

*Original Research***Molecular Identification of *Staphylococcus aureus* Isolates from Subclinical Mastitis in Cattle****P.V. Tresamol*, S. Suchithra, R. L. Rathish and M. R. Saseendranath**

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Abstract

Bovine mastitis is a major infectious disease that affects dairy cattle throughout the world and results in substantial economic losses to dairy farmers. The early and accurate diagnosis and control of subclinical mastitis is important to produce good quality milk and to prevent economic losses. The present study was aimed at molecular identification of *Staphylococcus aureus* isolates from bovine subclinical mastitis. The isolates obtained from milk samples of cows with subclinical mastitis were identified and *Staphylococcus aureus* isolates obtained were selected. DNA was extracted from cultures of *Staphylococcus aureus* using commercial DNA extraction kit and subjected to Polymerase chain reaction (PCR) using species specific primers targeting 23SrRNA gene of *S. aureus*. The species specific PCR yielded specific amplicons of 1318bp from all the isolates. It is concluded that PCR can be used as a rapid and accurate diagnostic tool for identifying isolates of *S. aureus* from mastitis cases.

Key words: Subclinical Mastitis, Bovine, *Staphylococcus aureus*, PCR

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Introduction

Mastitis is an important disease in dairy herds with considerable economic loss mainly due to reduced milk production and discarded milk. Subclinical states of mastitis are particularly dangerous in which milk looks normal and the udder or quarter affected appears healthy. But it can rapidly become clinical mastitis or can continue at subclinical levels, affecting milk production, milk quality, and spreading infection to other cows in the herd. Among the several bacterial pathogens causing clinical and subclinical bovine mastitis, *Staphylococcus aureus* is the most important one (Mork *et al.*, 2005). It causes a chronic and deep infection in the mammary glands that is extremely difficult to cure (Miles *et al.*, 1992).

Staphylococcus aureus cause damage to milk-producing tissues and thereby significantly reduces milk yield. Damage caused in the early stages of infection may be reversible, but tissue damage becomes excessive and irreversible, when diagnosis is delayed (Belschner *et al.*, 1996). Hence a rapid and accurate method of identifying the bacteria responsible for mastitis is important for prompt and timely management of mastitis in dairy herds (Taponen *et al.*, 2006). *Staphylococcus aureus* infections are also having public health significance because they can be transmitted through milk and milk products (Le Loir *et al.*, 2003). Identification of bacterial pathogens causing mastitis is done primarily using cultural isolation and identification. Even though it is considered as the gold standard test, it takes minimum of 24 to 72 hours and involves laborious procedures. Hence molecular methods are proposed nowadays for rapid identification of bacterial isolates. The present study was aimed at molecular identification of *Staphylococcus aureus* isolates from bovine subclinical mastitis.

Materials and Methods

A total of 547 quarter milk samples from 139 lactating animals were screened for subclinical mastitis using California mastitis test, electrical conductivity test and somatic cell count. The milk samples from positive cases were collected aseptically in sterile glass vials and were subjected to culture in Brain heart infusion agar. The isolates obtained were identified using the colony morphology, microscopical examination of Gram's stained smears of the culture and by using various biochemical tests as per Barrow and Feltham (2004). DNA was isolated from cultures of *Staphylococcus aureus* using commercial DNA extraction kit (QIAGEN) and Polymerase chain reaction (PCR) was performed using species specific primers targeting 23SrRNA gene of *S. aureus* (Table 1).

Table 1: Primers for *Staphylococcus aureus* species specific PCR

Primer	Sequence	Target Gene	Product Size
SAU 1	Forward: 5' GGA CGA CAT TAG ACG AAT CA 3'	23S r RNA	1318 bp (Amin <i>et al.</i> , 2011)
SAU 2	Reverse: 5' CGG GCA CCT ATT TTC TAT CT 3'	23S r RNA	

The PCR carried out on a total volume of 25µl (Table 2), under the cycling conditions as per Table 3 in an eppendorf thermocycler. The DNA isolated from known *S. aureus* was used as positive control and DNA isolated from *Escherichia coli* formed the negative control. The PCR products were subjected to 1.5% agarose gel electrophoresis for visualization of amplified products and documented.

