Prevalence and antibiogram profile of bacterial Isolates from clinical bovine mastitis

Sumathi B.R, Veeregowda B.M and Amitha R. Gomes

Department of Veterinary Microbiology, Karnataka Veterinary,
Animal and Fisheries Sciences University, Hebbal, Bangalore - 560024.

Abstract

This study investigated the current status of clinical mastitis among dairy cattle in and around Bangalore. The prevalence of mastitis was assessed by the results of bacteriological evaluation of milk samples collected from clinical mastitis cases. A total of seventy five bacterial isolates were recovered from sixty clinical cases of mastitis affected cows. The prevalence of major pathogens isolated was twenty four per cent for *Staphylococcus aureus* twenty per cent for *Escherichia coli* followed by sixteen per cent for *Staphylococcus epidermidis* and *Streptococcus spp* and ten per cent for *Klebsiella spp*. Antibiogram studies were also performed for these isolates and Gentamicin was found to be the most effective drug. It was concluded that microbiological and antibiogram studies are necessary for treatment and control of the disease.

Keywords: Bovine mastitis, Antibiotic sensitivity and Prevalence

Mastitis in bovines has become extremely complex and the costliest disease in India. It affects 50% of the herd population (1). It has been estimated that the mastitis alone can cause approximately 70% of all avoidable losses incurred during milk production. One important reason for treatment failure is assumed to indiscriminate use of antibacterials without testing in vitro sensitivity of causal organisms (2). This practice at one hand increases economic losses and on other results in development of resistance to commonly used antimicrobials (3). To chalk out suitable antibiotic therapy, bacterial isolation and antibiotic sensitivity studies are always essential. Keeping these points in view the present study was undertaken to identify the bacterial causes of mastitis and to select a suitable antibiotic for treatment.

Material and Methods

Source of milk samples: 60 Milk samples from clinical mastitis cases were collected from different dairy farms located in and around Bangalore and from cases that were presented in the clinical complex, Veterinary College, Bangalore.

Relevant information about the farm, breed and history of individual animal were recorded.

Media reagents and chemicals: The media and reagents were either obtained from Hi-media, Mumbai or prepared in the laboratory as per the standard procedures of (4).

Identification of isolates : The pure cultures of bacterial isolates were further subjected for identification and confirmation. Individual colony characteristics and haemolytic patterns on blood agar were noted. The staining and cellular morphological features of organisms were ascertained by microscopic examination of Gram stained smears. Further each culture was subjected to various biochemical tests according to Kreig and Holt (5). The bacteria isolated were identified on the basis of their cultural, morphological and biochemical characteristics as per the method of Cruickshank (4).

Antimicrobial susceptibility testing: Minimal inhibition concentration (MIC) values of the bacterial organisms were analyzed for thirteen different antimicrobials (M/s Hi Media Laboratories Ltd., Mumbai) namely chloramphenicol (30µg), cephalexin (30 µg), ciprofloxacin (10 µg), colistin (Methane sulphonate (25 µg), enrofloxacin (10 µg), nitrofurantoin (300 µg), penicillin, furazolidone (50 µg), gentamicin (30 µg), tetracycline (30 µg), neomycin (30 µg), streptomycin (10 µg) and sulphadiazine (300 µg).

The disc diffusion method as described by Miles and Amyes (6) was employed and the interpre-tation was made as per the zone size interpretation chart provided by the manufacturer of discs.

Results and Discussion

A total of 75 bacterial isolates were recovered from 60 milk samples obtained from 60 clinical cases

of mastitis cows. Out of 60 samples, 21 samples (35%) yielded pure cultures of which 6 were gram-positive and 15 were gram-negative organisms and the remaining 39 (65%) yielded mixed cultures.

