The Journal of Animal and Plant Sciences, 22(3 Suppl.): 2012, Page: 217-220
ISSN: 1018-7081

CLINICAL AND SUBCLINICAL *STAPHYLOCOCCUS AUERUS* IN DAIRY BUFFALOES: DISEASE CHARACTERISTICS AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF ISOLATES

A. Hussain, , A. Shakoor*, A. Yousaf**, S. U. Rehman*** and M. A. Zaman***

Livestock and Dairy Development, Azad Kahmir-Pakistan, *College of Veterinary and Animal Sciences, Jhang-Pakistan, **Department of Clinical Medicine and Surgery and ***Department of Microbiology, University of Agriculture Faisalabad, Pakistan
Corresponding Author: muhammadarfan2003@hotmail.com

ABSTRACT

This study was conducted to evaluate the disease characteristics of *Staphylococcus aureus* mastitis in buffaloes and antibiotic susceptibility of isolates. Visual inspection and palpation indicated as; hind quarters were asymmetric (n=2), udder and teat wounds (n=2), scar tissue (n=1), warts on teats (n=1) and all others parameters were normal. A total of 100 milk samples (20 clinical and 80 subclinical) from mastitic buffaloes were collected from different government and private livestock farms in and around Faisalabad. These samples were subjected to antibiotic susceptibility testing. Sixty isolates of Staphylococcocci were obtained on the basis of growth on Staph-110 medium, colony morphology and haemolytic pattern on 5% sheep blood agar plates. All sixty isolates were gram positive and catalase positive. Of these, 23 were slide coagulase test, Tube coagulase test and Staphytect plus test positive and remaining 37 were negative. Those 23 were identified as *Staphylococcus aureus* (coagulase positive Staphylococci) and the remainder were coagulase negative Staphylococci. All these 23 isolates of *Staphylococcus aureus* were subjected to antibiotic sensitivity testing against the most commonly used antibiotics in mastitis (i.e. enroflaxacin, ciprofloxacin, chloramphenicol, amoxicillin, ampicillin, gentamycin, novobiocin, oxytetracycline and cotrimaxazole). The antibiogram study of these antibiotics in the form of their percent sensitivity was as co-trimoxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamicin (86.95), ampicillin (82.60 %), ciprofloxacin (82.60%), chloramphenicol (82.60%), enrofloxacin (69.56%) and novobiocin (60.86%).

Key words: Mastitis, *Staphylococcus aureus*, Buffalo, antibiogram.

INTRODUCTION

Pakistan is an agricultural country and livestock is it's an important sector which contributes 52 % of the total agricultural value added and overall national GDP has steadily increased to 12 % GDP (Anonymous, 2008). Pakistan dairy industry is fragmentary in nature but the trend of organization is increasing day by day in response to a high demand of milk and milk products. Buffaloes and cattle are major dairy animals. Twenty nine million buffaloes and 31.5 million cattle are producing 42 million and 199 thousands tons fresh milk to be consumed all over the country. Buffaloes' share in this production is 71.30 %. So, buffalo is the mainstay of dairy industry in Pakistan. Of the major factors responsible for affecting the milk production of buffaloes, one is the mastitis which is considered the most costly disease (DeGraves and Fetrow, 1993; Kossaibati *et al*., 1998) and is a worldwide problem among dairy animals (Colditz and Watson, 1985; Lightner *et al*., 1988). A wide variety of micro-organisms have been associated with mastitis (Bramley and Dodd, 1984; Kapur *et al*., 1992 and Allore, 1993) of these, *Staphylococcus aureus* is the most prevalent as per findings of the studies undertaken between 1966 and 2002 in Pakistan (Razzaq, 1998; Memon *et al*., 1999). It also indicated that 50 % cases of mastitis in buffaloes are caused by *S. aureus*. This organism is able to produce a host of structural changes in udder and keeps on developing resistance against the most commonly used antibiotics. A voluminous body of literature is available about disease characteristics in cattle but information about this aspect is scanty in buffaloes. Against this backdrop, this study was designed with the following objectives

1. To study disease characteristics with relevance to *S. aureus*
2. To perform antibiogram study against *S. aureus* isolates from buffaloes.

MATERIALS AND METHODS

A total of 560 quarter milk samples from 140 buffaloes were screened for subclinical mastitis by Surf Field Mastitis Test (Muhammed *et al*., 1995) and 20 quarter milk samples were collected from 10 mastitic buffaloes on the basis of clinical signs and symptoms of mastitis.