Table 2: Composition of PCR mixture

Reagent	Volume
10X PCR buffer	2.5µl
dNTP	0.75µl
Mgcl ₂	3.0µl
Primer	2µl
Taq	0.5µl
Nuclease water	14.25µl
DNA template	2.0µl

Table 3: PCR protocol

Initial denaturation	95 ⁰ C for 5 minutes	
Denaturation	95 ⁰ C for 45 seconds	34 cycles
Annealing	62 ⁰ C for 50 seconds	
Extension	72 ⁰ C for 1.30 minutes	
Final extension	72 ⁰ C for 10 minutes	

Results and Discussion

Among 547 quarter milk samples from 139 lactating animals screened for subclinical mastitis, percent positivity of quarter samples comes to 55.6 per cent by California mastitis test. Electrical conductivity of more than 6 millisiemens (31.08 per cent) was obtained in 170 samples and 377 samples were having less than 6 millisiemens (68.92 percent). Among 139 animals 81 were positive (58.27 per cent) for subclinical mastitis and 58 were negative (41.73 percent) based on differential electrical conductivity. Among pooled milk samples from 139 animals only 22 animals were having a SCC of less than 2,50,000 cells/ml (15.83 per cent) and rest of the animals (84.17) were having a somatic cell count ranging from 2,50,000 to 60,00,000 cells/ml.

A total of 117 milk samples which were found to be positive for subclinical mastitis were subjected to cultural isolation in Brain heart infusion agar. A total of 98 isolates were obtained among which 97(98.98 percent) were Gram positive and one (1.02 percent) was Gram negative. Colonies of Gram-positive cocci were examined by catalase and coagulase test to identify *S. aureus* and were confirmed by inoculating on Baired Parker and mannitol salt agar media. Biochemical tests for identification of the isolates revealed 39 isolates of *Staphylococcus aureus*. Other isolates were identified as coagulase negative staphylococci (52), *Streptococcus* spp. (3), *Micrococcus* spp. (1) and *Staphylococcus intermedius* (2). Similar findings were also reported by Mekibib *et al.* (2010). *Staphylococcus aureus* is considered as the most important organism associated with clinical and subclinical mastitis worldwide because of persistent cow to cow spread through milking machines or hands of milkers (Su *et al.*, 1999). Sreenivasan *et al.* (2013) also reported *Staphylococcus* spp. as the major pathogen associated with subclinical mastitis.

The 23S r RNA PCR resulted in amplification of 1318 bp segment in all *Staphylococcus aureus* isolates. The result of the amplification is shown in Plate 1. No bands were observed with negative control.

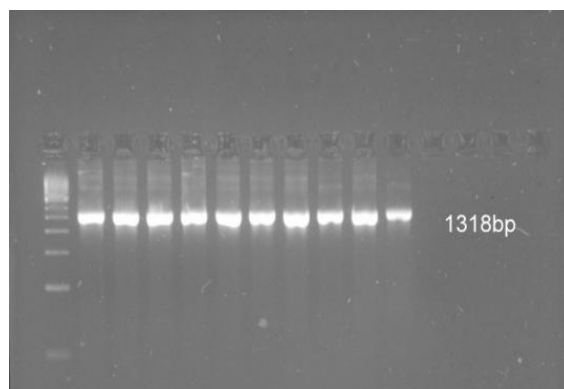


Plate 1

Lane 1-250bp ladder

Lane 2-Positive control (*Staphylococcus aureus* DNA)

Lane 12-Negative control (*Escherichia coli* DNA)

Lane 3to 11-Positive samples

The gene coding for the large subunit ribosomal RNA, 23S rRNA has been targeted for the development of PCR methods for bacterial detection (Hunt *et al.*, 2006). By the use of PCR the isolates were identified with less than 24 hours where as the conventional method of bacteriological culture and biochemical identification required more than 72 hours. Similar reports were given by Riffon *et al.* (2001) and Yamagishi *et al.* (2007). The higher sensitivity of PCR was observed by Amin *et al.* (2011), which was attributed to the fact that PCR is based on detection of specific DNA and hence was able to detect live and dead organisms where as culture detects only live organisms. The present study showed higher incidence of subclinical mastitis among dairy cattle which warrant appropriate control measures to reduce the level of infection. Also it showed PCR as a rapid and accurate diagnostic tool for identifying isolates of *S. aureus* in less than 24 hours. The pathogens responsible for clinical as well as subclinical mastitis should be identified rapidly and accurately for effective control and monitoring in dairy herds. Subclinical mastitis reduces milk quality and quantity resulting in great economic loss for producers (Halasa *et al.*, 2007). The early and accurate diagnosis and control of subclinical mastitis must be a priority for each dairy holder to produce good quality milk and to prevent economic losses.

Conclusion

Identification of pathogens responsible for clinical or subclinical mastitis is very important for effective control of mastitis in dairy herds. Early detection and management of mastitis is required for restoring the milk production. Molecular methods like PCR can be effectively used a rapid and accurate diagnostic tool for identifying isolates of *S. aureus* from mastitis cases.

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