European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 07, 2020

Bovine Mastitis: A Major Concern Of Dairy Industry

Manoj Kumar Jena

Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, 144411, India
Email : manoj.20283@lpu.co.in

ABSTRACT
Bovine mastitis is a major setback in the dairy industry causing huge economic loss to the dairy farmers. This disease is mainly the inflammation of mammary gland which may be acute and chronic. Mastitis can be either clinical (showing prominent symptoms) or sub-clinical (no visible symptoms found). The milk yield is reduced drastically after the cow suffers from mastitis. Besides the low milk yield, reproductive performance is also affected with infertility and abortions occurring in the affected animals. Antibiotics therapy is the existing therapy so far which has many drawbacks. The antibiotic residue come through the milk and enters the human food chain, besides the antibiotic resistance developed by the microorganisms. Some alternative therapies like phage therapy, nano-particle based therapy, bio-therapeutics have come into existence which need further research. Proper management of the disease and the animals with prompt treatment can reduce the loss rendered by the dairy farmers in future.

(Key words: Mastitis, bovine, dairy, mammary gland, and antibiotics)

1. INTRODUCTION

Bovine mastitis (mammary gland inflammation) is a disease of the mammary gland causing severe loss in the dairy industry as there is drastic reduction in the milk yield and rarely the affected cow regains its lactogenic potential (Supa-Amornkul*et al*,. 2019; Liang *et al.*, 2017; Vigueir *et al.*, 2009; Wall *et al.*, 2005).It is called the most expensive disease for the dairy farmers throughout the World (Zandkarimi *et al.*, 2018). The financial loss is found to the tune of approximately 35 billion US Dollars per year (Hertl *et al.*, 2010). The biological and environmental agents are responsible for this disease and the affected cattle show changes in their body physiology and behavior. The cows show low pregnancy rates, abnormalities in estrous cycle, and early embryonic death (Jamali *et al.*, 2018).

Many microbial agents are responsible for this disease. Bacterial, fungal, and algal agents are responsible for the mastitis and it is found 90 % of the mastitic cases are caused due to environmental bacteria (Kalinska *et al.*, 2017). *Staphylococcus aureus* is found to be the most common microorganism in mastitis. This microbe can reside in the mammary gland for long time causing chronic disease, and showing least response to the treatment (Sender *et al.*, 2017; Misra *et al.*, 2018). Besides, subclinical mastitis poses serious concern as the symptoms are not visible although the cows suffer from the infections. Antibiotics are used rampantly for the mastitis treatment and now time has come to take a break and think alternate strategies to tackle the issue. Antibiotics therapy has led to emergence of antibiotic resistance, carryover of antibiotic residues in milk and many other health problems. Proper

management of animals and early diagnosis of the disease can help prompt treatment of the disease and cause minimal loss to the farmers.

Causative microorganisms

Besides*Staphylococcus aureus, Streptococcus agalacteae, Streptococcus uberis, Escherichia coli, Serratia marcesens, Serratia liquefaciens* are also responsible for this disease. *E. coli* is found to have very important role in the clinical mastitis of dairy cattle (Shpigel et al. 2008). Recently, a study by Filioussis *et al.* (2020) revealed the existence of multi-drug resistant *E. coli* which is mcr-1 positive (colistin resistant) and also produces beta lactamase (extended - spectrum beta lactamase producing *E. coli*), isolated from bovine mastitic sample. This clearly shows the growing resistance developed by the microorganisms against the antibiotics therapy. Another microorganism *Serratia marcesens* and *Serratia liquefaciens* are also found affecting the mammary gland and complicating the mastitis disease (Schukken *et al.*,2012). Besides, their availability in the mastitic sample, they were also isolated from environment such as milking parlour, cow dung, animal beddings etc. They have the ability to produce heat resistant enzymes, enabling them to spoil the milk during processing (Friman *et al.,* 2019). Although the *Serratia* species cause mastitis, the *S. liquefaciens* sub species has beneficial effects in milk products processing due to their proteolytic activity which aids ripening of raw milk cheese products (Morales *et al.,* 2003).The other causative microorganisms are Pseudomonas spp., Corynebacterium, Mycoplasma, Mycobacterium tuberculosis (Kulkarni and Kaliwal, 2013). The microorganisms are generally found on the udder or surface of the teats and infection generally occurs during milking. Besides, the pathogens are also found in the saw dusts and beddings of the cows, manure, and the soil. The environmental microorganisms which cause the mastitis are generally opportunistic in nature and infect the animals when their immune system is compromised (Schukken *et al.,* 2005). Mastitis caused by Pseudomonas aeruginosa is found to be contaminated through water as endotoxaemia occurs as in the coliform mastitis. Another species such as Nocardia asteroides contaminates through soil (Kulkarni and Kaliwal, 2013). Bovine mastitis can also happen by microorganisms like Bacillus cereus, Streptococcus pyogenes, and Streptococcus pneumoniae which infect less frequently.

