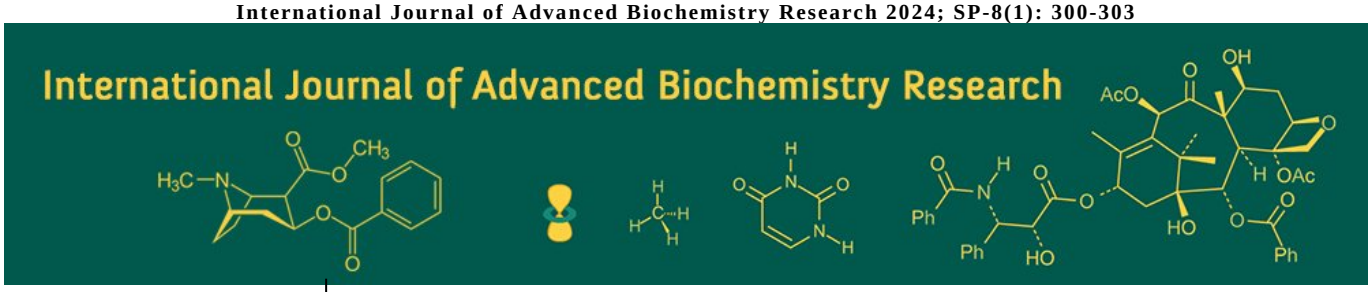


**ISSN Print:** 2617-4693
**ISSN Online:** 2617-4707
IJABR 2024; SP-8(1): 300-303
www.biochemjournal.com
Received: 23-10-2023
Accepted: 28-11-2023

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# Detection of *Staphylococcus aureus* from bovine mastitis milk

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**DOI:** https://doi.org/10.33545/26174693.2024.v8.i1Se.404

**Abstract**
Mastitis, an inflammation of the mammary gland. *Staphylococcus aureus* is the most common cause of economic loss in dairy cattle due to decrease quality and quantity of milk. This study was aimed to find-out the prevalence of *S.aureus* in case of bovine mastitis (clinical) through conventional method. Absolute diagnosis of pathogens plays great role in cure of disease. A total of 27 *S. aureus* isolates were isolated from 50 individual milk samples from dairy cows with clinical mastitis. The results showed that they were positive in gram staining (forming a bacteria cluster of purple cocci), catalase test, coagulase test, blood agar haemolysis, and DNase test. The mannitol, trehalose, sucrose, maltose, and lactose tests were also positive. The prevalence of bacterial isolates in conventional detection were 55%.

**Keywords:** Bovine, mastitis, prevalence, *Staphylococcus aureus*

## Introduction
India has vast resources in terms of livestock and poultry which play major role in improving the socio-economic condition of rural masses. Total livestock population in country is 535.78 million of which total bovine population (Cattle, Buffalo, Mithun and Yak) is 302.79 million have increased by 1.0% over the previous census. The Female Cattle (Cows population) is 145.12 million, increased by 18.0%. (20$^{th}$ livestock census, 2019) [1].
India is the largest producer of milk but mastitis is the most prevalent disease affecting dairy farms in country. It is defined as inflammation of parenchyma of mammary glands and is characterized by physical and pathological changes in glandular tissue, bacteriological changes in milk (Radostits, *et al*., 2000) [9]. Mastitis is a multi-etiological complex disease, which is affects milk quality directly in the technical characteristics and the hygienic quality of the milk, and indirectly through the intrinsic milk quality (Hogeveen *et al*., 2002) [4]. The overall prevalence of bovine mastitis in India has been reported to be 44.67% (Sharma *et al*., 2010) [11]. *Staphylococcus aureus* is one of the main etiological agent that cause contagious bovine mastitis. *S. aureus* mastitis is extremely difficult to control by treatment alone. (Pumipuntu *et al*., 2017) [8]. The bacteria persist in mammary glands, teat canals, and teat lesions of infected animal and infection is spread at the time of milking, when healthy gland come in contact with infected gland via milker's hand, infected utensils, milking machine etc. To date, successful control has been achieved only through the prevention of new infections and culling infected animals.

## Materials and Methods
### Collection of samples
The collection of milk samples was from cows affected with mastitis that had symptoms i.e. change in the colour of milk, presence of flakes, stickiness property in the milk, inflammation of the udder, and decreased production of milk. Before collection of milk, the udders of the affected cows were cleaned with water and then dried. Ethanol (70%) dipped cotton were used to disinfect the udder. Initially, the first few streams of milk were dropped and then the collected milk was kept in a cool carrier with ice and transported to the adjacent laboratory within 4 hrs. All samples were subjected for cultural examination and biochemical identification.

**Isolation of bacteria**

The milk samples were cultured on mannitol salt agar, 5% blood agar and incubated at 37 °C for 24 h.

- **Fermentation test on Mannitol Salt Agar medium (MSA):** The acid condition, as the color changes from pink to medium yellow, indicates the presence of *S. aureus*.
- **Coagulation Test:** Plasmatic clotting, showing the presences of *S. aureus*.
- **Hemolysis Test (Test Pathogenicity):** The pathogenic *S. aureus* is characterized by the ability to lyse red blood cells, with the presence of a transparent zone around the colonies on Blood Agar Media.

*S. aureus* identification was based on Gram's staining, conventional microbiological test including catalase test, oxidase test, coagulase activity, fermentation of various sugar by using API biochemical test strip, mannitol, and haemolysis of sheep blood agar. After confirmation of *S. aureus* colonies were sub-cultured on separate nutrient agar plates in order to obtain pure culture. All isolates were stored in 50% glycerol at -80 °C.

