RESEARCH ARTICLE

# Cultural and Biochemical Characterization of *Staphylococcus aureus* Isolates from Bovine Clinical and Subclinical Mastitis

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## Abstract

Bovine mastitis causes huge economic losses to the dairy industry all over the world. *Staphylococcus aureus* is a most common causative agent of bovine mastitis. The study was undertaken with an objective of isolation, identification and biochemical characterization of *Staphylococcus aureus* isolated from bovine mastitis cases. Total of 256 bovine milk samples (32 from clinical mastitis and 224 from suspected cases of subclinical mastitis) were collected from in and around Navsari. Of the 224 SCM suspected milk samples screened by California mastitis test (CMT), 108 (48.21 %) samples were found positive for subclinical mastitis, whereas all 32 clinical mastitis samples were CMT positive. Thus total 140 bovine milk samples (32 clinical and 108 subclinical mastitis) were used for detection of *Staphylococcus aureus.* Out of these, 17 (12.14%) isolates were identified as *Staphylococcus aureus* on the basis of cultural characteristics and biochemical tests. From 17 *S. aureus,* 16 (94.11%) isolates were categorized as mannitol fermenter and coagulase positive, while 1 (5.88%) isolate was mannitol non-fermenter and coagulase negative. Majority of the isolates 10 (58.82%) exhibited beta-haemolysis, whereas 5 (29.41%) isolates showed alpha-haemolysis and 2 (11.76%) isolates were non-haemolytic on 5% sheep blood agar. Among 17 *S. aureus* isolates, 14 (82.35%) isolates exhibited slime production, while 6 (11.76%) isolates were negative for slime production. This study showed overall 12.14 % incidence of bovine mastitis by *Staphylococcus aureus,* whereas cases of clinical mastitis and sub-clinical mastitis noted as 9.30% and 12.96%, respectively, by *S. aureus*.

**Key words:** CMT, Mannitol fermentation, Mastitis, Slime production, *Staphylococcus aureus.*

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## Introduction

Cows and buffaloes have been considered as main species that share major contribution in annual milk production of the country. Mastitis has been identified as a significant issue that causes major economic losses to dairy industry not just in India but also globally. Major economic losses occur due to the lowered milk production, poor milk quality and treatment costs (Parasana *et al*., 2024). Mastitis is an inflammation of the mammary gland characterized by physical, chemical and bacteriological changes in milk and pathological changes in the glandular tissues of the udder (Abd-Elrahman, 2013). It is divided into two main types as clinical and subclinical mastitis. Clinical mastitis is easily detected by visible abnormalities in udder and milk along with fever, depression and anorexia in animals (Cheng and Han, 2020). Subclinical mastitis (SCM) is asymptomatic (Hoque *et al*., 2015) and no gross abnormalities of the udder or milk are observed. It causes reduction in milk production up to 80% and 3-4 times more losses as compared to clinical mastitis (Mungube *et al*., 2005). California mastitis test (CMT) is considered as accurate, simple-to-use cowside screening test for subclinical mastitis at field level. It is used to identify udder inflammation in its early stages, preventing the spread of the infection across the herd (Bogni *et al*., 2011).

Bovine mastitis is multi-etiological condition. The most common mastitis pathogens are contagious and environmental pathogens, *viz*., *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli* and *Klebsiella* spp. Among the contagious pathogens, *Staphylococcus aureus* is the most common causative agents of bovine mastitis (Parasana *et al*., 2021). The ability of the bacteria to invade the immune host cells needs various virulence factors to begin growth and multiplication (Parasana *et al*., 2022). *Staphylococcus aureus* produces a variety of extracellular and cell wall associated virulence factors which are involved in the pathogenesis of

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mastitis (Momtaz *et al*., 2010). Conventional microbiological methods are considered as the gold standard for identification of bacteria from milk samples and routinely employed for confirmation of causative agents (Javia *et al*., 2018). So this study was designed to isolate, identify and determine the pathogenic *S. aureus* in cases of bovine mastitis using conventional laboratory methods.

## Materials and Methods

### Sample Collection

Milk samples were aseptically collected from cows and buffalos in and around areas of Navsari region (Gujarat, India). 224 milk samples (cows=86, buffaloes=138) from suspected cases of subclinical mastitis were collected and screened by California mastitis test (CMT) and graded as subclinical mastitis cases as per guidelines given on the reagent bottle. 32 milk samples of cows (n=26) and buffaloes (n=6) showing signs of clinical mastitis (CM) were also collected aseptically. All the samples were transported to the laboratory with chilled condition.

### Cultural Identification of *Staphylococcus aureus*

All the clinical and subclinica mastitis milk samples were streaked on 5% sheep blood agar and incubated aerobically at 37°C for 24 h for isolation of bacterial colonies. After reading colony characteristics the colonies were again streaked on brain heart infusion (BHI) plates for pure colony isolation, and then Gram staining was carried out for microscopic examination of organisms followed by catalase and oxidase tests (Yimana and Tesfaye, 2022).

### Mannitol Fermentation

The isolates of *Staphylococcus aureus* were inoculated on the mannitol salt agar and incubated at 37°C for 18-24 h. The yellow colored colonies were considered as mannitol fermenters (Parth *et al*., 2016). This occurs because *S. aureus* ferments mannitol and turns the medium yellow.

### Haemolysin Production

Haemolytic activity of the *S. aureus* was detected on 5 % sheep blood agar. All the isolates were streaked on the medium and incubated aerobically overnight at 37°C for 24 h. Haemolytic patterns of isolates were recorded on the basis of complete haemolysis, incomplete haemolysis and no haemolysis and graded as alpha, beta and gamma haemolysis, respectively (Darwish and Asfour, 2013).

