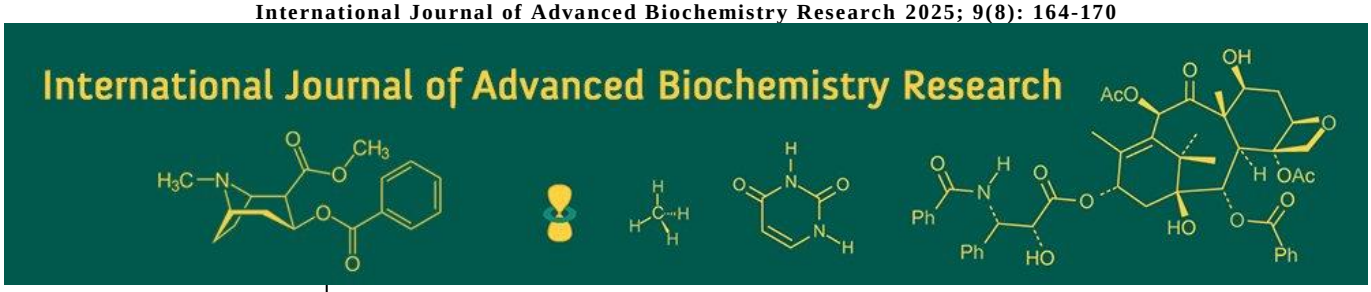

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Occurrence molecular characterization of Multidrug Resistant and methicillin resistant *Staphylococcus aureus* isolate from Bovine, Caprine milk and their handlers

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Abstract
Staphylococcus aureus is a zoonotic pathogen of public health significance commonly associated with bovine and caprine mastitis. Its ability to acquire antibiotic resistance, particularly methicillin resistance is mediated by the *mecA* gene that complicates treatment and further transmission by direct contact or through contaminated dairy products. This study was conducted to isolate *S. aureus* in the Jammu district and to assess their methicillin resistance and antibiotic susceptibility profiles. A total of 240 samples were collected, including raw milk (90), mastitis milk (90), teat skin swabs (30), and handler hand swabs (30). *Staphylococcus aureus* isolates were initially identified using standard bacteriological techniques, with molecular confirmation achieved through PCR amplification of the *nuc* and *mecA* genes. Out of 240 samples, 142 (59.16%) were positive for *S. aureus*. Among these, 90 isolates (63.38%) carried the *nuc* gene, confirming their identity as *S. aureus*, while 35 isolates (38.88%) were positive for the *mecA* gene, indicating methicillin resistance. Antimicrobial susceptibility testing was performed using the disc diffusion method against 15 commonly used antibiotics. High levels of resistance were observed against methicillin (71.14%), novobiocin (74.28%), and nalidixic acid (80%), whereas higher sensitivity was noted for bacitracin (85.71%), ciprofloxacin (77.14%), and gentamicin (77.14%). The presence of multidrug resistant and *mecA*-positive *S. aureus* isolates in milk, animals, and handlers underlines the potential public health risks associated with their transmission. The findings emphasize the need for improved hygiene practices on dairy farms, judicial use of antibiotics and continuous surveillance and monitoring of organised and unorganised sectors to mitigate the spread of MRSA in the food production chain.

