

Original Research Article

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## Detection of Phenotypic Variations and Biotypes of *Staphylococcus aureus* Obtained from Cattle Mastitic Milk

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### ABSTRACT

#### Keywords

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Cattle, Phenotypic  
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The present study was designed to find variations among *Staphylococcus aureus* on the basis of cultural and biochemical properties. From 59 samples of cattle clinical mastitic milk, 28 isolates were obtained and confirmed by species specific primers targeted against 23S rRNA with an amplicon of 1250bp. The genotypically confirmed isolates were subjected to determine phenotypic variations among them. In our study total seven biotypes were detected with variations in pigment production, haemolysis pattern and coagulase production.

### Introduction

Mastitis is one of the most important diseases affecting production in dairy industry worldwide and causes both direct and indirect losses to dairy farming (Petrovski *et al.*, 2006). *Staphylococcus aureus* is the main causative agent responsible for clinical mastitis in cattle (Kateete *et al.*, 2010). The organism exhibits variation on phenotypic properties and hence typing approaches are of significant importance to identify and understand the distribution of *S. aureus* strains among dairy herds. From the past few years, phenotypic methods such as antibiotic resistance typing, biotyping and phage typing are being used for typing of *S. aureus* isolates

(Wilson, 1987; Dallal *et al.*, 2010). To biotype and characterize *S. aureus*, phenotypic methods like pigment production, coagulase production and haemolysis are easy, rapid and economical techniques for epidemiological investigations (Momtaz *et al.*, 2011). The present study was designed to determine the biotypes on the basis of various pigment production, haemolysis, coagulase production and their ability to ferment mannitol.

### Materials and Methods

#### Collection of samples

A total of 59 milk samples from cows irrespective of breed and age, with clinical

mastitis were collected directly from teats in sampling tube, each about 5-10 ml in amount and then immediately taken to laboratory over ice for further processing on the same day. These animals belonged to different localities of Bikaner (Rajasthan).

### **Isolation and identification of *Staphylococcus aureus***

The organisms were isolated and identified as described by Cowan and Steel (1975) and Quinn *et al.*, (1994). Briefly, milk sample was swabbed on nutrient agar medium and then incubated overnight at 37°C. Next day different bacterial colonies were closely observed for their morphology, colour and consistency. Gram's staining, oxidase test and catalase test were used as primary identification tests and further the cultures were processed for confirmation by 23S rRNA gene-based genotyping. For genotypic confirmation a set of species specific primers was used *viz.* forward - 5'-ACG GAG TTA CAA AGG ACG AC-3' and reverse - 5'-AGC TCA GCC TTA ACG AGT AC-3' (Straub *et al.*, 1999).

### **Biotyping of *Staphylococcus aureus***

All genotypically confirmed isolates were further studied for cultural and biochemical properties. The isolates were cultured on mannitol salt agar and 5% sheep blood agar and incubated for 24-48 h at 37°C. Coagulase production test was carried out in tubes for production of free enzyme using human plasma according to Quinn *et al.*, (1994). Biotypes were determined as per biotyping methodology described by Devriese (1984).

### **Results and Discussion**

In the present study, out of 59 milk samples, 28 *Staphylococcus aureus* isolates were obtained with a recovery of 47.45% as

confirmed by species specific PCR method targeted against 23S rRNA with an amplicon of 1250 bp (Fig. 1).

Of the 28 isolates, seven biotypes were detected on the basis of pigment production, mannitol fermentation, haemolysis on blood agar and coagulase production (Table 1), *Staphylococcus aureus* isolates of the biotype-4 were found more prevalent (42.85%) whereas biotype-1, 3 and 6 were found with minimum prevalence (3.57%). All biotypes were mannitol fermenters (Fig. 2).

Out of seven biotypes, five biotypes produced golden yellow pigmentation (biotype-2, 3, 4, 5 and 6) while biotype-1 produced yellow pigmentation (3.57%) and biotype-7 produced white pigmentation (10.71%). Only biotype-3 was negative coagulase production in tube coagulase test (Fig. 3).