Biofilm production

Biofilms are the clusters of bacterial cells grown in an enclosed matrix produced by the bacteria themselves. Biofilms are produced due to genetic and physiological changes in the bacteria and in this mode of growth the bacteria become less sensitive to antibiotics therapy and also show tolerance to opsonophagocytosis (Stewart and Costerton 2001). Additionally, the biofilm induces the release of lysozymes enzymes by host tissue phagocytes and production of reactive oxygen and nitrogen species (Hermeyer *et al.,* 2011). As the biofilms existing in the mammary gland show less response to antibiotics therapy, it becomes difficult to eradicate the disease and causing economic loss to the farmers. It is observed sub optimal dose of antibiotics induces the biofilm production (Costa *et al.,* 2012). Additionally, biofilm production is influenced by milk composition, and it was found decrease of pH induces biofilm production (Atulya *et al.,* 2014). The microorganisms which have shown ability to produce biofilms in clinical mastitis and subclinical mastitis are *S. aureus, E. coli, E. faecalis, S. uberis, S. dysgalactiae,* and *S. agalactiae* (Gomes *et al.,* 2016).

Drawbacks of antibiotics therapy

Antibiotics therapy is the main therapy at present (Petitclerc *et al.,* 2007), but it has many drawbacks like gradual increase in antibiotics resistance, and residues of antibiotics secreted

through milk (Gomes and Henriques, 2016). The antibiotics residues are entering the human food chain in this way. It is high time now to think some alternatives to the antibiotics. Random use of antibiotics has prompted the microorganisms to develop biofilms. Antibiotics therapy should be followed with sensitivity tests after microorganism isolation and then administration of the particular antibiotics. It is recorded that mastitis is the major reason of antibiotics use in dairy industry (Moon *et al.,* 2006). Severe losses occur in terms of milk quality, health of animals, reproductive failure of animals, and affects human health as well.

Alternative Strategies to Antibiotics Therapy

a) Therapy using Nanoparticles

The nanoparticles derived from metal oxide are found to be effective antibacterial agents. Silver and Copper nano particles are found to be very effective against bacterial infection (Castellano *et al.,* 2007). Silver nano particles show the bactericidal and fungicidal action and can cause damage to the cell membranes through formation of silver ions in the microenvironment of the cell, denature the proteins and reactive oxygen species are formed (Kalinska et al. 2017). Zinc oxide nanoparticles (ZnO-NP) are widely used in biological field and experiments showed they are very much safe to be used in animals and humans (Fu et al. 2005). Study of Hozyen *et al.* (2019) showed that the potential of ZnO-NPs can be increased to make them suitable against the microorganisms causing mastitis. Different synthesizing method was followed in this aspect and capping agents used which influenced the agglomeration, size of the particles, and morphology. Sonochemical method was followed for particle synthesis and capping was done with starch which prevented the nanoparticles from agglomeration. This approach increased the antibacterial activity against *S. aureus, E. coli, and Klebsiella pneumoniae* isolated from milk of cows suffering from clinical mastitis (Hozyen *et al.,* 2019).