Keywords: *Staphylococcus aureus*, MRSA, occurrence, antimicrobial resistance

Introduction
Staphylococcal species are commensals that live both on humans and animals skin and occur globally. However, they can also be found in the digestive tract and on the mucous membrane of the lower urogenital and upper respiratory tract. They are relatively stable in the environment. They have been isolated from a wide range of food items, animals, humans and environment (Lee, et. al., 2015) [22]. However, *Staphylococcus aureus* is a zoonotic, food borne pathogen, primarily responsible for a number of infectious diseases, both in humans and animals, usually ranging from minor skin infections to more serious fatal conditions like blood infections (Bacteraemia/Septicaemia), endocarditis, toxic shock syndrome and necrotizing pneumonia (Suhaili *et al.*, 2018; Che Hamzah *et al.*, 2019) [11, 37]. *Staphylococcus aureus* is also found in dairy farms, and is capable of spreading among cows through contact with contaminated equipments results in large economic loses to dairy industry (Thiran *et al.*, 2017; Filipello *et al.*, 2018) [39]. In dairy animals, it is primarily responsible for causing subclinical and clinical form of bovine mastitis (Rehman *et al.*, 2017) [31] and caprine mastitis (Rizwan *et al.*, 2016) [32]. The *S. aureus* and coagulase negative *Staphylococci* (CNS) are common problem for milk producers (Wallenberg *et al.*, 2002) [43]. In subclinical mastitis, no signs and symptoms are observed; but it poses serious threat of disease transmission among the milk consumers.
Mastitis may cause changes in the composition of milk, and results in low quality milk, thus influences consumer demand (Little *et al.*, 2008) [23].

It is widely accepted that infected udder of milch animals is the primary source of *S. aureus* in a dairy herd. Bacteria are shed into milk from infected quarter (Blood and Henderson 1963) [8]. Animals with chronic infections serve as important pathogen reservoirs, allowing *S. aureus* infections to persists in herds, resulting in significant economic losses in dairy farms (Schukken *et al.,* 2011; Veh *et al.,* 2015) [33, 42].

Staphylococcus aureus developed the resistance against Penicillin in 1944 (Kirby, 1944) [20] and later on against Methicillin in 1962 (Livermore, 2001) [26] and thereby posing a serious threat in treatment of humans and animals (Kanagarajah *et al.,* 2017) [19]. Methicillin resistance in *S. aureus* is caused by the *mecA* gene, which produce a variant of penicillin binding protein (PBP) called PBP2a. The *mecA* gene found in MRSA, resides on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). This element provides resistance to several antibiotics. SCC*mec* carries both the mecA or mecC genes, regulatory genes and accessory genes that encode a specific PBP2a. This PBP2a may contain additional antimicrobial resistance determinants in Methicillin resistance *Staphylococcus aureus* (MRSA). Keeping in view the public health importance and traditional habits of consumptions of milk and milk products in Jammu province this study was carried out to find the occurrence of MDRSA among MRSA.

Materials and Method

Place of work

The research work was carried out in the Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S.Pura, Jammu. The period of study was from August, 2024 to July, 2025.

Sample collection: The milk samples were collected aseptically in pre-sterilized test tubes and transported over ice to the processing laboratory, while the swab samples transported in transport swab medium (Hi-media Pvt. Ltd.)

A total of 240 samples constituting apparently normal raw milk 90 (30 each from cow, buffalo and goat), mastitis milk samples 90 (30 each from cow, buffalo and goat), animal handlers hand swabs 30 and animal teat skin swabs 30 were randomly collected by applying a cross sectional study.

Isolation and Identification of *S.aureus*

The swab sample or 10 ml of milk sample was enriched in 90 ml sterile enrichment Muller Hinton Broth (Himedia Pvt. Ltd. Mumbai India) and incubated at 37 °C for 24 hours. A loopful of inoculum was streaked onto Mannitol Salt Agar (MSA) and incubated for 24-48 hour at 37 °C. Yellow colonies with yellow zones on MSA were considered as *S. aureus*. The suspected *S. aureus* colonies were sub-cultured onto nutrient agar supplemented with 6.5% sodium chloride (NaCl) and were incubated at 37°C for 24 hour. Identification of *S. aureus* was confirmed on the basis of Biochemical tests (Quinn, *et.al.,* 1994) [30]. The milk samples first subjected to different tests to detect the subclinical mastitis cases and then processed for isolation and confirmation of MRSA.

