International Journal of Science, Environment and Technology, Vol. 4, No 4, 2015, 1066 – 1072

ISSN 2278-3687 (O)
2277-663X (P)

BACTERIOLOGY AND ANTIBIOGRAM OF BOVINE MASTITIS IN RANCHI AND ITS VISINITY

Abhishek Kumar, S. Haque, K.K. Singh[1] and B.K. Roy[2]
Department of Veterinary Medicine, Ranchi Veterinary College, Kanke, Ranchi- 834006, Jharkhand, India
[1]Department of Pathology, RVC, Ranchi,
[2]Department of Pharmacology, RVC, Ranchi

[This paper is a part of Ph.D., the thesis submitted by first author to Birsa Agricultural University, Kanke, Ranchi-6.]

Abstract: The study was carried out to investigate the current antibiogram status of bovine mastitis in and around Ranchi. The prevalence of mastitis was assessed by the results of bacteriological evacuation of mastitic milk samples collected from mastitis cases. A total of 150 cases of mastitis were studied for isolation followed by antibiotic sensitivity test. The major prevalent pathogens isolated were *Staphylococcus aureus,* mixed infection*, Staphyloccus spp., E. coli, Streptococcus spp., Pseudomonas spp.* And *Klebsiella spp.* Antibiogram studies indicated that ciprofloxacin was the most effective antibiotic followed by Ceftriaxone, Enrofloxacin, Gentamicin, Amoxicillin+Sulbactam, Ampicillin+Cloxacillin and Ceftiofursodium.
Keywords: Bovine mastitis, antibiogram, bacteriology, ranchi.

INTRODUCTION

Mastitis or inflammation of mammary gland is the most common and most expensive disease of dairy cattle throughout the world. It is considered as a disease of major economic importance to the dairy farmers in India (Singh *et. al,* 1982). The estimated loss due to mastitis approximates Rs 60532 million/annum (Dua, 2001). Intramammary bacterial infection is the primary reason of bovine mastitis. Hence, Treatment of mastitis is solely based on control of infection by antibiotics. However, antibiotic treatment gets affected by difference in antibiotics sensitivity and emergence of drug resistant bacteria. Hence, identification of prevalent mastitis causing pathogens in particular area and its antibiotic resistance of the isolated bacteria are important prerequisites for implementation of effective control of mastitis. Considering these points in view the present study was undertaken to identify the mastitic agents and to study current antibiogram trend of mastitic bacteria in and around Ranchi, Jharkhand.

*Received July 3, 2015 * Published Aug 2, 2015 * www.ijset.net*

Materials and Methods

A total of 150 mastitic milk samples were collected from different locations in and around Ranchi on the basis of relevant history of individual animals. The bacteria were isolated in pure culture on Nutrient Agar. Further identification of bacteria was done on the basis of colony characters; Gram's staining reaction, growth patterns on Blood Agar and Mac Conkey Agar (i.e., hemolysis and lactose fermentation, respectively) followed by motility, morphology, arrangements and biochemical tests (Indol, MR, VP, Citrate and Catalase).

Minimal Inhibitory Concentration values of the bacterial organisms were analysed for different antimicrobials namely Enrofloxacin, Gentamicin, Ciprofloxacin, Amoxicillin+Sulbactam, Ampicillin+Cloxacillin, Ceftriaxone and Ceftiofur sodium. The disc diffusion method as described by Bauer *et al.*, 1966 was employed and the interpretation was made as per the interpretation chart provided by the manufacturer of discs depending on the diameter of zone of inhibition of bacterial growth.

Results and Discussion

In the present study mastitic agents were isolated from 68% cases, while no growth was evident in 32%. The failure of pathogens to grow *in-vitro* in high percentage of samples may be because of premedication of the animals with antibiotics, non-bacterial causes and the type of media that did not support the growth of whole range of bacteria associated with mastitis.

Microbiological study revealed that *staphylococcus aureus* was the most prevalent causative agents for bovine mastitis (37.25%) followed by mixed infection (17.65%), *staphylococcus spp.* (16.67%) and *E. coli* (11.76%). The lesser known cause of mastitis was found to be *streptococcus spp.* (7.84%), *pseudomonas spp.* (6.86%) and *klebsiella spp.* (1.96%). Our findings are in accordance with the findings of Mohini *et al.*, (2002); Turutoglu *et al.*, (2002) and Grewal *et al.*, (2005), who have also reported higher incidence of *staphylococcus aureus*. The prevalence of higher incidence of *staphylococcus aureus* may be attributed to the fact that the principal reservoirs of *staphylococcus aureus* are the skin of the udder and milk of the infected glands (Spencer and Lasmanis, 1952; Davidson, 1961). Further *staphylococcus aureus* is a contagious organism, with the capacity to penetrate into the tissue producing deep seated foci (Radostits *et al.* 2009). The organism thus, is protected by tissue barrier (Schalm *et al.*, 1971). Ability of *staphylococcus aureus* to establish within the mammary gland susceptibility to infection has been reported by Neave *et al.*, (1952) and Schalm and Woods, (1953) to be the major cause for higher incidence of *staphylococcus aureus* mastitis.

