Methicillin resistant *Staphylococcus aureus* mastitis in dairy cows in Thanjavur, Tamil Nadu

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**Abstract**

*Staphylococcus aureus* is an opportunistic contagious pathogen that causes clinical or subclinical mastitis in dairy cows throughout the world. It also has the potential to contaminate animal products during processing, preparation and storage. Injudicious use of antibiotics had led to the emergence of multi drug resistant strains, particularly Methicillin-resistant *S. aureus* (MRSA). The present study was conducted to investigate the prevalence of MRSA in dairy cows with clinical and sub-clinical mastitis brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu. Milk samples were collected in sterile containers from 35 cows with mastitis for isolation, identification and antibiogram of *S. aureus*. Briefly, the collected milk samples were enriched in nutrient broth and a loopful of inoculum from enrichment broth was streaked onto Mannitol salt agar (MSA) and incubated at 37 °C for 24 h. The characteristic golden yellow coloured colonies observed on MSA was confirmed by Gram’s staining, biochemical tests such as IMViC, oxidase and catalase tests, PCR for *mecA* gene (279 bp) of *S. aureus* and subjected to Methicillin sensitivity pattern. *S. aureus* was isolated and confirmed from 40% of the samples and all the isolates were resistant to Methicillin (100%). Thus, the high prevalence of Methicillin resistant *S. aureus* mastitis is an important concern for dairy industry and this an alarming concern for both animal and human beings on public health point of view, since the strains of this pathogen is becoming more resistant to commercially available antimicrobials.

**Keywords:** *S. aureus*, dairy cows, mastitis, methicillin resistance

**Introduction**

*Staphylococcus aureus* is a significant nosocomial pathogen responsible for causing mastitis in dairy cattle[1, 2]. Bovine mastitis is a significant clinical and economic problem in dairy cows. *S. aureus* strains resistant to Methicillin (methicillin resistant *S. aureus*, MRSA) is regarded as zoonotic and is responsible for causing numerous condition including sepsis, toxic shock syndrome, endocarditis and surgical wound infections in humans[3]. In addition, it is known to cause food-borne diseases and responsible for production of staphylococcal enterotoxins associated with food poisoning[4, 5]. *S. aureus* mastitis was reported to be around 41 to 57% in different states of India[6, 7] whereas MRSA level in cattle was reported to be 13%[8]. Milk acts as important source of *S. aureus* and numerous food-borne outbreaks have been documented to be linked with consumption of contaminated milk[9]. Additionally, milk is a good substrate for enterotoxin production, which can maintain their biological activity even after pasteurization[10, 11]. The aim of the present study was to document the prevalence of MRSA in dairy cows with clinical and sub clinical mastitis brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur district, Tamil Nadu.

**Materials and Methods**

**Sample collection and Microbiological analysis**

Milk samples were collected in sterile containers from 35 cows with signs of mastitis for isolation and identification of *S. aureus*. A volume of 2 ml of milk samples were transferred to nutrient broth and incubated at 37 °C overnight. After incubation, it was streaked on Mannitol Salt Agar (MSA) for isolation of *S. aureus*. Based on colony characteristics on specific medium, the isolates were characterized as presumptive *S. aureus* which was further subjected to Grams staining, biochemical tests and PCR.
**Biochemical characterization**

The characteristic golden yellow coloured colonies observed on MSA was subjected to Grams staining, catalase test, indole test, oxidase test, methyl red test, VP test, Simmon’s citrate utilization tests and triple sugar iron utilization tests.

**Polymerase chain reaction**

DNA was extracted from the isolated *S. aureus* colonies by boiling method. Briefly 1.5 ml of the sample in broth was centrifuged at 10,000 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the pellets were washed three times with sterile distilled water. After washing, pellets were reconstituted with 200 µl of sterile water and boiled in water bath at 100 °C for 10 minutes. The samples were centrifuged at 3,000 rpm for 5 minutes. The supernatant containing the DNA was used for DNA amplification with *S. aureus* specific primers [12, 13].

The PCR amplification against *nucA* gene (279 bp) of *S. aureus* containing forward primer (5' GCG ATT GAT GGT GAT ACG GTT 3') and backward primer (5' AGC CAA GCC TTG ACG AAC TAA AGC 3') is carried out in 25 µl reaction mixture consisting of 12.5 µl of PCR master mix, 1 µl of each primer, 1 µl of DNA and 9.5 µl of molecular grade water. Amplification was conducted in master-gradient thermocycler (Eppendorf). The cycling conditions for PCR were initial denaturation at 94 °C of 1 min, followed by 37 cycles of denaturation for 1 min at 94 °C, annealing for 0.5 minutes at 55 °C and extension for 1.5 minutes at 72 °C and final extension for 5 min at 72 °C. The amplified DNA products from *S. aureus* specific PCR products were analyzed on electrophoresis with 1.2% agarose gel and visualized by UV illumination.

**Methicillin and Vancomycin susceptibility pattern of isolated *S. aureus***

The isolated *S. aureus* from milk samples were subjected to Methicillin and Vancomycin susceptibility using disc diffusion methods [14]. The *S. aureus* cultures were streaked on Mueller Hinton agar plates (Himedia, India) using a sterile cotton swab and the Methicillin (5 mcg) and Vancomycin (30 mcg) discs were dispensed using a disc dispenser (Himedia, India). The agar plates were incubated at 37 °C for 16-18 h and the zones of inhibition for each antibiotic were measured.

**Results and Discussion**

The milk dairy cows with subclinical *S. aureus* mastitis may contain larger number of organisms in their milk and may pose severe threat to public health due to its ability to produce enterotoxins, invasiveness, and development of antibiotic resistance to Methicillin [15, 16]. Hence, this study was determined to study the prevalence of MRSA in dairy cows with mastitis and the result is provided in Figure 6. A prevalence of 40% *S. aureus* was observed based on colony morphology (Figure 1), Gram staining (Figure 2), IMViC (Figure 3). Further, the isolates were tested for PCR assay targeting *nucA* gene (279 bp) of *S. aureus* for further confirmation (Figure 4). Following PCR, isolates were tested for Methicillin and Vancomycin susceptibility using disc diffusion assay (Figure 5). All the isolated *S. aureus* strains were resistant to Methicillin and intermediate resistance to Vancomycin.

This research reports a prevalence of 40% MRSA in dairy cows affected with mastitis in Thanjavur region of Tamil Nadu. Prevalence of MRSA in milk and milk products varied significantly between different regions of India and different countries. For example, Shrivastava et al. (2016) studied the prevalence of MRSA in dairy cows in Jabalpur. They screened 85 mastitis milk samples and concluded a prevalence of 16.47% [17]. A prevalence level ranging from 5 to 27% has been reported previously by several researchers in India for MRSA [17, 18, 19, 20]. In African countries, it has been reported as high as 60% in Ethiopia. In Asian countries, it varied from around 1% in Japan to around 28% in Iran [21]. In European countries, US and Canada, low prevalence has been reported [21].

Presence of MRSA in milk is of public health significant due to resistance to methicillin and its potential to develop multiple resistances due to exchange of bacteria and its genes among humans and animals [22]. Presence of Methicillin resistance imparts resistance to all β-lactam antimicrobial agents like penicillins, cephalosporins and carbapenems [3]. In addition, it leads to economic losses due to increased cost of treatment associated with decreased treatment options and poor prognosis [23, 24].
Conclusion

Thus, the high prevalence of Methicillin resistant *S. aureus* mastitis is an important concern for dairy industry and this an alarming concern for both animal and human beings on public health point of view, since the strains of this pathogen is becoming more resistant to commercially available antimicrobials.

References

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