

**A COMPREHENSIVE AND ILLUSTRIOUS REVIEW ON CLINICAL AND
DIAGNOSTIC ASPECTS OF MASTITIS INFECTION IN HIGH YIELDING
LACTATING CATTLE**

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Article Received on
25 August 2014,

Revised on 18 Sept 2014,
Accepted on 13 Oct 2014

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ABSTRACT

Mastitis the chronic inflammation of the mammary gland of cattle and can have infectious and non-infectious etiology. It is characterized by physical, chemical and usually bacteriological changes in the milk and pathological changes in the glandular tissue of the udder and affects quality and quantity of milk. Mastitis is usually caused by bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland. Bovine mastitis is a multi-etio-pathogenic condition of mammary gland affecting dairy cows and remains the most economically important disease of dairy industries around the world. It is characterized by physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissues of the udder.

The changes in the milk include changes of colour, consistency and presence of abnormally large number of leukocytes. Production per cow due to the clinical and subclinical prevalence of mastitis is usually recognized as the main pathway in causing the economic losses due to the disease. The present exclusive review encompasses the clinical and diagnostic overview on mastitis recognized as an immensely important economically significant disease in cattle resulting to production loss.

KEY WORDS: Cattle, Clinical, Mastitis, Diagnosis, Etiology, Subclinical.

INTRODUCTION

Mastitis is characterized by physical, chemical and usually bacteriological changes in the milk and pathological changes in the glandular tissue of the udder and affects quality and

quantity of milk. Mastitis is usually caused by bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland. The present article highlights the mode of action of the active antigenic principles of the bacteria in causing the infection in cattle population in India. ^[1]

It remains the most economically important disease of dairy industries around the world producing great economic loss to farmers. There are two forms of mastitis *viz.*, clinical mastitis (CM) and Subclinical mastitis (SCM). ^[1]

Subclinical mastitis is 15-40 times more prevalent than clinical mastitis and it forms the basis of herd outbreaks. As no gross abnormality in milk and udder is noticed, subclinical mastitis goes unnoticed by the farmers. Therefore, subclinical mastitis is considered more important due to negative impact on the economy throughout the world.

Mastitis is the most economically important disease of dairy cattle, accounting for 38% of the total direct costs of the common production diseases and is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses. It is the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%). India is the highest milk producer in the world but the per capita availability of milk still remains half of the world average, demanding strategic intervention. One of the reasons for low productivity is poor animal health, particularly, mastitis which is single largest problem in dairy animal in terms of economic losses in India. It is proved by the reports that the annual economic losses due to bovine mastitis was increased 114 folds in about 4 decades from 1962 (INR 529 million/annum) to 2001 (INR 60532 million/annum). In addition to heavy losses in milk quality and quantity, it also causes irreversible damage to the udder tissue and less occasional fatalities. ^[2]

Etiology

Mastitis is a multi-etiological complex disease. The cow udder is an ideal environment for microbial growth and under optimum udder conditions, such as temperature, nutrition and freedom from outside influence, pathogenic organisms multiply astronomically and it is this factor that causes udder damage and triggers the response that is recognized as mastitis. Many studies from Asia countries have been reported that *Staphylococcus aureus* is the chief

etiological agent of mastitis in cattle and buffaloes. *Staphylococcus aureus* is ubiquitous and can colonize the skin as well as the udder. It is capable of causing peracute, acute, subacute, chronic, gangrenous and subclinical types of mastitis. The acute form of the disease usually occurs shortly after parturition and tends to produce gangrene of the affected quarters with high mortality. ^[1]

Large numbers of infectious agents are responsible in causing the disease in dairy animals. Bacterial agents like *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Corynebacterium spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, Mycoplasmal agents, fungal agents, viral agents are responsible for the disease. About 95% of intramammary infections are caused by *Staphylococcus spp.* and *Streptococcus spp.* The remaining 5% are caused by other organisms (NAIP Sub-Project at PD-ADMAS). *Staphylococcus aureus* is a major pathogen in dairy cattle mastitis. Streptococcal species isolated from dairy cows with clinical mastitis were obtained from mastitis research workers in Florida, Louisiana, New York, Vermont, Washington, and West Virginia. ^[2]

Disease Incidences with Involvement of Multi-Etiological Agents

Reports from different parts of India indicate high prevalence of both subclinical and clinical mastitis in dairy herds. Frequency of occurrence of mastitis is influenced by different managerial, environmental and genetic factors. Klastrup O, Bakken G, Bramley J and Bushnell R. (1987, *Bulletin of the International Dairy Federation*) estimated that 25% of susceptibility to infection is attributed to environmental factors, 20% to genetic factors and 50% to herd management. Therefore prevalence of mastitis can be used as a litmus test to judge the management practices and hygienic conditions of any organized dairy farm. Though there are a long list of microorganisms are held to be responsible for the disease, but the most common pathogens are *Staphylococci spp.*, and *Streptococci spp.* ^[3]

