

ISOLATION OF BACTERIAL PATHOGENS FROM CLINICAL CASES OF MASTITIS IN BUFFALOES

K. Lakshmi Kavitha¹, K. Sambasiva Rao², K.Rajesh³ and K. Satheesh^{1,*}

ABSTRACT

A total of 60 milk samples, from buffaloes showing clinical and recurrent type of mastitis, as confirmed by both California mastitis test and Wide side test, were collected for bacteriological examination. As many as 65 bacterial pathogens were isolated, using different selective media and their biochemical characterization revealed *Staphylococcus aureus* (20%), *Streptococcus* spp. (18.5%), *Escherichia coli* (4.6%), *Pseudomonas aeruginosa* (3%), *Salmonella* spp. (7.6%), *Klebsiella pneumonia* (1.5%), *Proteus vulgaris* (1.5%), *Actinobacillus* spp. (1.5%), *Actinomyces* (1.5%), *Corynebacterium* spp. (1.5%), *Bacillus* spp. (3%), *Nocardia* (1.5%), *Haemophilus* (3%), and other unidentified gram negative organisms (30%). Among the contagious pathogens, the prevalence of *Staphylococcus* and *Streptococcus* was almost equal whereas *Escherichia coli* and *Salmonella* were predominant among environmental pathogens.

Keywords: buffaloes, *Bubalus bubalis*, mastitis, bacterial pathogens, milk

INTRODUCTION

Mastitis is a complex disease having multiple aetiology and majorly involving contagious and environmental pathogens. It is responsible for 70% reduction in milk production, 14% in premature culling, 9% in veterinary expenses, 7% in discarded or low grade milk (National Mastitis Council 1996). Worldwide the estimated economic losses were about USD 35 billion (Sharma *et al.*, 2007) whereas in India a total of Rs 6000 crores per year monetary losses were due to mastitis (Sudhan and Sharma, 2010; Rajan *et al.*, 2008). The present study concentrated on the isolation of different etiological agents from milk of buffaloes in and around Krishna district showing clinical and recurrent type of mastitis.

MATERIALS AND METHODS

A total of sixty milk samples were collected from buffaloes showing clinical as well as recurrent mastitis were collected. These were first tested by California Mastitis Test and White Side Test (Barnumt and Newbouldt, 1960) and later attempted for the isolation of bacterial pathogens

¹Department of Veterinary Pathology, *E-mail: kommalapatiin@yahoo.co.in

²Department of Veterinary Microbiology,

³Department of Veterinary Medicine, NTR College of Veterinary Science, Gannavaram, Krishna district, Andhra Pradesh, India

using Blood agar, Mac Conkey Lactose agar (MLA) and other specific media. These isolates were later confirmed by using different biochemical tests.

RESULTS AND DISCUSSION

All the milk samples collected from suspected cases were positive when tested by California Mastitis Test and White Side Tests. For primary isolation 5% Sheep Blood agar was proved to be good for the isolation of fastidious pathogens of mastitis and is in accordance with the findings of Farooq *et al.* (2008). Biochemical characterization of 65 different bacterial isolates (Table 1) were found to be *Staphylococcus aureus* (20%), *Streptococcus* spp. (18.5%), *Escherichia coli* (4.6%), *Pseudomonas aeruginosa* (3%), *Salmonella* spp. (7.6%), *Klebsiella pneumonia* (1.5%), *Proteus vulgaris* (1.5%), *Actinobacillus* spp. (1.5%), *Actinomyces* (1.5%), *Corynebacterium* spp. (1.5%), *Bacillus* spp. (3%), *Nocardia* (1.5%), *Haemophilus* (3%), and other unidentified gram negative organisms (30%) (Figure 1) which is in concurrence with Farooq *et al.*, 2008; Ashfaq *et al.*, 2008; Ali *et al.*, 2011. Among the 70% organisms that were confirmed, the prevalence of *Staphylococcus* was highest (20%) followed by *Streptococci*. Ali *et al.* (2011) also found 28.32% isolates from mastitis cases were Staphylococci and also noted that *Staphylococcus aureus* being the top ranking pathogen.

The high incidence of Streptococci (18.5%) may be attributed to the micro environment, overcrowding, poor hygiene and mangemental practices as Streptococcus infection is contagious one. Khanal and Pandit (2013) also reported the predominance of *Streptococcus* in clinical mastitis than *Staphylococcus* and *Escherichia coli*. In

the present study it was observed almost equal prevalence of *Staphylococcus* and *Streptococcus* with little variation in degree and this may be due to the presence of more number of river type, graded murrah buffalo and managemental practices including teat dipping lead to the increased incidence of contagious mastitis. Further it was also observed that the prevalence of coagulase negative *Staphylococcus aureus* was 85%.

Among the environmental pathogens, the occurrence of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* was less when compared to contagious pathogens. The environmental pathogens *Salmonella* spp. and *Escherichia coli* are the predominant organisms responsible for mastitis which is in accordance with Khanal and Pandit (2013) Dhakal *et al.* (2007) reported that among the coliform pathogens *E. coli* has shared 7.1% whereas *Klebsiella* 3.6%. The present study also revealed the incidence of *E. Coli* and *Klebsiella* as 4.6% and 1.5% respectively. Ali *et al.* (2011) also reported the increased incidence of *E.coli* (16.18%) than *Klebsiella* (5.2%) and *Enterococcus* (3.17%) which is in agreement with the present study.

