# Multi-drug resistant enterotoxigenic *Staph. aureus* in Goat meat

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## Abstract

*Staphylococcus aureus* is recognized as an important cause of human food-borne intoxication through the consumption of raw, uncooked or contaminated meat. Raw meat serves as an ideal medium for the survival and transmission of drug-resistant *Staphylococcus aureus*, posing a significant public health risk.

A total of 65 swab samples of goat meat were collected from local meat shops in Bikaner city of Rajasthan and investigated for the frequency of *S. aureus* and classical and novel enterotoxin genes. The samples were cultured on Mannitol salt agar and subjected to confirmatory 23s rRNA typing, antimicrobial sensitivity test, and molecular detection of *coa*, *sea*, *seb*, *sec1, sed, see, seg* and *seh genes by* PCR.

The overall prevalence of *S. aureus* in goat meat was 46.2% (30/65). A 100% resistance was shown towards cefixime plus clavulanic acid followed by ampicillin (90%), penicillin-G (86%), and oxacillin (73%). Nine (30%) of the 30 *S. aureus* isolates were MDR strains while the MAR index of all the isolates ranged from 0.04 to 0.34, with 10 isolates having a MAR index between 0.2 and 0.3. All the isolates were positive for *coa* gene and showed polymorphism. A high prevalence of enterotoxin *seg* gene was detected in 23 (76.6%) isolates followed by *sec-1* in 13(43%) isolates; 13 (43%) isolates carried both *sec-1* and *seg* genes while *seb* gene was detected in only one isolate. Enterotoxin genes like *sea, seb, sed, see, and seh* were not detected in any of the isolates.

This study underscores the critical need for targeted interventions to mitigate the risks associated with *S. aureus* in the food chain, aligning with the global *One Health* concept.

**Keywords** *Staphylococcus aureus;* coagulase positive; Enterotoxin gene; Goat meat; multidrug resistance.

# Introduction

Asia has the largest population of goats with 55% of world goat population, mostly in India (35.2%), China (29.3%) and Pakistan (12%) (Devi *et. al*., 2014). In India, 95% of goat meat produced is consumed locally but poor hygiene (personal and process), quality of water, and unscientific slaughter practices and the meat being sold in open markets often leads to contamination of meat and its products with microbes (Muthusamy *et al.*, 2024). Since *Staphylococcus aureus* is a natural flora in skin and nasal cavity of human and animals (Pal *et. al*., 2020), it is recognized as one of the major food-borne pathogens in fresh and ready-to-eat products and responsible for various infections and food-borne diseases around world. Staphylococcal enterotoxins are often detected in food poisoning cases following ingestion of food, such as meat and dairy products (Fox *et. al*., 2017). In recent years, raw meat has been considered as an important means of transmission of *S. aureus* to people who have no contact with livestock (Carrel *et. al*., 2017).

Staphylococcal enterotoxins (*SEs*; *sea* to *see*, *seg* to *sei*, *ser* to *set*) belong to the broad family of pyrogenic toxin superantigens and these enterotoxins interact with the enteric nervous system by stimulating afferent neurons or induce enterochromaffin cells to release neurotransmitters which results in vomiting, diarrhea, or intestinal inflammation. These toxins are active in high nanogram to low microgram quantities and are resistant to conditions (heat treatment, low pH) that easily destroy the bacteria that produce them, and to proteolytic enzymes, hence retaining their activity in the digestive tract after ingestion (Larkin *et. al*., 2009).

The use of antibiotics can lead to the emergence of antibiotic-resistant strains in animals, which can be transmitted to humans through the consumption of meat. Therefore, there is special concern about the role of food of animal origin in the spread of antimicrobial resistance (Da Silva-Guedes *et. al*., 2022). Research has shown that an increasing number of food poisoning outbreaks are caused by multidrug resistant (MDR) *S. aureus* strains. In Asia, Europe, and North America, methicillin-resistant *S. aureus* (MRSA) has been found in retail meat and animals raised for food (Adesiji *et al*., 2011). Asymptomatic food-producing animal carriers and their meat can serve as potential threat for spread of MRSA associated human infections.