DNA extraction and molecular characterization of *S.aureus:* The bacterial colonies that grew on nutrient agar were subjected to DNA extraction by Snap Chill Method. Pure bacterial cultures of *S. aureus* grown on nutrient agar slants were inoculated into 5 ml of buffered peptone water and incubated at 28°C for 18 hours. After incubation, 1 ml of the broth culture was transferred into a microcentrifuge tube and centrifuged at 8000 rpm for 5 minutes. The supernatant was discarded, and the pellet was washed with 500 µl of sterilized nuclease free water. Following the wash, the pellet was re-suspended in 150 µl of sterilized nuclease free water and centrifuged again at 8000 rpm for 5 minutes. The microcentrifuge tubes were then placed in a boiling water bath for 10 minutes and immediately transferred to crushed ice for 20 minutes. After chilling, the samples were centrifuged at 8000 rpm for 5 minutes, and the supernatant containing bacterial DNA was used as a template DNA. The extracted DNA kept in -20°C in refrigeration for further use in polymerase chain reaction (PCR). PCR was carried out to screen the presence of *nuc* gene of *S.aureus*, and *mecA* gene of MRSA using the primers and protocol described by Brakstat, *et al*., (1992) [10]. Bacteria isolates with the presence of DNA bands of 270 bp were considered *S.aureus*, and the presence of DNA bands of 533 bp were considered as MRSA. All the *S. aureus* isolates confirmed by *nuc* gene detection by PCR were examined for presence of antibiotic resistance genes, responsible for resistance against methicillin (*mecA*), the primers used for amplification of the above genes are illustrated in Table-1.

Standardization of PCR assay for detection of *nuc* and *mecA* genes

A PCR assay was developed using specific primers targeting the *nuc* gene (specific to *Staphylococcus aureus*) and the *mecA* gene (a marker for methicillin resistance), following the protocol of Pereira, *et al.,* (2009) [29] with slight modifications. The PCR amplified fragments of *nuc* and *mecA* gene with a predicted band size of 270 and 533 bp respectively.

The reaction mixture was carried out in 0.5 ml microcentrifuge tubes in a total volume of 25 µl containing, Master Mix 12.5 µl, each primer 1 µl, Template DNA 2 µl, Taq polymerase 2.0 U, deoxyribonucleotide triphosphates 100 µM, and nucleic acid 150 µg, Nuclease free water 8.5 µl.

PCR amplification for *nuc* gene and *mecA* gene was carried out in a thermal cycler (Applied Biosystems). The Cyclic conditions used for PCR assay for *nuc* and *mecA* gene with some slight modifications are illustrated in table 2 and 3. Positive control and and negative control (sterile distilled water) was kept in every set of reactions.

The amplified products obtained was subjected to agarose gel electrophoresis in 2% agarose gel, prepared in 1X TAE buffer and stained with ethidium bromide (0.5 µg/ml). Gene Marker of 100 bp DNA Ladder was used as a molecular size reference to detect size of the DNA fragments. *S. aureus* isolates containing *mecA*, genes were classified as MRSA.

Antimicrobial susceptibility pattern of the MRSA isolates: The antimicrobial susceptibility profile of MRSA isolates was carried out using the Kirby-Bauer test on Mueller-Hinton agar (Hi-media Pvt. Ltd. Mumbai India), according to the Clinical and Laboratory Standards Institute (CLSI) [12] against the commonly used antibiotics in the field. MRSA isolates first suspended in sterile Mueller-Hinton broth (Himedia Pvt. Ltd. Mumbai India) adjusted to a 0.5 McFarland standard. The bacterial isolate from broth were then streaked on Mueller-Hinton agar (Himedia Pvt.

Ltd. Mumbai India) plates. Selected antibiotic disks commonly used in the field were placed on the streaked Mueller-Hinton agar plates and incubated at 37°C for 24 hours. The diameter of inhibition zones of each isolate was measured and compared to the antibiotic susceptibility breakpoints according to CLSI (2009) or discs manufacturer's guidelines. Phenotypically *S. aureus* resistant to one or more antimicrobial were classified as multidrug resistant *S. aureus* (MDRSA) as suggested by Magiorakos *et al.,* (2012) [27].

