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Prevalence and Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* in Milk and Meat of Small Ruminants in Southern Tamil Nadu

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Abstract
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a widely recognized pathogen of global concern. A total of 120 samples comprising raw milk (n=60) and meat (n=60) were collected from Tirunelveli, Tuticorin, Tenkasi and Kanniyakumari districts to study the prevalence and molecular characteristics of *S. aureus* in milk and meat of small ruminants. Isolation was performed using Baird Parker Agar followed by Gram's staining, biochemical tests and confirmation with HiStaph™ test kit. Genomic DNA was extracted and presumptive isolates were confirmed by PCR targeting 16S rRNA and nuc genes. Detection of *mecA* and *femB* genes was used to identify MRSA, while enterotoxin genes (*sea, seb, sec, sed, see*) were screened using multiplex PCR. Out of 120 samples, 31 (25.83%) were positive for *S. aureus*, including 23 from milk and 8 from meat. Among these, 17 isolates (54.83%) harbored both *mecA* and *fem B* genes, confirming MRSA, while enterotoxin genes *sea* and *sed* were detected in 6.45% and 3.22% isolates, respectively. A crucial prevalance of MRSA and enterotoxigenic *S. aureus* in small ruminant derived milk and meat in southern Tamil Nadu, is highlighted in our study. Hence, regular monitoring and implementation of food safety measures are essential to mitigate the threat of antimicrobial resistance transmission through the food chain.

Keywords: MRSA, Milk, meat, small ruminants, PCR, Enterotoxin genes

Introduction
Staphylococcus aureus is an opportunistic pathogen frequently associated with foodborne illnesses and development of antimicrobial resistance (Kadariya *et al* 2014). Its pathogenic potential is further enhanced by the continual emergence of new clonal lineages, rendering *S. aureus* a 'superbug' (Lakhundi and Zhang, 2018) [10]. MRSA is known to be a major threat to public health, as it accounts for 3 billion dollars in annual health costs (Lakhundi and Zhang, 2018) [10]. The emergence of MRSA in food producing animals has raised global concern, as contaminated milk and meat can act as reservoirs for transmission to humans through the food chain. Different LA-MRSA strains have been found in different foods, including milk, cheese, meat products and raw meat of chicken (Mostafa *et al* 2022) [12]. Goat milk contributes to 3 percent of total milk production across the Country. Small ruminants such as sheep and goats contribute substantially to milk and meat production in India, especially in Tamil Nadu, where these products are widely consumed (DAHD, 2023). While several studies have reported MRSA in various foods sources, such as poultry, pork, beef, milk and vegetables, suggesting that foods may serve as reservoirs (Wang *et al* 2014, Wu *et al* 2018) [17, 19], limited information exists on its occurrence in small ruminant derived food products. In addition to antibiotic resistance, *S. aureus* strains producing classical enterotoxins (*sea–see*) are of concern due to their involvement in staphylococcal food poisoning outbreaks worldwide (Argudin *et al* 2010) [1]. Detection of both resistance (*mecA*, *femB*) and enterotoxin genes in isolates from small ruminant products would provide essential insights into the dual risks of antimicrobial resistance and foodborne disease. Given the increasing global emphasis on antimicrobial resistance as a

"One Health" challenge (WHO, 2021), this study aims to investigate the prevalence, molecular characterization and enterotoxin gene profiling of MRSA in milk and meat from small ruminants in Tirunelveli, Tuticorin,Tenkasi and Kanniyakumari districts. Findings from this work will serve as baseline data for risk assessment and formulation of preventive strategies to protect public health.

Materials and Methods

Study Area and Sample Collection

The study was carried out in four southern districts of Tamil Nadu: Tirunelveli, Tuticorin, Tenkasi and Kanniyakumari. A total of 120 samples (60 raw milk and 60 meat samples) were collected from sheep and goats at local markets, retail outlets and farms. Approximately 10 mL of milk was collected aseptically in sterile screw cap containers and 25 g of meat samples were collected in sterile polythene bags. All samples were transported under refrigerated conditions (4–8 °C) to the Veterinary Public Health – Zoonoses Research Laboratory, Veterinary College and Research Institute, Tirunelveli and were processed within 24 hours of collection.