Firat and Uysal (1986, *Pendik Hayvan Hastaluklari Merkez Avastirma Enstitusu Dergisi*) isolated the organisms from 406 cows with clinical and subclinical cases of mastitis. Out of 390 isolates which showed CMT positive, 132 (34%) were coagulase positive Staphylococci, 63 (16%) coagulase negative Staphylococci, 51 (13%) gram negative bacteria, 28 *Streptococcus agalactiae*, 27 *Streptococcus uberis*, 22 *Streptococcus faecali* and 21 *Streptococcus dysgalactiae*. Konte M, Ndiaya AMS and Mbengue AB (1988, *Reven d'Elewage et de Medicine Veterinarire des pays tropicaux*) reported one year observation on mastitis in dairy herds of pure bred Monthbeliared and Pakistani Zebu near Dakar, 28 cases

yield 20 bacterial species, the commonest being *Staphylococcus aureus* (50%) and *E. coli* (36%). Nine cases of mixed infection with 2-5 species were considered to have developed after treatment failure in single infection.

Waage S, Ron I, Solberg I, Nordlokken H, Slettbakk T and Osteras O (1990, *Norsk Veterinaertidsskrift*) examined milk samples taken from 2491 quarters of 2233 cows with acute clinical mastitis and were found that *Staphylococcus aureus* predominated (48%), followed by enterobacteria (18%), *Streptococcus dysgalactiae* (12%), *Str. uberis* and *Actinomyces pyogenes* (3% each) and other streptococci (4%).

Lafi SQ, Al-Rawashdeh OF, Ereifej KI, Haitant NQ (1994, *Preventive Veterinary Medicine*) claimed that the most common organism isolated from clinical cases of mastitis was coagulase positive *Staphylococcus* spp. Turutoglu H, Atesoglu, Salihoglu H and Ozturk M (1995, *Pendik Veteriner Mikrobiyoloji Dergisi*) examined 1594 mastitis milk samples and yielded aerobic bacteria from 1126 samples. Among the positive samples *Staphylococcus aureus* was 28%, *Staphylococcus epidermidis* 23%, *Streptococcus agalactiae* 19%, *E. coli* 8%, *Streptococcus dysgalactiae* 4%. Pampori (2000, *The Veterinarian*) found that out of 190 milk samples, 174 samples were positive for bacterial growth. 88 (50.57%) yielded Staphylococci, 81 (45.55%) Streptococci and 5 samples (2.87%) yielded mixed growth of gram positive rods and gram negative cocci.

Ghose B, Sharda R, Chhabra D, Garg UK and Tiwari S (2001, *Indian Veterinary Medical Journal*) bacteriologically cultured 13 milk samples, 11 (84.6%) yielded one type of bacteria in the remaining 2 samples (15.4%) mixed infection were observed. Among the bacterial mastitogens *Staphylococcus aureus* (33.3%) was the major agent followed by *Streptococci agalactiae* (26.7%), *Corynebacterium* spp. (20%), *Streptococcus dysgalactiae* (13.3%) and *Staphylococcus epidermidis* (6.7%).

Ghose and Sharda (2004, *Indian Veterinary Medical Journal*) obtained 168 (15.85%) milk samples out of 1060 bacterial growth on cultural examination. A total of 224 isolates were obtained, out of which 74 (33.04%) were characterized as *Streptococcus* spp., i.e. *Str. agalactiae* (58.11%), *Str. pyogenes* (20.27%), *Str. dysgalactiae* (18.92%) and *Str. uberis* (2.7%). Balakrishnan G, Unny M, Dorairanjan D and Subramanian M. (2004, *Indian Veterinary Journal*) isolated a total of 40 bacterial isolates. The spectrum of organisms was *Staphylococcus aureus* (35%), *Escherichia coli* (27.5%), *Streptococcus agalactiae* (17.5%),

Pseudomonas aeruginosa (12.5%), *Streptococcus dysgalactiae* (2.5%), *Pasteurella haemolytica* (2.5%) and *Actinobacillus capsulatum* (2.5%). Kheirabadi P, Ebrahimi and Barati F (2008, *Indian Veterinary Journal*) reported that subclinical bovine mastitis in west Iran was mainly caused by coagulase positive *Staphylococcus aureus* and *Streptococcus agalactiae*.