Of the 75 isolates, 49 (65.33%) were gram-positive and remaining 26 (34.67%) were gram-negative. The predominant bacterial isolates recovered were *Staphylococcus aureus* (24%) and *Escherichia coli* (20%) followed by *Staphylococcus epidermidis*(16%),*Streptococcus sp.* (16%), *Klebsiella sp*. (10.67%), *Bacillus sp*. (5.33%), *Corynebacterium sp.* (4%), *Proteus sp.* (2.66%) and *Pseudomonas sp.* (1.33%). The frequency of isolation of different bacterial species from clinical mastitis cases is depicted in Table1.

The high prevalence of *Staphylococcus sp*. followed by *E. coli* in the present study is in accordance with work of several other workers7,8,9. Staphylococci are the most important and prevalent mastitis causing organism globally, including India. Higher incidence of *E. coli* mastitis may be due to poor hygienic conditions, as *E. coli* originates from the cow's environment and infect the udder via the teat canal10.

The *in vitro* antibiogram studies of the bacterial isolates from mastitis milk revealed gentamicin to be most effective drug (90%) followed by enrofloxacin (88%),ciprofloxacin (85%),chloramphenicol (75%), tetracycline (60%), colistin (57%), neomycin (50%), nitrofurantoin (50%), furazolidone (50%), cephalexin (47%), penicillin (45%), streptomycin (35%) and sulphadiazine (30%).

Gentamicin, enrofloxacin, ciprofloxacin and chloramphenicol are newer chemotherapeutic agents and are less commonly used for treatment of mastitis in the area of study resulting in higher efficacy of these drugs. Gentamicin proved to be the drug of choice in this study. Similar antibiogram pattern were reported by other workers also12,13,14. More number of isolates showed resistance to cephalexin, penicillin, streptomycin and sulpha-diazine. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Production of plasmids mediated beta-lactamase enzymes is supposed to be mainly responsible for resistance to penicillin. Since, streptomycin has been extensively used along with penicillin for treating mastitis; it may have led to the development of high resistance in bacteria against this antibiotic. Whereas the resistance to sulphadiazine could be due to either low affinity of the enzyme that uses the p-amino benzoic acid during folic acid synthesis or use of preformed folic acid from surroundings.

References

1. Garg DN (2001): Emergence of bovine mycoplasmal mastitis in India Round table Conference on mastitis (IAAVR). No.2:1-9.
2. Saxena RK, Dutta GN, Borah, P & Buragohain J (1993): Indian Vet J 70:201-203.
3. Owens WE, Ray CH, Watts JL & Yancey RJ (1997): J Dairy Sci 80:313-317.
4. Cruickshank R, Duguid JP, Marmion BP & Swain RHA (1975) Medical Microbiology. Vol. II, 12th edn, CrurchillLivingstone, New York, 31-57p & 96-218p.
5. Kreig NR & Holt JG (1984) Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore.
6. Miles & Amyes (1996): Practical Medical Microbiology, 151-178p.
7. Aarestrup FM, Wegener HC, Rosdahl VT & Jensen NE (1995): Acta Veterinaria Scandinavica 36: 475-487.
8. Bartlett PC, Miller GY, Lance SE & Heider LE (1992): Preventive Vet Med 12: 59-71.
9. Waage S, Mork T, Roros A, Aasland D, Hunshamar A & Odegaard SA (1999): J Dairy Sci 82: 712-719.
10. Mallikarjunaswamy MC & Murthy GVK (1997): Indian Vet J 74: 885-886.
11. Goswami P, Biswas S & Podder RC (2002): Indian J of Animal Health 41: 52-54.

Table1. Frequency of isolation of different bacterial species from clinical mastitis milk samples.

Sl. No	Bacterial species	No.of Isolates	Per cent
	Gram positive organisms		
1.	*Staphylococcus aureus*	18	24.00
2.	*Staphylococcus epidermidis*	12	16.00
3.	*Streptococcus sp.*	12	16.00
4.	*Bacillus sp.*	4	5.33
5.	*Corynebacterium sp.*	3	4.00
	Gram negative organisms		
1.	*Escherichia coli*	15	20.00
2.	*Klebsiella sp*.	8	10.67
3.	*Proteus sp.*	2	2.67
4.	*Pseudomonas sp.*	1	1.33