Isolation and Identification of *Staphylococcus aureus*

Samples were enriched in brain heart infusion (BHI) broth supplemented with 7% NaCl and incubated overnight at 37 °C. Selective plating was performed on Baird Parker Agar (BPA) and incubated for 45–48 hours at 35–37 °C. Colonies showing typical morphology (jet black colonies with a clear halo) were presumptively identified as *S. aureus*. Confirmation was carried out by Gram staining, catalase test, tube coagulase test and HiStaph™ Test Kit (HiMedia, India).

Genomic DNA Extraction

Genomic DNA was extracted from presumptive isolates using the HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia, India). DNA concentration and purity were assessed using a Nanodrop spectrophotometer and extracts were stored at –40 °C until further use.

Molecular Confirmation of *S. aureus*

PCR assays were carried out using published primers (Table1). Species specific confirmation was performed by amplification of the *nuc* gene (Brakstad *et al* 1992) [3] and *16S rRNA* gene (Fig 1) (Kohner *et al* 1999) [9].

Detection of Methicillin Resistance

The presence of the *mecA* and *femB* genes was determined by PCR (Mohanasoundaram & Lalitha, 2008) [11] to confirm MRSA isolates (Fig 2 and Fig 3)

Screening for Enterotoxin Genes

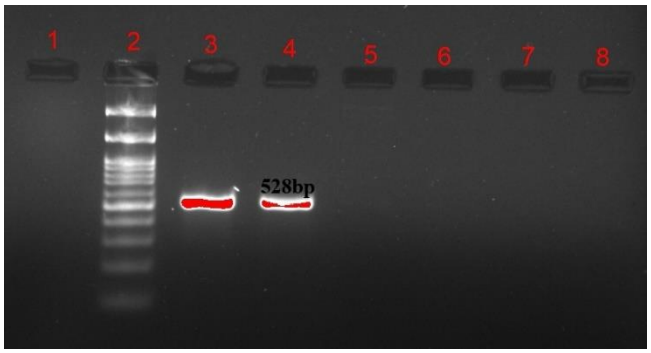
Multiplex PCR was employed to detect classical staphylococcal enterotoxin genes (*sea, seb, sec, sed, see*) following the method of Sharma *et al* 2000 [14] (Fig 4).

Results

Out of 120 samples (milk-60, meat- 60) screened for the presence of *Staphylococcus aureus* spp. About 23 milk samples (60) and eight meat samples (60) were positive for *Staphylococcus aureus* by conventional culture and biochemical tests. Upon further characterization of the isolates by *16s rRNA* and *nucA* gene PCR, all the 31 isolates were found to be positive. The overall prevalence of *Staphylococcus aureus* was only 25.83 percent in our study. of these *S. aureus* isolates 17 (54.83 %) were positive for both *mecA* and *fem B* genes. In the present study, the presence of five classical enterotoxin genes (*sea, seb, sec, sed* and *see*) were screened by Multiplex PCR and it was found that there was a prevalence of *sea* 6.45 % (2/31) and *sed* at 3.22 % (1/31) enterotoxin genes.