The present study was conducted to detect coagulase positive *S. aureus* strains from raw goat meat and to investigate their enterotoxin genes and antimicrobial resistance profile.

# Materials and methods

**Collection of samples:** Sterile cotton swabs moistened in 0.1% peptone water were used to collect samples. The samples were obtained from local meat shops, where lack of proper hygienic measures and unscientific slaughtering practices may contribute to contamination. A total of 65 swab samples were collected from freshly slaughtered goat carcasses at local meat shops in Bikaner (Rajasthan). These samples were taken from five different sites *viz* brisket, shoulder, flank, neck and rump. The collected samples were put in ice container and transported to the departmental laboratory for further processing.

**Isolation and identification of *Staphylococcus aureus*:** The swab samples were first inoculated in nutrient broth and kept at 37°C for 24 h. Next day growth was observed and a loop full of the inoculum was streaked on nutrient agar plates and incubated at 37°C for 24 h. Plates with pale yellow to golden yellow, small circular colonies were selected and re-streaked on Mannitol salt agar (MSA) and incubated overnight at 37°C. The isolates were identified as *S. aureus* by further biochemical characterization using Gram stain, catalase, coagulase, oxidase tests, and by *S. aureus* species specific 23s rRNA targeted PCR (Straub *et al*., 1999) using primers mentioned in table no.2 and as described earlier (Bhati *et al*., 2016).

**Antimicrobial sensitivity test:** All the *S. aureus* isolates were subjected to 22 different antibiotics of different classes using disc diffusion method to study the antibiotic sensitivity pattern of the isolates. In brief, the isolates were inoculated in sterile 5 ml nutrient broth tube, incubated for 18 h at 37°C and then the opacity was adjusted to 0.5 McFarland opacity standards. The inoculum was well spread over the agar surface with the help of sterilized swab and after drying, the antibiotic discs were carefully placed on the surface with enough space around each disc for diffusion of the antibiotic. Plates were incubated for 24 h at 37°C and the zones of inhibition were measured and compared with standard chart provided by disc manufacturer. Mutli drug resistant (MDR) strains were identified.

**Multiple antibiotic resistance (MAR) index of *S. aureus* isolates:** All multidrug resistant isolates were evaluated for their MAR index. Determination of MAR index followed the procedure (Krumperman, 1983) in which the number of antibiotics an isolate is resistant to (a) is divided by the total number of the antibiotics used in the study (b). The calculating formula is shown below:

MAR Index = a / b

**Detection of *coa* gene by PCR**: The genotypically confirmed isolates of *S. aureus* were subjected to amplification of *coa* gene (table no.1) as per the previously described method (Hookey *et al*., 1998). The cycling

conditions involved pre pre-denaturation at 94°C for 2 min, followed by 31 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min with final extension at 72°C for 2 min.

**Detection of staphylococcal enterotoxin genes using simplex PCR:** The classical enterotoxin genes (*sea, seb, sec1, sed and see*) amplification was done as per method described by (Johnson *et al*., 1991) with some modifications. The sequences of primers were used for different genes in a simplex PCR are shown in table no.1. The PCR was performed in Veriti Thermal Cycler (Applied biosystem) using following cycling parameters: pre-denaturation at 95°C for 5 min, followed by 31 cycles of denaturation at 94°C for 2 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min. The amplification ended by a final extension at 72°C for 5 min. The PCR products were resolved in 1.2% agarose gel prepared in 1X TBE buffer containing 0.5µg/ml of ethidium bromide and 100bp ladder was used as molecular marker. The novel genes *seg* and *seh* amplification was done as per method described by (Omoe *et al*., 2002) with some modifications using primers given in table no.1. The PCR conditions were: pre-denaturation at 95°C for 3 min, followed by 31 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min with final extension of 5 min.