Higher incidence of coagulase negative staphylococci in the present study may be due to the fact that they are the most prevalent organisms associated with udder skin, the streak canal and the interior of mammary glands. The coagulase negative staphylococci have been reported to be more susceptible to leucocyte enzymes than *staphylococcus aureus* by Cohn and Morse, (1959) and Melly *et al.*, (1960). Clearance of coagulase negative staphylococci by leucocyte enzyme might possibly be the reason for lower incidence of mastitis by these organisms as compared to *staphylococcus aureus*.

The incidence of streptococcal mastitis in the present study was found to be lower than *E. coli* mastitis. The finding is contrary to the report of Bhattacharya *et al.*, (1995); Shukla *et al.*, (1998); and Nagal *et al.*, (1999) who have reported higher incidence rate for streptococcal mastitis. *Streptococcus agalactiae* which is the most common cause of bovine streptoccal mastitis is the only mastitis pathogen totally dependent upon the mammary gland for their survival in nature i.e. *Streptococcus agalactiae* is an obligate parasite of the epithelium and tissue of ruminant mammary gland. Through detection and segregation of infected cows, use of hygienic milking practice and intramammary infusion of antimicrobial agents, S*treptococcus agalactiae* can be eliminated (Schalm *et al.*, 1971; Gyles and Thoen, 1993a). Proper hygienic measure and antimicrobial medication of streptococcal mastitis by the dairy farmers must have reduced the incidence of streptococcus mastitis as recorded in the present study.

In the present study, incidence of *E. Coli* mastitis was distinctly lower than the staphylococcal mastitis, although it superseded streptococcal mastitis. Despite of the wide presence of *E. coli* in the environment, low or sporadic incidence of *E. coli* has also been reported by various workers, Bhattacharya *et al.*, (1995); Krukowski *et al.*, (1998) and Shukla *et al.*, (1998) which is in accordance with present findings. The organisms generally invade the udder through teat canal and establish a local infection confined to udder. Beyond that, the organism may not find suitable environment in side mammary gland for its growth and subsequent inflammatory changes. Opsonisation of bacteria by IgM with subsequent phagocytosis and killing by neutrophils, lactoferrin inhibiting multiplication of *E. coli* by binding iron and rendering it unavailable to bacteria are some of the factor which prevent multiplication or establishment of *E. coli* mastitis. On the other hand, capsulated *E. coli* escape phagocytosis to bring about coliform mastitis (Gyles and Thoen, 1993b). These inherent properties of udder defence against *E. coli* infection might be responsible for reduced incidence of *E. coli* mastitis in the present study.

One of the significant causes of bovine mastitis in the present study was pseudomonas as an important cause of bovine mastitis has also been reported by Sarma and Boro, (1980); Ramchandra *et al.*, (1984); Sudhaona *et al.*, (1985) and Mohini *et al.*, (2002). The organism has its natural habitat in soil, water and sewage. Thus, improper personal hygienic and udder hygienic measures must have been the contributing factor for higher incidence of pseudomonas mastitis. Pseudomonas mastitis as a result of failure to maintain asepsis at the time of intramammary infusion of antibiotics has also been reported by Tucker, (1950). The source of herd infection with *Pseudomonas spp.* has been attributed to the use of contaminated water for washing udder (Pickens *et al.*, 1926; Redaelli and Perini, 1960) as well as intramammary therapy with contaminated equipment (Tucker, 1954).

Klebsiella spp., a member of coliform group of organism, was a minor cause of bovine mastitis in the present study. Klebsiella as a minor cause of mastitis has also been reported by Misra *et al.*, (1993); Erer *et al.*, (1996); Wadhwa *et al.*, (1996); Mohini *et al.*, (2002) and Dudco *et al.*, (2003).

In-vitro effectiveness of antibiotics against bacterial isolates showed that Ciprofloxacin (49.02%) was highest sensitive against all bacterial isolates followed by Ceftriaxone (42.16%), Enrofloxacin (37.25%), Gentamicin (34.31%), Amoxicillin+Sulbactam (33.34%), Ampicillin+Cloxacillin (18.63%) and Ceftiofursodium (12.75%).