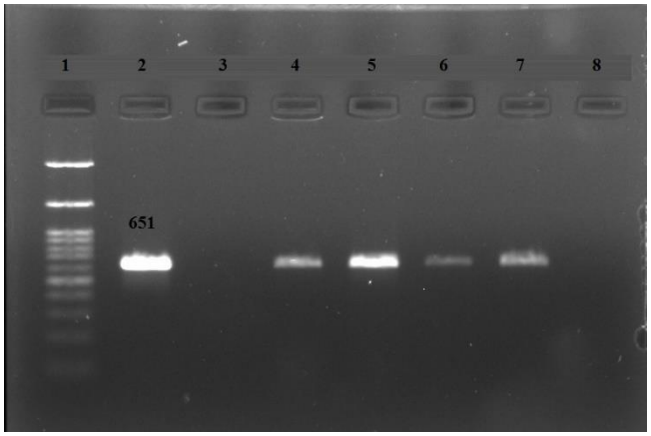
Table1: Details of oligonucleotide primers used in the present study

S. No.	Name	Sequence (5' -3')	Amplicon (bp)	Reference
1.	16S_Staph_F 16S_Staph_ R	CGA AAG CCT GAC GGA GCA AC AAC CTT GCG GTC GTA CTC CC	528bp	Kohner *et al*. 1999 [9]
2.	nuc A – F nuc A – R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	267 bp	Brakstad *et al*. 1992 [3]
3.	femB-F femB-R	TTACAGAGTTAACTGTTACC ATACAAATCCAGCACGCTCT	651bp	Mohanasoundram and Lalitha 2008 [11]
4.	mec A- F mec A- R	GAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310 bp	
5.	SA-U -Uni F	TGTATGTATGGAGGTGTAAC		Sharma *et al*. 2000 [14]
	SEA –REV	ATTAACCGAAGGTTCTGT	270 bp	
	SEB – REV	ATAGTGACGAGTTAGGTA	165 bp	
	ENT-C-REV	AATTGTGTTTCTTTTATTTTCATAA	102 bp	
	SED – REV	TTCGGGAAAATCACCCTTAA	306 bp	
	SEE – REV	GCCAAAGCTGTCTGAG	213 bp	



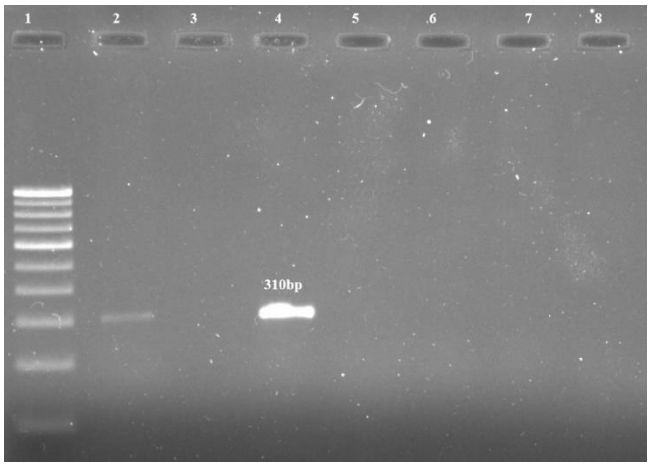
Lane1: Negtive control, Lane 2- 100bp DNA ladder, Lane 3- Positive control, Lane 4, 5, 6, 7, 8- Sample

Fig 1: *16 S rRNA* gene amplification



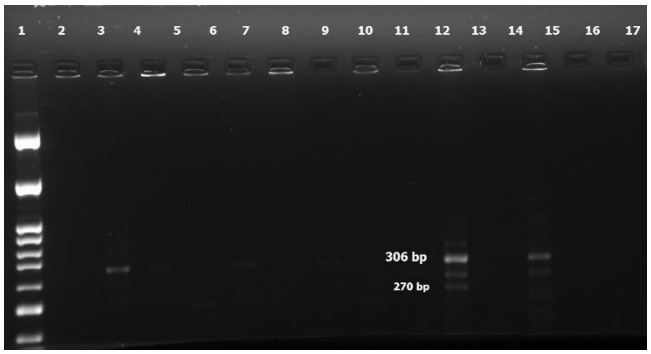
Lane1: 100bp DNA ladder, Lane 2- Positive control, Lane 3- Negative control, Lane 4, 5, 6, 7, 8- Sample

Fig 2: *femB* gene amplification



Lane1: 100bp DNA ladder, Lane 2- Positive control, Lane 3- Negative control, Lane 4, 5, 6, 7, 8- Sample