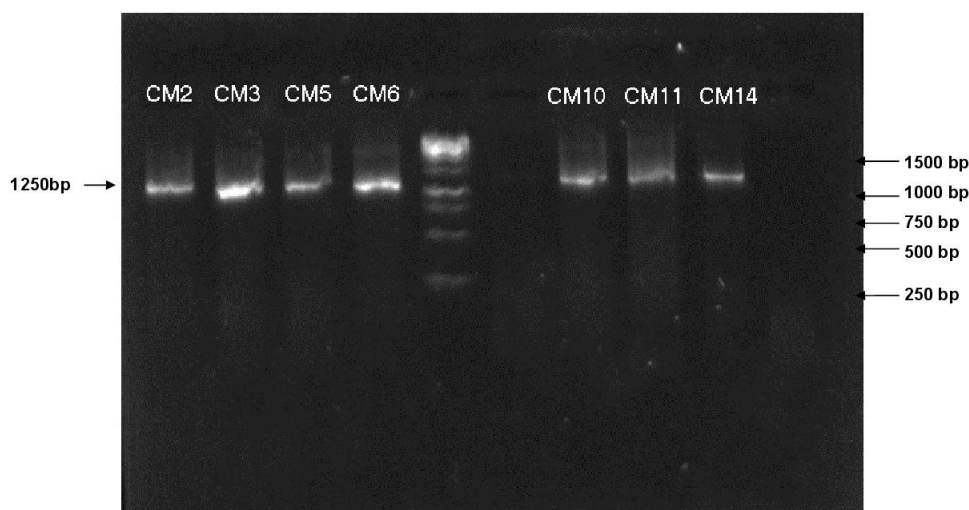
In present investigation, out of the total biotypes, biotype-5 produced complete haemolysis, biotype-1 and 4, produced incomplete haemolysis (Fig. 4) and biotype-6 produced both (complete and partial) haemolysis whereas biotype-2, 3 and 7 did not produce any haemolysis. When biotypes (1, 4 and 6) with incomplete and both haemolysis were incubated at 4<sup>0</sup> C to study hot-cold lysis, biotype-4 did not show hot-cold lysis whereas biotype-1 converted to complete haemolysis (hot-cold lysis) from incomplete haemolysis and biotype-6 from both haemolysis (complete and partial) changed to complete haemolysis.

In the present investigation, a biotyping, was used to determine phenotypic variations among *Staphylococcus aureus* isolates which may be used for epidemiological surveillance. The prevalence rate (47.45%) obtained in this study is in agreement with Seyoum *et al.*, (2017) who reported 42.9% occurrence of *S. aureus* from clinical mastitis milk samples of cattle.

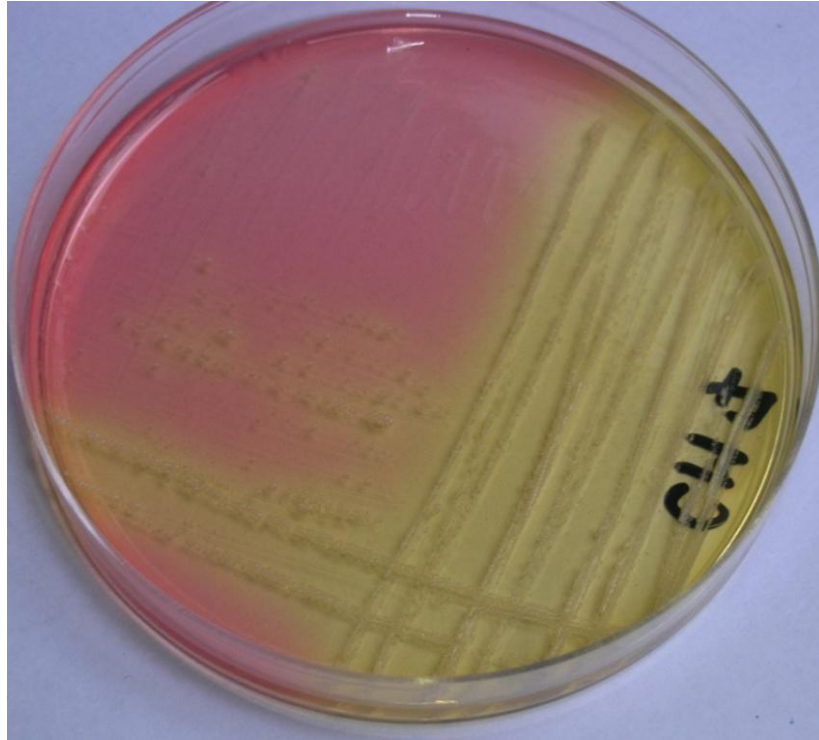
**Table.1** Cultural and biochemical variations among *Staphylococcus aureus* isolates via biotyping

S. N.	Biotype	Isolate (n)	Pigment Production	Mannitol Fermentation	5% Sheep Blood agar		Coagulase Production
					Haemolysis Phenomena	Hot Cold lysis Phenomena	
1.	1 Biotype1	CM2 (1)	Yellow	Fermentative	Incomplete	present (Convert in to Complete haemolysis)	Positive
2.	Biotype 2	CM1, CM12, CM16, CM20, CM26, CM28 (6)	Golden yellow	Fermentative	Negative	Negative	Positive
3.	Biotype 3	CM27 (1)	Golden yellow	Fermentative	Negative	Negative	Negative
4.	Biotype4	CM3, CM5, CM6, CM9, CM11, CM13, CM15, CM17, CM19, CM21, CM23, CM25 (12)	Golden yellow	Fermentative	Incomplete	Absent	Positive
5.	Biotype 5	CM4, CM8, CM14, CM18 (4)	Golden yellow	Fermentative	Complete	Complete	Positive
6.	Biotype 6	CM7 (1)	Golden yellow	Fermentative	Both	present	Positive
7.	Biotype7	CM10,CM22 CM24 (3)	White	Fermentative	Negative	Negative	Positive