Mastitis is a multi-etiological complex disease. The cow udder is an ideal environment for microbial growth and under optimum udder conditions, such as temperature, nutrition and freedom from outside influence, pathogenic organisms multiply astronomically and it is this factor that causes udder damage and triggers the response that is recognized as mastitis.^[4]

Many studies from Asia countries have been reported that *Staphylococcus aureus* is the chief etiological agent of mastitis in cattle and buffaloes. *Staphylococcus aureus* is ubiquitous and can colonize the skin as well as the udder. It is capable of causing peracute, acute, subacute, chronic, gangrenous and subclinical types of mastitis. The acute form of the disease usually occurs shortly after parturition and tends to produce gangrene of the affected quarters with high mortality. Reddy P, Chao Qi, Teresa Zembower, Gary A and Noskin Maureen Bolon (2007, *Emerging Infectious Diseases*) briefed a relative increase in methicillin resistance among 48 cases of *Staphylococcus aureus*-associated postpartum mastitis during 1998–2005. Of 21 cases with methicillin resistance, 17 (81%) occurred in 2005. Twenty (95%) isolates contained the *Staphylococcus* cassette chromosome *mec* type IV gene.

Moon J-S, Lee A-R, Kang H-M, Lee E-S, Kim M-N, Paik YH, Park YH, Joo YS and Koo HC (2007, *Journal of Dairy Science*) isolated 21 *Staphylococcus aureus* and 19 CNS strains which were resistant to methicillin. The *mecA* gene was also found in 13 methicillin-resistant *S. aureus* (MRSA) and 12 methicillin-resistant CNS (MRCNS) isolates. Bhandari BB, Roy A, Yadav MM and Joshi CG (2009, *The Royal Veterinary Journal of India*) isolated 43 *Staphylococcus aureus* from 121 clinical and subclinical mastitis cases of cows and buffaloes of Gujrat. All *S. aureus* were identified on the basis of morphological, cultural and biochemical properties. All 43 *S. aureus* were identified by PCR method using species specific oligonucleotide primer that encode the gene 23S rRNA. PCR based detection was carried out for virulence associated genes of *S. aureus* namely *clfA*, *spa*, *coa* and *mec* (methicillin resistant). Methicillin resistant gene of *S. aureus* isolates did not generate any amplified product. Sixteen (16) isolates were classified as methicillin resistant and 96 isolates as methicillin susceptible by disc diffusion and broth microdilution. The *mecA* gene was

identified in 15 of the 16 resistant isolates (Andrea T. Febler, Carmen Billerbeck, Kristina Kadlec, Stefan Schwarz. 2010, *Journal of Antimicrobial Chemotherapy*). Genotyping of the 17 methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from each cow revealed two staphylococcal cassette chromosome mec (SCC mec) types (IV and IVa), three spa types (t286, t324, and untypable), and two sequence types (ST1 and ST72) (Nam HM, Lee AL, Jung SC, Kim MN, Jang GC, Wee SH, Lim SK. 2011, *Foodborne Pathogens and Disease*).^[5]

Expression and Effect of Bacterial Antigenic Factors

Chronic form of Staphylococcal mastitis is not easily cured by antibiotic treatment. Despite of intensive control measures, it is difficult to eradicate the intramammary infection caused by such pathogen. Because, *Staphylococcus aureus* produces broad spectrum of surface active agents, proteins and exotoxins that act as virulence factors for the pathogenesis of the disease. This bacterium produces a variety of exoproteins that contribute to its ability to colonize mammary gland, with five of them being different membrane-damaging toxins, four hemolysins (alpha-, beta-, gamma- and delta-hemolysin) and leucocidin. Beta and alpha hemolysins are the most important in pathogenesis of the intramammary infections.

Enterotoxigenic *Staphylococcus aureus* in raw milk act as potential health hazard from public health point of view. So, the identification and characterization of protein extracted from *Staphylococcus aureus* isolated from mastitis milk is necessary for studying the biochemistry and pathogenesis of mastitis.^[4] Besides virulence factors, the increased resistance of *S. aureus* isolated from mastitic cows to several antimicrobial agents has been reported what impacts the effectiveness of therapy since control methods of this organism from dairy herds requires treatment of infected mammary glands with effective antimicrobial agents. Chronic form of Staphylococcal mastitis is not easily cured by antibiotic treatment. Despite of intensive control measures, it is difficult to eradicate the intramammary infection caused by such pathogen. Because, *Staphylococcus aureus* produces broad spectrum of surface active agents, proteins and exotoxins that act as virulence factors for the pathogenesis of the disease. This bacterium produces a variety of exoproteins that contribute to its ability to colonize mammary gland, with five of them being different membrane-damaging toxins, four hemolysins (alpha-, beta-, gamma-, and delta-hemolysin) and leucocidin. Beta and alpha hemolysins are the most important in pathogenesis of the intramammary infections. The beta-toxin is an Mg²⁺-dependent sphingomyelinase C, which degrades sphingomyelin in the outer

