Clinical and Subclinical *Staphylococcus aureus* Mastitis in Dairy Buffaloes: Disease Characteristics and Antibiotic Susceptibility Profiles of Isolates

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**Abstract:** Mastitis continues to be a major economic issue for dairy producers all over the world. *Staphylococcus aureus* is frequently isolated from bubaline mastitis and brings about glandular tissues changes obliterating the milk producing cells. Elimination of this organism from dairy herds requires treatment of infected mammary glands with antimicrobial agents and aggressive culling of refractory animals. The present study was designed to evaluate the disease characteristics of *Staphylococcus aureus* mastitis in buffaloes and antibiotic susceptibility profiles of isolates. Visual inspection and palpation indicated as, asymmetry between hind quarters (n = 3), symmetry between hind quarters (n = 3), asymmetry between front quarters (n = 1), symmetry between front quarters (n = 5), Clinical symptoms (n = 2), udder and teat’s wounds (n = 2), scar tissue (n = 1), warts (teat) (n = 1) and all others parameters were normal. A total of 100 milk samples (20 clinical and 80 sub-clinical) from mastitic quarters of buffaloes were collected. The antibiotic susceptibility profiles of recovered isolates *Staphylococcus aureus* were as, co-trimoxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (86.95%), ampicillin (82.60%), ciprofloxacin (82.60%), chloramphenicol (82.60%), enrofloxacin (69.56%) and novobiocin (60.86%).

**Keywords:** *Staphylococcus aureus*, mastitis, clinical and sub-clinical, antibiotic susceptibility

**INTRODUCTION**

There are many diseases affecting the milk yield of buffaloes. Of these, mastitis is at the top. It is the inflammation of the parenchyma of the mammary gland regardless of the cause and is characterized by physical and chemical changes in the milk and pathological changes in the glandular tissue. Inflammation may be caused by many types of injuries including infectious agents, their toxins, physical trauma or chemical irritants. Mastitis occurs in two forms i.e., clinical and subclinical. Clinical mastitis is recognized by abnormal milk, gland’s swelling and illness of the affected animal. Subclinical mastitis is recognized by apparently normal milk with an increase in somatic cell count (Radostits *et al.*, 2000). Subclinical mastitis is 15-40 times more common than the clinical mastitis and causes the greatest overall losses in most dairy herds (Schultz *et al.*, 1978). This disease is associated with a decrease in milk production, deterioration of milk quality, increased labour cost and culling (Dobbins, 1997; Fthenakis, 1994).

Mastitis may be caused by a wide variety of microorganisms including bacteria, fungi, yeast and mycoplasma etc. However, bacteria are described to be the most frequent pathogens of this disease. *Staphylococcus aureus* is held responsible for causing 50% cases of mastitis in buffaloes as per studies.
made thus for in Pakistan starting from 1966 to 2002 (Shakoor, 2006). It causes pockets in the depth of udder surrounded by fibrous tissue where antibiotics cannot gain access. The main reasons for the failure of any of the antibiotics therapy in most cases of Staphylococcal mastitis are inadequate concentration of antibiotic reaching at the site of infection, bacterial antibiotic resistance, L-forms of bacteria (sensitive to beta-lactam antibiotics), bacterial dormancy and tissue barrier as this organism lies in deep seated foci (Sandholm et al., 1991).

A lot of literature is available about the disease characteristics of mastitis in cattle (Vaarst and Enevoldsen, 1997; Morin et al., 1998; Radostits et al., 2000; Milne et al., 2003; Klaas et al., 2004), but the information about this aspect is lacking in buffaloes. In principle, antibiotic susceptibility against the available chemothapeutic agents in a region or locality should be done from time to time to enable the veterinarians to prescribe the most suitable antibiotics against the prevailing diseases.

To gain the maximum cure rate in the treatment of mastitis, the result of an antibiotic susceptibility testing should constitute the basis of antibiotics selection. There is a paucity of information about the comparative antibiotics susceptibility profiles of Staphylococcus aureus isolated from buffaloes. The proposed study was therefore planned to; (a) evaluate the disease characteristics of mastitic udder in buffaloes and (b) determine the antibiotics susceptibility profiles of Staphylococcus aureus isolates to 9 antibiotics.

MATERIALS AND METHODS

Screening of Lactating Buffaloes

A total of 560 quarter milk samples from 140 buffaloes were screened for detection of sub-clinical mastitis by Surf Field Mastitis Test (Muhammad et al., 1995) and 30 quarter milk samples were collected from 15 mastitic buffaloes on the basis of clinical signs and symptoms of mastitis.

