A Treatise on Bovine Mastitis: Disease and Disease Economics, Etiological Basis, Risk Factors, Impact on Human Health, Therapeutic Management, Prevention and Control Strategy

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Abstract

Mastitis is a disease complex that assumes highest clinical and economic significance in milch animals particularly medium to high yielding dairy cattle, usually in and around periparturient period. The disease is the infection of milk synthesizing alveolar tissue and is clinically manifested by gross abnormality of milk associated with pathological changes of udder in the form of pain, udder edema, fibrosis, and indurations, the extent depending on the severity of infection. Sub-clinical mastitis remains to be an obscure and latent form of this disease that poses more serious economic concern to the dairy livestock sector, as the incidence is much higher in a dairy herd than the clinical one. Sub-clinical mastitis accounts 40 times more than the clinical mastitis in dairy herds. Even though great technological advances have been made, mastitis continues to be a major economic issue for dairy producers, necessitating researchers and dairy advisors continue to refine the recommended mastitis control programme.

Keywords: Bovine mastitis; Economic impact; Mastitogens; Risk factors; Prevention and control

Etiological Basis of Bovine Mastitis

The etiology of the disease is in a continuous changing spree, with the evidence of new microbial species being incriminated. Round about 150 bacterial Spp. are isolated from bovine mastitic udders. Based on the pathogen involved, the disease is broadly classified into four types viz Bacterial mastitis; Mycotic/Fungal/algal mastitis; Mycoplasmal and Nocardial mastitis. The viruses as mastitogenic agents are of least clinical significance as most of the viral infections of the udder arise from general viraemias, characteristic of typical viral diseases primarily affecting the body elsewhere. The main viral affections of the bovine udder are ulcerative bovine mammilitis (mammary pustular dermatitis) due to bovine herpes virus, pseudo cowpox (milker's nodule) and cow pox viruses, which are truly the infection of epidermis/dermis of udder rather than its secretory alveoli. Therefore these lack the clinical significance unless otherwise superimposed by secondary invasion by bacteria, affecting the intramammary tissue.
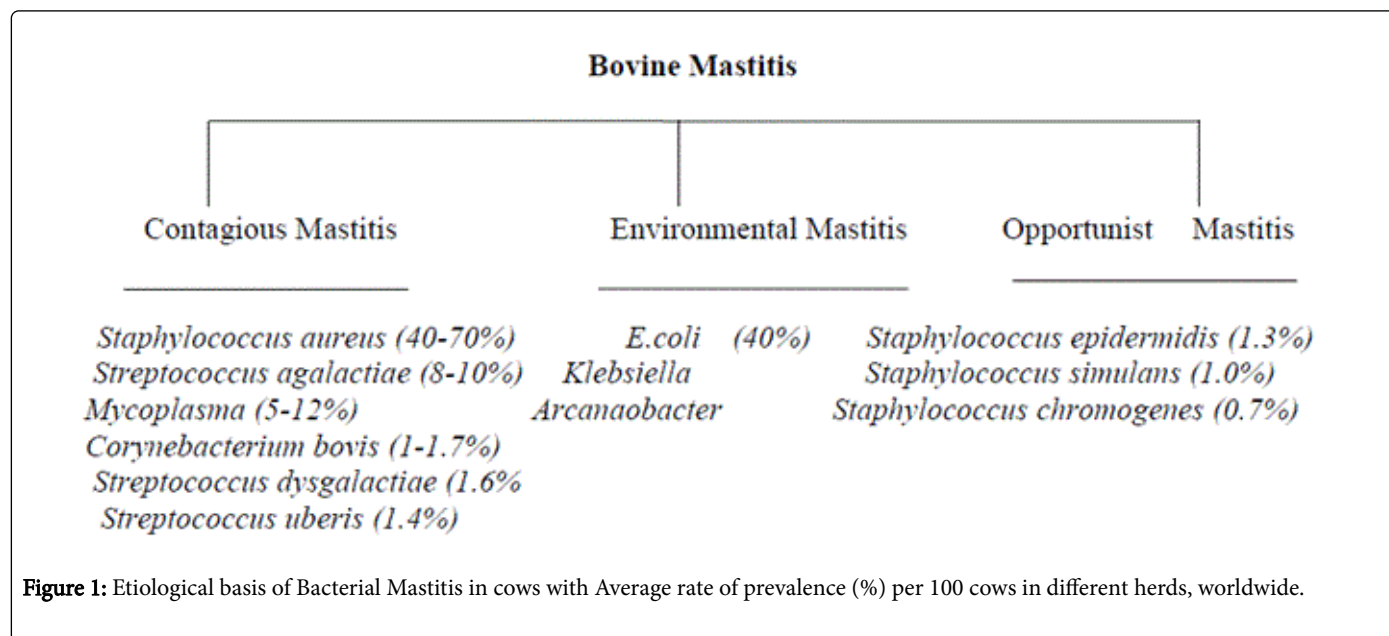
Bacterial Mastitis