b) Therapy using Lactococcus lactis

A different approach was attempted by Kitching *et al.* (2019) for treatment of bovine mastitis. They optimized a formulation of *Lactococcus lactis* DPC3147, which produces 2-component bacteriocin lacticin 3147, presented in the liquid form emulsion based on paraffin and termed as live biotherapeutic compound. The efficacy of this compound was compared with the antibiotics terrexine (contains combination of kanamycin and cephalexin), and it was found that these biotherapeutics showed comparative efficacy as the antibiotics to treat the clinical and subclinical mastitis. Additionally, the *L. lactis* bacteria remained viable in the emulsion form for up to 5 weeks showing that a single dose can work for a long time and show its therapeutic activity.

c) Bacteriophage Therapy

A recent concept of targeting the bacteria with bacteriophages has taken the upper hand (Varela *et al.,* 2018). Geng *et al.* (2019) studied in the mice model (mastitis induced by *S. aureus* strain Sau-XJ-21), using two bacteriophage strains like vBSM-A1 and vBSP-A2, and observed convincing results. The cocktail of these two strains gave better result (synergistic effect) in reducing the population of S. aureus than the individual one.

Subclinical Mastitis

Very few symptoms are observed in this type of mastitis although infection persists in the mammary gland. There is reduction in milk yield with high somatic cell count (SCC), poor quality of the milk produced, and also affects the reproductive performance. Mostly *S.*

aureus, *S. agalacteae* etc. affect the mammary gland in this disease. It is found that biofilm is developed by *S. agalacteae* as observed in the isolates of milk from dairy cows affected with subclinical mastitis (Bonsanglia *et al.*, 2019). It is proposed that recurrent infections may attribute to biofilm growth and enhance resistance to host defense system and antimicrobial therapy. (Rosini and Margarit, 2015). Besides, *S. uberis* is also an important microorganism causing both the clinical and subclinical mastitis in dairy animals (Calonzi *et al.*, 2020). The cost effective multiplex PCR assays have been developed by Calonzi *et al.* (2020) to detect the virulence genes in these species and characterize the particular strain.A study in Switzerland on the microorganism *S. uberis* found 153 strains isolated from mastitic milk and the antibiotic susceptibility test was performed with 11 antibacterial agents including penicillin (Kappelli *et al.*, 2019). The researchers showed the importance of genetic characterization and their eradication. The importance of milk and milk products have been described thoroughly and stress is given on maintaining a disease-free health in human being (Nagpal et al., 2012). Various beneficial effects of milk has been discussed in many research findings and it is necessary to consume healthy milk for healthy life (Nagpal 2011; Nagpal et al., 2010; Kumar et al., 2009; Agarwal et al., 2020; Vise et al., 2018).

Preventive and Control Measures

The mastitis incidences can be minimized by proper management of the dairy herd. The measures to be taken are healthy rearing of animals, adopting sanitary practices, culling of the infected animals suffering from chronic infection, isolation of the infected animals in separate house etc. The somatic cell count (SCC) of the milk should be monitored at regular interval as SCC above 2, 00000 indicates unhealthy mammary gland. The animals once found to be diseased, should be promptly treated to cure from the disease. Personal hygiene should be maintained by the workers during milking, and during care and management of the animals. The animal houses should always be kept clean and dry. Strategies for clean milk production should be followed. The milking equipments should be kept clean and if possible, should be sterilized for subsequent use.

2. CONCLUSION

Mastitis has been the major concern in dairy industry due to the huge economic losses caused to the dairy farmers. Antibiotics therapy is found to have many drawbacks besides the development of resistance by the microorganisms. The antibiotic residues enter in human food chain raising major concern on human health. Some alternative therapies are considered at present which have potential to replace the antibiotic therapy. The therapies like bacteriophage therapy, nanoparticle based therapy, biotherapies, can take the upper hand in future for mastitis management. More research work is needed for early detection of the subclinical mastitis and clinical mastitis which can promote prompt treatment of animals with minimal loss to the farmers.