Fig 3: *mec A* gene amplification



Lane1: 100bp DNA ladder, Lane 2-17 – samples, Lane 12- *sed* gene and *sea* gene specific amplicon

Fig 4: Classical eneterotoxin gene amplification by multiplex PCR

Discussion

In the present study an overall prevalence of 25.83% was observed. Similar studies in Tamil Nadu, wherein nasal swabs were collected from small ruminants like goats and sheep, depicted 28.57% prevalence (Rao *et al* 2023) [13]. This is slightly higher than our study report. Majority of the study focused only on bovine mastitis associated with *S. aureus.*

Another study in Bareilly, Uttar Pradesh analysed various samples viz., milk sample, nasal swabs and wound swabs from healthy animals viz., cattle, buffalo and pigs and observed about 17.73% prevalence in cattle followed by buffaloes (15.90%) and pigs (6.94%) (Das *et al* 2025) [5]. Another similar study in goat meat reported 46.2% prevalence, which is higher than our report (Dhruw *et al* 2025). Different prevalence has been reported in different studies throughout India. This difference might be contributed by multiple factors like farm management system, sampling methodology, sampling strategies, geographic location, hygienic practices during handling (Venugopal *et al* 2023). In the present study, milk from small ruminants showed higher prevalence of 38.3% than meat 13.3%. However, *S. aureus* is one among the predominant cause of subclinical mastitis, poor udder hygiene or contamination during milking may contribute to the increased prevalence in milk. In the present study about 17 isolates were found to carry *mec A* gene, depicting about 54.83% (17/31) prevalence. About half of the isolates (54.83%) were confirmed as MRSA by detection of *mec A* and *fem B* genes suggesting that small ruminant derived food may act as a potential reservoir for antibiotic resistant staphylococci. Another concern for MRSA transmission and development of antimicrobial resistance suggests poor farm sanitation and water management (Yam *et al* 2019) [20]. In the present study, the five classical enterotoxins (*sea, seb, sec, sed* and *see*) were screened by multiplex PCR. The distribution of enterotoxigenic *S. aureus* from food varies in many similar studies due to detection methods, source of food etc. Among the classical enterotoxins, *sea* and *seb* are associated with food explicitly linked to contamination of human origin whereas *sec* and *sed* are associated with contamination stemming from animals (Dhruw *et al* 2025 and Wu *et al* 2019) [6, 19]. In addition, enterotoxins *sea* and *sed* have been implicated in various outbreaks of Staphylococcal food poisoning worldwide (Argudin *et al* 2010) [1]. In the present study, about 6.45% (2/31) isolates were found to carry *sea* and 3.22% (1/31) isolates carry *sed* suggesting contamination of human origin. Though lower prevalence has been recorded in our study, it highlights the potential food safety risk of enterotoxigenic *S. aureus.* Balaban and Rasooly (2000) in their study reported that *sea* as most common enterotoxin which can occur either alone or with other enterotoxins suggesting high resistance to proteolytic enzymes. Many epidemiological studies have highlighted the significance of staphylococcal enterotoxins in food, hence even at a low frequency, continuous monitoring is very important as ingestion of preformed toxin can itself cause illness irrespective of bacterial viability. This study emphasize the need for routine surveillance, strict hygienic practices during milking and slaughter. The results also support the One Health approach to ensure food safety.

Conclusion

This study is the first to document the prevalence of MRSA in milk and meat of small ruminants in southern Tamil Nadu. The overall prevalence of *mecA* gene was high among the *Staphylococcus aureus* isolates obtained confirming MRSA. The detection of enterotoxin genes, although at low frequency, highlights the potential food safety hazards associated with small ruminant products. These findings underline the urgent need for regular monitoring, improved hygienic practices, and rational antimicrobial use in livestock to mitigate the risk of transmission of resistant and toxigenic *S. aureus* to humans.

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