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Study the prevalence of *S. aureus* in subclinical mastitis and their antibiogram in buffaloes

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Abstract
The purpose of this study was to investigate sub-clinical mastitis in dairy farm and prevalence of *Staphylococcus aureus* with its antibiogram pattern. A total of 80 buffaloes (320 quarters) were investigated for sub-clinical mastitis. Samples positive for sub-clinical mastitis were subjected for isolation and biochemical characterization. Isolates that was confirmed as *S. aureus* were subjected for antibiotic-susceptibility testing. The overall quarter wise incidence of sub clinical mastitis among 80 buffaloes was 2.4 per cent (90 quarters out of 320 quarters). After biochemical characterization, *S. aureus* was confirmed in 90 samples. Antibiotic resistance pattern revealed that the highest resistance to the antimicrobials used was to observed in ampicillin (75.00%), followed by chloramphenicol (72.50%), cefotaxime (62.50%), levofloxacin (55.00%) gentamicin (55.00%), cepholexin (50.00%), ceftriaxone and ciprofloxacin (45.00%), penicillin G (40.00%), and tetracycline (42.50 %). This study confirms the importance of *S. aureus* as a major mastitis causing bacterium and existence of alarming level of resistance to frequently used antibiotic by *S. aureus* and a potential risk for human health from nearly possible transmission of the *S. aureus* strains as milk borne pathogen.

Keywords: Sub clinical mastitis, California mastitis test (MCMT)

1. Introduction
Mastitis is an economically important disease due to its high morbidity, loss of milk production, high cost of treatment and major adverse effects on the quality of by products made from contaminated milk. It is one of the most costly diseases of dairy cattle resulting in the reduction of milk yield and quality (Atakisi *et al.*, 2010) [1]. Bansal and Gupta (2009) [2] have been estimated Rs. 4151.16 crore annual economic losses due to subclinical mastitis in India.

In dairy farming Mastitis continues to be one of the economically most important diseases**.** Bovine mastitis is one of important production diseases of dairy animals that affect the economy of farmers and thereby affecting the economy of country. The severe economic losses in dairy cattle mastitis is due to reduced milk yield, treatment cost, labour cost, milk withheld following treatment and premature culling (Miller *et al.*, 1993) [11]. Dua (2001) [6] reported annual losses due to clinical and sub-clinical mastitis (SCM) to the tune of Rs 6053.21 crores in developing country like India.

The sub-clinical form of mastitis in buffaloes is important because this form is 10 to 30 times more prevalent than the clinical form, usually precedes the clinical form, long duration, difficult to detect, reduces milk production, and adversely affects milk quality (Seegers *et al.*, 2003) [17]. The diagnosis of sub-clinical mastitis is more problematic since the milk appears normal but usually has an elevated somatic cell count. Early diagnosis of mastitis is vital because changes in the udder tissue take place much earlier than they become apparent. One of the main etiological agents of sub-clinical mastitis is *Staphylococcus aureus*. It has been recognized as the most important cause of ruminant mastitis. As a remedy antimicrobials were used frequently as therapeutic purpose against *S. aureus* infection especially in mastitis cases. But the outcome is poor due to versatile nature of pathogen and multidrug resistance strains.

The aim of the present study was to investigate the incidence of sub-clinical mastitis cases in buffaloes and further to determine the antimicrobial resistance pattern of isolates.

2. Materials and Methods

Buffaloes are used in this study, they are provided with green grass and concentrate diet and are kept together in common shed but at peri-parturient period (advanced pregnancy and early lactating stage) they are maintained in separate byres. All buffaloes were subjected to post-milking teat disinfection, those buffaloes were dried off approximately two months before expected calving and all quarters of buffaloes were infused with an antibiotic preparation approved for use in non-lactating buffaloes following the last milking of lactation.

2.1 Sampling

A total of 80 lactating buffalo females were tested for sub-clinical mastitis. Milk samples from each quarter were collected aseptically from apparently healthy lactating Buffaloes females. Teats were washed thoroughly and dried. The first three streams of milk from each teat were discarded. The teat end and orifice was sanitized with cotton swabs soaked in 70% ethanol.

2.2 Sub clinical mastitis detection by California mastitis test

California mastitis test (CMT) was carried out as described by Schalm *et al.,* (1971) [16]. Briefly, from all the quarters (N=320) 1.5 ml of milk samples was collected in each shallow cup in the CMT paddle. An equal amount of CMT reagent was added. The milk reagent mixture is swirled in a circular motion with presence of gel or slime being recorded for each quarter.

2.3 Culture and identification of microorganisms

The samples were inoculated on nutrient agar and blood agar at 37^0C for 24 to 48 hours. Identification of isolates was done on basis of colony morphology, Grams' staining, catalase test, Oxidase test, Indole production, Citrate utilization, Methyl Red test, Voges Proskauer test (Cheesbrough, 1994) [3]. The colonies confirmed as *Staphylococcus aureus* were further tested for antimicrobial susceptibility.