Results

Molecular detection of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* gene

The present study was conducted to assess the occurrence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus.* Two hundred forty (240) samples randomly collected in a cross sectional study from diverse sources were analysed. These include 90 samples from raw milk, 90 from mastitis milk, 30 each from teat skin swab and handlers hand swab. 142 isolates of *S. aureus* were obtained and identified on the basis of cultural, morphological and biochemical characteristics (Quinn, *et al.,* 2004) [30]. To confirm the identity of the isolates at the molecular level, the presence of the species specific *nuc* gene, that encodes a thermostable nuclease, was detected using polymerase chain reaction (Brakstat *et.al.,* 1992) [10]. Detection of the *nuc* gene is considered a reliable molecular marker for the confirmation of *S. aureus*. The *S. aureus* isolates obtained were further processed for the detection *mecA* gene using polymerase chain reaction, as described by Pereira *et al.,* 2009 [29] with slight modifications. The *in-vitro* antibiotic pattern of the Methicillin Resistant *Staphylococcus aureus* isolates was also studied. All the results are simplified in table-4 and 5.

***In vitro* antibiotic sensitivity/resistance pattern of MRSA isolates:** All the thirty five MRSA isolates were subjected to antibiotic susceptibility testing against 15 commonly used antimicrobials/antibiotics by using disc diffusion technique (Bauer *et al.,* 1966) [5]. The study revealed high percentage of resistance with Nalidixic acid (80%), Novobiocin (74.28%), Methicillin (71.14%). On the other hand MRSA isolates were found to be most sensitive to Bacitracin (85.71%), Gentamicin (77.14%), and Ciprofloxacin (77.14%). Intermediate resistance was shown by Amoxicillin (28.57%), Azithromycin (28.57%), Norfloxacin (20%) as shown in the table 5.

Discussion

Occurrence of *Staphylococcus aureus* in Raw Milk.

In the present study, *Staphylococcus aureus* was detected in 58.88% of raw milk samples. Among different animal species, the highest occurrence was observed in cow milk (73.33%), followed by goat milk (60%) and buffalo milk (43.33%). Additionally, 64.15% of the *S. aureus* positive samples were coagulase positive, indicating the presence of potentially pathogenic strains. This high occurrence suggests that raw milk serves as an important reservoir for *S. aureus*, which may be due to factors such as poor udder hygiene, contaminated equipment, improper milking procedures, and sub-clinical mastitis infections. The current findings are in agreement with several previous reports from India and abroad. Bharathy *et al.,* (2015) [6] reported *S. aureus* prevalence in cow milk (68%), goat milk (62.5%), and buffalo milk (40%) in Chennai. Similarly, Kou *et al.,* (2021) found 61.7% positivity in cow milk from Chinese retail markets. In contrast, some studies showed lower prevalence rates, likely reflecting better hygiene practices. Fagundes *et al.,* (2010) [14] and Lee *et al.,* (2012) [21] reported *S. aureus* in only 6.7% and 5.5% of individual cow milk samples in Brazil. The high contamination rate observed in the current study emphasizes the urgent need for improved sanitation during milking, routine microbial screening, and proper training of dairy workers to reduce the risk of food-borne infections caused by *S. aureus*.

Occurrence of *Staphylococcus aureus* in Mastitis Milk

In the present investigation, *Staphylococcus aureus* was detected in 62.22% of mastitis milk samples, with the highest occurrence observed in cow milk (80%), followed by goat (56.66%) and buffalo milk (50%). This elevated detection rate indicates the significant role of *S. aureus* as a principal causative agent of both clinical and subclinical mastitis in dairy animals. The predominance in cow milk may be attributed to factors such as higher milk yield, frequent handling, increased susceptibility to intramammary infections and environmental or managerial stress. Comparable findings have been reported in various national and international studies. Abebe *et al.,* (2016) [1] documented 62.6% prevalence of *S. aureus* in mastitis milk samples in Ethiopia. Liu *et al.,* (2022) [25] recorded a 58.33% prevalence in raw goat milk from a Chinese dairy farm, that is in proximity to our findings in goat mastitis milk. A study by Ariffin *et al.,* (2019) [2] in Pakistan also reported *S. aureus* in 63.4% of mastitic caprine milk samples. In contrast, several studies have documented lower prevalence rates. Fagundes *et al.,* (2010) [14] reported *S. aureus* in only 7.3% of milk samples from cows with subclinical mastitis. Sharma *et al.,* (2011) [36] found a prevalence of 21.73% in cattle milk in Meerut. These variations may reflect differences in regional husbandry practices, animal health management, hygiene standards, and mastitis control strategies. Gandhale *et al.,* (2017) [15] reported *S. aureus* in 54.5% of milk samples from clinically healthy animals in Maharashtra, India, highlighting the potential for asymptomatic carriage and the risk of contamination during milking. This finding is in agreement to our findings.