Pathologically and thus economically the most significant is bacterial mastitis. Pathogenic bacteria of bovine udder originate either as environmental pathogens or contagious microbes. The so called minor pathogens of mastitis include species of *Micrococcus, Bacillus, Pneumococcus* organisms and certain fungi. The minor pathogens of bovine mastitis are mostly recorded in cases of mastitis with mixed infection, though independently capable of inducing pathology to glandular tissue [1-3]. Their incrimination in mastitis increases the chances of treatment failure due to multidrug response. Besides, *Pneumococcus, Listeria* and *Lactobacilli* are occasionally reported from cases of bovine mastitis and their origin is traced to human and other animal sources. Another group of mastitogens is the opportunist pathogens involving all coagulase negative Staphylococci, lining the teat and udder skin. Broadly two types of bacterial mastitides are reported worldwide; Gram-positive and Gram-negative, of which the major/chief agents of mastitis are the Gram-positive bacteria including *Streptococcus agalactiae, Staphylococcus aureus* and *Mycoplasma bovis*. These agents are responsible for contagious mastitis in cattle including *Corynebacterium bovis*. The strain of *Staph.aureus* is transmitted in most cases as nosocomial infection (hospital acquired/ human transmitted) [4]. The opportunist mastitis is mainly due to infection with *Staph. epidermidis*, upon gaining entry into the teat canal.

Methicillin resistant *Staphylococcus aureus* (MRSA) incriminated in bovine mastitis has been evidenced [5], resulting in multidrug resistant mastitis. *Staphylococcal mastitis* accounts for single largest cause of economic losses in terms of depressed milk production, cost of treatment and culling of high genetic vigor animals, particularly in rural South-Asia, where it remains to be the predominant mastitis agent because of hand milking practice, while in Europe, use of penicillin derivatives has allowed Streptococcus Spp. to supersede this pathogen [6]. Some variants of *S. aureus* are associated with toxic shock syndrome (TSST-1), resulting in severe and per acute form of toxic mastitis in affected herds. Second highest prevalence is attributed to esculin positive and negative *Streptococcus Spp.* [7] While *Streptococcus agalactiae* and *S. dysgalactiae* are well known mastitides in the Asian sub-continent; *Streptococcus uberis* caused havoc in Scandinavian cattle herds and was considered a single cause of serious economic loss in dairy industry [8]. Because of adoption of stringent control programme, this pathogen was overcome but only paving a way for Coliform mastitis due to drug resistance menace and low somatic cell count on account of poor polymorph recruitment [9]. Low somatic cell count herds produce low levels of the neutrophil lysosomal

enzyme, aceloxyacyl hydrolase (AOAH) that increases the susceptibility of cows to Coliform mastitis in early lactation, subsequently rendering low rate of detoxification of endotoxin through deacylation of neutrophils pathogen kill via respiratory burst and phagocytosis [10] (Figure 1).

Figure 1: Etiological basis of Bacterial Mastitis in cows with Average rate of prevalence (%) per 100 cows in different herds, worldwide.

The chief agents of gram negative mastitis (environmental mastitis) include *E.coli*, Proteus and Klebsiella species. The Coliform mastitis or the endotoxic mastitis is increasingly emerging in today's intensive organized dairy systems due to introduction of stringent mastitis control programmes that render the first line udder defense; the cell mediated immunity, dormant. Closed winter housing is a significant risk factor for gram negative mastitis in cows [11]. The coliforms are often incriminated as mixed pathogens along with gram positive bacteria in mastitis. This adds to the complications in the treatment of bovine mastitis, owing to the fact that drugs effective against gram positive bacteria are not usually effective against the gram negatives and vice versa. It is therefore imperative that cases showing involvement of both types of bacteria need to be treated with more than one antibiotic simultaneously, or a mega spectrum single antibiotic having in-vivo efficacy against both the types of pathogens. The most notorious agent among gram negatives is *Pseudomonas aeruginosa* which is often found resistant to several antibiotic generations [12] and is of nosocomial importance on dairy farms and veterinary hospitals. It occurs usually in mixed infection of bovine udder; there hence the antibiograms of such milk samples are often misleading. Thus *Pseudomonas Spp*. when isolated from mixed infections needs to be separately tested for drug sensitivity.

Mycotic Mastitis

Fungal infection of bovine mammary tissue is attributable to super infection by certain fungal species as consequence to strict mastitis control programmes that render natural udder immunity quiescent. Indiscriminate antibiotic therapy might also be a contributory factor for fungal super infection of udders. Moreover factors like micronutrient inadequacy particularly that of vitamin A and zinc in cattle are precipitating. Contamination of teat dips, intramammary infusions and moldy surroundings play significant role. The important mycotic mastitogens are *Aspergillus fumigatus* and *Candida albicans*. Some other fungi such as *Cryptococcus neoformans*, Trichospron,

Toluropsis and Saccharomyces species are reviewed [13]. Fungal invasion of bovine mammary tissue often occurs as mixed infection; however fungi can be isolated as independent mastitogens as well from udder infections. Algal agent like *Prototheca zopfii* is also incriminated in bovine mastitis, as a result of algal contamination of feed and fodder, drinking water, and cattle premises by house hold sewage, discarded food items including bread, rotten vegetables, and fruits and thrown out junk food items. The disease is more prevalent in the regions where cattle are often grazed in the vicinity of public parks, lakes and tourist places [14].