As per guidelines of National Mastitis Council (Naitonal Mastitis Council, Inc., 1990) for collecting the samples to culture and isolate the *S. aureus*, a total of 100 quarters' mastitic milk samples (20 clinical and 80 subclinical) were collected from buffaloes, aseptically in sterilized screw capped glass vials from subclinical and clinical cases of mastitic buffaloes from the Government and different private livestock farms around Faisalabad.

All the milk samples were cultured on Staph. 110 Medium as a selective medium for *S. aureus* and 5% sheep blood agar for checking the haemolytic properties of Staphylococcal species.

Identification of *S. aureus* was made by Gram's staining, Catalase test, Coagulase test (slide and tube) and Staphytect plus (Latex Slide Agglutination test) as per recommendations of Essers and Radebold (1980)

Staphytect plus (Oxoid, Basingstoke Hampshire, UK) is a slide agglutination test for the identification of *S. aureus* by detection of clumping factor, protein A and certain polysaccharides found in *S. aureus*.

Palpation of mastitic quarters along with teats was made to find the disease characteristics relanvant to *S. aureus*. Clinically infected teats along with their quarters were manipulated and visualized immediately after complete milking as per method desribed by Klaas *et al*., (2004) i.e. clinical variables asymmetry between front quarters, asymmetery between hind quarters (slight, pronounced, complete atrophy of on quarter), acute clinical mastitis. Udder oedema (slight, larger area of udder, most of the udder), Knotty tissue (present or not), fibrosis (present or not), udder shape (normal, small, long abdominal, backward bulging, slanting, deep and slanting), teat shape (normal, short, conical, fleshy, udder and teats' wounds, scar tissue in teat canal, skin quality of udder and teats, warts on teat, wounds on warts and soiling on udder and teats.

In vitro antibiotic susceptibility of *S. aureus* to 9 antibiotics (ampicillin, amoxicillin, oxytetracycline, chloramphenicol enrofloxacin, crprofloxacin, cotrimoxazole, gentamycin and novobiocin) was determined by using disk diffusion method. Antibiotic susceptibility was done according to the standards of National Commettee for Clinical Laboratory Standards (NCCLS), now called Clinical Laboratory Standards Institute (CLSI, 2005). *S. aureus* ATCC 25923 (American Type Culture Collection, Rockiville, Maryland, USA) was used as the sensitive quality control organism. Data of the disease characteristics of *S. aureus* mastitis and antibiotic susceptibility profiles of *S. aureus* is presented in numerical number and percentages

RESULTS AND DISCUSSION

The disease characteristics of *S. aureus* clinical mastitis were as, hind quarters were asymmetric (n =2), clinical symptoms (n=2), udder and teat wounds (n=2), udder and teat wounds (n=2), one scar tissue (n=1), one having warts on teat and all others parameters were normal. These wounds may be ascribed to the presence of *S. aureus* infection because of harboring the skin microflora especially *S. aureus*, which also causes the mastitis sometimes (Sears, 1993). This is in line with the findings of Miline *et al*. (2003) who recorded similar clinical features in cows like swollen or hard udders along with others but these were not correlated with *S. aureus* and were in general. Such disease characteristics of *S. aureus* mastitis are not reported in buffaloes so far.

Of 20 milk samples of clinical mastitis, only two yielded growth on Staph-110 medium. This may be ascribed to instantaneous use of antibiotics in the animals by the owners themselves. Of 80 milk samples of subclinical mastitis, only 21 grew on Staph-110 medium and blood agar, indicating the nature of organism as haemolytic *S. aureus*.This lack of growth on the medium may also be due to indiscriminate use of antibiotics at the farm by the owners as a remedial measure just at the outset. This practice is quite common.

Staph-110 medium was used as a selective medium for the isolation of *S. aureus* and 5% sheep blood agar to assess the type of hemolytic activity of different *S. aureus* isolates. These findings are in agreement with the findings of several workers (Cruckshank *et al*., 1975; Buxton and Frasser, 1977 and Power, 1988).

Of the 100 mastitic quarters' milk samples, only 60 grew on Staph.110 medium. Among these 60, only 33 were found haemolytic and 27 non-haemolytic. Of these 33 haemolytic, 20, 11 and 2 were found alpha, Beta, and alph-beta haemolytic, respectively.

