International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2017 Vol.7 (3) September-December, pp.69-74/Saini et al.
Research Article

PREVALENCE OF COAGULASE POSITIVE *STAPHYLOCOCCUS AUREUS* IN MILK SAMPLES OF LIVESTOCK IN SEKHAWATI REGION, RAJASTHAN, INDIA

Deepika Saini[1]*, Dau Lal Bohra[2] and Ikram Quresi[3]
[1]Department of Biotechnology, JJTU, Jhunjhunu
[2] PG Department of Zoology, Seth G B Podar College, Nawalgarh, Jhunjhunu
[3]Department of Biotechnology, JJTU, Jhunjhunu
**Author for Correspondence: saini.deepika12@gmail.com*

ABSTRACT
Mastitis is an economic problem. The economic damage caused by mastitis, either clinical or sub-clinical, can be summarized as, milk production losses, drugs, discarded milk, veterinarian, labour, milk quality, culling, clinical mastitis, sub-clinical mastitis and other diseases. The costs for these factors might differ from farm to farm. The present study describes the presence of *Staphylococcus aureus* in the milk samples of ruminants. The study showed the isolation of 81 cultures of *S. aureus* from 240 normal and clinical cases of milk samples. A total 57 *Staphylococcus* isolates were found coagulase positive. Cases of Mastitis showed 100% bacterial load in 24 milk samples with *Staphylococcus* species. All cases reported with mastitis were observed during winter, summer, and rainy season. The highest cases were reported in 19 milk samples in winter season and 2 were found in summer and 3 were found in rainy season in all animals. On the behalf of sugar fermentation (D-Xylose, Sucrose, D-Trehalose, Maltose, Manitols) and haemolysis, 6 different species were identified, namely, *Staphylococcus aureus* (52), *Staphylococcus epidermis* (10), *Staphylococcus haemolyticus* (7), *Staphylococcus lugdunensis* (3), *Staphylococcus xylosus* (5), *and Staphylococcus caprae* (4) respectively.

Keywords: S. aureus, coagulase positive, Mastitis

INTRODUCTION

Milk is an example of ideal culture medium for different microorganisms because milk is rich in proteins, lipids and sugars, hence we can say that milk is a readymade vehicle for the omnipresent microorganisms. Some of the bacteria contained in milk (like as *Lactobacillus spp.* or *Bifido bacterium spp.*) are also present in the healthy human gastrointestinal tract, assisting in digestion and protection from other infections, while other external bacteria can be extremely harmful to human health *(Revathi et al., 2012).* The bacterial contamination of milk not only decreases the nutritional quality but also utilization of this type of milk terrorizes health of the society. The presence of these pathogenic microorganisms in milk poses a main public health threat, usually for those people who still drink raw milk *(Fadaei, 2014).* Animal Husbandry is an extensive economic business of the rural community, mostly in the arid and semiarid regions of the Rajasthan. Enlargement of livestock zone has an important advantageous impact in initiating employment and reducing poverty in rural areas.

Mastitis is a multi etiological complex disease with inflammation of mammary gland parenchyma, representing significant economic losses to dairy producers because of high morbidity, discarded milk, treatment costs and reduced milk production *(Chandrasekaran, 2013, Biffa et al. 2005).* Mastitis is associated with apoptosis of mammary epithelial cells and has a significant impact in averting the full genetic potential of an animal to produce milk. It can be characterized by many physical and chemical changes in milk, and pathological changes in the glandular tissues. The losses mainly arise from decreased milk production and quality, therapeutic interference, loss of antibiotic contaminated milk and extra labour. This disease has been described as the most imposing disease facing dairy producers around the world. Mastitis has a great impact on the economic loss in dairy sector. Aside from the decrease in

milk production and alterations in milk composition, studies have indicated an association between reproductive failure and clinical mastitis in lactating dairy cows.