2.4 Antimicrobial susceptibility testing

The antibiotic-susceptibility profile of isolates for 10 different antibiotics was prepared using the disk diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (2008) [4]. *S. aureus* isolates were grown overnight on blood agar at 37°C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard.

The suspension (100 µl) was spread over the medium plate. Then, the antibiotic disk was transferred aseptically on to the surface of the inoculated medium, and was incubated further at antibiotics at 37°C, for a period of 24 h. The antibiotics and their concentrations used are as follows: Ampicillin (25 µg), Ceftriaxone (30 µg), Cefotaxime (10 µg), Cephalexin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Gentamicin (20 µg), Levofloxacin (5 µg), Penicillin G (10 units) and Tetracycline (30 µg).

3. Results and Discussion

The present study was conducted to investigate the quarter wise incidence of sub clinical mastitis in bovine. The overall quarter wise incidence of sub clinical mastitis among 80 apparently healthy Buffaloes was 12.5 per cent (40 quarters out of 320 quarters) all 40 quarters (12.5 %) were found positive for sub -clinical mastitis and normal quarters were 280 (87.5 %).

The 40 *S. aureus* isolates were tested for antimicrobial susceptibility. The sensitivity and resistance of isolates to antibiotics is shown in Table 1. The highest resistance to the antimicrobials used was to observed in ampicillin (75.00%), followed by chloramphenicol (72.50%), cefotaxime (62.50%), levofloxacin (55.00%) gentamicin (55.00%), cepholexin (50.00%), ceftriaxone and ciprofloxacin (45.00%), penicillin G (40.00%), and tetracycline (42.50 %).

The overall quarter wise prevalence of sub clinical mastitis was 12.50 per cent and normal healthy quarters were 87.50 per cent. In contrast to present study, several reports (de Medeiros *et al.,* 2009; Hussein, 2012) [7] of very high quarter wise prevalence of subclinical mastitis were recorded. Higher prevalence rate (36.36% and 21.7 %) was also reported by Islam *et al.,* (2011) [9] and Mdegela *et al.,* (2009) [10], respectively. These differences in the prevalence of subclinical mastitis are perhaps due to difference in managemental and hygienic practices adopted in different dairy herds. The low prevalence (12.5%) in this study is due to extensive management practices followed at this farm including use of teat dip antiseptic solutions before and after milking of lactating Buffaloes, proper washing of milkers' hands, milking parlor using disinfectants, separate milking parlor etc.

After biochemical characterization, *Staphylococcus aureus* was confirmed in 40 samples (87.5 %). A high percentage of subclinical mastitis milk samples were positive for *S. aureus*. Similar, reports was also recorded by Patel *et al.,* (2000) [14] and Hussein (2012) [7].

During milking, organisms spread from animal to animal. This organism is capable of causing peracute, acute, chronic, gangreneous and subclinical mastitis.

Table 1

Sr. No.	Antibiotics	Sensitive (%)	Resistant (%)	Total
1	Ampicillin	10(25.00%)	30(75.00%)	40
2	Cefotaxime	15(37.50%)	25(62.50%)	40
3	Ceftriaxone	22(55.00%)	18(45.00%)	40
4	Cephalexin	20(50.00%)	20(50.00%)	40
5	Chloramphenicol	11(27.50%)	29(72.50%)	40
6	Ciprofloxacin	22(55.00%)	18(45.00%)	40
7	Gentamicin	18 (45.00%)	22(55.00%)	40
8	Levofloxacin	22(55.00%)	18(45.00%)	40
9	Penicillin G	24(60.00%)	16(40.00%)	40
10	Tetracycline	23(57.50%)	17(42.50%)	40

From the Table 1, it is observed that the higher resistance of *S. aureus* to various anitmicrobials agents like ampicillin, followed by chloramphenicol, cefotaxime, levofloxacin gentamicin, cepholexin, ceftriaxone and ciprofloxacin, penicillin G, and tetracycline. Similar findings was also reported by various scientest (Nathawat *et al.,* 2013; Patel *et al.,* 2000; Pavulraj *et al.,* 2013) [13, 14, 15].

Ikiz *et al.,* (2013) [8] reported that cefoperazone antibiotic is resistance (24.95%) and also reported higher sensitivity (91.66%) to gentamicin. Highest sensitivity of *Staphylococcus aureus* isolates to Gentamicin and Ampicillin was reported by Mubarack *et al.,* (2012) [12] and Pavulraj *et al.,* (2013) [15]. Some of the antibiotics are indiscriminate used in the animals and human and causes resistant against that particular antibiotics.

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