# Results

In the present study, a total of 30 samples of goatmeat out of 65 (46.2%) were found positive for *S. aureus* and all the 30 isolates produced an amplicon of 1250 bp when subjected to 23s rRNA ribotyping. All the isolates were gram positive cocci arranged in grape like clusters, catalase positive, oxidase negative, and mannitol fermenters. It was observed that 17 (56%) out of 30 *S. aureus* isolates in the present study were positive for coagulase production using plasma from sheep, cattle, camel and human.

**Antimicrobial sensitivity test**: All the 30 isolates were 100% sensitive to piperacillin tazobactam and imipenem while high efficacy of gentamicin (96.6%), amoxicillin plus clavulanic acid and nitrofurantoin (96% each), vanconycin (93.3%), oxytetracycline (93%) and norfloxacin (90%) was observed. On the other hand 100% resistance was observed against cefixime plus clavulanic acid followed by ampicillin (90%), penicillin-G (86%) and oxacillin (73%) (table no.2).

**Multiple antibiotic resistance (MAR) index of *S. aureus* isolates** In the present investigation, out of 30 *S. aureus* isolates, nine (30%) isolates were MDR strains *i.e.* they exhibited resistance to at least one antibiotic in three or more than three different antimicrobial category. The MAR value of all 30 isolates ranged from 0.04 to 0.34, while 10 isolates exhibited MAR index value from 0.2 to 0.3.

**Detection of *coa* gene and *coa* gene polymorphism**: All the 30 isolates carried the *coa* gene. Despite of the fact that all of them harbored the *coa* gene, only 17 (56%) isolates were positive for tube coagulase test whereas 13 (43.3%) isolates were negative. All 30 *S. aureus* isolates were grouped into seven coagulase types based on the amplicon size of *coa* gene produced (table no. 3). The size of *coa* gene amplicons were in the range of 350-750 bp. Majority of the isolates *i.e*. seven (23.3%) carried 680 bp amplicon size followed by five isolates showing an amplicon size of 650 bp while only one isolate (R11) produced an amplicon of 610bp.

**Table 1**: List of primers used in the study

| S. No | Gene | Primer sequence (5' to 3') | Size (bp) | Reference |
|---|---|---|---|---|
| 1. | *23S rRNA* | F-5' -ACGGAG TTA CAA AGG ACG AC-3'<br>R-5' -AGC TCA GCC TTA ACG AGT AC-3' | 1250 | Straub *et al*., 1999 |
| 2. | *coa* | F-5'-ATAGAGATGCTGGTACAGG-3'<br>R-5'-GCTTCCGATTGTTCGATGC-3' | Variable | Hookey *et al*., 1998 |
| 3. | *sea* | F-5'-TTGGAAACGGTTAAAACGAA-3'<br>R-5'-GAACCTTCCCATCAAAAACA-3' | 120 | Johnson *et al*., 1991 |
| 4. | *seb* | F-5'-TCGCATCAAACTGACAAACG-3'<br>R-5'-GCAGGTACTCTATAAGTG CC-3' | 478 | |
| 5. | *sec-1* | F-5'-GACATAAAAGCTAGGAATTT-3'<br>R-5'-AAATCGGATTAACATTATCC-3' | 257 | |
| 6. | *sed* | F-5'-CTAGTTTGGTAATATCTCCT-3'<br>R-5'-TAATGCTATATCTTATAG GG-3' | 317 | |
| 7. | *see* | F-5'-TAGATAAAGTTAAAACAAGC-3'<br>R-5'-TAACTTACCGTGGACCCTTC-3' | 170 | |
| 8. | *seg* | F-5'-AAGTAGACATTTTTGGCGTTCC-3'<br>R-5'-AGAACCATCAAACTC GTA TAG C-3' | 287 | Omoe *et al*., 2002 |
| 9. | *seh* | F-5'-GTCTATATGGAGGTACAACACT-3'<br>R-5'-GACCTTTACTTATTTCGCTGTC-3' | 213 | |