Of the 60 *S. aureus* isolates, all were catalase positve but 23 were coagulase positive and 37 were negative. Upon subjecting to Staphytect plus kit (Latex agglutination test) 23 were positive and 37 were negative. These confirmed 23 S.aureus isolates were subjected to the touch stone of antibiotic sensitivity testing.

According to Essers and Redebold (1980), Latex agglutination test is a rapid and reliable test for the identification of *S. aureus*. It is based on the detection of clumping factor, protein A and certain polysaccharides of *S. aureus*.

A total of 23 isolates of *S. aureus* from mastitis were evaluated through disk diffusion method for antibiotic susceptibility testing. The tested chemotherapeutic agents, cotrimoxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (86.95%), ampicillin (82.60%), ciprofloxacin (82.60%) chloromphenicol (82.60%), enrofloxacin (69.56%) and novabiocin (60.86%) showed sensitivity. It was contrary to the findings of Zahid (2004) who found gentamycin as the drug of choice on the basis of drug sensitivity for the treatment of clinical mastitis in buffaloes, while Aziz *et al*., (1977) reported that *S.*

aureus deriving from cattle was sensitive to oxytetracycline. On the other hand, Rashid (2001) concluded gentamycin sensitive 62 % against *S. aureus* mastitis in buffaloes. Conducting similar studies, Fazal-ur-Rehman (1995) concluded that gentamycin, chloromphenicol, cotrimaxazole, amoxicillin and oxytetracycline showed an in vitro efficiency over 90%. Novobiocin had efficiency between 80 to 90 % and ampicillin efficiency was less than 80%. Rossetti (1993) found that *S. aureus*, the most commonly isolated organism from mastitis was 100% sensitive to gentamycin, chloromphenicol and cotrimoxazole and were on percent sensitive to oxytetracyline. The results of this study do not correspond to the findings of present study. Hodges *et al*.(1984) also observed a similar pattern of sensitivity while conducting studies on bovine mastitis in Newzealand, *S. aureus* isolates were 80-90% sensitive to oxytetracycline and novobiocin. Chanda *et al*. (1989) reported that gentamycin was the most effective antibiotic for Staphylococci followed by ampicillin, oxytetracycline and chloromphenicol. Khan *et al*. (2005) drew a conclusion that the antibiogram analysis of gentamycin, ciprofloxacin, chloromphenicol, cotrimoxazole showed more than 90% sensitivity to *S. aureus* derived from buffaloes and cows. Iqbal *et al*. (2004) made an antibiotic susceptibility testing in buffaloes and cattle and they concluded that gentamycin and enrofloxacin were the most effective drugs against *S. aureus*. The intermittent changing pattern of antibiotic susceptibility against *S. aureus* may be ascribed to the extent of different antibiotics to be used from locality to locality.

This study has divulged the co-trimoxazole as the most sensitive chemotherapeutic agent against mastitis in buffaloes. So, it is mandatory that antibiogram study be made from time to time in a locality to have an idea of the most effective drugs against the prevailing mastitogens i.e. bacteria.

REFERENCES

Allore, H. G., (1993). A review of the incidence of mastitis in buffaloes and cattle. Pakistan Vet. J., 13: 1-7.

Anonymous, (2008). Economic Survey of Pakistan 2008-2009. Finance Division, Economic Advisor's Wing. Government of Pakistan, Islamabad.

Aziz, M. A., M. A. Ghani and M. T. Muhammad (1977). Studies on Staphylococcocal subclinical bovine mastitis with special reference to treatment by penicillin, Mardadal combination (Masticillin M). J. Egyptian Vet. Med. Assoc., 36: 69-78

Bramley, A. J. and F. H. Dodd (1984). Mastitis control: progress and prospects. J. Dairy Sci., 5: 481-485

Buxton, A and G. Frasser (1977). Animal Microbiology, Immunology, Bacteriology and Mycology. 1: pp 241-246. Blackwell Scientific Publisher, Oxoid, UK.

Chanda, A., C. R. Roy, P. K. Banerjee and C. Ghha, (1989). Studies on incidence of bovine mastitis, its diagnosis, etiology and in vitro sensitivity of the isolated pathogens. Indian Vet. J., 66: 277-282.

Clinical Laboratory Standards Institute (CLSI), (2005). Performance Standards for Antimicrobial Susceptibility testing. CLSI Approved Standards M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA. CDC. USA.

