis. We have isolated phages that are toxic and specific for *Staphylococcus aureus*. The optimal infection multiplier is 0.01. Electro-microscopic examination showed that IME-SA1 belongs to the Myoviridae family with the same head (98 nm) and a long tail (200 nm). Experimental lysis experiments showed a phage incubation time of 20 minutes and a burst size of 80. If the host bacterium is in the early stages of exponential growth, the multiplicity of infection is 0.01, resulting in complete lysis of the bacterium after 9 hours. We cloned the endricin gene (804 bp) into the pET-32a bacterial expression vector and succeeded in obtaining recombinant Trx-SA1 endricin with a molecule size of about 47 kDa. Preliminary results from a milk treatment study indicated that Trx-SA1 can effectively control mild clinical mastitis caused by *Staphylococcus aureus*. Endolicin Trx-SA1 may be another strategy for the treatment of infections (including MRSA) caused by *Staphylococcus aureus* [60].

### 6.4 Mesenchymal stem cells (MSCs)

Although many methods are effective against bovine mastitis, they do not address the problem of udder tissue regeneration and are associated with increased antibiotic resistance worldwide. Experimentally gold in terms of the safety and efficacy of staphylococcus, given the need for alternative therapies that have a large economic impact on the disease, and reports of mesenchymal stem cell (MSC) regeneration and antibacterial effects. We evaluated this intramammary therapy based on color-induced allogeneic MSCs. In a safety study, heifers received a 2.5 x $10^7$ AT-MSCs on day 1 and 10. The animals are clinically examined and blood samples were taken for testing. In efficacy studies, Holstein black-and-white cows were vaccinated with *Staphylococcus aureus*, carrier (NEG; days 4 and 10), antibiotics (ATB; days 4 and 5), or 2.5 x $10^7$ AT-. MSC (MSC; 4th and 5th day). Cows are clinically examined daily and somatic cell count (SCC) and colony forming units (CFU) are collected from milk samples. Blood samples are collected to measure serum haptoglobin and amyloid A. Two intra-mammary injections of AT-MSC into healthy dairy animals do not cause changes in clinical or hematological parameters, and pro-inflammatory cytokines. Compared to a quarter of the ATB or NEG infected cows, a quarter of the cows in the MSC group had a similar log/ ml SCC of milk. However, compared to a quarter of NEG cows, a quarter of MSC cows have a lower log CFU/ml. Re-inoculation with 2.5 x 10 allogeneic AT-MSC in the udder does not elicit a clinical or immune response in healthy cows. In addition, anti-inflammatory treatment with MSC reduced the number of bacteria in the milk of cows with clinical *Staphylococcus aureus* mastitis compared with untreated cows. This study provides preliminary evidence for the safety and efficacy of emulsions based on allogeneic intra-mammary MSCs for the treatment of bovine mastitis [61].

### 6.5 Bacteriophage as therapeutic agent

The lytic effects of bacterial deposition on *Staphylococcus aureus* isolates in milk have been investigated in vitro, and their possible applications in the treatment of udder infections caused by different bacteria have been discussed. The host range of the sequenced lytic phage was determined for 92 strains of *Staphylococcus aureus*. These isolates were taken from a quarter of the forehead samples in cases of clinical and subclinical mastitis. A point test followed by plaque analysis is used to determine the range of phage hosts. Three bacterial products STA1, ST29, EB1, ST11 and EB1, ST27 were selected according to host range, reproductive properties and storage properties to prepare a phage mixture (1: 1: 1) and tested for their lytic activity against *Staphylococcus aureus* in cold sterilized raw milk. It has been found that at least two-thirds of the phage can lyse almost two-thirds of the isolate. The phage mixture can reduce the density of *Staphylococcus aureus* bacteria in cold sterilized milk and retain their regenerative capacity in raw milk. Compared to pasteurized milk, the regenerative capacity is only moderately reduced. The significant decreasing capacity of the mixture of phages in raw milk facilitated further in vivo studies [62].

