**Pakistan Vet. J., 2006, 26(1): 20-22.**

# STAPHYLOCOCCAL MASTITIS IN BOVINES AND SOME PROPERTIES OF STAPHYLOCOCCAL ISOLATES

M. ARSHAD, G. MUHAMMAD[1], M. SIDDIQUE, M. ASHRAF AND H.A. KHAN

Department of Veterinary Microbiology, [1]Department of clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad, 38040, Pakistan

## ABSTRACT

Milk samples of 100 clinically mastitic bovines (50 each from buffaloes and cows) were processed for bacterial isolation. A total of 90 bacterial isolates, including 33 of staphylococci and 57 of other bacterial species were recovered. Of the 33 staphylococcal isolates, 23 were identified as *Staphylococcus aureus* and 10 as *Staphylococcus epidermidis*. All *Staphylococcus aureus* isolates were positive for deoxyribonuclease and mannitol fermentation test and produced ß-haemolysis on blood agar. All *Staphylococcus epidermidis* isolates were negative to these tests and failed to produce ß-haemolysis on blood agar containing 5% sheep erythrocytes. Penicillin resistance was detected in 13(56.5%) isolates of *Staphylococcus aureus* and 8(80%) isolates of *Staphylococcus epidermidis*. Penicillinase was produced by 4 of the 13 penicillin resistant isolates of *Staphylococcus aureus* and 2 of the 8 penicillin resistant *Staphylococcus epidermidis* isolates.

**Key words**: Mastitis, staphylococci, biochemical properties.

## INTRODUCTION

Bovine mastitis is caused by various bacteria. *Staph. aureus* and *E. coli* are often present in the cow's skin and in the environment around the cow (Jubb *et al.*,1993). Staphylococci are currently the most frequently isolated microorganisms from bovine mammary glands of cattle and buffaloes (Watts, 1988; Kupur *et al.*, 1992). The genus staphylococcus consists of 28 species (Birgerssen *et al.*, 1992), 14 of which have been isolated from the bovine udder (Watts, 1988). In most countries, *Staph. aureus* is the predominant cause of subclinical mastitis (Singh and Buxi, 1982) and is also frequently isolated from the clinical cases (Kupur *et al.*, 1992). These isolates show haemolytic activity on sheep blood agar, Deoxyribo-nuclease activity, and coagulase activity (Patrick *et al*., 2002). The frequent association of these organisms with increased somatic cell counts in milk with considerable production losses (Timms and Schultz, 1987; Rainard *et al.*, 1990), as well as their occasional association with clinical mastitis, has spawned considerable interest in these organisms.

There is considerable evidence of drug resistance reflected by strains of staphylococci other than *Staph. aureus* and mostly it is thought to be mediated by penicillase production (Owens and Watts, 1988). Recently, these organisms have been shown to act as donors of antibiotic resistance genes for *Staph. aureus* (Muhammad *et al*., 1993).

A detailed knowledge of the resistance determinants and biochemical characteristics of staphylococci is important not only in assessing the clinical significance of these organisms, but also in their epidemiological typing. The purpose of the study reported here was to investigate the relative prevalence, and to determine the biochemical profiles, of staphylococcal isolates from cattle and buffaloes suffering from mastitis.

## MATERIALS AND METHODS

Milk samples of 100 mastitic cases comprising 50 buffaloes and 50 cattle were collected aseptically and processed for bacteriological examination, using standard techniques recommended by Collee *et al.* (1989) and National Mastitis Council of United States (Anonymous, 1990). Deoxyribonuclease (DNAase) activity, coagulase production and mannitol fermentation tests were performed and haemolytic properties of staphylococcal isolates were studied on blood agar containing 5 percent sheep blood, as described by Cruickshank (1975) and Cheesbrough (1989). Sensitivity to penicillin was tested by disc diffusion method following the recommendations of National Committee for Clinical Laboratory Standards. Penicillin-resistant staphy-lococcal isolates were screened out and resistance to penicillin mediated by penicillinase production was detected by nitrogen hydrolysis (Cheesbrough, 1989).