Mycoplasmal and Nocardial Mastitis

Among several species *M. bovis* and to some extent *M. bovirhinis* and possibly to a lesser extent *M. canadense* are causal agents of contagious bovine mastitis (CBM). At least 17 mycoplasmae are incriminated in bovine mastitis. Being the main culprit *Mycoplasma bovis* is isolated from majority of mycoplasma mastitis cases in India [15]. The infection is often a herd problem and may assume epidemic proportions when introduced in a herd. Sub-clinical infections can prevail in a premmune herd for some time and after the immunity is lowered the disease may assume clinical form. This type of mastitis finds its origin from increasing involvement of mycoplasmae in respiratory infections, arthritis, vulvovaginitis, keratoconjunctivitis and metritis of cattle [16]. The disease is prevalent all over the world and the vaccine is not found effective. Many antimycoplasmal drugs are not licensed to be used in lactating cows. The disease is predisposed by pre-existing IBR in dairy herds [17].

Nocardial mastitis is saprophytic bacteria in origin; the causal agents, *Nocardia asteroids*, *N. braziliensis* and *N. farcinicus* are involved in several chronic and granulomatous forms of mastitis. Bovine mastitis due to nocardia occurs as a result of poor environmental hygienic conditions, soil contamination of udders, teat dips and intramammary infusions. Nocardial mastitis is usually refractory to conventional antibiotics, except Amikacin and Ceftiofur

[18]. Indiscriminate antibiotic use is suggestive of causing super infection with Nocardia.

Tuberculous Mastitis

Bovine mastitis associated with mycobacterium infection is usually the reflection of tuberculosis of other organs. However disease of the milk gland can occur independently, posing potential zoonotic threat. Tuberculous mastitis is caused by *M. bovis* and *M. tuberculosis*. While the former is purely of animal origin, the latter may be acquired from tuberculous humans. Agents such as *M. lacticola* is also isolated from the cases of bovine mastitis previously treated with intramammary therapeutic agents in oil. Mycobacterial mastitis has highest incidence in the South-Asian subcontinent. The prevalence is highest in Bangladesh (30%), followed by India (28.5%) and Pakistan (27%) [19-21]. Tuberculous mastitis is a chronic form with grannulomatous/ caseous degeneration of the milk gland. The disease has a very long duration and is refractory to conventional antimicrobial therapy.

Disease Economics

Mastitis is perhaps the costliest disease of dairy cattle accounting for economic losses on account of depressed milk production, culling of high vigor breeds and losses on account of veterinary medical expenses. Globally, the losses due to mastitis amount to about $53 3billion [22], Rs. 6053.21 crores per annum in India [23] and much higher, ascribed to lack of mastitis prevention practices in Pakistan [24]. Economic losses owing to mastitis in Bangladesh are Takka 122.6 (US $ 2.11) million per year [25]. Two billion dollar losses occur annually in United States, due to mastitis [26]. The disease impact is very serious as it affects 50% dairy herd population in India [27], 47.5% herd population in Pakistan [28] and 51.3% cows in Bangladesh [29]. Mastitis affects 54.3% dairy cows in Zhejiang province of China [30] and 52.5% dairy cows due to *Staphylococcus aureus* infection in Assuite Egypt [31]. Mastitis, costs about estimated 301 US dollars per cow per year, above the desired baseline in European dairy herds. The estimated loss of milk per cow per one lactation cycle is 70% of the total losses and the cost of cows lost due to premature culling is 14%, while the cost of milk downgraded/ discarded due to mastitis is 7% and the cost incurred on medical treatment and other veterinary expenses amount to 8%, of the total losses, reported worldwide [32]. Mastitis low prevalence herds can save up to 25% cost on losses than the high prevalence herds (Figure 2).

Figure 2: Effect of Mastitis on Various Economic parameters.
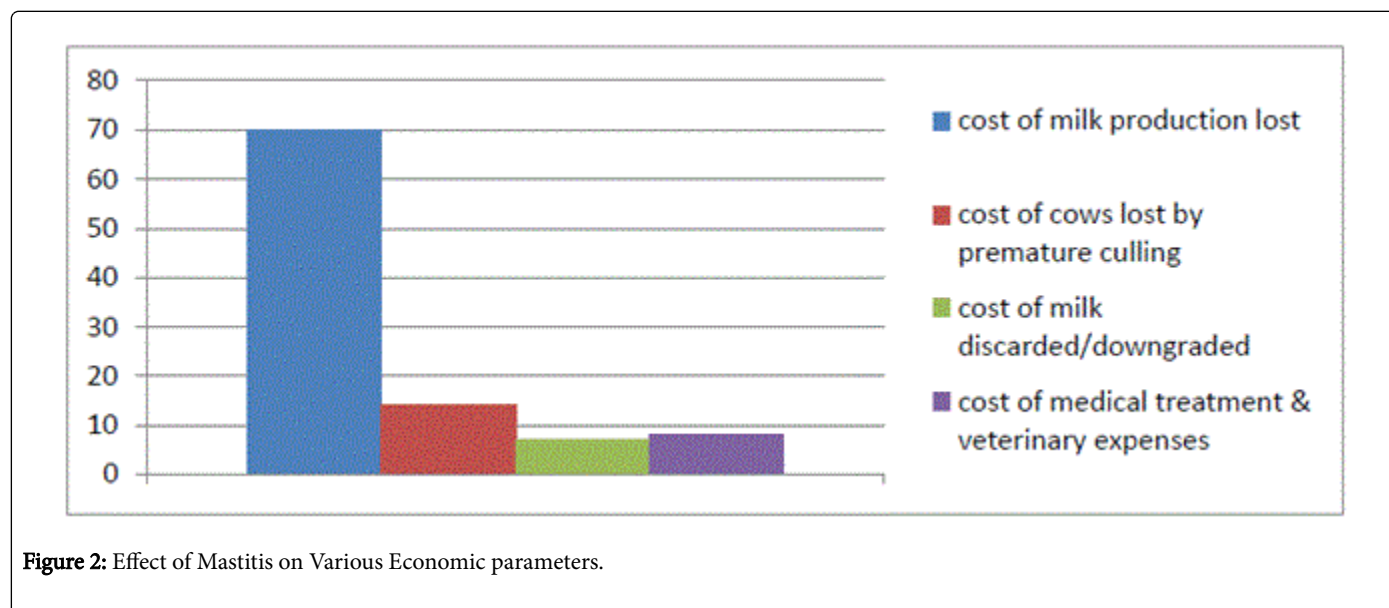
Effect of Mastitis on Various Economic Parameters