### 6.6 *Taraxocum mongolicum* as therapeutic agent

*Taraxocum mongolicum* is widely used as a traditional Chinese medicine for the treatment of various inflammations and infectious diseases, as well as clinically in the treatment of mastitis. The aim of this study was to investigate the protective effect of *T. mongolicum* against *S. aureus* mastitis and its underlying mechanism. Female ICR mice were given 2.5, 5 and 10 g/kg *T. mongolicum* extract twice daily for 6 consecutive days and infected with *Staphylococcus aureus* via the teat canal to induce mastitis. Anti-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) levels were measured by ELISA. The activity and distribution of myeloperoxidase (MPO) was measured using a kit and immunohistochemistry. Observe histo-pathological changes in udder tissue with H&E staining. Western blotting was used to demonstrate the expression of talk like receptor 2 (TLR2), phosphorylation of related nuclear factor-κB (NF-κB) proteins, and mitogen-activated protein kinase (MAPK) signaling pathway. *T. mongolicum* reduces TNF- and agr, IL-6- and IL-1. Serum and udder levels of mastitis infected with *Staphylococcus aureus* reduce the activity and spread of MPO. In addition, *T. mongolicum* is effective in reducing histo-pathological damage and cell necrosis in udder tissue infected with *Staphylococcus aureus*. In addition, *T. mongolicum* suppress TLR2 expression and phosphorylation of κBα (IκBα), p65, p38, extracellular signal kinase 1/2 (ERK1/2), and N-terminal c-Jun kinase (JNK). This study showed that *T. mongolicum* prevents mastitis caused by *Staphylococcus aureus* infection by exerting an anti-inflammatory effect by inhibiting the TLR2-NF-κB/MAPK signaling pathway [63].

## 7. Role of probiotics and prebiotics in prevention of *S. aureus* infection

As mastitis is the most dangerous and costly disease of dairy industry. The use of antibiotics leads to the development of drug resistance due to which it becomes untreatable disease. Also, the presence of antibiotic residues in milk and dairy products render it unused able for consumer. So, there is another approach for prevention and treatment of mastitis [64]. Many successful experiments have been performed in past by using bacteriocin-based products. Nisin has been used as a

commercial product for the disinfection of teats. And lacticin is used in the dry cow therapy for sealing teat canal at the time of drying off of cow [54, 65].

### 7.1 Probiotics

Recently, there is need to use other sources in order to reduce antibiotic administration because antibiotic administration is a major cause of lethal infections in dairy industry. Probiotics are live microorganisms which when administered in sufficient amount provides cure from diseases. The probiotics which prevent diseases are called as probiotic drugs. So, mammary probiotics are recently being used as alternatives to antibiotic for the treatment of mastitis. One of the most useful probiotics are LABs (lactic acid bacteria) which interferes with bacteria associated with mastitis, or interact with mammary epithelial cells. Many experiments were performed and claims the therapeutic and preventive effectiveness of probiotics [66]. Results evaluated by using lactic acid bacteria showed that LABs are pro-inflammatory for the mammary glands and it causes an influx of neutrophils into the milk and at drying off of animals. So, it provides protection against mastitis causing *S. aureus* and their ability to provide cure from mastitis remains to be established [67]. Probiotics interferes with the teat microbiota and prevents adherence and colonization of harmful bacteria with the teat canal. However, oral probiotics provides no cure, but intra mammary preparations can be used with caution to prevent mastitis [66].

Some strains of *Lactobacillus casei* and *weisella* produces some compounds which are active against persistence of *S. aureus* bacteria with the epithelial wall of udder tissues, and thus resisting *S. aureus* bacterial pathogenicity by producing hydrogen peroxide, competing nutritional components, changing of host immune system and its utilization. Prolong use of these probiotics and their metabolites seems to be effective alternatives for the control and prevention of mastitis [66, 68].

There are many mechanisms which explain the mode of action of probiotics.