**Fig.1** Agarose gel electrophoresis of amplicons of 23S rRNA ribotyping of *S. aureus* isolated from cattle with clinical mastitis



**Fig.2** Mannitol fermentation by *S. aureus* isolated from cattle with clinical mastitis on Mannitol salt agar

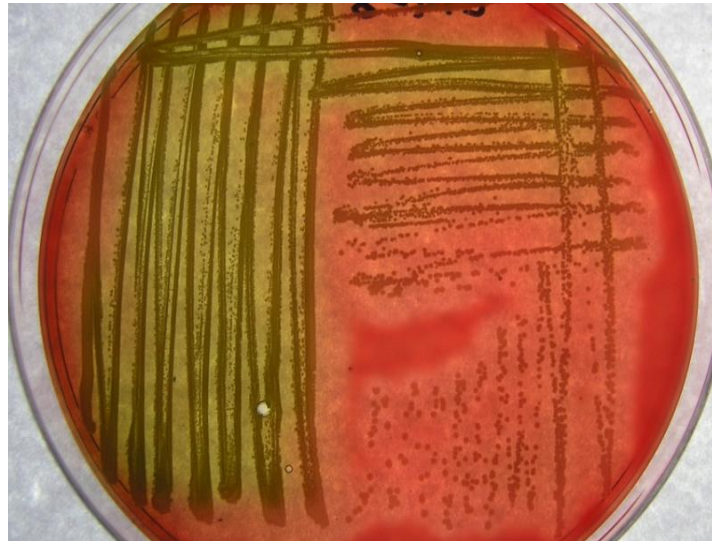


**Fig.3** Tube coagulase test for *S. aureus* isolates obtained from cattle with clinical mastitis with human plasma





**Fig.4** Partial haemolysis by *S. aureus* isolated from cattle with clinical mastitis on Sheep blood agar



The mannitol fermentation of isolates on MSA obtained in our study is similar to earlier observations of Lange *et al.*, (1999), Sanjiv *et al.*, (2008), Sharma *et al.*, (2013) and Jahan *et al.*, (2015) who had also isolated *S. aureus* from different sources like subclinical mastitis milk, clinical mastitic milk, nasal discharge from pneumonic camels and raw cow milk, respectively. The observations made in this study is in accordance with the study of Pumipunto *et al.*, (2017) who reported sensitivity of MSA fermentation is 100% effective in detection of *S. aureus*. The production of staphyloxanthin- a membrane bound carotenoid pigment imparts colour to the colonies in variable amounts (Clauditz *et al.*, 2006) and protects *S. aureus* against oxidative stress. In this study only biotype 1 comprising of a single isolate and biotype 7 comprising of three isolates were recorded as yellow and white, respectively, while all other five biotypes were recorded with golden yellow pigment. Study on 143 of *S. aureus* isolates from humans and bovine raw milk samples in an Iranian province also showed that 85, 38 and 20 isolates produced purple, yellow and white coloured colonies (Alini *et al.*, 2016). El-

Jakee *et al.*, (2010) isolated *S. aureus* from mastitic cows and buffalo and obtained three types of pigments *viz.* golden yellow, creamy and white. Quereshi and Kataria (2006) also characterized *S. aureus* from skin wounds in camel with colony pigmentation as golden yellow, yellow and white. Bhati *et al.*, (2016) obtained 38 *S. aureus* from subclinical mastitis in cattle and recorded 35 isolates with golden yellow pigmentation and three with white colonies. Hence, mannitol fermentation and pigment production can be effective tests to find variations among isolates.

In our results 27 of the 28 isolates were coagulase positive which is similar to the results reported by Kateete *et al.*, (2010) who reported 29 out of 32 *S. aureus* positive for human plasma. Sanjiv *et al.*, (2008) also reported coagulase negative *S. aureus* isolates from clinical mastitis in cattle from the same area of study. However, other workers (Khichar and Kataria, 2015; Al-Ratha and Sekhi, 2016 and Bhati *et al.*, 2016) did not record coagulase negative isolates from cattle mastitis. The results of haemolysis corroborated earlier reports by Yadav *et al.*, (2015) who investigated 32 *S. aureus* isolates

from milk of cattle and buffalo with clinical mastitis of which five, 20, four and three showed complete, partial, both and no haemolysis, respectively, on 5% sheep blood agar. The results in our study showed hot-cold haemolysis similar to reports of Bhati *et al.*, (2016) who observed 33.33% of 12 isolates from subclinical mastitis in cattle to show hot-cold lysis.

Phenotypic variations among *Staphylococcus aureus* obtained from bovine mastitis were recorded and biotyping can be an important tool to study variations among *S. aureus*.

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