Colditz, I. G. and D. L. Watson (1985). The immunophysiological basis for vaccinating ruminant against mastitis. Aust. Vet. J., 62: 145-153.

Cruckshank, R., J. P. Duguid, B. P. Marmion and R. H. A. Swain (1975). Medical Microbiology. 11: pp. 356-360. Churchill Livingstone, USA.

Degraves, F. J. and J. Fetrow (1993). Economics of Mastitis and mastitis control. Vet. Clinics of North America. Food Animal Practice, 19: 421-434.

Essers, L. and K. Redebold (1980). Rapid and reliable identification of *Staphylococcus aureus* by Latex Agglutination test. J. Clin. Microbiol., 12: 641-643.

Hodges, R. T., Y. S. Jones and J. T. Holland (1984). Characterization of Staphylococci with clinical and subclinical bovine mastitis. Newzealand Vet. J., 32: 141-145.

Iqbal, M., M. A. Khan, B. Daraz and U. Saddique (2004). Bacteriology of mastitic milk and in vitro antibiogram of the isolates. Pakistan Vet. J., 24:161-164.

Kapur, M., P. Anshusharma and R. M. Bhardwal (1992). Bacteriology of clinical mastitis in Buffaloes. Buffalo Bulletin, 1: 32-35.

Khan, A. Z., G. Muhammad, T. Ali and A. Khan (2005). Prevalence of mastitis in cross bred cows and buffaloes and antibiotics sensitivity profiles of isolates. Pakistan Vet. J., 26: 74-76.

Klaas, I. C., C. Enevoldsen, M. Vaarst and H. Houe (2004). Systematic clinical examinations for identification of latent udder health types in Danish dairy herds. J. Dairy Sci., 87: 1217-1228.

Kossaibati, M. A., M. Hovi and R. J. Esslemont (1998). Incidence of clinical mastitis in dairy herds in England. Vet. Rec., 143: 649-653.

Lightner, J. K., G. Y. Miller, W. D. Hueston and C. R. Dorn (1988). Food animal economics: estimation of the costs of mastitis using National Animal Health Monitoring system and milk somatic cell count data. J. Amer. Vet. Med. Assoc., 192: 1410-1413.

Memon, M. I., K. B. Mirbahar, M. R. Memon, N. Akhtar, S. A. Soomro and P. Dewani (1999). A study on the etiology of subclinical mastitis in buffaloes. Pakistan J. Agri., Agril. Engg., Vet. Sci., 15: 34-36.

Miline, M. H., A. M. Biggs, J. L. Fitzpatrick, G. T. Innocent and D. C. Barrett (2003); Use of clinical information to predict the characteristics of bacteria isolated from clinical cases of bovine mastitis. Vet. Rec., 152: 615-617.

Munahammad,, G., M. Athar, A. Shakoor, M. Z. Khan, Fazal-Ur-Rehmanan and M. T. Ahmad (1995). Surf Field Masititis Test: An inexpensive new to tool for evaluation of wholesomeness of fresh milk. Pakistan J. Food Sci., 3: 91-93.

National Mastitis Council, INC., (1990). Microbiological procedures for the diagnosis of bovine udder infection. National Mastitis Council, Inc. Wilson Boulevard Arlington, Virginia, USA.

Power, D. A., (1988). Manual of BBLR Products and Laboratory Procedures, 6th ed. Bacton Dickinson Microbiology Systems, Cockeysville, MD.

Rashid, A., (2001). Studies on mastitis among dairy buffaloes. PakistanVet. J.,21: 220-221.

Razzaq, A., (1998). Comparative efficacy of Vetimast®, Tetra-Delta®, and Akamycin-D® in mastitis of buffalo in and around Lahore. MSc. Thesis, College of Vet. Sci. Lahore.

Rossetti, C. A., (1993). Prevalence of subclinical mastitis caused by *Staphylococcus aureus* in Buenosaires dairy area and its susceptibility to antibiotics. Veterinaria argentia, 10: 323-326.

Sears, P. M., (1993). *Staphylococcus aureus* mastitis. Proc. 32nd Annu. Meet. Natl. Mastitis Conc. Inc., Kansas City Missouri, Feb. 15-17. pp.182-193.

Zahid, I. A., (2004). Studies on comparative incidence of subclinical and clinical mastitis and in vitro antibiotics susceptibility of isolates from Holstein-Friesian, Jersey cows and buffaloes. Pakistan Vet. J., 24: 76-81.