Long-term surveys and reviews on mastitis carried out in different herds have shown the significance of *S. aureus* in the dairy industry losses, which have remained unchanged. *S. aureus* causes economic loss higher than for an average case of mastitis with other agent and disadvantages include, reduction in yield and quality of milk, veterinary treatment costs, premature culling and loss of genetic potential. Infections due to *S. aureus* are difficult to remove from animal and herd. Being an endemic disease on dairy farms all over the world, mastitis is an important cause of a less efficient milk production. Additional, mastitis affects milk quality directly through a change in technical and hygienic milk quality and indirectly through the intrinsic milk quality. Therefore mastitis is a huge concern for the dairy industry. Mastitis management therefore should have the goal of improving milk quality and the efficiency of milk production and thus make the production of milk more sustainable. Given the multi-factorial nature of mastitis, management consists of a wide range of activities, amongst others the treatment of diseased animals, dry cow therapy, prevention of transmission of infection and improvement of the immune system *(H. Hogeveen, 2005).*

The research work was carried out on *S. aureus* isolates from a variety of milk and dairy products in *shekhawati* area from different animal species. The present investigation examines phenotypic characterization of coagulase *positive S. aureus* from normal and clinical mastitis in cattle (cow, buffalo, goats etc.) in terms of cultural characterization.

MATERIALS AND METHODS

A total of 240 milk samples consisting of cow (n=60), Buffalo (n=60), Goat (n=60), sheep (n=60) were collected during winter (W), summer (S) and rainy (R) season of year 2015-16, from Jhunjhunu, Sikar, Churu, district. Economy of these areas mainly depends upon livestock animals for milk and milk products. The present study was carried for one year (Jan 2016 to Dec 2016). Different clinical and non clinical mastitis case which were interpreted for *S. aureus* positive were collected from Jhunjhunu, Sikar, Churu, districts of Rajasthan. The samples were screened for S. aureus on phenol red mannitol salt agar in sterilized petri plates by streaking. The culture plates were incubated at 37°C for 24-48 hours in incubator. Yellow pigmented colony was selected as positive isolate and further screened for gram staining. The gram positive cocci colonies were further screened for catalase and coagulase tests. For screening of MRSA, culture (0.5 Mac Farland) was streaked on Mueller-Hinton agar supplemented with 4% NaCl and 6 mcg/ml Methicillin to incubation overnight at 37°C.

RESULTS AND DISCUSSION

Staphylococcus is a versatile microorganism with several virulent characteristics and resistance properties. The name '*Staphylococcus'* for cocci shaped bacterial Colonies arranged in clusters was derived from the Greek word 'Staphyle' meaning a bunch of grapes. Clustering of staphylococci is magnified by growth on solid medium *(Kent B. Crossley et al., 2009).* Staphyloccoci are facultative anaerobes capable of generating energy by aerobic respiration, and by fermentation which produce principally lactic acid. *Staphylococcus species* is catalase positive, a feature differentiating them from *Streptococcus* species, and these are oxidase-negative. *Staphyloccoi* requires complex nutrients such as, many amino acids and vitamins B, for growth *(Konrad et al., 2009). Staphylococci* and *Streptococci* both genera are Gram positive and have the similar spherical cell shape, however there are called cocci. Both are having round, spherical cell shape, but the arrangement of cells is different due to a different binary fission. *Streptococci* form a chain of round cells, because their division occurs in one linear direction, whereas *staphylococci* divide in various directions forming grape-like clusters. When these bacteria divide, they do so along two axes, so form clumps of bacteria. This is as opposed to *streptococci*, which divide along one axis, so form chains *(Jin M.et al., 2005).* The *staphylococci* are non-motile, non-spore forming *(Harris et al., 2002)* and usually are an encapsulated or have limited capsule formation (Friedrich

et al., 2006). Most species have a complex nutritional requirement; in generally they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, such as arginine, valine, and B vitamins, including thiamine and nicotinamide. *Staphylococci* are tolerant to high concentrations of salt and show resistance to heat. Pathogenic staphylococci are commonly identified by their ability of coagulase production, and thus blood clotting. *S. aureus, S. intermedius* and *S. hyicus* are the coagulase positive species, and other staphylococcal species such as *S. epidermidis*, are coagulase-negative. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most characterised and studied strains *(Harris et al., 2002).*