While considering the efficacy of antimicrobial agents on the basis of highly sensitive and moderately sensitive nature, Ceftriaxone (79.41%) was found to be most effective followed by Ciprofloxacin (76.47%), Enrofloxacin (71.57%), Gentamicin (70.59%), Amoxicillin+Sulbactam (55.88%), Ceftiofursodium (50.00%) and Ampicillin+Cloxacillin (49.02%). On the contrary antibiotics showing higher rate of resistance patterns were Ampicillin+Cloxacillin (31.37%) followed by Amoxicillin+Sulbactam (23.53%), Gentamicin (22.55%), Ceftriaxone (9.80%), Ciprofloxacin (8.82%), Enrofloxacin (8.82%) and Ceftiofursodium (4.90%).

While judging the antimicrobial agents by high to moderate sensitivity it was found that amongst the effective antimicrobials, Gram positive cocci was more sensitive to Ciprofloxacin (79.37%) followed by Ceftriaxone (79.37%), Enrofloxacin (76.19%), Gentamicin (68.25%), Amoxicillin+Sulbactam (65.08%), Ampicillin+Cloxacillin (60.38%) and Ceftiofursodium (47.62%) than the Gram negative bacilli. On the other hand, antimicrobials like Ceftriaxone (80.95%) followed by Gentamicin (76.19%), Ceftiofursodium

(71.42%), Ciprofloxacin (66.67%), Enrofloxacin (61.90%), Amoxicillin+Sulbactam(38.09%) and Ampicillin+Cloxacillin(33.34%) were found to be more effective against Gram negative bacilli than the Gram positive cocci. Moreover, highest sensitivity was observed for Ceftriaxone (79.42%) followed by Ciprofloxacin (76.47%) in both Gram positive and Gram negative organisms.

It was also observed that amongst Gram positive organisms, antimicrobials like Ciprofloxacin, Ceftriaxone and Enrofloxacin was more effective against *staphylococcus aureus* than other *staphylococcus spp.* Moreover, *staphylococcus spp.* showed more resistant to antimicrobials than *streptococcus spp.* except against Enrofloxacin and Ceftiofur sodium which were found to be more effective against *staphylococcus spp.* than *streptococcusspp.*

With regard to Gram negative organisms *E. coli* found to be more sensitive to Gentamicin (66.67%) followed by Ciprofloxacin (41.67%) and Enrofloxacin (41.67%) as compare to other Gram negative bacilli. Moreover *pseudomonas spp.* was more sensitive to Ampicillin+Cloxacillin (42.86%) than the other Gram negative bacilli. It was also noticed that Gram negative bacilli had developed higher drug resistant tendency towards Amoxicillin+Sulbactam and Ampicillin+Cloxacillin than other drugs under trial. *Klebsiella spp.* was highly resistant to Ampicillin+Cloxacillin than Gentamicin and Enrofloxacin however, it was highly sensitive to Ciprofloxacin and Ceftiofur sodium.

Finally it was noticed that majority of isolates both Gram positive and Gram negative were found highly sensitive to Ciprofloxacin except *E. coli* which was most sensitive to Gentamicin followed by Ciprofloxacin.

References

[1] Bansal, B.K.; K.B. Singh; R. Rohan; D.V. Joshi; D.C. Nauriyal and Rajesh Mohan (1995). Incidence of subcinical mastitis in some cow and buffalo herds in Punjab. *J. Res. PAU.* 32 (1): 79- 81.

[2] Bauer, A.M.; W.M.M. Kirby; J. C. Sherris and M. Turk (1966). Antibiotic susceptibility testing using a standard single disc method.*Am. J. clin.Pathol.* 45: 493- 496.

[3] Bhattacharya, D. and H. Rahman (1995). Antibiogram of pathogens isolated from case of bovine mastitis. *Indian Vet. J*. 72(4): 414-415.

[4] Buswell, J. (1995). Simple mastitis bacteriology for the practice.*In Practice,* 17: 426- 432.

[5] Cohn, Z.A. and S.T. Morse (1959). Interactions between rabbit polymorphonuclear leukocytes and staphylo cocci.*J.xp. Med.* 110: 419.

[6] Davidson, I. (1961). Observations on the pathogenic staphylococci in dairy herd during a period of six years. *Res. Vet. Sci.* 2: 22.

[7] Davidson, I. (1961). Observations on the pathogenic staphylococci in dairy herd during a period of six years. *Res. Vet. Sci.* 2: 22.

[8] Dudco, P. (2003). The microbiological examination of milk samples results conducted in the Northern great Poland region during the bovine mastitis control. *Annoles-Univ.-Moriae-Curie-Sklodowska-Sectio-DD,-Medicino-Veterinaria. 58: 103-116.*

[9] Erer, H.; M. Ates; M.M. Kiran.; M.K. Cifttci and O. Kaya (1996). Pathological and bacteriological studies on bovine mastitis.*VeterinerBilimleriDergisi*., 12 (1): 123-133.