### Coagulase Test

Aseptically 3 mL sterile distilled water was mixed with the contents of vials (FD248, Hi Media) as per instruction given by manufacturer. From this, 0.5 mL of solution was taken (rehydrated FD248) in a tube. Then 0.05 mL of overnight broth culture of *S. aureus* was added, mixed and incubated at 37°C in incubator for 4 h. Tubes were observed for clot formation and results were recorded (Makwana *et al*., 2012).

### Slime Production Assay

For slime production assay, each isolate was streaked on the Congo red agar medium and incubated aerobically at 37°C for 24 h. The production of black or red colonies by the isolates was recorded and interpreted according to Parth *et al*. (2016).

## Results and Discussion

In the present study, out of 224 SCM suspected milk samples from cows and buffaloes screened by CMT, 108 (48.21%) samples were found positive for subclinical mastitis. Out of 86 cow samples 37 (43.02%), and out of 138 buffalo samples 71 (51.44%) samples were found positive for subclinical mastitis. Similar to this, earlier workers have reported 51 to 54 % prevalence for subclinical mastitis in bovines (Reddy *et al*., 2014; Kabir *et al*., 2017; Mohammed *et al*., 2019).

In the present study, 50 % of the total isolates were identified as *Staphylococcus* spp. from cases of clinical and subclinical mastitis. All the isolates exhibited Gram positive cocci in group morphology microscopically, catalase positive and oxidase negative. Similarly, Jeykumar *et al*. (2013) and Mehmeti *et al*. (2016) reported 44.44 % and 58.5 % *Staphylococcus,* respectively, from bovine mastitis cases. Previous reports also stated *Staphylococcus* spp. as one of the most frequently isolated bacteria as compared to other organisms in bovine mastitis in India as well as worldwide (Kurjogi and Kaliwal, 2011; Javia *et al*., 2018).

Overall incidence of *Staphylococcus aureus* from cases of clinical and subclinical mastitis was found to be 12.14% (17 out of 140) in which number of isolates of *S. aureus* in clinical mastitis was recorded as 9.30 % (03/32), while from subclinical mastitis cases it was 12.96 % (14/108).

Species wise, in cows 9.52 % (06/63) incidence of mastitis was recorded (clinical mastitis 11.53 % and subclinical mastitis 8.10 %), while in buffaloes, 14.28 % (11 /77) incidence of mastitis (Subclinical mastitis 15.49 %) was noted due to the *S. aureus*. Compared to present study, higher incidence of *Staphylococcus aureus* was reported by many workers (Parth *et al.,* 2016; Gayatri *et al*., 2017; Abed *et al*., 2021; Lubna *et al*., 2023) from bovine mastitis. This might be due to involvement of other etiological agents in mastitis condition and other factors like season, breed of the animal, anatomy of the udder, lactation stage of animal and hygienic condition related with different geographical locality.

Mannitol fermentation is one of the useful characteristics for screening and differentiation of *S. aureus* from other species (Pumipuntu *et al*., 2017). Due to fermentation there is change in the pH of phenol red indicator which imparts yellow color to the colonies and medium. In present study, 94.11% (16/17) isolates were mannitol fermenters and 5.88% (1/17) isolates were mannitol non-fermenters, whereas Bhanderi (2007) recorded 74.41% isolates as mannitol fermenters and 25.58% mannitol non-fermenter *S. aureus*.

Out of 17 *Staphylococcus aureus* isolates, 10 (58.82%) were beta haemolytic, 5 (29.41%) were alpha haemolytic, while 2 (11.76%) were found non-haemolytic. Similar to this, Parth

*et al*. (2016) studied alpha, beta and gamma haemolysis in *Staphylococcus aureus* and found 33.96%, 49.06% and 7.55% isolates with this pattern, respectively. In the present investigation more isolates of beta haemolytic *Staphylococcus aureus* were observed (58.82%) in the clinical and subclinical mastitis cases which supports the previous reports of Morandi *et al*. (2009) and Parth *et al*. (2016). In contrast to this, Bhanderi (2007) reported 62.79% alpha haemolytic isolates and Xiaohong and Yanjun (2011) reported 43.4% alpha, 34.11% beta and 22.48% gamma haemolytic isolates of *S. aureus* from bovine mastitis.

In this investigation, 16 (94.11%) isolates were found coagulase positive on the basis of clot formation in overnight broth culture and 1 (5.88%) isolate was coagulase negative in which there was no clot formation detected. Similarly, Makwana *et al*. (2012) and Parth *et al*. (2016) reported 94.00% and 92.45% coagulase positive *Staphylococcus aureus*, respectively, from bovine mastitis cases. While Pankaj *et al*. (2013) noted lower number of coagulase positive isolates of *Staphylococcus aureus* (31.30%) from mastitic milk.

Slime production ability is a good indication of pathogenic ability of *Staphylococcus aureus* which aid in survival and colonization in host tissue. In the present investigation slime production was observed in 82.35% (14/17) isolates, which produced black colonies on Congo red agar while 11.76% (2/17) isolates found negative (red colonies on Congo red agar) for slime production. Similarly, Parth *et al*. (2016) recorded slime production in 79.25% isolates, while Darwish and Asfour (2013) recorded slime production in 70.4% isolates of *Staphylococcus aureus*.

## Conclusion

The findings of the present study revealed overall incidence of bovine clinical and subclinical mastitis, caused by *Staphylococcus aureus* as 12.14 % in and around Navsari. Mannitol fermentation test, haemolytic activity, coagulase production and slime production tests were valuable for biochemical identification and phenotypic characterization of pathogenic *Staphylococcus aureus*. CMT can be considered as simple and cost effective test for regular screening of sub-clinical mastitis.

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