Species of *staphylococci* are separated into two large groups on the basis of ability to produce the extracellular coagulase enzyme. Bacteria that produce coagulase are known as coagulase-positive *staphylococci* and those not producing are referred as coagulase-negative. The presence of coagulase can be estimated by assessing broth medium for secreted enzyme, which reacts with coagulase-reacting factor in plasma and results in formation of a fibrin clot, or by testing for cell-bound enzyme, which results in clumping when a suspension of organisms is incubated with plasma *(Bradley and Nizet, 2015).* The *staphylococci* make up the family of Gram-positive cocci, Staphylococaceae, *Staphylococcus aureus* was one of the first bacterial pathogens identified, and causes a very broad range of infections including impetigo, folliculitis, superficial and deep skin abscesses, wound infections, osteomyelitis, suppurative arthritis, pneumonia, pleural emphysema, meningitis, septicemia and endocarditis, toxic shock syndrome, scalded skin syndrome, and food poisoning.

Further characterization of *Staphylococcus haemolyticus* and Staphylococcus lugdunensis on the behalf of, β-D- Fructose, Urease Production and Ornithine decarboxylase were estimated. In this study we also tried to find more variety of Staphylococcus on the behalf sugar utilization characterized for confirmation test. On the behalf of sugar fermentation (D-Xylose, Sucrose, D-Trehalose, Maltose, Manitols) and haemolysis classified into six different species were found. The present values of occurrence of different species including *Staphylococcus aureus* was 64.2%, *Staphylococcus epidermis* 12.4%, *Staphylococcus haemolyticus* 8.6%, *Staphylococcus lugdunensis* 3.7%, *Staphylococcus xylosus* 6.2%, and *Staphylococcus caprae* was found 4.9% in total 81 *Staphylococcus* species isolates. Highest occurrences of *Staphylococcus* spp. were found in clinical and non- clinical cases. *S. aureus* was higher than *S. lugdunensis* (Fig.1)

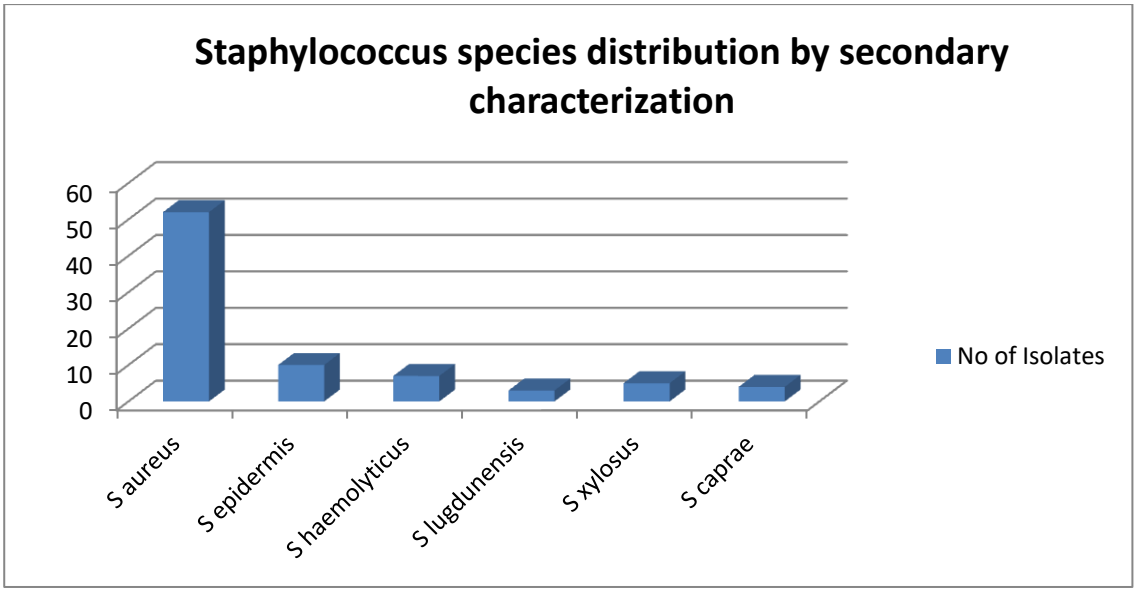

Figure 1: Staphylococcus species distribution by secondary characterization

Characterizations of Staphylococcus aureus

Test of coagulase production: The coagulase production is considered an important criterion for identification of S. aureus. However, non-production of coagulase by S. aureus has also been reported by various workers (Prasad and Yadav, 2000; Wani and Bhatt, 2003; Singh 2006). Difference in coagulation of plasma from various species was observed by Qureshi et al. (2004), Bohra et al. (2009) who recorded that S. aureus isolates coagulated the plasma from rabbit, human, buffalo, horse, cattle, goat, camel and sheep in decreasing order of superiority (Table1)