1. The change of the composition of local bacteria and production of bacteriocins and metabolites helps in the efflux of pathogenic bacteria by competing for nutrients.

2. By increases the barriers of epithelium, either by improvement of epithelial junctions or new formation of epithelial cells and introduction of antimicrobial peptides.

3. Enhancement of general immune response against bacteria. By interacting with many cells like monocytes, macrophages, and dendritic cells and train them for innate immunity.

4. Quick actions of systemic responses, like endocrine modulations or central nervous system via signaling mediators.

Different experiments are going on to unmask the details of action mechanism of probiotics in mastitis alongside boosting up the welfare and production aspects of the animals [67, 69].

### 7.2 Prebiotics

Antibacterial properties of prebiotics were studied *invitro*. To investigate further efficacy against bacteria studies were conducted in lab animals and their success for treatment and to prevent against bacteria was determined by evaluating liver

enzymes (aspartate transaminase and alanine transaminase), bacterial colony count of liver and lungs, and also histological changes. In some studies, raisin was used as prebiotic. But it was less effective than others. So, prebiotics are less effective against *S. aureus* than other things [70]. Synbiotics are also tested against mastitis causing bacteria. Especially, these are more effective against *E. coli* and *listeri*a but less effective against *S. aureus* which is highly resistive to the synergistic effect [66, 70].

## 8. Vaccination against *S. aureus* udder infection

Mastitis is an important disease of the dairy industry that affects production and has economic losses, losses are due to high medicine cost and unusable milk which goes wasted as a result lowers producers' profit. For control of this disease it is necessary to follow some recommendations like teat sanitization, use of cloth to clean udder before and after milking, antibiotic treatment of clinically ill cases, dry cow therapy and proper management and nutrition of dairy animals [71]. In addition to these, vaccination against many pathogens is recommended for prevention and elimination of disease. One of the most important pathogenic entity in mastitis etiologies is *S. aureus*. In order to improve the general health, welfare, production as well as reproductive efficiency of dairy animals, many therapeutic as well as preventive approaches has been in use with less satisfactory results [72].

*Staphylococcus aureus* mastitis is found in many herds of dairy cows. Due to the predominant infectivity of this organism, many herds have been able to maintain a low prevalence of IMI caused by this organism. This varies greatly between herds and geographic regions, but it has been shown that calves can be infected with this pathogen during calving [73]. This pathogen usually causes only a small fraction of cases of clinical mastitis, and subclinical infections usually become chronic and refractory to treatment. The ability of this pathogen to establish a long lasting IMI varies from strain to strain. However, many toxic factors increase the viability of microorganisms in the host tissue. For longer periods of infection, fibrin deposits and abscess formation further reduce the effectiveness of the immune response. The ability of phagocytic cells to survive intracellularly affects humoral immunity and drug therapy. In addition, for example, infection with *Staphylococcus aureus*. It rarely elicits a significant innate immune response compared to *E. coli*. This avoids an acute immune response that could compromise the presence of infected tissue [74]. An effective vaccine against this pathogen must overcome some major hurdles. [1] Conservative and universal antigens are required for large variation in strains. [2] Toxic factors of "immunity", especially cell survival and the ability to not be exposed to antibodies. [3] Difficulty in assessing the effect of vaccines on reducing infections and the deleterious clinical effects of actual IMI status. The last point reflects the nature of *Staphylococcus aureus* IMI. It can be regularly excreted by bacteria in milk from infected glands. Due to L-type transformation (no cell wall mutations), *Staphylococcus aureus* can relapse in milk up to 80% of the quarter within 28 days after treatment with careful continuous sampling. IMI One or two samples are less sensitive and can correctly identify negative quarters [75].

