International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 2 (9): 804-811, 2007
ISSN 1816-4897

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

[1]Abid Hussain, [1]Abdul Shakoor, [2]Muhammad Akbar Shahid,
[3]Muhammad Numan and [2]Fahad Gulraiz
[1]Department of Clinical Medicine and Surgery,
[2]Department of Veterinary Microbiology,
Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan
[3]Veterinary Research Institute, Ghazi Road, Lahore Cantt-13, Pakistan

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-trimaxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (86.95%), ampicillin (82.60%), ciprofloxacin (82.60%), chloramphenicol (82.60%), enrofloxacin (69.56%) and novabiocin (60.86%).

Key words: *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

Corresponding Author: Muhammad Akbar Shahid, Department of Veterinary Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan
Tel: +92-0685881227 +92-0333-7453426

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 chemotherapeutic 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 informations 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 cogulase test
- Tube Coagulase test at 4, 12 and 24 h (Anonymous, 1990)
- *Staphylococcus aureus* were further confirmed by using latex slide agglutination Test (Staphytect plus, Oxoid, Basingstoke Hampshire, UK) (Essers and Radebold, 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 gold in colour. After obtaining pure cultures, different tests were conducted for the identification of isolates.

Table 1: Disease characteristics of *Staphylococcus aureus* mastitis in dairy buffaloes (n = 6)

Disease characteristics	Frequency
Asymmetry between hind quarters	3
Asymmetry between front quarters	1
Symmetry between hind quarters	3
Symmetry between front quarters	5
Clinical symptoms (swelling, pain, warmth, hardness of udder tissue)	2
Udder oedema	-
Udder shape	Normal
Teat shape	Normal
Udder and teat wounds	2
Scar tissue (teat canal)	1
Skin quality of udder and teat	Normal
Warts on teat	1

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

Fig. 4: Teat wound with special reference to *Staphylococcus aureus* mastitis in buffaloes

Table 2: Comparative biochemical characteristics of *Staphylococcus aureus* isolated from mastitic buffaloes

	Gram's staining		Catalase		Slide Coagulase test		Tube Coagulase test		Staphytect plus	
Species	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Staphylococcus aureus	60	-	60	-	23	37	23	37	23	37

Table 3: *In vitro* antibiotic susceptibility profiles of *Staphylococcus aureus* isolates from mastitic buffaloes against different antibiotics

	Cotrimexazole			Amoxicillin			Ampicillin			Enrofloxacin			Ciprofloxacin		
Species	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus aureus (n = 23)	23	-	-	20	-	3	19	-	4	16	3	4	19	2	2

	Gentamycin			Oxtetracycline			Chloramphenicol			Novabiocin		
Species	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus aureus (n = 23)	20	1	2	22	-	1	19	2	2	14	3	6

S = Sensitive, I = Intermediately sensitive, R = Resistant

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 Staphytect 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 dairies 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-trimaxazole (100%), oxytetracycline (95.65%), amoxicillin (86.95%), gentamycin (86.95%) ampicillin (82.60%), ciprofloxacin (82.60%), chloramphenicol (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*. (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, cotrimaxazole, 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 aualysis of gentamycin, ciprofloxacin, chloramphenicol, cotrimaxazole showed more then 90% sensitivity to *Staphylococcus aureus* derived 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 (34.41%), oxytetracycline (28.25%), cotrimaxazole (12.35%) and ampicillin (2.43%) were sensitive. Similar study was made by Lafi and Hailat (1998) in buffaloes and they concluded that cotrimaxazole (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 cotrimaxazole (83.3%), pencillin (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 cotrimaxazole (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 antibiotics to be used from locality to locality. From this study, it is concluded that the co-trimaxazole 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

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

Aziz, M.A., M.A. Ghani and M.T. Muhammad, 1997. Studies on *Staphylococcal* subclinical bovine mastitis with special reference to treatment by pencillin, Mardadal combination (Masticillin M). J. Egyp. Vet. Med. Assoc., 36: 69-78.

Chaudhary, T.M. and M.W. Azam, 1995. Sensitivity of various organisms isolated from clinical cases of mastitis in cattle, buffaloes and goats. Pak. Vet. J., 1: 74-76.

