INTRODUCTION

Pakistan is an agricultural country and livestock is its 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 tons fresh milk to a high demand of milk and milk products. Buffaloes are 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 Redebold (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 described by Klaas et al., (2004) i.e. clinical variables asymmetry between front quarters, asymmetry 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, ciprofloxacin, cotrimoxazole, gentamycin and novobiocin) was determined by using disk diffusion method. Antibiotic susceptibility was done according to the standards of National Committee for Clinical Laboratory Standards (NCCLS), now called Clinical Laboratory Standards Institute (CLSI, 2005). S. aureus ATCC 25923 (American Type Culture Collection, Rockville, 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 alpha-beta haemolytic, respectively.

Of the 60 S. aureus isolates, all were catalase positive 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 novobiocin (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 \textit{S. aureus} mastitis in buffaloes. Conducting similar studies, Fazal-ur-Rehman (1995) concluded that gentamycin, chloromphenicol, cotrimoxazole, 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 \textit{S. aureus}, the most commonly isolated organism from mastitis was 100\% sensitive to gentamycin, chloromphenicol and cotrimoxazole and were on percent sensitive to oxytetracycline. The results of this study do not correspond to the findings of present study. Hodges \textit{et al}. (1984) also observed a similar pattern of sensitivity while conducting studies on bovine mastitis in Newzealand, \textit{S. aureus} isolates were 80-90\% sensitive to oxytetracycline and novobiocin. Chanda \textit{et al}. (1989) reported that gentamycin was the most effective antibiotic for Staphylococci followed by ampicillin, oxytetracycline and chloromphenicol. Khan \textit{et al}. (2005) drew a conclusion that the antibiogram analysis of gentamycin, ciprofloxacin, chloromphenicol, cotrimoxazole showed more than 90\% sensitivity to \textit{S. aureus} derived from buffaloes and cows. Iqbal \textit{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 \textit{S. aureus}. The intermittent changing pattern of antibiotic susceptibility against \textit{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**