The economic losses are more associated with sub-clinical mastitis which is 40% more prevalent than clinical mastitis [33]. However the cost of treatment of sub-clinical mastitis is much low compared to that of Clinical mastitis accounting for 10-20 times higher (Figure 3).
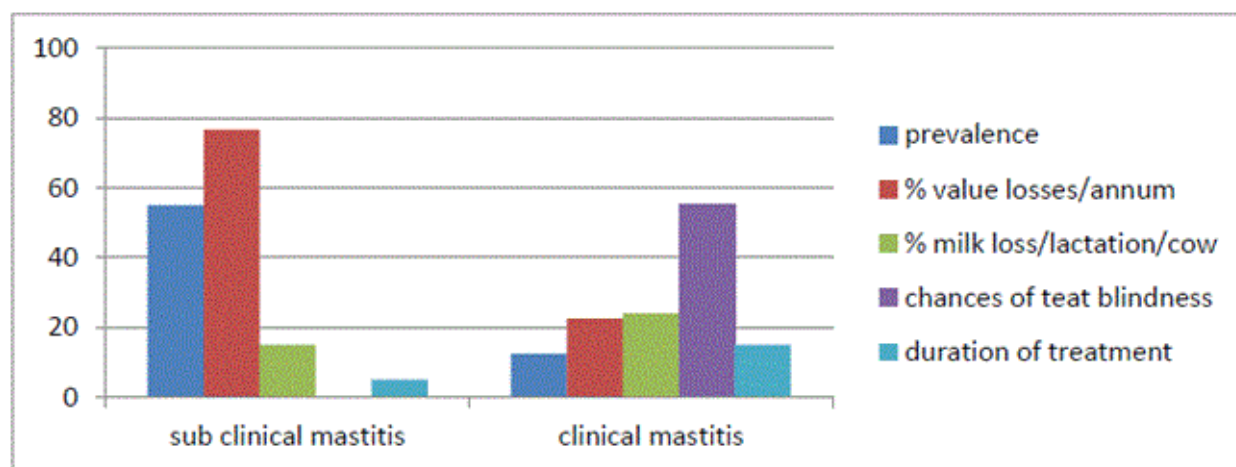
Figure 3: Comparison of Economic Fallout of Two Mastitis Forms.

Comparison of Economic Fallout of Two Mastitis Forms

Risk Factors

Bovine mastitis is predisposed by several epidemiological risk factors that play significant role in causing mammary incompetence to protect it from the invasion of infectious agents. These should receive due consideration in the course of developing an integrated mastitis control programme. The risk factors include the host factors, environmental factors and the pathogen factors.

Host Factors

The somatic cell count: (SCC) should be optimum viz. more than 2×10^5/ml of milk but not 5×10^5 /ml of milk which indicates sub-clinical infection. Depressed SCC may predispose udders easily to infection particularly by coliforms. Initial low somatic cell counts indicate dormant udder immunity. However in a pathogen free milk herd, age of the cow, production level and stage of lactation, significantly influence SCC. Variation in SCC within the herd can be attributed to nutritional imbalance, debility, concurrent disease, convalescence, early lactation and drying off, parasiticism and lack of minimum exposure to intramammary infection. Several cows showing pathogen free milk samples might have abnormally high SCC [34] due to factors other than infection.

Breed hierarchy: Of high yielders, in a herd increases the chances of genetic vulnerability to mastitis. The pure breeds and cross bred heavy milkers appear to be genetically more vulnerable than the local medium yielders. Multiparous cows having calved more than twice are more vulnerable to intramammary infection due to immunoincompetence.The incidence of mastitis in crossbred Murrah is considerably high as compared to indigenous breeds of buffaloes and cattle in India [35]. Mastitis rate was lower in Jersey than in Holstein cattle [36]. The involvement of genetic factors causing susceptibility to mastitis has also been established as incidence rates showing higher among relatives of affected individuals than the general populations. The risk of mastitis increases with the increase in parity, attributable to mammary immunocompromization [37]. The best and worst sire families have been shown to differ by 10% in mastitis rate per lactation when analyzed for predicted transmitting abilities [38]. Other host related factors like age, parity, production level and stage of lactation have significant influence on the occurrence of infection of the milk gland. The, High milk yield and peak stage of lactation along with deep and drooping morphology of udders are associated with more incidence of mastitis in cows and buffaloes [39].