3. REFERENCES

[1] Atulya, M., Mathew, A.J., Rao, J.V., Mallikarjuna Rao, C. Influence of milk components in establishing biofilm mediated bacterial mastitis infections in cattle: a fractional factorial approach. Res Vet Sci; vol. 96, 2014, pp. 25–7.

[2] Bonsaglia, E.C.R., Latosinski, G.S., Rossi, R.S., Rossi, B.F., Possebon, F.S., Pantoja, J.C.F., Fernandes, Júnior A., and Rall, V.L.M.Biofilm production under different atmospheres and growth media by Streptococcus agalactiae isolated from milk of cows with subclinical mastitis.Arch Microbiol., 2019, doi: 10.1007/s00203-019-01727-8. [Epub ahead of print].

[3] Calonzi, D., Romano, A., Monistero, V., Moroni, P., Luini, M.V., Biscarini, F., Castiglioni, B., and Cremonesi, P. Technical note: Development of multiplex PCR assays for the molecular characterization of Streptococcus uberis strains isolated from bovine mastitis. J Dairy Sci.; vol. 103, No. 1, 2020, pp. 915-921.

[4] Castellano, J.J., Shafii, S.M., Ko, F., Donate, G., Wright, T.E., Mannari, R.J., Payne, W.G., Smith, D.J., and Robson, M.C.Comparative evaluation of silver-containing antimicrobial dressings and drugs.Int Wound J.; vol. 4, no. 2, 2007, pp. 114-22.

[5] Costa, J.C.M., Espeschit, I.F., Pieri, F.A., Benjamin Ldos, A., and Moreira, M.A. Increased production of biofilms by *Escherichia coli* in the presence of enrofloxacin. Vet Microbiol; vol. 160, 2012, pp. 488–90.

[6] Filioussis, G., Kachrimanidou, M., Christodoulopoulos, G., Kyritsi, M., Hadjichristodoulou, C., Adamopoulou, M., Tzivara, A., Kritas, S.K., and Grinberg, A.Short communication: Bovine mastitis caused by a multidrug-resistant, mcr-1-positive (colistin-resistant), extended-spectrum β-lactamase-producing Escherichia coli clone on a Greek dairy farm.J Dairy Sci. vol. 103, no. 1, 2020, pp. 852-857.

[7] Friman, M.J., Eklund, M.H., Pitkälä, A.H., Rajala-Schultz, P.J., and Rantala, M.H.J.Description of two Serratia marcescens associated mastitis outbreaks in Finnish dairy farms and a review of literature.Acta Vet Scand.; vol. 61, no. 1, 2019, p. 54.

[8] Fu, G., Vary, P.S. and Lin, C.T. Anatase TiO2 nanocomposites for antimicrobial coatings. J. Phys. Chem. B, vol. 109, no. 18, 2005, pp. 8889-8898.

[9] Geng, H., Zou, W., Zhang, M., Xu, L., Liu, F., Li, X., Wang, L., and Xu, Y. Evaluation of phage therapy in the treatment of Staphylococcus aureus-induced mastitis in mice. Folia Microbiol (Praha), 2019. doi: 10.1007/s12223-019-00729-9. [Epub ahead of print].

[10] Gomes, F., Saavedra, M.J., and Henriques, M. Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms.Pathog Dis.,vol. 74, no. 3, 2016, pp. 1-7.

[11] Gomes, F. and Henrique, M. Control of bovine mastitis: Old and recent therapeutic approaches. Curr. Microbiol., vol. 72, no. 4, 2016, pp. 377-382.

[12] Hermeyer, K., Jacobsen, B., Spergser, J., Rosengarten, R. and Hewicker-Trautwein, M.Detection of Mycoplasma bovis by in-situ hybridization and expression of inducible nitric oxide synthase, nitrotyrosine and manganese superoxide dismutase in the lungs of experimentally infected calves. J Comp Pathol; vol. 145, 2011, pp 240–50.

