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Staphylococcus aureus associated with bovine mastitis in Quetta, Pakistan

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Abstract

Staphylococcus Mastitis is a one of the costly and major problem for dairy animals in Pakistan and worldwide. Aim: The main aim of this study was to isolate and identify the *Staphylococcus aureus* species, responsible for the bovine mastitis from dairy animals in different areas of Quetta city, Pakistan. Total of 20 affected samples were collected in which 14 (70%) infected samples were from Buffalo and 6 (30%) infected samples of cow were collected from 11 different dairy herds of Quetta city. Sample were identified on the basis of cultural and biochemical tests. Antibiogram study was performed. Staphylococcus *aureus* associated with were 66.66% from Buffalo and 33.33% were from cow indicating the higher prevalence in buffalo than cow. Udder-wise prevalence 28.57% of right front (RF) was positive. All isolate were resistance to colistin sulphate. Not any staph was isolated from Left Front (LF). *S. aureus* is sensitive to antibiotic, can be eradicated and thus will prevent the economic loss and health of domestic and dairy animals.

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Introduction

Mastitis can be defined as "mammary gland inflammation caused by different bacteria, that enter the udder and produce harmful toxin (Schroeder 1997). In dairy animals, It is the most important and costly disease, causing great economic losses due to reduction in milk yield, lowering its nutritive value (Lightner *et al*. 1988; Ali *et al*. 1989).Subclinical mastitis causes high economic losses in dairy herds than clinical mastitis about (Schultz *et al*. 1978).

Mastitis decrease the shelf life of processed milk, milk protein, sugar contents, fat and increase somatic cell count, results in substandard, sub-optimal output of fermented products (Urech *et al*. 1999). The affected animals like sheep, goat cattle and buffalo provide a mechanism of spread of diseases like tuberculosis, leptospirosis, sore throat, brucellosis and render it unfit for human consumption (Ratafia 1987).

The organisms causes the mastitis are staphylococci (*aureus* and. *epidermidis*), Streptococci (*dysgalactiae, agalactiae, bovis and Uberis*) coliforms (*Klebsiella pneumoniae* and *E. coli*) frequently *Nocardia, Pseudomonads,* yeast and *Mycoplasma (Mcdonald 1979)*. Coagulase negative staphylococcus (CNS) (Lafi *et al*. 1994).

The common mastitis pathogens are environmental and contagious, *S aureus, Str. agalactiae* are common contagious pathogens and *S. aureus* is the predominant organism responsible for bovine mastitis (Kapur *et al*. 1992). Streptococci, Staphylococci followed by coli-form and coryne bacterium were the abundant groups of microorganisms in different areas of Pakistan and elsewhere, responsible for mastitis in dairy animals with prevalence ranges from 20-80%, *the* large capsule protects the *S. aureus* from attack by the host immunological defenses (Ghuman 1967; Chander and Baxi 1975; Hashmi *et al*. 1980; Anwar and Chaudhri 1983; Ahmad *et al*. 1991; Iqbal *et al*. 2004; Khan *et al*. 2004; Cenci-Goga *et al*. 2003).

Staphylococcus *aureus,* in Latin's *aureus* means "golden" a facultative anaerobic, Gram positive cocci, about 1 micrometer in diameter, under microscope its appears as grape-like clusters, often cause with hemolysis when grown on blood agar. Produce large golden yellow round colonies on rich medium, catalase positive- able to convert hydrogen peroxide (H_2O_2) to water and oxygen, the enzyme catalase differentiate it from streptococci. *S. aureus* is primarily coagulase positive- can produce "coagulase", a protein product which cause clot formation while most other *Staphylococcus* species are coagulase negative (Todar 2004).

Mastitis caused great losses because of lack of mastitis prevention practices like antibiotic therapy and teat dipping procedure in Pakistan, the prevalence of mastitis on the basis of surf field mastitis test was 58.7% while animal-wise prevalence 77.98% in buffaloes was recorded and it is an important cause of premature culling in imported Holstein Friesian cattle and in local born cattle; accounting for 22.5% of all culling in last decade (Arshad 1999; Bachaya *et al*. 2005; Samiullah *et al*. 2000). The present study was designed to identify *Staphlocoocus aureus* responsible for Bovine mastitis from different dairy herds of Quetta city, Pakistan.