Its need of time to control *S. aureus* for profitable business of dairy industry and for comfort of consumers with good quality milk and dairy products. In the past years, much progress was made by the researchers but in spite the use of different strategies to control mastitis, *S. aureus* is still a problem. In the dairy industry, anti-staphylococcus vaccines give different results depending upon types of immunization, route of administration, adjuvant used and involvement of some other factors [76]. Considerable effort, encompassing numerous antigens, virulence factors, and bacterial strains, has been made to develop an efficacious and practical *S. aureus* vaccine.

### 8.1 Vaccines available in market

Many types of vaccines are in use like commercial and herd specific (autogenous) vaccines. The purpose of vaccines is to protect new infections and to stimulate cows' immune system which may provide protection against clinical mastitis [71, 77]. Vaccination may result in the increased rate of antibodies in blood circulation against *S. aureus* pathogens which decreased bacterial growth rate after entering into the udder. The resulting increased immunity may decrease pathogen damage to milk producing tissues, decreased inflammation, and enhance tissue repair. Commercial preparations of vaccine against mastitis caused by *S. aureus* are available in the market [54]. Currently there are only 2 commercially available vaccines are in use for bovine mastitis control. Lysigin® is available in the United States and Starvac® (hipra) is available in Europe and Canada. Many others are in trials and local practices with no wide range application [6].

#### *8.1.1 Lysigin®*

Bacteria containing lysed polyvalent phage-type cultures (including several types of capsular sera) are commercially available in the United States (Lysigin; Boehringer Ingelheim, Ridgefield, CT, USA). This product was derived from a Louisiana study conducted twice every 6 months at 2-week intervals for coagulase-negative staphylococcus (CNS) and *Staphylococcus aureus* calves with decreased IMI. However, the problem that arose was that infection studies showed that this bacteriocin did not prevent IMI, did not increase IMI clearance, and did not affect SCC or post-exposure lactation. The clinical score in heifers treated with Lysigin improved and the clinical course of mastitis was short. Immunization with Lysigin increased the level of bovine serum against *Staphylococcus aureus* IgG1, but did not affect the concentration of IgG1, IgG2 or IgM in milk [75].

Lysigin® is a multivalent vaccine which is prepared by using the mastitis causing strains of *S. aureus*, disintegrated into smaller particles. Early studies showed that this vaccine reduces the risk of new infections, lowers somatic cell count and thus lowers clinical mastitis effects. It is evident from experimental studies that by following a proper vaccination schedule early in life of heifer staphylococcal mastitis can be avoided. Moreover, in some recent studies animals in which this vaccine was administered showed clinical symptoms of mastitis. But these animals recovered earlier than non-vaccinated animals; also, there was no difference in somatic cell count and *anti-S. aureus* antibodies. So, this vaccine failed to provide sufficient antibodies in milk to help leukocytes in the elimination of *S. aureus* bacteria from mammary glands. But Lysigin® vaccinated animals have a higher cure rate as compared to other animals [6, 78].

#### *8.1.2 Starvac®*

It's a multivalent vaccine which is mixture of inactivated *E. coli* and inactivated *S. aureus* which shows SAAC (slime associated antigenic complex). This preparation helps reduce clinical mastitis cases to some extent that are caused by *S. aureus* and *E. coli* bacteria. This vaccine increases antibodies but does not provide complete protection from mastitis, but decreases intramammary infections and also decreases rate of transfer of infections [79, 80].