[10] Grewal, K.D.; Gupta, M.P. and Singh, K.B. (2005). Therapeutic efficacy of Gentamycin in clinical cases of mastitis in buffaloes. *Indian Vet. J.,* 82 (2): 123-125.

[11] Gyles, C.L. and C.O. Thoen (1993a). Streptococcus, In: Pathogenesis of bacterial infection in animal. 2nd Edn. International book distributing Co., Lukhnow. Pp: 3-20.

[12] Gyles, C.L. and C.O. Thoen (1993b). E. Coli, In: Pathogenesis of bacterial infection in animal. 2nd Edn.International book distributing Co., Lukhnow. Pp: 164-187.

[13] Krukowski, H.; Majewski, T. and Popiolek, M. (1998). Changes in IgG concentration in bovine mastitis. *Medycyna-Weterynaryjna*. 54 (11): 770-771.

[14] Melly, M.A.; J.B. Thomison and D.E. Rogers (1960). Fate of staphylococci in leukocytes. *J. Exp. Med.* 112: 1121.

[15] Mohini, K.P.; R. Janaki and B. Gupta (2002). Diagnosis and therapy of udder mcroflora obtained from clinical mastitis cases in cross bred cows. *Indian Vet. Med. j*. 25: 365-366.

[16] Nagal, K.B.; Sharma, M.; Katoch, R.C. and Sharma, M. (1999). Etiology of bovine mastitis in and around Palampur in Himachal Pradesh.*Indian J. Anim. Sci.* 69 (3): 150-152.

[17] Neave, F.K.; M. Philips and A.T.P. Mattick (1952). Clinical mastitis in six herds free from streptococcisagalactiae. *J. Dairy Res.* 19: 14.

[18] Pickens, E.M.; M.F. Welsh and L.J. Poelma (1926). *Pyocyaneusbacillosis* and mastitis due to *pseudomonas aeuruginosa. Cornell Vet.* 16: 186.

[19] Radostits, O.; C.C. Gay; K.W. Hinchcliffand P.D. Constable (2009). Veterinary Medicine- A text book of the diseases of cattle, horse, sheep, pig and goats.10th Edn.Elsevier, a division of Reed Elsevier India (P) Ltd., Noida, U.P. (India).

[20] Redaelli, G. and G. Perini (1960). Contributoallo studio dellamastite bovina da *pseudomonasaeuruginosa. Arch. Vet. Ital.* 11: 273.

[21] Sarma, G. and B.R. Boro (1980). Isolation and sensitivity test of etiological agents from bovine mastitis. *Indian J. Anim. Hlth*. 19: 47-49.

[22] Schalm, O.F. and G.M. Woods (1953). The mastitis complex.*J. Am. Vet. Med. Ass.* 122: 462.

[23] Schalm, O.W.; E.J., Carroll and N.C. Jain (1971) Bovine mastitis. *1st Edn., Lea and Frbiger, Philadelphia*, USA. Pp. 102-108.

[24] Shukla, S.K.; D.C. Dixit; D.C. Thapliyal and A. Kumar (1998). Bacteriological studies of mastitis in dairy cows. *Indian Vet. Med. J.* 22: 261-264.

[25] Singh, N.; V.K. Sharma; H.B. Ranjan and Y.R. Sinha (1982). Incidence, economy and test efficacy of subclinical mastitis in dairy animals.*Ind. Vet. J.* 59: 693- 696.

[26] Spencer, G.R. and J. Lasmanis (1952). Reservoirs of infection of *micrococcus pyogenesis*in bovine mastitis. *Am. J. Vet. Res.* 13: 500.

[27] Sudhaona, O.; G.K. Nair; RM. Piliai and S. Sulochana (1985) *Kerela J. Vet. Sci*. **16**: 99.

[28] Tucker, E.W. (1950). *Pseudomonas* infection of bovine udder apparently contracted from contaminated treatment equipment and materials. *Cornell Vet.* 40: 95.

[29] Tucker, E.W. (1954). Studies on bovine udder infection with *pseudomonas auregunesa*and other closely related organisms. *Cornell Vet.* 44: 110.

[30] Turutoglu, H. and S. Mudul (2002). Is *E. coli* 0157: H_7 an etiological agent of bovine mastitis. *Israel J. Vet. Med.* 57 (2): 82-83.

[31] Wadhwa, D.R.; V.N. Rao; B. Prasad; Mamdeep Sharma and M. Sharma (1996). Clinical mastitis in cows in Palam Valley of Himachal Pradesh. *Indian Vet, J.* 73 (12): 1271-1273.