Materials and Methods

Collection of milk samples

All the possible hygienic measures were adapted to collect 20 milk samples in pre labelled test tube of 05ml from different dairy herds of Quetta city of Pakistan. Each teat was separately scrubbed with a pledged of cotton moistened with 70% ethyl alcohol. About 05ml of milk from effected teat were collected after discarding the first few streams. In an ice box the collected samples were immediately cooled and transferred to the *Centre for Advance Studies in Vaccinology and Biotechnology* (CASVAB) University of Balochistan, Brewery Road Quetta, Pakistan for microbiological examination.

Surf Field Mastitis Test (Sfmt) Preparation

In 100 ml of common water adding 03grams of commonly used detergent powder (Surf Excell®, Lever Brothers, Pakistan), 03% of Surf solution was prepared.

In petri plate, equal volume of surf solution and milk sample was mixed. Gel formation were the indication of positive mastitis and the gel formation was graded into four categories from higher to lower intensity as (++++), (+++), (++) and (+) (Muhammad *et al*. 1995; Muhammad *et al*. 2010).

Preparation of media and reagents

The Brian heart infusion broth (Oxoid), Brian heart infusion agar (Oxoid), Staphylococcus medium no 110 (Oxoid), Mannitol salt agar (Lab M), were used for isolation, purification and biochemical characterization. As per manufacturer's specification, all the said media's were mixed with distilled water in 500 ml quantity and were autoclaved at 15 lb/in^2 pressure per square inch (PSI) for 15 minutes at 121°C and allowed to cool down to 45°C. The solid media were aseptically poured into Petri plates, were allowed to solidify Liquid media were dispensed in clean pre-sterile test tubes having cotton wool plugs. The culture media(s) were incubated at 37°C for 24 hours to confirm sterility of the media. Sterile media were stored at 4°C.

Processing of samples

Milk samples were first inoculated in a brain heart infusion broth (BHI) and incubated at 37°C for 24 hours then transferred from BHI broth tubes to BHI agar plates and incubates at 37°C for 24 hours. The plates were checked for any bacterial growth next day. A suspected colony is picked up and further streaked on staphylococcus medium no 110 and mannitol salt agar for further confirmation of isolate. Similarly another colony was picked and gram stained. The isolate is identified as staphylococcus on the basis of their cultural, morphological characteristics (Hargitai *et al*. 1992) Staphylococcus produce small tiny colonies on staphylococcus medium no 110 and on mannitol salt agar produce yellow colonies with yellow zones. Pathogenic strain of staphylococcus. *Staphylococcus aureus* ferment mannitol salt and produce yellow coloration to the medium

Antibiotic sensitivity test

The Isolates were suspended in a normal saline solution and the suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard (1.5 x 10^8 colony forming unit (CFU)/ml). Drug susceptibility testing was performed by the agar disk diffusion method by spreading pure culture on Mueller Hinton agar and applying antibiotic-impregnated disc onto agar surface (Bauer *et al*. 1966). Plates were then inverted and incubated at 37°C for 18-24 hours and checked for confluent lawn of growth and uniform, circular zones of inhibition, was measured to nearest mm.

Results

Morphological characteristics of the isolates

Among Twenty (20) different milk samples 06 (30%) isolates produced circular, small, cream color colonies on Staphylococcus medium-110 after 24 hours incubation at 37°C. These small circular cream colonies were picked and further streaked on Staphylococcus medium-110 to achieve pure growth as shown in (Fig. 1-A). The *Staphylococcus aureus* produced typically small rounded yellow color colonies on Mannitol Salt Agar after 24 hours of incubation at 37°C (Fig. 1-B).