Stress: seems to be one of the potential risk factors causing predisposition of dairy herds to mastitis. Whether nutritional, parturition or lactation stress, each has its own aftermath on the udder health in the form of declined local and systemic resistance to infections. Stress often causes depression of udder defense mechanism, paving way to intramammary infections. Nutritional stress especially due to diets inadequate in micronutrients such as trace minerals (copper, zinc, selenium, cobalt, chromium, iron) and antioxidant vitamins (A,C,E, β- Carotene, Lycopene),amino acids (L-histidine, lysine) and certain bioactive ingredients (Lactoferin, Probiotics) are likely causes of diminishing the udder immunity both at cellular and humoral level in cows particularly during risk period of onset lactation [40,41]. Normal Zinc level is essentially needed for optimum cell mediated immune responses. Serum trace mineral concentration in respect of copper, zinc and iron decreased owing to Staphylococcal mastitis [42]. An optimum level of macro elements such as calcium is also essential for optimum udder immunity during parturition and lactation stress. In females negative calcium balance occurring in late pregnancy and early lactation, is a calculated fact. Depression of ascorbic acid concentration is associated with bovine mastitis and its inadequacy causes mammary tissue lipid per oxidation [43]. Vitamin A and E supplementation in diets improve mammary gland host defense and effective neutrophil recruitment [44]. Humoral and cellular factors are important in inhibiting bacterial growth in greater concentration in mastitic milk and Cell-free normal milk with a variable degree of growth inhibitory activity for Streptococci, Staphylococci and Coliform organisms. Lactoferin, immunoglobulin and transferin like soluble factors in milk prevent bacterial multiplication and establishment of infection. Lactoferin is bacteriostatic for a variety of bacteria owing to its iron-chelating ability. The main function of immunoglobulins in secretions of the bovine mammary gland is opsonization of microorganisms for phagocytosis but they also play a

role as antitoxin [45]. Intercurrent diseases, stress and malnutrition reduces considerably the defensive response of these bioactive components in affected dairy herds.

Blood Group Factor

Association of the M blood group system with bovine mastitis has been evidenced [46]. Associations of the 11 bovine blood group systems with mastitis were examined in Red Danish dairy cattle. A significant effect of the M blood group system on the incidence of mastitis was observed in the first and second lactation periods and a lower frequency of mastitis was found among animals lacking the "M" factor as compared to those having the M blood group factor.

Growth Hormones (bST)

There is a positive correlation between milk production of cows and the level of growth hormone (bovine Somatotrophin) in their blood. Growth hormone causes redistribution of nutrient within the cow's body to favour nutrient utilization towards milk production. However, bST is not directly involved in milk secretion process. Interpretation of a direct effect of recombinant bovine Somatotrophin (bST) on mastitis incidence is confounded by the higher incidence of mastitis in cows of higher milk production [47].

Environmental Factors

The environmental mastitis is associated with two main groups of pathogens, the Coliforms (*E.coli and Klebsiella*) and environmental Streptococci (*S. uberis* and *S. dysgalactia*), present in the environment of the cow. Association of *Arcanobacterium pyogenes* with mastitis is also established. The environment of cow forms the source of infection. The major methods of transmission include inadequate management of the environment such as wet bedding, dirty lots, milking wet udders, inadequate premilking teat preparation, and poor housing system and fly control. Milkers hand introduced infection has the largest impact on the incidence of Staphylococcal and Streptococcal mastitis particularly the methicillin resistant *Staphylococcus aureus* (MRSA) mastitis. Priory sanitized human hands and premilking udder preparation considerably reduces the incidence of the disease [48]. In several instances contagious mastitis in cattle was shown to be human introduced. This is particularly true with our conditions in South Asian subcontinent, where hand milking practice is more common. Other pathogens of bovine mastitis such as Micrococcus and Pneumococcus species are also human transmitted through oral and nasal secretions.

Bedding material: Materials used to bed dairy cows allow bacteria to accomplish growth and nourishment. Many organic materials provide adequate nutrition for both Coliforms and environmental Streptococci to reach populations in excess of 107 colony forming units per gram of bedding [49]. Common organic bedding materials such as sawdust and straw usually allow very few mastitis pathogens to thrive before use as bedding. Bacterial populations tend to remain in stationary growth phase for up to 7 to 10 days and then start to decline due to the exhaustion of nutrients in bedding. Finely chopped materials support greater bacterial numbers than the same bedding with larger particle sizes as the finely chopped materials adhere more readily to teat skin than larger materials, thus increasing exposure of the teat end to mastitis pathogens.

Climate: Has a significant influence on the occurrence of mastitis. Hot and humid climate gives opportunity to flies/insects to breed and thus spread bacterial agents from herd to herd. Summer mastitis (diptheroid mastitis) due to *Corynebacterium* Spp. is one example [50]. Closed housing appears to increase the risk of mastitis particularly winter mastitis due to Coliforms and Mycoplasmae, as consequence to more chances of floor contamination and wet bedding with preservance of infectious agents than the dry bedding. Monsoon season causes more incidence of Mycotic mastitis, however in many cases the seasonal variation is non-significant [51]. *Staphylococcal mastitis* has little seasonal variation. Growth rates of coliforms and environmental streptococci are greatest during warm, wet weather. The effects of season on bacterial populations in bedding are quite dramatic in regions that experience a wide variation of temperatures within a year. In general, the impact of bedding on exposure of cows in confinement housing decreases during cold weather and increases as temperatures and humidity increase. Therefore, a proper ventilation of barn is essential to moderate the effects of heat and humidity in housing areas. Amount of environmental heat and rainfall has direct bearing both on the cow's immunity as well as the premises where it is kept and fed. Dry and hot summer reduces the bacterial load while wet conditions increase the bacterial population around the animal's surroundings.