The uncommon pathogens isolated from the clinical cases which cause mastitis sporadically were *Haemophilic*, *Pseudomonas*, *Bacillus*, *Actinobacills*, *Actinomyces*, *Nocardia* and these findings were in concurrence with Ali *et al.* (2011) and Sudhan and Sharma (2010). The present study revealed that the incidence of mastitis in Krishna district was mainly due to contagious pathogens *Staphylococcus* and *Streptococcus*. Whereas among the environmental and other pathogens *E. coli*, *Salmonella* had shown the predominance which have public health significance.

Table 1. The list of the organisms isolated from the milk samples.

S. No.	Organism
1. <i>Staphylococcus</i> sp.	13 (20%)
2. <i>Streptococcus</i> sp.	12 (18.5%)
3. <i>Actinomyces</i>	1 (1.5%)
4. <i>Corynebacterium</i>	1 (1.5%)
5. <i>Nocardia</i>	1 (1.5%)
6. <i>Bacillus</i>	2 (3%)
7. <i>Salmonella</i>	5 (7.6%)
8. <i>Escherichia Coli</i>	3 (4.6%)
9. <i>Pseudomonas</i>	2 (3%)
10. <i>Klebsiella</i>	1 (1.5%)
11. <i>Haemophilus</i>	2 (3%)
12. <i>Proteus</i>	1 (1.5%)
13. <i>Actinobacillus</i>	1 (1.5%)
14. Other Gram negative organisms isolated and not characterized	20 (30%)

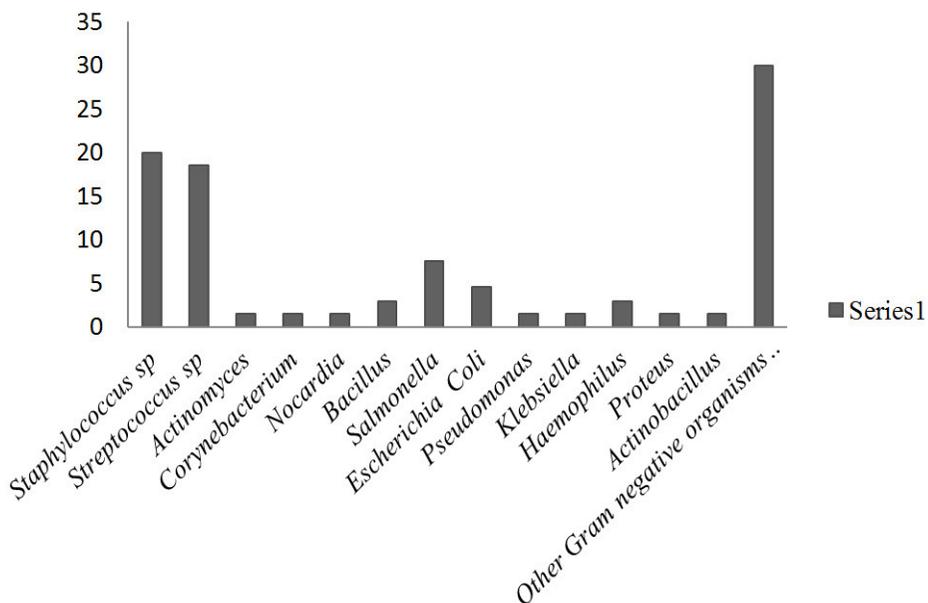


Figure 1. Percentage incidence of pathogens from clinical cases of mastitis.

REFERENCES

- Ali., M.A., M.D. Ahmad, K. Muhammad and A.A. Anjum. 2011. Prevalence and subclinical mastitis in dairy buffaloes of Punjab, Pakistan. *J. Anim. Plant. Sci.*, **21**: 477-480.
- Ashfaq. K. and G. Muhammad. 2008. Pathogens associated with bovine and bubaline mastitis in Peri-Urban areas of Faisalabad, Pakistan. *Pak. J. Life. Soci. Sci.*, **6**(2): 86-88.
- Barnumt, D.A. and F.H.S. Newbouldt. 1960. The use of California mastitis test for the detection of bovine mastitis. *Canadian Veterinary Journal*, **2**: 83-90.
- Dhakal, I.P., P. Dhakal, T. Koshihara and H. Nagahata. 2007. Epidemiological and bacteriological survey of buffalo mastitis in Nepal. *J. Vet. Med. Sci.*, **69**: 1241-1245.
- Farooq, A.A., S. Inayat, M.S. Akhtar and M. Mushtaq. 2008. Prevalence of mastitis and antibiotic sensitivity of bacterial isolates recovered from Nili Ravi buffaloes. *J. Anim. Plant. Sci.*, **18**: 76-77.
- Khanal, T. and A. Pandit. 2013. Assessment of sub-clinical mastitis and its associated risk factors in dairy livestock of Lamjung, Nepal. *Int. J. Infect. Microbiol.*, **2**: 49-54.
- National Mastitis Council 1996. *Current concepts in Bovine Mastitis*. <http://www.cowmasittis.com> Accessed September 9, 2012.
- Rajan, R., M.K. Gupta, K.K. Dingh and D.K. Jha. 2008. Comparative efficacy of Papanicolaou stain and Leishman stain in cytological study of bovine mastitis. *Indian J. Vet. Pathol.*, **32**: 277-279.
- Sharma, N., S.K. Maiti and K.K. Sharma. 2007. Prevalence, etiology and antibiogram of microorganisms associated with subclinical mastitis in Burg, Chattisgarh stat (India). *Int. J. Dairy Sci.*, **2**: 145-151.
- Sudhan. N.A. and N. Sharma. 2010. Mastitis- An important production disease of dairy animals, p. 72-88. *In Farm Management and Diseases*, Serva Manav Vikas Samiti, India.