## RESULTS AND DISCUSSION

A total of 90 bacterial isolates of different species were recovered on processing 100 mastitic cases of cows and buffaloes. Of these, 44 were recovered from buffaloes and 46 from cattle. Staphylococcal isolates accounted for 36.67 percent (33/90) of all bacterial isolations; being 40.9 percent in buffaloes and 32.6 percent (15/46) in cattle. Of the 33 staphyloccocal

isolates, 23 were found to be *Staph. aureus* (14 from buffaloes and 9 from cattle) and 10 *Staph. epidermidis* (4 from buffaloes and 6 from cattle). Aguilerd (1987) recorded the relative prevalence of *Staph. aureus* as 27.8 percent in bovines, whereas the relative distribution of *Staph. epidermidis* was reported to be 14.6 percent by Filho *et al.* (1985).

All *Staph. aureus* isolates were ß-haemolytic and positive for coagulase, DNAase production and mannitol fermentation test, whereas all the isolates of *Staph. epidermidis* were negative for these tests. These findings concur with those of Devries and Oeding (1976), who reported that production of coagulase and DNAase remained a constant property of bovine strains of *Staph. aureus*. With respect to DNAase production, our results are in conflict with those of Langlois *et al.* (1990), who found that 42 percent of *Staph. epidermidis* isolates were positive for DNAase activity. For the mannitol fermentation, our results are in disagreement with those of Oyekunle and Adetosoye (1988). These workers reported that a higher percentage (100%) of coagulase-negative isolates than coagulase-positive isolates (88.2%) of bovine staphylococci fermented mannitol.

Higgins and Chartier (1984) reported that coagulase-positive staphylococci were mostly positive to DNAase and mannitol fermentation reaction. These tests alongwith the test for hyaluronidase production were found satisfactory for differentiation of *Staph. aureus* and coagulase-negative staphylococci. Erasmus (1985) also found that coagulase-positive staphylococcal species fermented mannitol and produced catalase. According to Devries and Oeding (1976), characteristics of staphylococci derived from certain hosts can vary between geographic regions and strains recovered from different animal species may also differ from one another. In addition, the biochemical reactions of staphylococci have been shown to vary within the same gland over time (Maisi and Riipinen, 1991).

Resistance to penicillin among staphylococci isolated from mammary glands is wide spread (Sing and Buxi, 1982). Three types of resistance to antibiotics are recognized in staphylococci. Of these, penicillinase production mediated by plasmids is considered to be the most common form of penicillin resistance among staphylococci, although the percentages of such strains vary between countries (Anderson, 1983). In the present study, 56.5 percent of the isolates of *Staph. aureus* and 80 percent of *Staph. epidermidis* were found resistant to penicillin. Resistance to penicillin through penicillinase production was detected in 30.8 and 25.0 percent of the *Staph. aureus* and *Staph. epidermicis*, respectively.

Iqbal *et al*. (1984) found that 92.86 percent of *Staph. aureus* isolates from cow milk were resistant to penicillin. In 84.6 percent of these isolates, resistance to penicillin was associated with pencillinase production. Almost similar results have been reported by Owens and Watts (1988). English workers (Jones and Health, 1985) found that 66.1 percent of 221 *Staph. aureus* isolates tested were positive for penicillinase, as assayed by the nitrocefin test which is more than two fold of the magnitude recorded in the present study. This difference in the proportions of penicillin resistant *Staph. aureus* isolates in the present and other studies cited above could be related to differences in the quantum of penicillin used in different parts of the world.

Penicillinase-negative penicillin resistant staphylococcal strains might develop resistance to penicillin by virtue of other mechanisms. In view of the fact that a large proportion (15/21) of staphylococci in the present study showed resistance to penicillin by some mechanism(s) other than penicillinase production, further work is needed to determine nature of such mechanism(s).

## REFERENCES

Aguilerd, R., 1987. Clinical and microbiological studies of mastitis in dairy cows at the end of lactation. Revrista de Salud Animal, 9(4): 318-323.

Anderson, J. C., 1983. Veterinary aspects of staphylococci. In: Staphylococci and Staphylococcal Infections. Vol. 1. Adlam, C. and C. S. F. Easmon (eds), Academic Press, New York, USA, pp: 193-241.

Anonymous, 1990. Microbiological Products for the Diagnosis of Bovine Udder Infection. 3rd Ed., National Mastitis Council, Avlington, Virginia, USA.