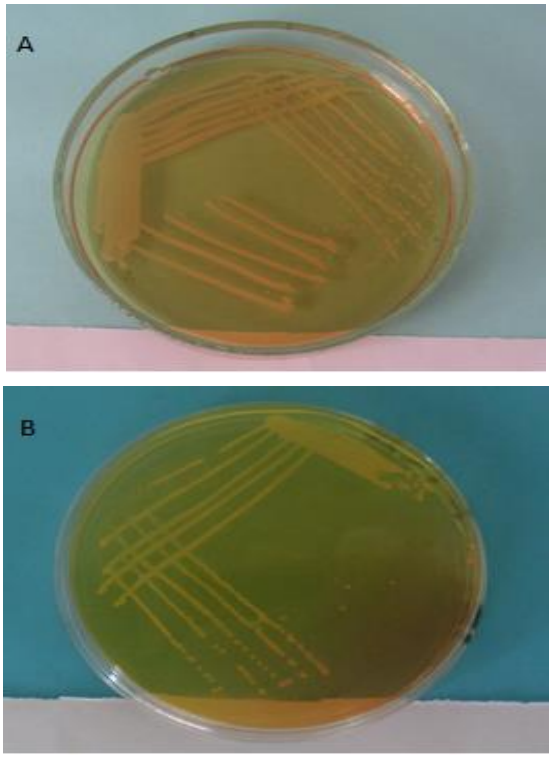

Fig. 1. (A) Colonies of *Staphylococcus aureus* on Staphylococcus medium 110 plates. (B) Typical yellow coloration of *Staphylococcus aureus* on Mannitol Salt Agar plate.

Table 1. Results of Growth on different medium with staining characters Catalase and coagulase test.

Sample No	BHI Broth	BHI Agar	Staph 110 Media		Manitol Salt Agar		Catalase Test	Coagalase Test
			Growth	Morphology	Growth	Morphology		
1.	+	+	+	Cocci	+	Cocci	+	+
2.	+	+	-	-	-	-	-	-
3.	+	+	+	Cocci	+	Cocci	+	+
4.	+	+	-	-	-	-	-	-
5.	+	+	+	Cocci	+	Cocci	+	+
6.	+	+	+	-	-	-	+	-
7.	+	+	-	-	-	-	-	-
8.	+	+	-	-	-	-	-	-
9.	+	+	-	-	-	-	-	-
10.	+	+	-	-	-	-	-	-
11.	+	+	+	Cocci	-	-	+	-
12.	+`	+	+	Cocci	+	Cocci	+	+
13.	+	+	-	-	-	-	-	-
14.	+	+	+	Cocci	+	Cocci	+	+
15.	+	+	-	-	-	-	-	-
16.	+	+	-	-	-	-	+	-
17.	+	+	+	-	-	-	-	-
18.	+	+	+	Cocci	-	-	+	-
19.	+	+	+	Cocci	+	Cocci	+	+
20.	+	+	+	Cocci	-	-	+	-

Gram staining of the isolates

Organisms were found in bluish clusters showing gram positive reaction under oil immersion (100X) lens (Fig. 2). Majority was clusters but some chains and diplococci were also observed.

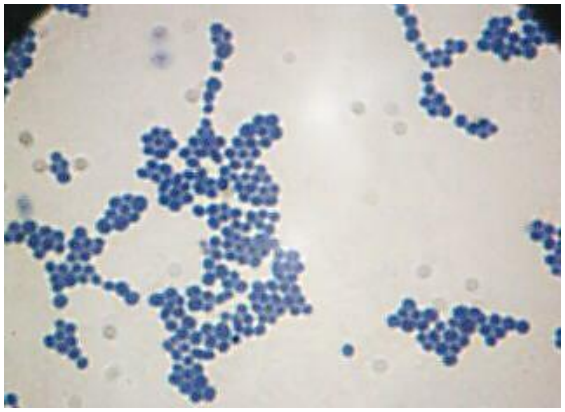

Fig. 2. Structure and staining characteristics of *Staphylococcus aureus under* microscope.

Antibiotic sensitivity test

After incubation different antibiotics (Table, 1) exhibited varying degree of inhibition zones as shown in (Fig. 6). Isolate were considered as sensitive, intermediate or resistance to a particular antimicrobial agent on the basis of inhibitory zone that match the criteria of the manufacturer interpretive table which follow the recommendation of National Committee for Clinical Laboratory Standard (Standards 2004).

Among these five antibiotics Sulphamethoxazole Trimethoprim exhibited high zones ranging from 30-26mm diameter. And Colistin showed least sensitivity against theses isolates.

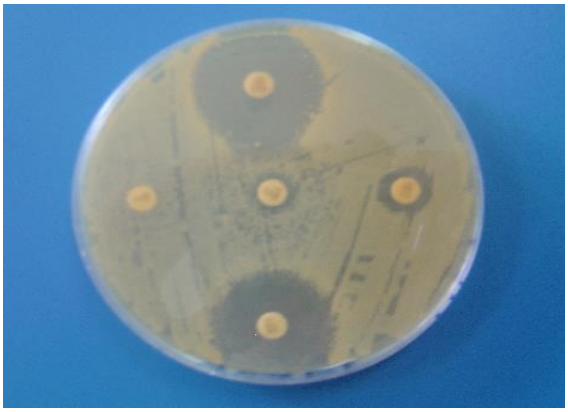

Fig. 3. Antibiotic sensitivity test by Disc diffusion method.