Occurrence of *Staphylococcus aureus in* Handler's Hand Swabs: In the present study, *Staphylococcus aureus* was detected in 63.33% of hand swab samples collected from dairy handlers, with 36.84% of isolates being coagulase positive. This high occurrence indicates the critical role of dairy workers as significant contributors to milk contamination, particularly in environments lacking strict hygiene standards. Ballah *et al.,* (2022) [4] also observed a substantial prevalence (23.81%) in food and hand swabs in Bangladesh. The significantly higher contamination rate in the present study reveals the necessity of hand hygiene training, proper sanitation and use of gloves during milking to mitigate the risk of contamination.

Occurrence of *Staphylococcus aureus in* Teat Skin Swabs

In the present study, *Staphylococcus aureus* was isolated from 46.66% of teat skin swab samples, the teat skin represents a critical reservoir and point of contact during milking, facilitating the direct transmission of *S. aureus* into

milk. This finding is in close agreement with previous reports by Gandhale *et al.,* (2017) [15], who documented 46.91% prevalence on udder and teat surfaces and Gwida *et al.,* (2021) [16], who observed a slightly higher prevalence of 50%. Comparable findings were also reported by Tsehayneh *et al.,* (2021) [41] in Ethiopia (45.7%) in udder or teat skin swabs. The high occurrence rate observed in this study insights the necessity of effective pre and post milking teat sanitation, especially in small-scale or manually operated farms, to prevent both direct milk contamination and the recurrence of intramammary infections.

Molecular detection of *Staphylococcus aureus nuc* gene and MRSA *mecA* gene

Molecular identification of *Staphylococcus aureus* plays an important role in surveillance, and monitoring of livestock farms. In present study (59.16%) samples were positive for *S. aureus* by culture, among which (63.38%) were confirmed by PCR targeting the *nuc* gene- Furthermore, (38.88% of *nuc*-positive strains), found possing *mecA,* gene indicating the presence of methicillin-resistant *S. aureus* (MRSA) across animal and environmental samples.

The *nuc*- gene positivity was highest among mastitis milk samples (69.64%), followed by raw milk (64.15%), highlighting the pathogenic role of *S. aureus* in both subclinical and clinical mastitis cases. Teat skin and hand swab isolates also showed PCR-confirmed *S. aureus*, at lower proportions (50% and 52.63%, respectively), suggesting their role as reservoirs and sources of contamination.

Sample wise, *mecA* gene occurence was highest in raw milk isolates (47.05%), particularly from cow milk (53.33%) and goat milk (45.45%), and to a lesser extent in buffalo milk (3.75%). Mastitis milk samples showed an overall *mecA* positivity of 38.46%, consistent with previous studies that have reported frequent MRSA isolation from mastitic milk (Shah *et al.,* 2019; Brahma *et al.,* 2022) [9, 34]. Notably, goat mastitis samples exhibited a particularly high prevalence (54.54%), which aligns with Papadopoulos *et al.,* (2018) [28], who reported up to 80% contamination in caprine milk in Greece.