**Table 2**: Antimicrobial sensitivity test of *S. aureus* isolates

| Class of antibiotics | Antibiotics | Conc. (mcg/disc) | Antibiogram pattern (%) | | |
|---|---|---|---|---|---|
| | | | Sensitive | Intermediate | Resistance |
| Beta –lactams | Oxacillin | 1 | 23 | 3 | 73 |
| | Penicillin G | 10 IU | 15 | 0 | 86 |
| | Piperacillin-tazobactam | 100/10 | 100 | 0 | 0 |
| | Ampicillin | 10 | 10 | 0 | 90 |
| | Cefixime plus clavulanic acid | 5/10 | 0 | 0 | 100 |
| | Ceftriaxone | 30 | 70 | 30 | 0 |
| | Amoxicillin plus clavulanic acid | 30 | 96 | 3 | 0 |
| | Imipenem | 10 | 100 | 0 | 0 |
| | Methicillin | 5 | 63 | 25 | 15 |
| Chloramphenicol | Chloramphenicol | 30 | 78 | 13.3 | 10 |
| Quinolones | Ciprofloxacin | 5 | 53 | 46 | 0 |
| | Norfloxacin | 10 | 90 | 0 | 10% |
| | Levofloxacin | 5 | 76 | 16 | 6 |
| Sulphonamides | Co –trimoxazole | 25 | 70 | 20 | 10 |
| Tetracyclines | Oxytetracycline | 30 | 93 | 0 | 6.6 |
| Polypeptides | Polymyxin B | 300 IU | 73 | 0 | 26 |
| Macrolides | Erythromycin | 15 | 0 | 80 | 20 |
| | Azithromycin | 15 | 70 | 0 | 30 |
| | Clindamycin | 2 | 10 | 76.6 | 15 |
| Aminoglycosides | Gentamicin | 10 | 96.6 | 3.3 | 0 |
| Glycopeptides | Vancomycin | 30 | 93.3 | 0 | 6.6 |
| Nitrofurans | Nitrofurantoin | 300 | 96 | 3.3 | 0 |

**Table 3**: Polymorphism in *coa* gene in *S. aurues* isolates

| S. No. | Isolate numbers | Total solates | *coa* gene amplicon size(bp) |
|---|---|---|---|
| 1 | R23,R24,R25,R26 | 4 | 750bp |
| 2 | R21,R22 | 2 | 700bp |
| 3 | R1,R2,R3,R4,R7,R8,R9,R18 | 7 | 680bp |
| 4 | R10, R12,R15,R16,R17 | 5 | 650bp |
| 5 | R11 | 1 | 610bp |
| 6 | R19,R20 | 2 | 600bp |
| 7 | R27,R28,R29,R30 | 4 | 350bp |

**Table 4**: Detection of staphylococcal enterotoxins from goat meat

| S.No. | Enterotoxin gene | Prevalence (%) |
|---|---|---|
| 1 | *sea* | 0 |
| 2 | *seb* | 1(3.3%) |
| 3 | *sed* | 0 |
| 4 | *sec-1* | 13 (43.3%) |
| 5 | *see* | 0 |
| 6 | *seh* | 0 |
| 7 | *seg* | 23(76.6%) |
| 8 | *seb+sec- 1+seg* | 1(3.3%) |
| 9 | *sec-1+seg* | 13 (43.3%) |

**Detection of staphylococcal enterotoxin (SE) genes:** In the present study, classical enterotoxin genes like *sea, seb, sec-1, sed, see* and novel enterotoxin genes like *seg and seh* were targeted for detection in *S. aureus* isolated from goat meat (table no.4). The *seg* gene was the most prevalent, identified in 23 isolates (76.6%), followed by the *sec-1* gene, detected in 13 isolates (43.3%). Additionally, 13 isolates (43.3%) harbored both *sec-1* and *seg* genes. The *seb* gene

was found in only one isolate (3.3%), while enterotoxin genes such as *sea*, *seb*, *sed*, *see*, and *seh* were not detected in any of the isolates.