### *8.1.3 Other formulations/approaches*

After experimental infection, it was found that different formulations of bacterial drugs that kill whole cells can reduce the number of infections and a quarter of SCC, but this effect was only reported 13 days after infection. Subsequent field reports of two doses at the same time, lower doses in cattle and additional doses during the subsequent lactation period may produce higher antibodies against *Staphylococcus aureus*. Researchers also report that vaccinated animals consume an average of 0.5 kg of milk per day and have lower SCC levels. In this study, Freund's incomplete adjuvant was used as part of the first biphasic administration. This adjuvant is known to cause significant injection site reactions and is not commonly used in commercial products. More interesting developments from the same research group have identified targets for RNAIII-activated protein (TRAP), a highly conserved membrane protein in many *Staphylococcus* species, including *Staphylococcus aureus*. This antigen can become a specific and universal vaccine against *staphylococcus* [81]. *Staphylococcus aureus* produces adhesin, a pathogenic factor that promotes attachment of host tissues and subsequent attachment between bacterial cells, creating a biofilm that can resist feeding. Surface polysaccharides are an important component of staphylococcal biofilm, and strains expressing high levels of extracellular polysaccharides (surface-associated antigenic complex [SAAC]) have been isolated [82]. A commercial formulation combining SAAC *Staphylococcus aureus* and *E. coli* has been approved by the European Union. Clinical reports have shown that this product can improve udder health by reducing re-infection and SCC in vaccinated animals. The use of vaccines against *Staphylococcus aureus* may be restricted in many dairy farms, especially herds with low IMI prevalence, such as herds with SCC <200,000 cells/ml. Thus, *Staphylococcus aureus* bacteriosin cannot significantly influence the successful control of infectious mastitis through the use of correct milking techniques and milking machine maintenance. Conversely, people who have been vaccinated with the right treatment can experience disappointing results. As previously mentioned, this varies greatly between herds and geographic areas. If the herd has *Staphylococcus aureus*, bacteriosin can also reduce the shedding of bacteria in the milk of infected animals. Researchers have administered *Staphylococcus aureus* bacteriosin to improve antibacterial treatment, but the results are mixed [83].

## 8.2 Autogenous vaccine against *S. aureus* mastitis

These vaccines are the preparations having specific strains of bacteria obtained from mastitis suffered by animals and used to immunize the herd for protection against further new udder infections with the same strain of bacteria. There are evidences which shows that the use of autogenous *S. aureus* vaccines enhances antibody titer in vaccinated animals as compared to non-vaccinated herd and reduce the risk of both clinical and sub-clinical mastitis [71]. Some studies also show that autogenous vaccines provide almost 70% protection from infection and provide protection from clinically ill mastitis cases challenged with *S. aureus* [80].

Early studies suggest that vaccines for *S. aureus* will increase cure rate and lower SCC but in actuality, it does not work against adult cows. Experimental success was also seen with commercial *S. aureus* vaccine Lysigin® in the young dairy animals. When serum samples from vaccinated animals were checked they showed higher antibody titer as compared to non-vaccinated animals to combat against *S. aureus* infections [71]. So experimental and commercial preparations of *S. aureus* vaccine

provide protection against mastitis. Efficacy of these preparations against *S. aureus* ranges between 44%–66% and this strategy for prevention of *S. aureus* in the future, some new antigens and adjuvants are added to the vaccine preparations to enhance their effectiveness [72, 84].

### 8.3 Vaccine response in vaccinated dairy animals

*Staphylococcus aureus* is a predominant organism causing mastitis in different species. So, different experiments were conducted in different animal species to determine vaccine efficacy. The production and implementation of *S. aureus* vaccine in milk animals has a great impact towards public health. Inactivated vaccine was prepared and checked by using different adjuvants against *S. aureus* [85].

She camel having sub-clinical mastitis, vaccinal isolates were taken from her having alpha and beta hemolysin toxin, also some were multidrug resistant. Inactivated alum precipitated *S. aureus* vaccine (APSV) and oil adjuvant *S. aureus* vaccine (OASV) were prepared after confirming its antigenicity in rabbits. Experiments showed that APSV and OASV were safe, effective and expressed immunogenic responses in experimental rabbits [86, 87].

*S. aureus* is a major cause of mastitis in dairy cows causing mild to severe and chronic infections having drastic effects on cow's wellbeing, lifespan and milk production. Irrespective of years of research on mastitis issues still there is no production of an effective vaccine against *S. aureus mastitis.* Experimental studies showed that it's possible to vaccinate *S. aureus* naïve cattle and also this experimental immunization leads to humoral immune response which is different from response that occurs after natural exposure [79, 88].