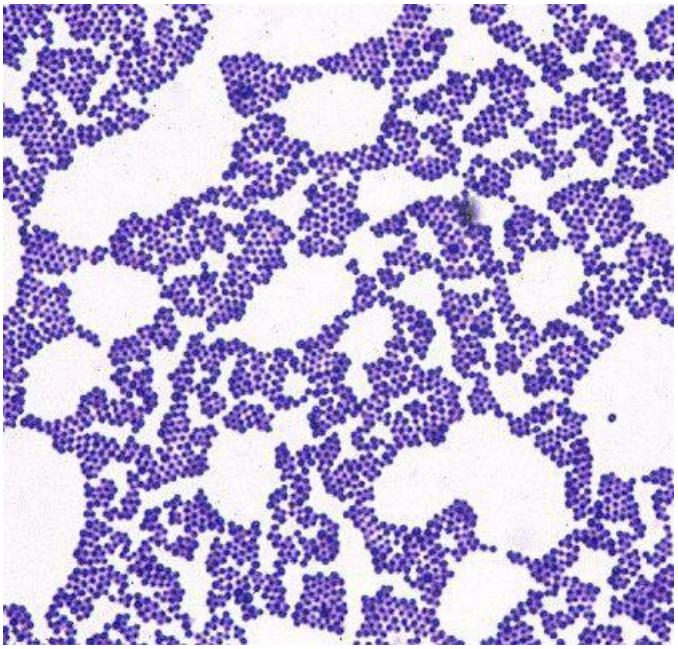

Figure 2: Cluster morphology of *Staphylococcus aureus in milk sample*

Table 1: Test of coagulase production

s. no	Species	Coagulase
1	*Staphylococcus aureus*	+
2	*Staphylococcus epidermis*	_
3	*Staphylococcus haemolyticus*	_
4	*Staphylococcus lugdunensis*	_
5	*Staphylococcus xylosus*	_
6	*Staphylococcus caprae*	_

Haemolytic properties (Test for toxin production of Staphylococcus aureus)

In the present study from 42 cattle isolates of *S. aureus* (Fig.2) subjected to haemolysis on sheep blood agar, 22 produced α haemolysis (complete haemolysis), 14 produced β haemolysis (partial haemolysis) and 6 of the isolates produced both α and β type of haemolysis on blood agar. Almost similar results were obtained with 10 sheep and goat isolates where 6 isolates produced α haemolysis, 3 produced β

haemolysis and 1 produced both α and β type of haemolysis. Hence, out of total 52 isolates in the present study, 45 isolates were found to produce either α or β haemolysis and 7 isolates produced both α and β haemolysis. The plates with incomplete haemolysis incubated at 4°C turned into complete lysis (Hot-cold lysis) by next morning.

Table 2: Haemolytic properties in different milk sample

S. No.	Samples	No of *Staphylococcus aureus*	α-haemolysis	β haemolysis	Both α and β haemolysis
1	Cattle (Cow + Buffalo) n=120	47	22	19	6
2	Sheep and Goat n=120	10	6	3	1

CONCLUSION

It was found that 57 *Staphylococcus* species isolated including coagulase positive and negative and as confirmatory 24 clinical cases with mastitis 100% bacterial load was found within 24 milk samples with *Staphylococcus* species. A total 47 *Staphylococcus aureus* including (α, β haemolysis and α and β both haemolysis) occur in cow and buffalo milk samples. From small ruminant sheep and goat, only 10 *Staphylococcus aureus* including α, β haemolysis (Table.2). The present study was conducted with the aim to characterize *S. aureus* isolated from milk samples from cattle and goat with clinical mastitis with better treatment.

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