The occurrence rate of *mecA* in this study aligns with intermediate values observed globally, such as 25% in mastitis cases in Hyderabad (Brahma *et al.,* 2022) [9] and 12.2% in food- contact surfaces in Bangladesh (Shahid *et al.,* 2021) [35]. The findings also echo Yang *et al.,* (2020) [44], who confirmed 100 per cent *mecA* carriage in MRSA isolates from subclinical mastitis in China, emphasizing the growing threat of multi-drug resistance among foodborne *S. aureus* strains.

Antimicrobial Resistance Patterns of isolates

The antimicrobial susceptibility testing of isolates in the present study revealed significant variability in resistance patterns, underlining the growing challenge of antimicrobial resistance (AMR) in dairy environments. High levels of resistance were observed against methicillin (71.14%), nalidixic acid (80%), and novobiocin (74.28%), while the isolates remained largely sensitive to bacitracin (85.71%), gentamicin (77.14%), ciprofloxacin (77.14%), and levofloxacin (74.28%). The elevated methicillin resistance is indicative of a considerable presence of methicillin-resistant *S. aureus* (MRSA), a finding supported by the detection of the *mecA* gene in 38.88% of isolates. These observations are in agreement with reports by Dweba *et al.* (2019) [13] in South Africa (94.5%) and Bissong *et al.* (2020) [7] in Cameroon (74.3%). The high resistance to nalidixic acid aligns with Badawy *et al.,* (2022) [3], who reported 86.6 per cent resistance in Egyptian dairy environments, indicating rising resistance to quinolones, potentially due to their overuse at the farm level. In contrast, earlier studies by Sharma *et al.,* (2011) and Liu *et al.,* (2018) [24, 36] documented significantly lower resistance, suggesting an emerging temporal trend. Gentamicin and ciprofloxacin were among the most effective antibiotics, each with 77.14 per cent sensitivity, in line with studies by Thaker *et al.,* (2013), and Tibebu *et al.,* (2021) [38, 40], all reporting sustained efficacy of these drugs.

Table 1: Primers used for PCR amplification of *nuc* gene and *mec* A gene in study

S.No.	Primers	Primer sequence (5`-3`)	Product size (bp)	Reference
	nuc	F: GCGATGATGGTGATAGGGTT R:AGCCAAGCCTTGACGAACTAAAGC	270	Brakstat, *et.al.* 1992 [10]
1.	*mecA*	F: AAAATCGATGGTAAAGGTTGG R: AGTTCTGCAGTACCGGATTTG	533	Pereira, et.al. 2009[29]

Table 2: Cyclic conditions used for *nuc* gene

S.No	Steps	Temperature	Duration	No. of cycles
1.	Inital denaturation	94 °C	5 minutes	1
32.	Denaturation	94 °C	1 minutes	
3.	Annealing	55 °C	30 seconds	35
4.	Extension	72 °C	1 minutes	
5.	Final Extension	72 °C	7 minutes	1
6.	Hold/ stand by	4 °C	10 minutes	

Table 3: Cyclic conditions used for *mecA* gene

S.No	Steps	Temperature	Duration	No.of cycles
1.	Inital Denaturation	95	5 minutes	15
2.	Denaturation	94	30 seconds	
3.	Annealing	55	30 seconds	30
4.	Extension	72	30 seconds	
5.	Final Extension	72	10 minutes	1
6.	Hold/ stand by	4	10 minutes	

Table 4: Screening and Molecular Detection of *Staphylococcus aureus* and MRSA

S.No	Nature of Sample	Number of samples (n)	Number of samples positive *for S. aureus* phenotypically	PCR positive for *nuc* gene for *S. aureus*	PCR positive for *mecA* gene for *S. aureus*
1.	Raw Milk	90	53 (58.88%)	34 (64.15%)	16 (47.05%)
	a) Cow milk	30	22 (73.33%)	15 (68.18%)	8 (53.33%)
	b) Buffalo milk	30	13 (43.33%)	8 (61.53%)	3 (3.75%)
	c) Goat milk	30	18 (60%)	11 (61.11%)	5 (45.45%)
2.	Mastitis Milk	90	56 (62.22%)	39 (69.64%)	15 (38.46%)
	a) Cow milk	30	24 (80%)	19 (79.16%)	7 (36.84%)
	b) Buffalo milk	30	15 (50%)	9 (60%)	2 (22.22%)
	c) Goat milk	30	17 (56.66%)	11 (64.70%)	6 (54.54%)
3.	Handlers hand swab	30	19 (63.33%)	10 (52.63%)	3 (30%)
4.	Teat skin swab	30	14 (46.66%)	7 (50%)	1 (14.28%)
	Total	240	142 (59.16%)	90 (63.38%)	35 (38.88%)