Birgerssen, A., P. Jonsson and O. Holmberg, 1992. Species identification and some characteristics of coagulase-negative staphylococci isolated from bovines udders. Vet. Microbiol., 31: 181-189.

Cheesbrough, M., 1989. Medical Laboratory Manual for Tropical Countries, Vol. 11, ELBS, London, UK.

Collee, J. G., J. P. Duguid, A. G. Fraser and B. P. Marmion, 1989. Mackie and McCartney Practical Medical Microbiology, Vol. 2, 13th Ed., Churchill Livingston, London, UK.

Cruickshank, R., J. P. Duguid and R.H.A. Swain, 1975. Medical Microbiology, Vol. 2, 12th Ed., The English Language Book Society, Churchill, Livingstone, Edinburgh, UK.

Devries, L. A. and P. Oeding, 1976. Characteristics of *Staphylococcus aureus* strains isolated from different animal species. Rev. Vet. Sci., 21: 284-294.

Erasmus, J. A., 1985. Some features of coagulase positive staphylococci from bovine milk. I. Carbohydrate metabolism, comparison of conventional techniques and the API 50 CH system. Onderrstepoort. J. Vet. Res., 52: 25-29.

Filho, A. N., R. P. Schocken-Inturrino, O. D. Jr. Rossi and E. M. Cembranelli, 1985. Prevalence and aetiology of bovine mastitis in the Ribeirao Preto region, Saopaul. Pesquisa Veterinaria Brasileria, 5(2): 53-56.

Higgins, R. and P. Chartier, 1984. Identification of coagulase positive staphylococci of animal origin. Medicine Veterinarie du Quebec, 14(2): 61-65.

Iqbal, J., A. S. Hashmi and M. Ashfaq, 1984. Incidence of penicillin resistant staphylococci. Pakistan Vet. J., 4: 169-170.

Jones, T. O. and P. J. Heath, 1985. ß-lactamase production in *Staphylococcus aureus* isolated from bovine mastitic milk. Vet. Rec., 28: 1985.

Jubb, K. V. F., P. C. Kennedy and N. Palmer, 1993. The mammary gland. In: "Pathology of Domestic Animals" 4th Ed. Vol. 3. Academic Press, San Diego, California, USA, pp: 454-470.

Kupur, M., P. Anthusharma and R. M. Bhardwal, 1992. Bacteriology of clinical mastitis in buffaloes. Buffalo Bulletin, 11: 32-35.

Langlois, B. E., A. K. Parlindungan, R. J. Harmon and K. Akers, 1990. Biochemical characteristics of staphylococcus species of human and bovine origin. J. Food Protec., 53(2): 119-126.

Maisi, P. and L. Riipinen, 1991. Pathogenicity of different species of staphylococci in caprine udder. British Vet. J., 147: 126-132.

Muhammad, G., K. H. Hoblet, D. J. Jackwood, S. Bech-Nielsen and K. L. Smith, 1993. Interspecific conjugal transfer of antibiotic resistance among staphylococci isolated from the bovine mammary gland. Amer. J. Vet. Res., 54: 1432-1440.

Owens, W. S. and J. L. Watts, 1988. Antimicrobial susceptibility and ß-lactamase testing of staphylococci isolated from dairy herds. J. Dairy Sci., 71(7): 1934-1939.

Oyekunle, M. A. and A. I. Adetosoye, 1988. Biochemical characterization of staphylococci isolated from Nadama Cattle. Trop. Vet., 6: 48-54.

Patrick, B., P. Kuhnert, D. Hüssy and M. Schaellibaum, 2002. Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. J. Clin. Microbiol., 41(2): 767-771.

Rainard, P., M. Ducelliez and B. Poutrel, 1990. The contribution of mammary infections by coagulase negative staphylococci to the herd bulk milk somatic cell count. Vet. Res. Commun., 14: 193-198.

Singh, K. B. and K. K. Buxi, 1982. Studies on etiology, *in vitro* sensitivity and treatment of subclinical mastitis in milch animals. Indian Vet. J., 59: 191-198.

Timms, L. L. and L. H. Schultz, 1987. Dynamics and significance of coagulase negative staphylococcal intramammary infections. J. Dairy Sci., 70: 2648-2657.

Watts, J. L., 1988. Etiological agents of bovine mastitis. Vet. Microbiol., 16: 41-66.