# Discussion

This study aimed to determine the prevalence of *S. aureus* in goat meat, assess their antimicrobial resistance, and identify enterotoxigenic strains. The hygiene in the slaughterhouse plays important role in the quality of meat (Besana & Paller 2020). In this study, *S. aureus* was detected in 30 out of 65 samples (46.1%), a finding consistent with those of Adesiji *et al*. (2011) and Herve *et al*. (2017) who reported *S. aureus* prevalence rates of 46% (138/300) and 46.61% (40/86), respectively, in various meat sources, including raw chicken, beef, goat, and pork. Higher prevalence rates have been reported by Rahimi *et al*. (2013) at 60.3% from Iran (223/370) and Savariraj *et al*. (2021) at 66.67% (80/120) from India. In contrast, Sanlibaba, (2022) and Choudhary *et al*. (2022) documented lower prevalence rates of 21.23% (96/452) in Turkey and 19.17% (23/120) in India, respectively, in animal-derived food products. The variation observed within the same country and globally can be attributed to multiple factors, such as differences in sampling techniques, isolation methods, sampling locations on the carcass, types of meat cuts, contamination during and after slaughter, meat storage conditions, and processing practices. Food contamination by *S. aureus* can occur directly through workers with skin lesions harboring the bacteria or through sneezing and coughing. Around 50% of humans are carriers of *S. aureus* as commensals. Additional sources of contamination include soil, water, dust, and air (Rahimi *et al*., 2013).

In the current study, all 30 isolates were found to carry the *coa* gene. However, despite the presence of the *coa* gene in all isolates, only 17 (56%) tested positive for the tube coagulase test, while 13 (43.3%) tested negative. This contradiction results between traditional and molecular method were also reported in a former study by Javid *et al*. (2018). The findings emphasize the use of molecular methods in the identification and detection of *S. aureus.* This study outlines the antimicrobial resistance profiling of *S. aureus* isolates subjected to 22 different antibiotics. A 100% resistance was observed against cefixime plus clavulanic acid followed by ampicillin (90%), penicillin-G (86%) and oxacillin (73%). These results align with the observations of Mechesso *et al.* (2021) and Owusu *et al*. (2024), which emphasized the high level of resistance to penicillin among *S. aureus* isolates from goat carcasses. The frequent resistance to penicillin in goat meat isolates could be linked to its extensive use in animal husbandry practices. The resistance of *S. aureus* to several clinically significant antibiotics is a growing concern due to the risk of transmission to humans through close contact with infected animals and the food chain (Chang *et al*., 2015). Dorjgochoo *et al*. (2025) observed that contamination in retail environments may involve not only more resistant strains but also inadequate sanitation and disinfection practices.The antibiotic sensitivity pattern for tetracycline observed in this study differed from the findings of Hemlata *et al*. (2015) in the same study area, where a significantly higher resistance rate of 86.96% was reported compared to the 6.6% resistance observed in this study. This variation could be attributed to differences in sampling periods, changes in antibiotic usage practices over time, variations in the isolates tested and it may also reflect improved antimicrobial stewardship or reduced reliance on tetracycline in livestock management in recent years. In this study, 30% of the isolates were multi-drug resistant (MDR).and Multiple Antibiotic Resistance (MAR) index ranged from 0.04 to 0.34, with 10 isolates scoring between 0.2 and 0.3, indicating exposure to high antibiotic pressure. Antimicrobials are widely utilized in livestock for disease prevention, control and to support sustainable production. However, the use of antibiotics in food animal production has been identified as a major contributor to the development of antimicrobial resistance (AMR) in humans.