phospholipid layer of the membrane. Staphylococcal protein A is a membrane-bound exoprotein characterized and well known for its ability to bind to the Fc region of immunoglobulins of most mammalian species. Coagulase protein has the ability to turn fibrinogen into fibrin threads by a mechanism different from natural clotting. Coagulase has also been shown to be a virulence factor in intramammary infection. As these agents are injurious to milk producing cells, impairs gland and immune defence mechanism of the host body, which make them capable to reside intracellularly and to establish a chronic infection that persists for life of the animal. Enterotoxigenic *Staphylococcus aureus* in raw milk act as potential health hazard from public health point of view. So, the identification and characterization of protein extracted from *Staphylococcus aureus* isolated from mastitis milk is necessary for studying the biochemistry and pathogenesis of mastitis.^[5, 6] The prevalence of Staphylococcal mastitis was highest i.e. 53.33%. The overall incidence of coagulase positive Staphylococcus spp. i.e. *S. aureus* was 52.50% higher than coagulase negative *S. Epidermidis* (47.50%).^[6] Besides virulence factors, the increased resistance of *S. aureus* isolated from mastitic cows to several antimicrobial agents has been reported what impacts the effectiveness of therapy since control methods of this organism from dairy herds requires treatment of infected mammary glands with effective antimicrobial agents. The determination of antimicrobial susceptibility of clinical isolates is required not only for therapy but also for monitoring the spread of resistant strains throughout the populations.^[7]

Mastitis, inflammation of the mammary gland, can have an infectious and non-infectious etiology. It is characterized by physical, chemical and usually bacteriological changes in the milk and pathological changes in the glandular tissue of the udder and affects quality and quantity of milk. The whole cell protein profile analysis of *S. aureus* and *S. epidermidis* by SDS-PAGE technique showed broad similarity in band pattern except 4 bands (36.35 KDa, 31.16 KDa, 24.05 KDa and 19.49 KDa and 2 bands (69.09 KDa and 32 Kda) respectively were highly expressed may be due to some strain variation or mutation or antibiotic resistance and virulence.^[7] The determination of antigenic interaction of pathogenic bacteria with the host tissue helps in understanding the mode of action of the antigenic principles of the microorganisms responsible in causing mastitis and in proper antibiotic formulations to be effective systemically in the host.^[8] Out of total 60 samples, 38 samples (63.33%) were positive for staphylococci and 10 samples (16.66%) were positive for streptococci. Coagulase positive staphylococci organisms were identified by reacting the isolates with rabbit plasma in the ratio of 1:10 dilution. It was observed that 84.21% isolates were coagulase positive.

89.5% staphylococci isolates were resistant to sulphamethizole, 84.2% to ampicillin, 79% to colistin, 60.5% to penicillin G, 52.63% to methicillin, 42.1% to ciprofloxacin, 26.3% to tetracycline, 23.7% to ceftizoxime and enrofloxacin and 13.2% to ceftriaxone by disc diffusion method. All the streptococci isolates were resistant to penicillin G. [8]

From the epidemiological point of view also, it is important to determine the origin of the organisms involved in the etiology of the disease. Therefore an exact identification of bacterial pathogens is an unavoidable requirement in order to detect the reservoirs and sources of infection between animal populations. There is considerable genetic heterogeneity through natural populations of *S. aureus*. Many different techniques are available for tracing the spread of single *S. aureus* strain of human and animal origin, such as antibiotyping, biochemotyping the phage typing, protein electrophoresis by SDS-PAGE, plasmid profiling and PCR. Despite the presence of all these methods, each of these techniques has advantages and disadvantages in their discriminatory power, reproducibility and type ability. The analysis of whole cell profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has recently been established as a useful method for the identification of Staphyococcal spp. Treatment and eradication of diseases caused by *Staphylococcus aureus* and its multidrug resistance require precise typing of various pathogenic strains so as to find out the common clones, their origins, sources and routes of transmission. [9, 10]

CONCLUSION

The determination of antimicrobial susceptibility of bacterial isolates from subacute clinical cases of mastitis is required not only for therapy, but also for monitoring the spread of resistant strains throughout the populations. It has been estimated that mastitis reduces milk yield by approximately 21% and butter fat by 25% in affected cattle as compared to normal one. The milk of the infected cow turns inferior in quality and the milk of the affected cow is unfit for human consumption. The determination of antigenic interaction of pathogenic bacteria with the host tissue helps in understanding the mode of action of the antigenic principles of the microorganisms responsible in causing mastitis and in proper antibiotic formulations to be effective systemically in the host.

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