Specie, Sex and Age Wise Prevalence of Staphaureus

In this study out of 20 samples 14 (70%) were collected from Buffalo and 6 (30%) were from cow. Out of 06 positive samples 04 (66.66%) were from Buffalo and 02 (33.33%) were from cow indicating the higher prevalence in buffalo than cow. udder-wise prevalence out of 20 samples 7 (35%) were from right front (RF), 2 (10%) were from right rear (RR) , 5 (25%) were from left front (LF), and 6 (30%) were from left rear (LR). out of 7 samples of right front

(RF) 2 (28.57%) were positive. 02 samples were found positive from each of the right front (RF) right rear (RR) and left rear (LR) quarter while not any staph were isolated from Left Front (LF). Age wise 03 samples were found positive each from 1-5 years and above 05 years age.

Table. 2. Effect of different antibiotics on *Staphylococcus aureus* by Disc diffusion method.

Sample ID	Mean zone of inhibition (mm)				
	Novobiocin NV5	Amoxicillin AML10	Gentamycin CN10	Sulphamethaxzole-trimethoprim SXT25	Colistan sulphate CT25
01	23	8	18	26	R
03	29	R	12	28	R
05	R	20	21	27	7
12	25	10	24	26	R
14	20	26	18	30	8
19	20	30	20	28	7

Discussion

Mastitis is the most important disease of all the domestic animals that affects both quality and quantity of milk yield. It directly affects the economy of the farmers in conjunction with the poor health of the animals. The losses due to mastitis might be higher in Pakistan because the mastitis prevention practices like teat dipping and dry period antibiotic therapy are not in practice (Arshad 1999). *Staphylococcus aureus* is both catalase and coagulase positive. The colonies were verified by biochemical tests i.e. catalase and coagulase test (Sears and McCarthy 2003). However there are certain species of *Staphylococcus aureus* that are coagulase negative. Out of 06 positive samples 04 were from buffalo and 02 were from cow exhibiting the higher prevalence of *Staphylococcus aureus* in buffalo (Farooq *et al.* 2008) (Hussain *et al.* 1984).

Udder-wise prevalence out of 6 positive samples, 02 samples were from right front (RF), 02 from right rear (RR) and 02 samples from left rear (LR) quarter while not any *Staphylococcus aureus* were isolated from Left Front (LF).These finding suggests that There is high prevalence in hind quarters than for quarters (Khan and Muhammad 2005) and (Sharif and Ahmad 2007).

The data of antibiotic sensitivity test of *Staphylococcus aureus* showed that it is resistance to colistin sulphate but is sensitive to Sulphamethaxzole-trimethoprim, Gentamycin, Amoxicillin and Novobiocin.

Among these antibiotics Gentamicin is very active against *Staphylococcus aureus* (Adesiyun 1994) and (Bezek 1998). Sulphamethaxzole-trimethoprim also shows great bactericidal activity against *Staphylococcus aureus* (Kaka *et al.* 2006). In our study *Staphylococcus aureus* shows great resistance against colistan sulphate (Efuntoye and Adetosoye 2003).

The use of antibiotics for the treatment of mastitis helps to a large extent in avoiding economic losses from the disease. Most of the *S. aureus* strains are normally sensitive to majority of antibiotics but antibiotic resistance is becoming a problem so it is advisable to perform antibiotic sensitivity test to minimize the hazard of drug resistance and to avoid economic loss on treatment. If a new *Staphylococcus aureus* is not treated, the bacteria penetrate the mammary gland tissue and the cow attempts to wall off the area, forming an abscess and eventually scars tissue (Belschner, *et al*, 1996)

Antibiotics sensitivity test on different strains of *S. aureus* in this study shows that all the strains of *S. aureus* were sensitive to Sulphamethaxzole-trimethoprim, Gentamycin, Amoxicillin and Novobiocin. So it is recommended that these antibiotics can be used for the treatment of mastitis caused by *Staphylococcus aureus*, more importantly managers of dairy herds should adopt good milking technique, thus it is our social responsibility to save dairy animals and can also prevent the losses while adapting mastitis preventing practices.

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