Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 164-167

Received: 16-11-2019
Accepted: 18-12-2019

K Manimaran
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

S Balakrishnan
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

A Sangeetha
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Atmakur Venkatesh
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

M Dhanalakshmi
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

T Sivakumar
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Corresponding Author:
MA Laichattiwar
Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

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

K Manimaran, S Balakrishnan, A Sangeetha, Atmakur Venkatesh, M Dhanalakshmi and T Sivakumar

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 *nuc*A 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 diary 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 Mannital 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 intermediately 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 [7, 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].

Fig 1: Growth of *S. aureus* on MSA agar with characteristics golden yellow colonies

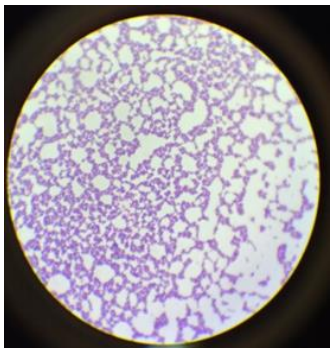

Fig 2: *S. aureus* colony showing Gram positive cocci arranged like bunch of grapes in Gram's staining

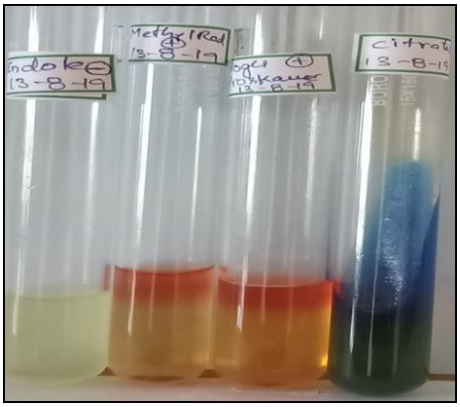

Fig 3: IMViC (-/+/+/+) pattern of *S. aureus* isolates

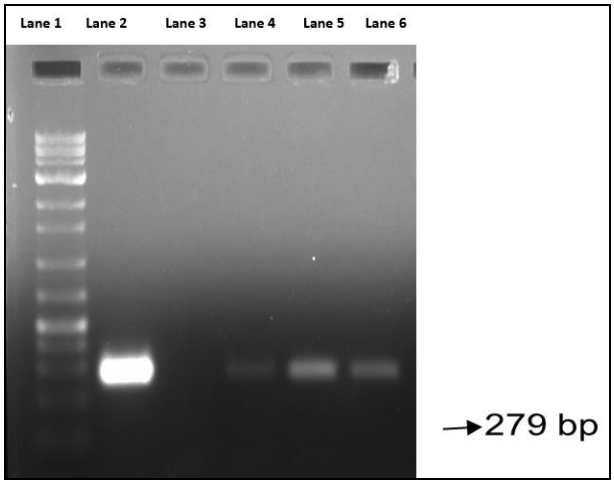

Fig 4: PCR amplification of *nucA genes of S. aureus* isolates. Lane 1 represents 100 bp molecular weight markers, lane 2 represents positive control, lane 3 negative control and lane 4, 5 and 6 represents *S. aureus* isolates

Fig 5: Methicillin and Vancomycin susceptibility pattern of *S. aureus*

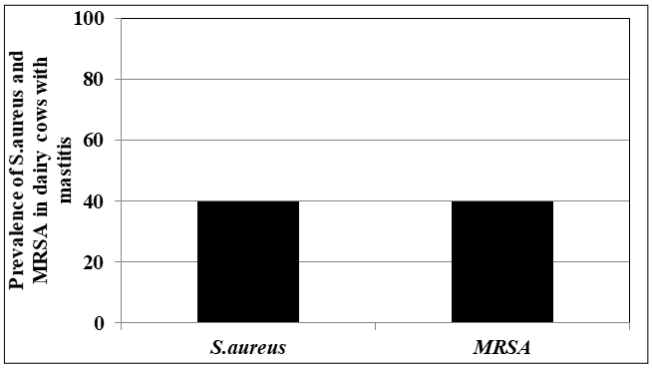

Fig 6: Prevalence of *S. aureus* and MRSA in dairy cows with mastitis

Conclusion

Thus, the high prevalence of Methicillin resistant *S. aureus* mastitis is an important concern for diary 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

1. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN *et al*. Phenotypic and genetic antibiogram of methicillin-resistant *Staphylococci* isolated from bovine mastitis in Korea. Journal of Diary Science. 2007; 90(3):1176-1185.
2. Quinn PJ, Carter ME, Markey B, Carter GR. Bacteriology. *Staphylococcus* species. St. Louis: Mosby, Harcourt Publishers Limited. Clinical Veterinary Microbiology, 2000; 118-126.
3. Weese JS, van Duijkeren E. Methicillin resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Veterinary Microbiology. 2010; 140(3-4):418-429.
4. Normanno G, Corrente M, La Salandra G, Dambrosio A, Quaglia NC *et al*. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. International Journal of Food Microbiology. 2007; 117(2):219-22.
5. De Buyser ML, Dufour B, Maire M, Lafarge V. Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. International Journal of Food Microbiology. 2001; 67(1-2):1-17.
6. Sudhan NA, Singh R, Singh M, Soodan JS. Studies on prevalence, etiology and diagnosis of subclinical mastitis among crossbred cows. Indian Journal of Animal Research. 2005; 39(2):127-130.
7. Kutar K, Verma AK, Sharma B, Kumar A, Yadav SK. Analysis of *mecA* gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. Analysis of *mecA* gene and antibiotic resistance in *Staphylococcus aureus* isolates from bovine mastitis. 2015; 36(1):22-27.
8. Kumar R, Yadav BR, Singh RS. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. Journal of Biosciences. 2011; 36(1):175-188.
9. Fetsch A, Contzen M, Hartelt K, Kleiser A, Maassen S *et al*. *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. International Journal of Food Microbiology. 2014; 187:1-6.
10. Asao T, Kumeda Y, Kawai T, Shibata T, Oda H *et al*. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiology and Infection. 2003; 130(1):33-40.
11. Rall VL, Vieira FP, Rall R, Vieitis RL, Fernandes A *et al*. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Veterinary Microbiology. 2008; 132(3-4):408-413.
12. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. Journal of Clinical Microbiology. 1992; 30(7):1654-1660.
13. Saadat S, Solhjoo K, Norooz-Nejad MJ, Kazemi A. VanA and vanB positive vancomycin-resistant *Staphylococcus aureus* among clinical isolates in Shiraz, South of Iran. Oman Medical Journal. 2014; 29(5):335-339.
14. Bauer AW, Kirby MM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4):493-6.
15. Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR, Hegde NV. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. Journal of Dairy Science. 2004; 87(10):3561-3573.
16. Silva NCC, Guimaraes FF, Manzi MP, Fernandes A,

Gomez-Sanz E *et al*. Methicillin-resistant *Staphylococcus aureus* lineage ST398 as cause of mastitis in cows. Letters in Applied Microbiology. 2014; 59(6):665-669.
17. Shrivastava N, Sharma V, Nayak A, Shrivastava AB, Sarkhel BC *et al*. Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) mastitis in dairy cattle in Jabalpur, Madhya Pradesh. Journal of Animal Research. 2017; 7(1):77-84.
18. Prashanth K, Rao KR, Vivek Reddy PV, Saranathan R, Makki AR. Genotypic characterization of *Staphylococcus aureus* obtained from human and bovine mastitis samples in India. Journal of Global Infectious Diseases. 2011; 3(2):115-22.
19. Singh RS, Kumar R, Yadav BR. Distribution of pathogenic factors in *Staphylococcus aureus* strains isolates from intra-mammary infections in cattle and buffaloes. Indian Journal of Biotechnology. 2011; 10(4):410-416.
20. Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS *et al*. Pattern of antibiotic resistant mastitis in dairy cows. Veterinary World. 2014; 7(6):389-394.
21. Pexara A, Solomakos N, Govaris A. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in milk and dairy products. Journal of Hellenic Veterinary Medical Society. 2013; 64(1):17-34.
22. Sampimon OC, Lam TJ, Mevius DJ, Schukken YH, Zadoks RN. Antimicrobial susceptibility of coagulase-negative *staphylococci* isolated from bovine milk samples. Veterinary Microbiology. 2011; 150(1-2):173-179.
23. Barkema HW, Schukken YH, Zadoks RN. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science. 2006; 89(6):1877-1895.
24. Bardiau M, Yamazaki K, Duprez JN, Taminiau B, Mainil JG *et al*. Genotypic and phenotypic characterization of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk of bovine mastitis. Letters in Applied Microbiology. 2013; 57(3):181-186.