Table 5: Antibiogram of the MRSA isolates against commonly used antimicrobials

S.No.	Antimicrobials	Disk Potency	Sensitive	Intermediate	Resistant
1.	Gentamicin	10 µg	27 (77.14%)	5 (14.28%)	3 (8.57%)
2.	Amoxicillin	30 µg	18 (51.42%)	10 (28.57%)	7 (20%)
3.	Chloramphenicol	30 µg	22 (62.85%)	7 (20%)	6 (17.14%)
4.	Ciprofloxacin	5 µg	27 (77.14%)	5(14.28%)	3 (8.57%)
5.	Bacitracin	10 µg	30 (85.71%)	3 (8.57%)	2 (5.71%)
6.	Azithromycin	30 µg	18 (51.4%)	10 (28.57%)	7 (20%)
7.	Erythromycin	15 µg	20 (57.14%)	4 (11.42%)	11(31.42%)
8.	Methicillin	5 µg	4(11.42%)	6 (17.14%)	25 (71.14%)
9.	Novobiocin	30 µg	6 (17.14%)	3 (8.57%)	26 (74.28%)
10.	Vancomycin	30 µg	23 (65.71%)	8 (22.85%)	4 (11.42%)
11.	Norfloxacin	10 µg	22 (62.85%)	7 (20%)	6 (17.14%)
12.	Tetracycline	30 µg	24(68.57%)	4 (11.42%)	7 (20%)
13.	Levofloxacin	5 µg	26 (74.28%)	5 (14.28%)	4 (11.42%)
14.	Nalidixic acid	30 µg	2 (5.714%)	5 (14.28%)	28 (80%)
15.	Cephradine	30 µg	18 (51.42%)	7 (20%)	10 (28.57%)