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.

Cruickshank, R., J.P. Duguid, B.P. Marmion and R.H.A. Swain, 1975. Medical Microbiology. 12th Edn., Vol. II. Churchill Livingstone, USA., pp: 356-360.

Dobbins, C.N., 1997. Mastitis losses. J. Am. Vet. Med. Assoc., 170: 1129-1132.

Erskine, R.J., R.D. Walker, C.A. Bolin, P.C. Bartiett and D.G. White, 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J. Dairy Sci., 85:1111-1118.

Essers, L. and K. Radebold, 1980. Rapid and reliable identification of *Staphylococcus aureus* by latex agglutination test. J. Clin. Microbiol., 12: 641-643.

Fazal-ur-Rehman, 1995. Studies on: (1) Evaluation of Surf Field Mastitis Test for the detection of sub-clinical mastitis in buffaloes and cattle and (2) antibiotics susceptibility of the pathogens. M.Sc. (Hons) Thesis, Univ. Agric. Faisalabad, Pakistan, pp: 170-173.

Fthenakis, G.C., 1994. Prevelance and etiology of sub-clinical mastitis in ewes of South Greece. Small Rum. Res., 13: 293-300.

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

Khan, A.Z., G. Muhammad, T. Ali and Asmatullah Khan, 2005. Prevalence of mastitis in cross bred cows and buffaloes and antibiotics sensitivity profiles of isolates. Pak. 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.

Lafi, S.Q. and N.Q. Hailat, 1998. Incidence and antibiotic sensitivity of bacteria causing bovine and ovine mastitis in Jordan. Pak. Vet. J., 18: 88-94.

Milne, 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.

Morin, D.E., P.D. Constable and G.C. McCoy, 1998. Use of clinical parameters for differentiation of gram-positive and gram-negative mastitis in dairy cows vaccinated against lipopolysaccharide core antigens. J. Am. Vet. Med. Assoc., 212: 1423-1431.

Muhammad, G., M. Athar, A. Shakoor, M.Z. Khan, Fazal-ur-Rehman and M.T. Ahmad, 1995. Surf field mastitis test: An inexpensive new tool for evaluation of wholesomeness of fresh milk. Pak. J. Food Sci., 3: 91-93.

National Committee for Clinical Laboratory Standards, 1990. Performance Standard for Antimicrobial Disc Susceptibility Test. 4th Edn., Approved standard M2-A3. National Committee for Clinical Laboratory Standards, Villanova Pathogens, USA.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edn., W.B. Saunders Co., Philadelphia, USA., pp: 603-610.

Ramachandraiah, K.K., S. Kumar and O. Sreemannarayana, 1990. Survey of mastitis in pure Jersey herd. Indian Vet. J., 67: 103-106.

Rashid, A., 2001. Studies on mastitis among dairy buffaloes. Pak. Vet. J., 21: 220-221.

Sandholm, M., M. Louhi and J. Espinasse, 1991. Bovine mastitis: Why does antibiotic therapy fail? Mammites Desvaches Laitieres, 9: 88-106.

Saxena, R.K., G.N. Dutta, P. Borah and J. Buragohain, 1993. Drug susceptibility and treatment of bovine sub-clinical mastitis. Indian Vet. J., 79: 201-203.

Schultz, L.H., R.W. Brown, D.E. Jesper, M.W. Berger, R.P. Natzke, W.N. Philpot, J.W. Smith and P.D. Thompson, 1978. Current Concept of Bovine Mastitis. 2nd Edn., The Natl. Mastitis Council, Inc. Washington DC., USA., pp: 6-9.

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

Shakoor, A., 2006. Preparation and Evaluation of *Staphylococcus aureus* vaccines for the control of mastitis in dairy buffaloes (*Bubalus bubalis*), Ph. D Thesis, Dep. Clin. Med. Surgery, Univ. Agric. Faisalabad, Pakistan, pp: 2-5.

Vaarst, M. and C. Enevoldsen, 1997. Patterns of clinical mastitis manifestations in Danish organic dairy herds. J. Dairy Res., 64: 23-37.

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