**Results and Discussion**

The isolates of *Staphylococcus aureus* were gram positive which morphologically are cocci and was clustered together like grapes (Fig.1). They were non motile, catalase positive and oxidase negative. Catalase enzyme functions as a catalyst, which diffused hydrogen peroxide ($H_2O_2$) into $H_2$ and $O_2$; thus, when the bacteria colonies are mixed up with $H_2O_2$, gas bubbles are produced. These isolates were showing golden yellow color on mannitol salt agar (Fig.2) because during fermentation of mannitol, phenol red turned into yellow color by formation of acidic by-product.

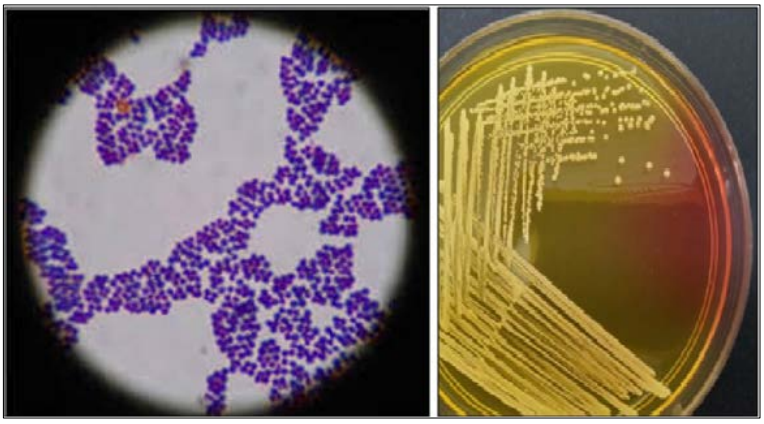

**Fig 1 & 2:** Morphology of *S. aureus* and fermentation of Mannitol salt agar (golden yellow) by *S. aureus*

Coagulase testing is the reliable method for identifying *Staphylococcus aureus* (Koneman *et al*., 1997) [7]. The phenotypically identified isolates were confirmed by production of coagulase revealed that 70% of isolates were produced coagulase (Table 1). Some of the isolates showed weak coagulation reaction even after 5 hr. of incubation however, they all showed a strong reaction at the 24 h reading. The production of the coagulase enzyme becomes the pathogenic factor of *S. aureus*, differentiating it from other types of Staphylococci. The coagulase enzyme is able to clot blood plasma, since it resembles prothrombin, which can convert fibrinogen to fibrin (Fig.3). Our results are in agreement with Turkyilmaz and Kaya (2005) [13]; Kateete *et al*. (2010) [5] and Yadav *et al.* (2015) [15].

In the present study, test result showed that twenty out of the 27 isolates (75%) were able to had DNase activity. Deoxyribonuclease (DNase) is an extracellular enzyme and virulence factor produce by *S. aureus*. It is capable of destroying deoxyribonucleic acid (Subathra *et al*., 2016) [12]. Kateete *et al*., (2010) [5] also found that 75% (24/32) of *S. aureus* isolates were showed DNase activity. Similar findings were reported by Rao *et al.* (2002) [10].


**Fig 3:** Coagulase positive *S. aureus*

Following the identification of target organism, the colonies were then streaked onto sheep blood agar (BA), in order to observe the its ability to induce hemolysis of blood erythrocyte, to visualize the hemolytic zone around colonies. In the present study, 6 isolates produced alpha haemolysis (complete), 16 produced beta haemolysis (incomplete) and 5 isolates not produced haemolysis on sheep blood agar. Staphylococcus produces alpha haemolysin causes a narrow zone of complete haemolysis and beta haemolysin produces a wider zone of partial or incomplete heamolysis or both (double haemolysis) or no haemolysis in some other bacteria (Cowan, 1993) [2]. The results of the present study are in complete agreement with that of Khichar and Kataria (2007) [6]; Upadhyay and Kataria, (2010) [14], Gangwal *et al.* (2016) [3] who did record hemolytic activity of *S. aureus* obtained from milk samples of cattle and goats.

The sugar fermentation studies were carried out by using API biochemical test kit for *S. aureus*. In our investigation, all the isolates of target organism fermented mannitol, lactose, sucrose, 92.5% of the isolates fermented Trehalose and Maltose. Whereas more than 90% of the isolates not fermented arabinose and raffinose (Table 1). The observations of the present study with respect to fermentation of mannitol, maltose, fructose, dextrose, sucrose, mannose is similar to findings of Upadhyay (2010) [14] who carried out fermentation studies with *S. aureus* obtained from milk of mastitic cattle and goat.

**Table 1:** Biochemical characterization of *Staphylococcus aureus*

| | Positive | | |
|---|---|---|---|
| **Test** | **Number** | | **Percentage** |
| DNase test | 20/27 | | 75% |
| Haemolysis | 22/27 | | 82% |
| | 6 α | 16 β | |
| Coagulase | 19/27 | | 70% |
| Mannitol | 27/27 | | 100% |
| Trehalose | 25/27 | | 92.5% |
| Lactose | 27/27 | | 100% |
| Maltose | 25/27 | | 92.5% |
| Sucrose | 27/27 | | 100% |
| Arabinose | 3/27 | | 11% |
| Raffinose | 2/27 | | 7.4% |

## Conclusion

On the basis of our study, it can be concluded that the incidence of *S. aureus* mastitis in a studied area was quite high which were 55%. It can be diagnosed on the basis of phenotypic characterization. It was little bit laborious but helpful to find-out the proper cause of mastitis which play tremendous role in cure out the disease. *S. aureus* is a contagious bacterium, which can be isolated from the skin surface of humans or animals. Its role as a cause of mastitis in cows is related to environmental sanitation, milking hygiene, improper diagnosis, lack of awareness about prevention and control. Abundantly use of antibiotic is maybe a major cause of its prevalence in field condition.

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