[13] Hertl, J.,Gröhn,Y., Leach, J. et al. Effects of clinicalmastitis caused by gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows. J Dairy Sci., vol. 93, 2010, pp. 1551–1560.

[14] Hozyen, H.F., Ibrahim, E.S., Khairy, E.A., and El-Dek, S.I. Enhanced antibacterial activity of capped zinc oxide nanoparticles: A step towards the control of clinical bovine mastitis.Vet World., vol. 12, no.8, 2019, pp. 1225-1232.

[15] Jamali, H., Barkema, H.W., Jacques, M., Lavallée-Bourget, E.M., Malouin, F., Saini, V., Stryhn, H., and Dufour, S. Invited review: incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows. J Dairy Sci., vol. 101, no. 6, 2018, pp. 4729–4746.

[16] Kalinska, A., Gołębiewski, M., and Wójcik, A. Mastitis pathogens in dairy cattle – a review. World Scientific News, vol. 89, 2017, pp. 22-31.

[17] Käppeli, N., Morach, M., Zurfluh, K., Corti, S., Nüesch-Inderbinen, M., and Stephan, R. Sequence Types and Antimicrobial Resistance Profiles of Streptococcus uberis Isolated From Bovine Mastitis. Front Vet Sci., vol. 6, 2019, p. 234.

[18] Kitching, M., Mathur, H., Flynn, J., Byrne, N., Dillon, P., Sayers, R., Rea, M.C., Hill, C., and Ross, R.P. A Live Bio-Therapeutic for Mastitis, Containing Lactococcus lactis DPC3147 With Comparable Efficacy to Antibiotic Treatment. Front Microbiol.; vol. 10, 2019, p. 2220.
[19] Kulkarni, A.G., and Kaliwal, B.B. Bovine Mastitis: A Review. International Journal of Recent Scientific Research, vol. 4, no. 5, 2013, pp. 542 -548.
[20] Liang, D., Arnold, L. M.,Stowe, C. J.,Harmon, R. J. and Bewley,J. M.Estimating US dairy clinical disease costs with a stochastic simulation model. J. Dairy Sci., vol. 100, 2017, pp. 1472–1486.
[21] Misra, N., Wines, T.F., Knopp, C.L., Hermann, R., Bond, L., Mitchell, B., McGuire, M.A., and Tinker, J.K. Immunogenicity of a Staphylococcus aureus-cholera toxin A2/B vaccine for bovine mastitis. Vaccine vol. 36, 2018, pp. 3513–3521.
[22] Moon, J.S., Lee, A. R., Kang, H. M., Lee, E. S., Joo, Y. S., Park, Y. H., Kim, M. N. and Koo, H. C. Antibiogram and coagulase diversity in staphylococcal enterotoxin producing Staphylococcus aureus from bovine mastitis. J.Dairy.Sci., vol. 90, 2006, pp. 1716-1724.
[23] Morales, P., Fernández-García, E., and Nunez, M. Caseinolysis in cheese by Enterobacteriaceae strains of dairy origin. Lett Appl Microbiol.; vol. 37, 2003, pp. 410–4.
[24] Petitclerc, D., Lauzon, K., Cochu, A., Ster, C., Diarra, M.S., and Lacasse, P. Efficacy of a lactoferrin-penicillin combination to treat {beta}-lactam-resistant Staphylococcus aureus mastitis. J Dairy Sci., 90, 2007, pp. 2778–2787.
[25] Rosini, R., and Margarit, I. Biofilm formation by Streptococcus agalactiae: influence of environmental conditions and implicated virulence factors. Front Cell Infect Microbiol., vol. 5, 2015, p. 6.
[26] Schukken, Y., Chuff, M., Moroni, P., Gurjar, A., Santisteban, C., Welcome, F., and Zadoks, R.The "other" gram-negative bacteria in mastitis: Klebsiella, Serratia, and more.Vet Clin North Am Food Anim Pract., vol. 28, no. 2, 2012, pp. 239-56.
[27] Schukken, Y. H., Tikofsky, L. and Zadoks, R. N. Environmental control for mastitis prevention, milk quality and food safety. In: Hogeveen, H. (ed.), Mastitis in dairy production Current knowledge and future solutions, Wageningen Academic Publishers, The Netherlands, 2005, pp.109-114.
[28] Sender, G., Pawlik, A., and Korwin, K.A. Current concepts on the impact of coagulase-negative staphylococci causing bovine mastitis as a threat to human and animal health – a review. Anim Sci Paper Rep., vol. 35, no. 2, 2017, pp. 123–135.
[29] Shpigel, N. Y., Elazar, S.and Rosenshine,I.Mammary pathogenic Escherichia coli. Curr. Opin. Microbiol., vol. 11, 2008, pp. 60–65.
[30] Stewart, P.S., and Costerton, J.W. Antibiotic resistance of bacteria in biofilms. Lancet vol. 358, 2001, pp. 135–8.
[31] Supa-Amornkul, S., Mongkolsuk, P., Summpunn, P., Chaiyakunvat, P., Navaratdusit, W., Jiarpinitnun, C. and Chaturongakul, S.Alternative Sigma Factor B in Bovine Mastitis-Causing Staphylococcus aureus: Characterization of Its Role in Biofilm Formation, Resistance to Hydrogen Peroxide Stress, Regulon Members.Front Microbiol.; vol. 10, 2019, p. 2493.
[32] Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., and O'Kennedy, R. Mastitis detection: current trends and future perspectives. Trends Biotechnol., vol. 27, 2009, pp. 486–493.
[33] Wall, R. J., Powell, A.M., Paape,M. J., Kerr, D. E., Bannerman, D. D., Pursel, V. G., et al. Genetically enhanced cows resist intramammary Staphylococcus aureus infection. Nat. Biotechnol. Vol. 23, 2005, pp. 445–451.