Collection and Handling of Milk Samples

A total of 110 quarter milk samples (5 mL of each) positive to Surf Field Mastitis Test were collected aseptically from dairy buffaloes maintained at private and Government farms around District Faisalabad, Pakistan, during 2006. Thus collected samples were transported to the Mastitis Research Laboratory in the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan for bacteriology. The procedures for collection, transportation of milk samples and culturing of microorganisms were followed as per recommendations of National Mastitis Council Inc. USA (Anonymous, 1990).

Disease Characteristics of Mastitic Udder in Buffaloes

Clinical cases of mastitis (n = 15) were observed by visual inspection and palpation for the determination of disease characteristics of mastitic udder as described by Klaas et al. (2004).

Culturing of Milk Samples

The milk samples were streaked onto the following culture media and incubated at 37°C for 48 h for the isolation of Staphylococcus aureus. Staph. 110 medium: A selective medium for the isolation of Staphylococcus spp. (Cruickshank et al., 1975); Blood agar: To assess the haemolytic activity. (Anonymous, 1990).

The following tests were performed for the identification of Staphylococcus aureus:

- Gram’s staining (Anonymous, 1990)
- Catalase test (Anonymous, 1990)
- Slide coagulase test
- Tube Coagulase test at 4, 12 and 24 h (Anonymous, 1990)
- Staphylococcus aureus were further confirmed by using latex slide agglutination Test (Staphytest plus, Oxoid, Basingstoke Hampshire, UK) (Essers and Radobold, 1980).
Antibiotic Susceptibility Profiles of Staphylococcus aureus Isolates

In vitro antibiotic susceptibility of *Staphylococcus aureus* to 9 antibiotics (ampicillin, amoxicillin, oxytetracycline, chloramphenicol, enrofloxacin, ciprofloxacin, trimethoprim + sulphonamide, 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, 1990), now called Clinical Laboratory Standards Institute (CLSI, 2005). *Staphylococcus aureus* ATCC 25923 (American Type Culture Collection, Rockville, Maryland, USA) was used as the sensitive quality control organism.

RESULTS

Screening of Lactating Buffaloes

A total of 560 quarters milk samples from 140 buffaloes were screened by Surf Field Mastitis Test (SFMT) for sub-clinical mastitis. Of these, 80 samples were positive to Surf Field Mastitis Test (Fig. 1). On the other hand, 30 quarters milk samples were taken from the 15 buffaloes clinically affected with mastitis.

Disease Characteristics of Staphylococcus aureus Mastitis

A total of 30 quarters milk samples were collected from clinical cases of mastitic buffaloes. Of these, *Staphylococcus aureus* was isolated from six quarters belonging to six buffaloes and disease characteristics of these clinical cases of *Staphylococcus aureus* mastitis were observed by visual inspection and palpation. Disease Characteristics are shown in Table 1 and Fig. 2-4.

Culturing of Mastitic Milk Samples on Different Media

**Staph. 110 Medium**

The mastitic milk samples were cultured on Staph. 110 medium (Difco Laboratories, Detroit, Michigan, USA) for 24-48 h of incubation at 37°C. Of the 110 samples, only 60 samples showed growth on this medium and the remaining 50 did not grow. The colonies on Staph. 110 plates showed smooth, circular, moist, creamy or golden yellow appearance.

**Blood Agar Medium**

The same sixty samples grown on Staph. 110 medium were further streaked onto blood agar for checking the haemolytic patterns of *Staphylococci*. Of these, 33 were haemolytic and 27 were non-haemolytic. Of haemolytic isolates, 20, 11 and 2 were α, β and αβ haemolytic, respectively. The colonies of *Staphylococci* on blood agar were circular, 1-2 mm in diameter, raised, convex having entire margins and yellow or creamy in colour. After obtaining pure cultures, different tests were conducted for the identification of isolates.

<table>
<thead>
<tr>
<th>Table 1: Disease characteristics of <em>Staphylococcus aureus</em> mastitis in dairy buffaloes (n = 6)</th>
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<tr>
<td>Disease characteristics</td>
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<td>Asymmetry between hind quarters</td>
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<td>Symmetry between hind quarters</td>
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<td>Symmetry between front quarters</td>
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<td>Clinical symptoms (swelling, pain, warmth, hardness of udder tissue)</td>
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<td>Udder oedema</td>
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<td>Udder shape</td>
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<td>Teat shape</td>
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<td>Udder and teat wounds</td>
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<td>Scab tissue (teat canal)</td>
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<td>Skin quality of udder and teat</td>
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<td>Warts on teat</td>
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Fig. 1: One receptacle of mastitis detecting paddle of SFMT showing the mastitic milk and gel formation.

Fig. 2: Theilitis (Inflammation of the teats) related to Staphylococcus aureus mastitis in buffaloes.

