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Prevalence of *Staphylococcus aureus* in Lactating Cows with Subclinical Mastitis and their Antibiogram in Organized Dairy Farm, Maharashtra, India

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A B S T R A C T

The purpose of this study was to investigate sub-clinical mastitis in organized dairy farm and prevalence of *Staphylococcus aureus* with its antibiogram pattern. The present study was carried out at Research Cum Development Project on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. A total of 1296 lactating crossbred cows (5184 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 1296 crossbred cows was 3.62 per cent (188 quarters out of 5184 quarters). After biochemical characterization, *S. aureus* was confirmed in 152 samples (80.85 %). Antibiotic resistance pattern revealed that the highest resistance to the antimicrobials used was to observed in Cephalexin (86.84%), followed by Co-trimoxazole (82.89%), Penicillin G (82.23%), Ampicillin (81.57%), Cefoperazone (76.97%), Ceftriaxone (69.73%), Cefotaxime (65.13), Amoxyclav (63.15%), and Tetracycline (54.60 %) Chloramphenicol (46.71%), Ciprifloxacin (42.10%), Gentamicin (38.81%), Enrofloxacin and Levofloxacin (19.73 % each). 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.

Introduction

In livestock (dairy) farming Mastitis continues to be one of the economically most important diseases**.** Especially 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). Dua (2001) 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 dairy cows is important because this form is 15 to 40 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). 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 dairy cows and *S. aureus* in bovine sub-clinical mastitis cases and further to determine the antimicrobial resistance pattern of isolates.

Materials and Methods

Animals

The whole investigation was carried out on lactating dairy cows of Research cum Development Project (RCDP) on Cattle, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra State (India).

Husbandry and management system

Crossbred dairy cows used in this study were managed under loose housing system at RCDP on Cattle. All the cows were vaccinated twice in a year for Hemorrhagic Septicemia, Black Quarter and Foot and Mouth Disease. Twenty one days prior to vaccination deworming was carried out. 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 cows were subjected to post-milking teat disinfection, those cows were dried off approximately two months before expected calving and all quarters of cows were infused with an antibiotic preparation approved for use in non-lactating cows following the last milking of lactation.

Sampling

A total of 108 lactating crossbred females were tested for sub-clinical mastitis per month i.e 1296 animals (5184 quarters) were tested for period of 1 year. Milk samples from each quarter were collected aseptically from apparently healthy lactating crossbred 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.

Sub clinical mastitis detection by California mastitis test

California mastitis test (CMT) was carried out as described by Schalm *et al.,* (1971). Briefly, from all the quarters (N=5184) 2 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.

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). The colonies confirmed as *Staphylococcus aureus* were further tested for antimicrobial susceptibility.

Antimicrobial susceptibility testing

The antibiotic-susceptibility profile of isolates for 14 different antibiotics was prepared using the disk diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (2008). In brief, *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), Amoxyclav (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), Cefotaxime (10 µg), Cephalexin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Co-Trimoxazole (30 µg), Enrofloxacin (10 µg), Gentamicin (20 µg), Levofloxacin (5 µg), Penicillin G (10 units) and Tetracycline (30 µg).

Results and Discussion

The present study was conducted to investigate the quarter wise incidence of sub clinical mastitis in crossbreds of bovine and *S. aureus* in Sub-clinical mastitis cases at Research cum Development Project on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri (India). The overall quarter wise incidence of sub clinical mastitis among 1296 apparently healthy crossbred cows was 3.62 per cent (188 quarters out of 5184 quarters) whereas 62 quarters (1.19 %) were found positive for Clinical mastitis and normal quarters were 4825 (93.07 %). Out of 188 quarters positive for sub-clinical mastitis, single quarter involvement per cow is seen in 166 cows (88.30 %), whereas 11 cows (11.70%) showed sub-clinical mastitis in two quarters.

As compare to clinical mastitis (1.19%), the incidence of subclinical mastitis (3.62%) is nearly 3 times. From the total of 188 milk samples from cows with sub-clinical mastitis, 172 samples showed growth of aerobic bacteria. From 8 milk samples no growth was observed.

The 152 *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 Cephalexin (86.84%), followed by Co-trimoxazole (82.89%), Penicillin G (82.23%), Ampicillin (81.57%), Cefoperazone (76.97%), Ceftriaxone (69.73%), Cefotaxime (65.13), Amoxyclav (63.15%), and Tetracycline (54.60 %).

For successful breeding from the health and economic point of view, it is paramount importance that the udder of lactating animals should remain healthy.

The overall quarter wise prevalence of sub clinical mastitis was 3.62 per cent whereas 1.19 per cent was found positive for Clinical mastitis and normal healthy quarters were 93.07 per cent. Out of 188 quarters positive for sub-clinical mastitis, single quarter involvement per cow is seen in 88.30 per cent, whereas 11.7 per cent showed sub-clinical mastitis in two quarters. In contrast to present study, several reports (de Medeiros *et al.*, 2009; Hussein, 2012) 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) and Mdegela *et al.*, (2009), 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 (3.62%) 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 cows, proper washing of milkers' hands, milking parlor using disinfectants, separate milking parlor etc.

As compare to clinical mastitis (1.19%), the prevalence of subclinical mastitis (3.62%) is nearly 3 times. Thus losses due to subclinical mastitis are more as compare to clinical mastitis. It also reflects that subclinical mastitis is responsible for 60-70 per cent of total economic losses associates with total mastitis infections. Moreover, subclinical mastitis frequently goes unnoticed and it is also impairing milk productions and reduced reproductive performance and increases culling of lactating animals.