[34] Zandkarimi, F.,Vanegas, J., Fern, X.,Maier, C.S., and Bobe, G. Metabo types with elevated protein and lipid catabolism and inflammation precede clinical mastitis in prepartal transition dairy cows. J Dairy Sci., vol.101, no. 6, 2018, pp. 5531–5548.
[35] Nagpal R., Behare P.V., Kumar M., Mohania D., Yadav M., Jain S., Menon S., Parkash O., Marotta F., Minelli E., Henry C.J.K., Yadav H. Milk, Milk Products, and Disease Free Health: An Updated Overview. Critical Reviews in Food Science and Nutrition, 52(4), 2012, 321-333.
[36] Nagpal R., Behare P., Rana R., Kumar A., Kumar M., Arora S., Morotta F., Jain S., Yadav H. Bioactive peptides derived from milk proteins and their health beneficial potentials: An update. Food and Function, 2(1), 2011.
[37] Nagpal R., Kumar A., Arora S. In vitro probiotic potential of lactobacilli isolated from indigenous fermented milk products. International Journal of Probiotics and Prebiotics, 5(2), 2010.
[38] Kumar M., Mohania D., Poddar D., Behare P.V., Nagpal R., Kumar A., Aggarwal P.K. A probiotic fermented milk prepared by mixed culture reduces pathogen shedding and alleviates disease signs in rats challenged with pathogens.International Journal of Probiotics and Prebiotics, 4(3), 2009.
[39] Agarwal A., Pathera A.K., Kaushik R., Kumar N., Dhull S.B., Arora S., Chawla P. Succinylation of milk proteins: Influence on micronutrient binding and functional indices. Trends in Food Science and Technology, 97, 2020.
[40] Vise E., Garg A., Ghatak S., Karam A., Bhattacharjee U., Sen A., Shakuntala I., Puro K., Sanjukta R., Sharma N.R., Das S. Molecular Speciation of Mycobacterial Isolates from Raw Cow Milk Reveals Predominance of Mycobacterium chelonae.Proceedings of the National Academy of Sciences India Section B - Biological Sciences, 88(4), 2018.