Numerous studies on the distribution of enterotoxigenic staphylococci isolated from food vary from one report to another in the percentage of enterotoxigenic strains and the distribution of enterotoxin genes. Types of sample or source and detection methods also affect their occurance. In the present study, 23 out of 30 isolates (76.6%) carried one or more enterotoxin genes, which is consistent with findings from other studies- Ozdemir and Keyman (2016) found 77.1%, Savariraj *et al*. (2019) observed 82.61% enterotoxigenic isolates, and Haghi *et al*. (2021) identified 58.1% of meat isolates as enterotoxigenic. In contrast, Wu *et al*. (2019) reported a significantly higher prevalence of SEs, with 99.07% detected in retail raw red meat samples collected in China. Among the classical staphylococcal enterotoxins (SEs), *sea* and *seb* are explicitly linked to contamination of human origin. Conversely, *sec* and *sed* are associated with contamination stemming from animals. Notably, *sec* can also signal human contamination (Wu *et al*., 2019). In this study, 43.3% of the isolates were found to carry the classical *sec-1* gene, suggesting potential human contamination.

Enterotoxin genes are commonly located either individually or in clusters on diverse mobile genetic elements (MGEs). These include prophages, plasmids, transposons, *Staphylococcus aureus* pathogenicity islands (SaPIs), and enterotoxin gene clusters (*egc*) (Malachowa and DeLeo, 2010). In our study, we detected only the *seb*, *sec*, and *seg* genes, which are located on pathogenicity islands and gene clusters in *S. aureus* isolates. Other classical and novel

enterotoxins, typically associated with mobile genetic elements, were not detected in this study. The *seg* gene (76.6%) was predominant novel SE gene detected in our study. Similarly, Sahin *et al.* (2020) and Chen *et al*. (2023) reported that enterotoxin gene cluster (*egc*) encoded enterotoxin genes, *seg* and *sei* were determined most frequently in *S. aureus* isolates from different animal's meat (sheep, goat, cattle and chicken), retail foods, hands of staff and surfaces in contact with food. It has been reported that this is probable because both *seg* and *sei* genes are in the same cluster (egc) and have a structural similarity. Identification of one is sufficient to indicate the presence of others (Jarraud *et al*., 2001). Enterotoxin genes like *sea, sed, see*, and *seh* were not detected in any of the isolates in our study. Similarly, Özdemir and Keyvan (2016) and Savariraj *et al*. (2019) reported zero prevalence of *see* gene whereas Sanlibaba (2022) did not detect *see* and *seh* genes in any of the isolates. In contrast to the present findings, Haghi *et al*. (2021) found *sea* and *see* genes with higher frequency than others in both meat and clinical samples. The differential expression of enterotoxin genes may be influenced by variations in contamination pathways, host transmission dynamics, and other epidemiological risk factors associated with *S. aureus* contamination in goat meat (Dorjgochoo *et al*., 2025). Epidemiological studies have highlighted the significant role of enterotoxigenic staphylococci in foodborne intoxications. Therefore, caution is advised when consuming raw or undercooked meats or using them in the preparation of processed foods.

The isolates exhibiting pathogenic potential, as evidenced by the presence of enterotoxin genes and distinct antibiogram profiles, present challenges in determining their precise origin as the presence of *S. aureus* may not solely originate from the animal itself but can also arise from environmental sources such as food handlers, cross-contamination during slaughter and processing and retail environments, further contributing to the observed differences. Additionally, future studies should aim to determine the key risk factors associated with *S. aureus* contamination and foodborne illness outbreaks to improve the effectiveness of food safety measures.

**Conclusion**

This study highlights the prevalence of coagulase positive *S. aureus* in goat meat, its antimicrobial resistance, and the distribution of enterotoxin genes. The findings revealed a significant presence of enterotoxigenic *S. aureus* isolates, with *seg* being the most frequently detected novel SE gene. High resistance to commonly used antibiotics was observed, raising concerns about antimicrobial resistance and its potential transmission through the food chain. The results underscore the importance of adopting molecular methods for accurate detection, implementing stringent hygiene practices during meat processing, and promoting responsible antibiotic use to mitigate public health risks.

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