Experiments are still in progress for the use of vaccine against *S. aureus* mastitis in small ruminants. Still, there is gap in using mastitis vaccine for prevention of *S. aureus* mastitis which is a major issue in dairy industry and causes huge economic losses every year. Perhaps there might be bright future for farmers because trails of mastitis vaccine in lab animals are showing satisfactory results, which is a hope [43, 50].

### 8.4 Vaccine success rate

Mastitis is one of the most dangerous disease of dairy industry. Vaccination and other managemental practices are the tools to prevent mastitis caused by contagious as well as environmental pathogens. The success rate of immunization depends upon type of vaccine, adjuvant used and route of administration of vaccination regardless of type of vaccine, only vaccine is not enough in the large herds with high mastitis cases. For achieving success, it is necessary to use up to date manage mental practices along with vaccine and culling of chronically ill cases to reduce intra mammary infections [50, 89].

Experiments conducted from last 15 decades show that experimental *S. aureus* vaccines as well as commercial vaccines reduce new infections in dairy heifers. *S. aureus* vaccine was prepared by focusing on two major components of *S. aureus* (pseudo-capsules and alpha toxins). 2 and 4 weeks before calving heifers were given injections in the supra-mammary lymph node of mammary glands. Injections were given subcutaneously. After caving these heifers were challenged with *S. aureus* infections. These heifers showed 46% reduction in *S. aureus* infections as compared with control group of animals. There was almost 70% success rate from infection in vaccinated animals and less than 10% in non-vaccinated animals. Clinical signs of mastitis were also mild in vaccinated herds compared to control group of animals [6, 72].

## 9. Conclusion

*Staphylococcus aureus* is a major mastitis causing pathogen which is contagious in nature and persist in the mammary epithelial cell for long period of time and cause further infections. About 74% quarter prevalence of *S. aureus* in bovine udder with overall prevalence exceeding 61% in dairy animals. Cure rate in *S. aureus* mastitis is merely 25–50% during the lactation. A wide array of genotypic variations has been observed with great genetic diversity in the isolates of bovine as well as caprine origin. 17 different pulsotypes of dairy originated *S. aureus* have been reported with 24 virulence coding genes for leukocidins (lukED/lukM), pyrogenic toxin superantigen (PTS Ag), haemolysins (hla-hlg), toxic-shock syndrome toxin (tst), enterotoxins (sea-seo, seu), exfoliative toxins (eta, etb), and genes for methicillin (mecA) and penicillin (blaZ) resistance. The magnitude of yield loss in 30% cases of clinical mastitis reached up to 950-1050 kg per lactation. Attainment of refuge inside the macrophages and neutrophils is a major cause of *S. aureus* mastitis persistence. The antimicrobials cannot penetrate these structures to reach the mastitis causing organisms. This limits the use of antimicrobials to secondary importance in relation to immediate need of supportive treatment. Mammary probiotics are recently being used as alternatives to antibiotic for the treatment of mastitis. One of the most useful probiotics are lactic acid bacteria which interferes with bacteria associated with mastitis, or interact with mammary epithelial cells. Antibacterial properties of prebiotics are also studied *invitro*. Literature showed anti- staphylococcus vaccines with different results depending upon types of immunization, route of administration, adjuvant used and involvement of some other factors. Many types of vaccines are in use like commercial and herd specific vaccines. Commercial vaccines against mastitis caused by *S. aureus* are available in the local markets with variable efficacy around the globe. Studies conducted from last 15 decades show that experimental herd specific *S. aureus* vaccines as well as commercial vaccines reduce new infections in dairy heifers. Experiments are still in progress for the use of vaccine against *S. aureus* mastitis with optimal efficacy and reliability. Still, there is knowledge gap in using vaccines for prevention of *S. aureus* mastitis, needed to be research in focus. Perhaps, there might be bright future for farmers because of highly satisfactory trail results of mastitis vaccines in the lab animals.

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