Pathogen Factors

Non-pathogenic microbes inhabiting the udder surface play a vital role in inducing colonization resistance and thereby preventing the introduction of pathogenic organisms via teats. The survivability of such eco-friendly organisms is largely dependent on the environmental cleanliness, comfortable housing and hygienic milking practices. Poor or no milk ductular tissue attachment of *C. bovis, Micrococci, Streptococcus fecalis* and non-pathogenic *E. coli* is highly suggestive of non-involvement of these agents in bovine mastitis, which usually occupy teat canal in order to prevent entrance of major mastitides. It seems to result from investigation that *C. uberis* is able to protect the cow udder against mastitis caused by bacteria [52]. Lactobacillus species inhabiting udder and teat skin induce protection from invading pathogenic agents. These have been shown to possess antagonistic activity against mastitis causing *S. aureus* [53]. Mastitis causing pathogens particularly *S. agalactiae* and *S. aureus* are potentially selective in attachment to ductular tissue and if the dose of infection supersedes the population of normal skin flora of udder, intramammary infection results. Virulence of major mastitis agents like Streptococci, Staphylococci and perhaps mycoplasmae is determined by the source of infection, potential of infectivity (antigenic expression) and host immune status. Mycoplasmal mastitis spreads eventually from cow to cow, so that the whole herd is affected over a given time, whereas *S. aureus* from both human and animal origin is virulent and the foci of infection remain well established in the form of micro-abscesses within the milk alveoli, spreading through environment, milking machines and the herdsmen to cows.

Effects of Mastitis on Human Health

With increasing public awareness of food safety issues, the role of mastitis pathogens as possible zoonotic agents has received due consideration. An outbreak of gastroenteritis in human consumers of unpasteurized mastitic milk with *Campylobacter jejuni* from one herd is documented [54]. The need for further research into risks to human health from Staphylococcal enterotoxin contamination of mastitic milk

is highly desirable, although most outbreaks of food poisoning due to Staph. aureus are thought to be caused by contamination by human handlers of food. Numerous hospital borne infections arise due to methicillin-resistant *Staph. aureus* (MRSA), and these too have been associated with enterotoxin production. The MRSA expressing *Staph. aureus* strains are identified in bovine milk [55]. Milk Zoonoses are of particular importance in developing countries where there is an increased level of consumption of untreated milk. The risk of zoonosis on account of *Prototheca zopfii* causing mastitis in dairy cows is described [56]. The risk to human health from *Mycobacterium bovis*, the causal agent of tuberculous mastitis is of grave concern particularly in developing countries [57]. South-Asian subcontinent is exposed to this menace. It is suggested that milk-borne infection is the principal cause of non-pulmonary tuberculous disease in humans. It has been claimed that the current pandemic of HIV/AIDS in parts of the world rose new questions regarding the epidemiological impact of immunosuppression on the transmission of *M. bovis*. The increase in prevalence of *M. bovis* infected dairy cows in the UK makes the possibility of disease transmission through milk a matter of concern. Mastitis due to *Pseudomonas Spp.* is considered a potential risk to human consumer health owing to the fact that the pathogen is emerging as a multiple drug resistant (MDR) strain that is life threatening to man. Field surveys of major livestock diseases in Pakistan have indicated that it is one of the important health hazards in that country [58]. Sampling bulk tank milk has revealed the presence of *Listeria monocytogenes*, *Salmonella Spp.*, *Campylobacter Spp.*, verocytotoxigenic *E.coli* in 2.7%, 0.2%, 0.5% and 0.9% of milk samples, respectively. Yeast called *Cyrptococcus neoformans*, causing human Cryptococcosis is associated with acute mastitis in cattle and buffaloes. Infection to humans has a fair chance to occur if unpasteurized milk is consumed and the farm families are more likely to be exposed to some risk.

Therapeutic Management of Bovine Mastitis

Antimicrobial approach

The widespread use of conventional antibiotics in the medical treatment of mastitis in cows has not met much success, though antimicrobial therapy is currently the last resort to treat mastitis. Many antibiotics kill the micro-organisms during replication where as non-replicating pathogens in the udder tissue remain unaffected and some others gain resistance [59]. Clinical mastitis has varying response to treatment, assigned to three reasons- Impermeability of blood-udder barrier to several drugs, presence of milk and accumulation of inflammatory debris in the milk alveoli and lack of milk fat/lipid solubility of many other drugs. The clinical efficacy of antibiotics vary in response among the herds and within the herds due to type of mastitogens involved, location and extent of the infection, degree of udder pathology, physiochemical property and kinetics of the drug used, site of administration and sensitivity pattern of pathogens. More over the affinity to mammary tissue, blood-udder penetrability and milk lipid solubility of the drug are the criteria to be given due consideration. Also increasing the dose and duration of treatment is not without consequences. It has resulted to improve the cure rates particularly by the simultaneous use of parenteral and intramammary routes (Table 1). Thus important criteria at the initiation of treatment of mastitis may include-

- Form of mastitis (Sub-clinical/Clinical)
- Extent of pathological damage to udders