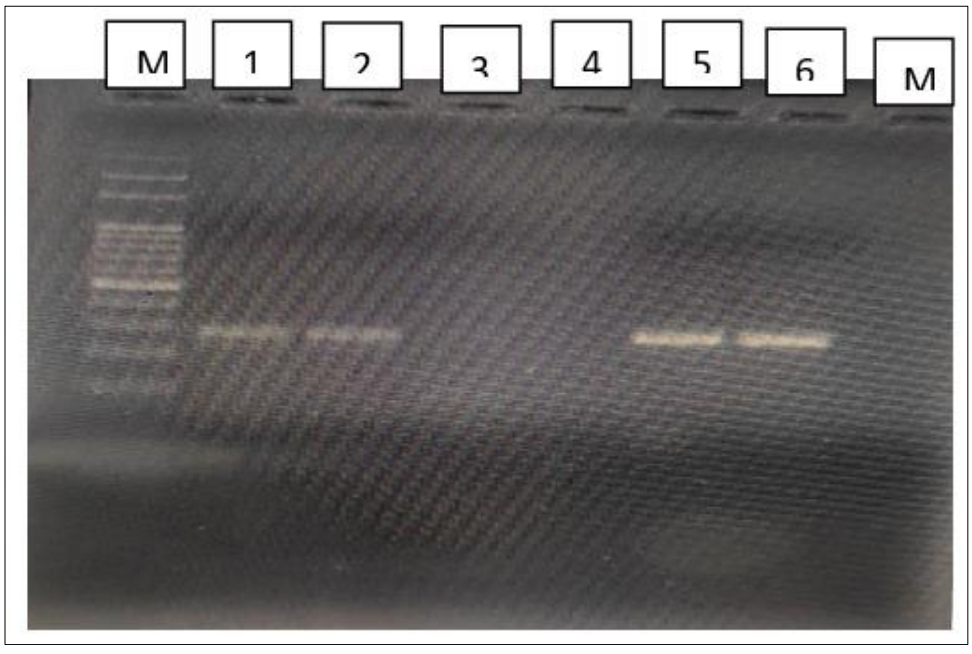

Fig 1: PCR assay for detection of *nuc* gene of *Staphylococcus aureus.*

Lane M: 100bp DNA ladder; Lane 1: positive control; Lane 2, 5,6 positive isolates; Lane 3, 4 negative isolates; Lane 7: Negative control

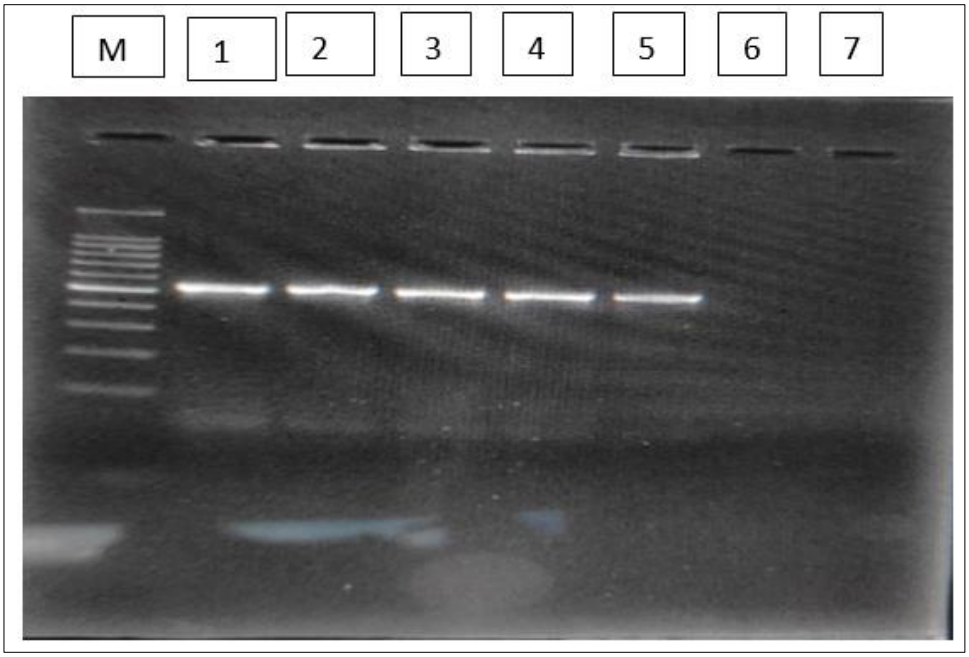

Fig 2: PCR assay for detection of *mec*A gene of *Staphylococcus aureus.*

Lane M: 100bp DNA ladder; Lane 1 positive control. Lane 2, 3, 4, 5 positive isolates; Lane 6: Negative isolates Lane 7: Negative control

Conclusion

This study provides critical insights into the occurrence and molecular profile of multidrug resistant *Staphylococcus aureus* in dairy environments. The detection of the *mecA* gene in a substantial proportion of isolates from both animal and human-associated sources confirms the presence of methicillin resistant *S. aureus* (MRSA) in the studied population. The high occurrence of *S. aureus* in raw and mastitic milk, combined with significant resistance to commonly used antibiotics, poses a potential threat to public health and milk hygiene. The findings emphasize the importance of routine molecular surveillance and antimicrobial resistance monitoring in dairy farms. Furthermore, rigorous hygienic practices during milking and rational use of antibiotics are essential to limit the spread of MRSA and ensure the safety of milk and milk products. Future research should focus on exploring alternative control strategies, such as bacteriophage therapy or probiotic interventions, to mitigate the burden of antibiotic resistance in the dairy sector.

Conflict of interest

The authors declare that there is no conflict of interest.

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