Fig. 3: Udder and teats wounds as disease characteristics with special reference to Staphylococcus aureus in buffaloes.
Identification of *Staph. aureus* Isolates

The colonies of *Staphylococci* were subjected to Gram’s staining and catalase test. It showed that all the 60 isolates were Gram positive, cocci, usually occurred in irregular often grape like clusters and all the 60 isolates were catalase positive. After the application of other identification tests; slide coagulase test, tube coagulase test and Staphytest plus test, out of 60 isolates, 23 were confirmed as *Staphylococcus aureus* and other 37 were coagulase negative *Staphylococci* (Table 2).

**Antibiotic Susceptibility Profiles of *Staphylococcus aureus***

The percent sensitivity of *Staphylococcus aureus* against different antibiotics was: co-trimaxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (86.95%), ampicillin (82.60%), ciprofloxacin (82.60%), chloramphenicol (82.60%), enrofloxacin (69.56%) and novobiocin (60.86%). Results are shown in Table 3.

**DISCUSSION***

*Staphylococcus aureus* mastitis is a common problem facing dairy practitioners. Approximately 80% of dairy have some level of infection with this pathogen. *Staphylococcus aureus* is a contagious
pathogen which spreads rapidly from animal to animal in some herds, while it can exist at a very low level with minimal control practices in other herds. Many dairies see few signs of clinical mastitis, while subclinical infection silently steals economic profits. However, other herds may experience outbreaks of clinical mastitis cases, some of which can progress to gangrenous mastitis. Therapy results are equally variable, with success for lactation therapy ranging from 15 to 70%. In this study disease characteristics of Staphylococcus aureus from clinical mastitis were observed as, hind quarters were asymmetric (n = 3), asymmetry between front quarters (n = 1) clinical symptoms (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 Staphylococcus aureus infection because these harbor the skin micro flora especially Staphylococcus aureus. The clinical mastitis is also sometimes caused by Staphylococcus aureus (Sears, 1993). These finding are also in line with the findings of Milne et al. (2003) who also recorded certain clinical features in cows like swollen or hard udders along with others but these were not correlated with Staphylococcus aureus infection and were in general.

For Staphylococcus aureus, the tested chemotherapeutic agents, co-trimoxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (85.95%) ampicillin (82.60%), ciprofloxacin (82.60%), chloramphenicol (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. (1997) reported that Staphylococcus aureus deriving from cattle was sensitive to oxytetracycline. On the other hand, Rashid (2001) concluded gentamycin sensitive 62% against Staphylococcus aureus mastitis in buffaloes. Conducting similar studies, Fazal-ur-Rehman (1995) concluded that gentamycin, chloramphenicol, cotrimoxazole, amoxicillin and oxytetracycline showed an in vitro efficiency over 90%. Novobiocin had efficiency between 80 to 90% and ampicillin efficiency was less then 80%. Khan et al. (2005) drew a conclusion that the antibiogram analysis of gentamycin, ciprofloxacin, chloramphenicol, cotrimoxazole showed more then 90% sensitivity to Staphylococcus aureus derive from buffaloes and cows. Chaudhary and Azam (1995) made a similar study about various species of Staphylococcus aureus deriving from buffaloes and concluded that gentamycin (62.94%), chloramphenicol (34.11%), oxytetracycline (28.25%), cotrimoxazole (12.35%) and ampicillin (2.43%) were sensitive. Similar study was made by Lafi and Haslat (1998) in buffaloes and they concluded that cotrimoxazole (75.67%), amoxicillin (72.29%), chloramphenicol (70.20%), gentamycin (70.20%), oxytetracycline (70.20%) and ampicillin (62.16%) showed sensitivity against Staphylococcus aureus. Similarly Iqbal et al. (2004) made an antibiotic susceptibility testing in buffaloes and cattle and they concluded that gentamycin and enrofloxacin were most the effective drugs against Staphylococcus aureus. Ramachandraiah et al. (1990) made a study about antibiotic susceptibility in India and concluded that gentamycin and chloramphenicol were 100% effective followed by cotrimoxazole (83.3%), penceillin (83.3%) and oxytetracycline (66.6%) against Staphylococcus aureus mastitis. Saxena et al. (1993) construed that Staphylococci derived from bovine mastitis were highly sensitive to gentamycin. Having conducted a similar study, Erskine et al. (2002) made a conclusion that cotrimoxazole (99.4%), gentamycin (98.9%), oxytetracycline (91.5%) and ampicillin (50.4%) were sensitive to various species of Staphylococcus aureus deriving from cattle.

The intermittent changing pattern of antibiotic susceptibility against Staphylococcus aureus may be ascribed to the extent of different antibodies to be used from locality to locality. From this study, it is concluded that the co-trimoxazole is the most sensitive chemotherapeutic agent. So, it is mandatory that antibiogram study is made from time to time in a locality to be on the lookout of the most effective drug against the prevailing mastitogens i.e., bacteria.
REFERENCES


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