- Type of pathogen involved (Single/mixed infection)
- Area specific experience of drug response in mastitis
- Drug sensitivity pattern
- Selection of antimicrobial drug with ideal pharmacological properties
- Maintenance of appropriate dose/interval

Drug	Dose	Route	Interval/hr
Procaine penicillin G	66000 iu/kg	IM	12
Benzathine	66000 iu/kg	IM/SC	48
penicillin	11-22 mg/kg	IM/SC	12
Ampicillin	11 mg/kg	IM/SC	12
Amoxycillin	55 mg/kg	SC	6
Cephalothin	1 mg/kg	IM	24
Ceftiofur	3-5 mg/kg	IV/IM	8
Gentamicin			

Table 1: Recommended dosage and route of administration of some antimicrobials in Mastitis [60].

The treatment of bovine mastitis in peak lactating phase is a weak spot and very often fails with conventional antibiotics because of continuous drainage during milking, absence of immunocompetance of the affected udder and milk losses due to withdrawal. The immediate prompt treatment has so far been considered the only effective way to avoid much pathological damage to the udder tissue. In our experience Chloramphenicol proved to be highly effective than Gentamicin, Enrofloxacin and Amoxycillin in inducing bacteriological elimination in Gram negative mastitis in cows but milk withdrawal period of 3 days post treatment is necessary, because the drug is deleterious to consumer health. The use of Amoxicillin along with suicide inhibitors like Tazobactum is 80% effective in the treatment of mastitis [61] while some have shown that Penicillin and Tazobactum provide increased spectrum of activity (Table 2) in clinical mastitis. Cefquinome given intramammary along with ascorbic acid @20gm intramuscularly proved effective in eliminating sub-clinical intramammary infection [62]. Intramammary route alone during lactation, due to presence of pus and milk does not allow the drug to be diffused [63].

Alternative approach

Phytotherapy or the herbal remedies are assuming pertinence in recent years in view of the development of resistance to antibiotics and persistence of drug residues in the milk. However the herbal derivatives as gel are more often used for topical application. Other topical applications of soothing, nonirritant herbal gels derived from *Cedrus deodara*, *Curcuma longa*, *Glycyrrhiza glabra* and *Eucalyptus globulus* are effective in the treatment and prophylaxis of Sub-clinical mastitis. The antibacterial and immunomodulatory propensity of *Ocimum sanctum* leaf extract, reduced somatic cell count and total blood count significantly in Sub-clinical mastitis [65]. Likewise, Nisin, an antimicrobial polypeptide produced by *Lactococcus lactis* offered higher cure rate of *S. aureus* mastitis in cows than Gentamicin [66]. However phytotherapy alone is not widely acceptable to replace antibiotics in the mastitis cure. These are mostly used concomitantly

with antibiotics. The use of bacteriophage particularly against *S. aureus* has been researched extensively but only with low or moderate therapeutic success in clinical mastitis. The ability of lytic *S. aureus* bacteriophage K is poor in eliminating *S. aureus* intramammary infection [67]. However it offers strong prophylaxis in healthy quarters as the phage is excreted in milk and the somatic cell count rises so that phagocytic potential of the udder increases to combat infection.

Antimicrobials	Lipid solubility	Milk: Plasma ratio
Penicillin G	Low	0.13
Ampicillin	Low	0.24
Cephaloridin	Low	0.24
Aminoglycosides	Low	0.5
Polymixin	Very Low	0.3
Sulfadimethoxine	Moderate	0.21
Sulfadiazin	Moderate	0.59
Oxytetracycline	Moderate	0.75
Doxycycline	High	1.53
Trimethoprim	High	2.9
Tylosin	High	3.5
Erythromycin	Very High	8.7

Table 2: Milk: Plasma concentration and Lipid solubility of conventional antibiotics used in Mastitis treatment [64].

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Prevention and Control Strategy

Integrated Mastitis control programme

Sanitization and udder hygiene: Mastitis control depends on reducing the exposure of teat ends to pathogens and increasing the resistance of cows to infection. This includes udder hygiene, environmental hygiene and sanitization measures for milker's hands or milking machines. Dissemination and proliferation of pathogenic microbes is brought well under control by proper and regular disposal of excreta, disinfection of housing premises and sanitization of cow's udders and milker's hands or the milking machines prior to use. Prior and post milking teat dips with isopropyl alcohol, chlorhexidine and 1% sodium hypochlorite solution is effective. These measures have proved to reduce the risk of environmental mastitis caused by Coliforms and contagious mastitis due to *S. aureus*.

Milking order: Thumb rule is to milk primiparous cows' first and multiparous cows latter. Among these healthy cows are to be milked first and mastitic cows milked at last. Hind quarter milking should preferably follow fore quarter milking.

Dry Cow Therapy: Use of intramammary infusions of long acting antibiotics on drying off is of paramount importance in minimizing the incidence of mastitis at freshening. Drugs of choice include Benzathine penicillin, Benzathine cloxacillin, Benzathine cephapirin, Novobiocin and sustained release formulations of Eythromycin and penicillin. Teat sealing with acrylic latex can also be done by dipping teats into the solution. Tilmicosin intramammary infusion has successfully eliminated *S. aureus* [68].