From the total of 188 milk samples from cows with sub-clinical mastitis, 172 samples showed growth of aerobic bacteria. Failure to detect pathogens in 8 milk samples may be due to intermittent excretion of the organisms or their disappearance because of spontaneous recovery. Also the possibility of mycoplasmal mastitis cannot be ruled out in such cases, since the organism cannot be cultivated on common bacteriological media. After biochemical characterization, *Staphylococcus aureus* was confirmed in 152 samples (80.85 %). A high percentage of subclinical mastitis milk samples were positive for *S. aureus*. Similar, reports was also recorded by Singh *et al.*, (1982), Shukla *et al.*, (1998), Patel *et al.*, (2000) and Hussein (2012).

The higher infection rate of *S. aureus* is due to its ubiquitous nature and its ability to colonize the skin as well as the udder.

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

Table.1 Antibiogram results for *S. aureus* isolated from sub-clinical mastitis milk

Sr. No.	Antibiotics	Sensitive		Resistant		Total
		No.	%	No.	%	
1	Ampicillin	28	18.43	124	81.57	152
2	Amoxyclav	56	36.85	96	63.15	152
3	Cefoperazone	35	23.03	117	76.97	152
4	Cefotaxime	53	34.87	99	65.13	152
5	Ceftriaxone	46	30.27	106	69.73	152
6	Cephalexin	20	13.16	132	86.84	152
7	Chloramphenicol	81	53.29	71	46.71	152
8	Ciprofloxacin	88	57.90	64	42.10	152
9	Co-Trimoxazole	26	17.11	126	82.89	152
10	Enrofloxacin	122	80.27	30	19.73	152
11	Gentamicin	93	61.19	59	38.81	152
12	Levofloxacin	122	80.27	30	19.73	152
13	Penicillin G	27	17.77	125	82.23	152
14	Tetracycline	69	45.40	83	54.60	152

From the Table 1, it is observed that the higher resistance of *S. aureus* to various anitmicrobials agents like Ampicillin, Amoxyclav, Co-trimoxazole, Cephalexin, Cefoperazone, Ceftriaxone, Cefotaxime, Penicillin G and Tetracycline. Similar findings was also reported by various authors (Miller *et al.,* 1993; Mubarack *et al.,* 2012; Nthawat *et al.,* 2013; Patel *et al.,* 2000; Pavulraj *et al.,* 2013).

The moderate resistance to the antimicrobials used was noticed in Chloramphenicol (46.71%), followed by Ciprifloxacin (42.10%) and Gentamicin (38.81%). Lower resistance was observed in Enrofloxacin and Levofloxacin (19.73 % each). Relatively moderate to low resistance of *S. aureus* was reported to Chloramphenicol, Ciprofloxacin, Enrofloxacin, Gentamicin, Levofloxacin was reported by Abera *et al.,* (2013); Adwan, (2006); Awandkar *et al.,* (2013); Nthawat *et al.,* (2013). Our results are in agreement to these reports.

Higher resistance to ampicillin, amoxyclav and penicillin G is due to production of beta lactamase enzyme by isolates.

Resistance (24.95%) to cefoperazone antibiotic is reported by Ikiz *et al.,* (2013), who also reported higher sensitivity (91.66%) to Gentamicin, which is in contrast to our findings of low sensitivity (61.19%). Highest sensitivity of *Staphylococcus aureus* isolates to Gentamicin and Ampicillin was reported by Mubarack *et al.,* (2012) and Pavulraj *et al.,* (2013). Resistance to cefoperazone antibiotic is unusual as this antibiotic is recently introduced in Veterinary medicine in this area. But high resistance to all the cephalosporins may be attributed to its indiscriminate use in therapeutics in animals and human, irrespective of the infection may not be of bacterial origin. In contrast to present findings, Sharma *et al.,* (2012) reported 100 per cent sensitivity of *Staphylococcus aureus* isolates to Ceftriaxone, Cefoperazone and Tetraacycline and high (90.90 to 100.0%) sensitivity towards Enrofloxacin, Cephalexin). Difference in findings towards newer and older antibiotics may be due to rationale use of these antibiotics at the farms under study of latter.

Antibiotic resistant strains of *S. aureus* of animal origin are posing a growing, worldwide, especially in developing countries like India. The effectiveness of current treatment regime for mastitis control in animals may become hazardous not only to animals but also to human health. The indiscriminate and injudicious administration of antibiotics / antimicrobials and irrational treatment of bovine mastitis with different antibiotics have invited severe complications like multiple drug resistance (Ganguly *et al.,* 2016). Although innovations in various therapeutic regimens and improved management practices, mastitis unfortunately has remained ever green disease to Dairy industry (Mahapatra *et al.,* 2018).

Impacts

The present investigation demonstrates the presence of *S. aureus* in high percentage in subclinically positive mastitis milk samples.

The existence of alarming level of resistance to frequently used antibiotic by *S. aureus* isolates in the farm.

It is therefore, very important to implement a systemic application of *in vitro* antibiotic susceptibility testing prior to the use of the antibiotics in both treatment and prevention of intra-mammary infections.

Antimicrobial resistant pathogens in animals have been incriminated as a potential risk for

human health from nearly possible transmission of the *S. aureus* strains as milk borne pathogen.

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