Lactation Cow therapy: Sub-clinically mastitic cows are subjected to short term treatment with effective antibiotics at least for 3-5 days preferably through intramammary route, though clinical mastitis might have longer treatment duration. Amoxycillin, Erythromycin, Cloxacillin, Pirlimicin and Cephalonium infusions offer substantiative treatment results in sub-clinical mastitis [6,69]. It is imperative to undertake udder status screening through various indirect tests and bacteriological culture on paired milk samples from every quarter of individual cows of a lactating herd at least three to four times a year. This would guide in designing future control practices. In larger herds' bulk tank SCC and culture tests can be performed.

Maintenance of optimum udder immunity: Udder's local defense system, involving cellular reaction to infectious agents and enzyme as well as non-enzyme based antioxidant system requires activation. Activation and interplay of these factors is largely dependent on nutritional status of the animal. Dietary/ancillary supplementation of micro elements such as, Zinc, Copper, Cobalt, Iron, Manganese, Cromium and Selenium is essentially required for optimization of udder immunity. Micronutrients such as Beta-carotene, Vitamin A, C and E, lactoferrins, L-histidine provide enhanced immunity and anti-oxidative effect, which prevent alveolar tissue degeneration [42]. Therefore during lactation, it is necessary that a vulnerable cow should have normal blood levels of all these nutrients.

Autogenous mastitis vaccine: A successful vaccination programme for mastitis must furnish one of three goals: 1) Elimination of chronic intramammary infections 2) prevention of new intramammary infections 3) reduction of the incidence or severity of new intramammary infections. Mastitis bacterins are prepared from the prevalent mastitogenic bacteria such as *Staph. aureus* or *E.coli* and aluminium hydroxide gel is used as a vehicle. The commercial

bacterins may not contain the mastitis strains endemic to a particular dairy herd. The dairy cows must not receive any of the gram negative vaccines such as *Pasturella*, *Salmonella*, *Campylobacter*, *Haemophillus*, *E. coli* or *Moraxella* species bacterins within 5-7 days of mastitis bacterins, to prevent any antigenic cross reactivity. The core antigen of the inner cell wall provides more immunogenicity than the somatic O antigen of coliforms. Thus the rough mutant *E. coli* 0111 B4 (J5) with its antigen of the lipopolysaccharide exposed core provides cross-protection against Gram negative coliform bacteria [70]. The vaccine is usually given 4 weeks before parturition. Marketed vaccine may contain, *Streptococcus uberis*, *S. agaglactiae*, *Staph. aureus* and *E. coli*. However multietiological nature of mastitis is the main constraint in the development of an efficacious vaccine. Generally mastitis vaccines are not accepted as prophylactic substitute and that vaccines should be seen as an adjunct to supplement other effective control practices.

Breeding for resistance to mastitis: Many criteria for breeding dairy cows are positively correlated with clinical and subclinical mastitis, suggesting that new approaches may be required to reduce mastitis while selecting for high milk production. Selection indices have recently included health-related traits, including longevity. The incorporation of SCC and clinical mastitis records in selection of dairy cattle for breeding has the potential to reduce mastitis. Identification of genes those are associated with reduced mastitis has focused to date mostly on the Major Histocompatibility Complex (MHC) genes. The most promising region of the MHC is DRB where associations with certain haplotypes and reduced SCC or clinical mastitis have been described by a many of researchers. It is likely that marker-assisted selection of cattle for breeding may be exploited in future, and breeding for mastitis resistance may become easier with new genomic technologies. Advances in the ability to produce transgenic animals make it likely that such animals will become important components of animal farming in future. Genetic selection for mastitis is not possible without records of clinical mastitis, subclinical mastitis, type traits associated with teat and udder health, and markers of immunity relevant to the defense of the udder. Demand for more milk yield causes more incidence of mastitis, and that selection for improved udder health is sacrificed for improvement in production traits [71]. Genetic evaluations based on SCC or somatic cell score (SCS) associated with selection for production, should enhance, genetic resistance to mastitis. Milk somatic cell counting has been incorporated into recording systems in many countries and provides a source of easily accessible data at relatively low cost. Different mastitis pathogens induce different numbers of somatic cells for differing durations. Organisms such as *Staph. aureus* and *S. agalactiae* produce high cell numbers that often persist over many months while coliform infections may also induce very high cell counts but they often reduce quickly [72]. This suggests that mean lactation SCC may better reflect infections with *Staph. aureus* and *S. agalactiae* rather than Coliform infections. Genetic selection based on low SCC may, therefore, be more likely to reduce contagious mastitis rather than environmental mastitis. Type traits are currently employed in assessing cows for breeding and include, body characteristics, foot angle and udder and teat anatomy.

Gene therapy: The goal of this novel tool is to enhance mastitis resistance of dairy cows by enabling the cells of the mammary gland to secrete additional antibacterial proteins. A mouse model has been shown to produce varying levels of lysostaphin in their milk [73]. This protein is believed to have potent anti-staphylococcal activity and its secretion into milk confers substantial resistance to *S. aureus* mastitis. Lysostaphin is produced by nonpathogenic cousin strain of *S. aureus*. Additional antibacterial proteins are being sought that can complement lysostaphin. A potential benefit of transgenic application of antibacterial proteins is the sparing of use of antibiotics in farm animal health. Research efforts are still in the stage of infancy. The transgenic approach to insertion of new genetic material into agriculturally important animals is feasible but requires extensive prior evaluation of the transgene and transgene product in model systems.

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