RESEARCH ARTICLE

Molecular Detection and Antibiotic Sensitivity Patterns of *S. aureus* Isolates from Fish Samples of Retail Outlets in Tirupati Town

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Abstract

Staphylococcus aureus is one of the ESKAPE pathogen which is widely recognized for antibiotic resistance and responsible for food poisoning. Inadequate hygienic practices at fish market act as a source for the transmission of *S. aureus* from fish meat to human. This study was aimed to determine the prevalence and antimicrobial resistance profile of *S. aureus* from retail meat outlets in Tirupati. A total of 120 fish meat samples were collected and examined for *S. aureus* and methicillin resistant *S. aureus* (MRSA) using cultural and molecular methods. Of the 120 samples, 38 (31.6%) were found positive by culture methods and 21(17.5%) were confirmed as *S. aureus* by targeting the *nuc* gene using PCR. These 21 isolates were subjected to antibiotic sensitivity test. The highest antibiotic resistance was observed for oxacillin (100%), followed by ciprofloxacin (86%). Presence of *mecA* gene was detected among 16 isolates. Sea food contaminated with MRSA accelerates the spread of drug resistant strains resulting in significant public health concerns.

Key words: Fish, Fish meat, *mecA,* MRSA. *nuc*, *S. aureus.*

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Introduction

Fish meat is considered as a highly nutritious food as it is a natural source of protein and omega-3 fatty acids that exhibit significant anti-inflammatory properties which contribute to cardiovascular health (Mei *et al*., 2019). Several food borne pathogens have been reportedly associated with fish contamination during post-harvest management (Akbar and Anal, 2011). *S. aureus* is amongst one of the leading cause of food contamination, which can spoil the food by producing lethal enterotoxin, and is a major threat to food safety and public health (Rashid *et al*., 2023). These enterotoxins are super antigens, highly heat stable and often associated with staphylococcal food borne intoxication (Dabassa *et al*., 2019). Prepared foods containing more than 10^3 CFU/g of *S. aureus* are considered unsatisfactory and counts exceeding 10^4 CFU/g render the food potentially harmful for consumption. Consuming food contaminated with staphylococcal enterotoxins in amounts as small as nanograms to micrograms can cause severe illness ranging from skin infections to life threatening conditions (Seo *et al.,* 2007)

Staphylococcal infections have been generally treated with the commonly used antimicrobials against Gram positive bacteria with the choice of beta-lactam antibiotics either alone or with aminoglycosides (Thakuria and Lahon, 2013). The widespread use of beta-lactam antibiotics has evolved the emergence of multidrug resistant strains especially methicillin resistant *S. aureus* strains (MRSA) that makes its eradication more difficult and its incidences are also increasing steadily. Owing to the ability of the bacteria to develop resistance against various antibiotics, the incidence of MRSA has increased from 2% in 1974 to 64% in 2004 (Emily *et al*., 2010). Fish are highly vulnerable to microbial contamination during its processing and storage, and failure to maintain proper refrigeration conditions during processing of sea food promotes the growth of microbial organisms especially *S. aur*eus (Getnet *et al*., 2025). Contamination of sea food with MRSA has zoonotic importance capable of producing disease in both humans and animals particularly in humans it causes food poisoning, suppurative pneumonia, osteomyelitis, pyogenic endocarditis, and otitis media etc (Algammal *et al*., 2020).

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Although several studies have been conducted to detect the prevalence, antibiotic resistance of *S. aureus* in dairy and meat products, there were few or no reports of *S. aureus* in fish meat samples. Interestingly, Zarei *et al.* (2012) have reported higher prevalence of *S. aureus* in sea food than that of *Listeria monocytogenes, Vibrio parahaemolyticus, and Salmonella* spp. Since sea food is handled by various people before reaching consumers and as the *S. aureus* is the common inhabitant of nares, skin, throat and GIT of human, the possibility of occurrence of multiple drug resistant and methicillin resistant *S. aureus* in sea food is relatively high. Hence, the present study was designed to detect the incidence of *S. aureus* in fish meat samples purchased at retail outlets of Tirupati, Andhra Pradesh.

Materials and Methods

The current research was performed in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati (India).

Sample Collection, Isolation and Identification

A total of 120 fish samples were procured from retail fish outlets in Tirupati town. Each sample was collected under aseptic conditions and placed in a sterile zip-lock bag. The cold chain was maintained until the samples were transported to the laboratory.

Approximately 10-20 g of each sample was taken and homogenised using a mortar and pestle by adding 90 mL peptone water. A 3 mL aliquot was pre-enriched in nutrient broth and incubated at 37°C for 18-24 h. For isolation of *S. aureus,* the pre-enriched sample was streaked on Mannitol salt agar (HI-Media Laboratories) and incubated at 37°C for 24-48 h. Identification of *S. aureus* colonies was based on their morphology and fermentation of mannitol.

Gram staining was performed for all suspected *S. aureus* colonies and their morphology was examined under the microscope. Typical *S. aureus* appeared as Gram positive cocci arranged in grape-like clusters (Getnet *et al*., 2025). To assess catalase activity a pure colony of *S. aureus* was transferred to a clean glass slide using a sterile loop and a drop of 3% hydrogen peroxide was added. Bubble formation indicated the presence of *S. aureus* (Quinn *et al.,* 2002). The suspected colonies were preserved as nutrient agar slants for further antibiotic susceptibility testing and molecular confirmation.

Molecular Detection of Methicillin Resistant Gene

The DNA was extracted from the suspected colonies using boiling and snap chilling method. For molecular confirmation the *nuc* gene (Table 1) was targeted as it is species-specific for *S. aureus*. The PCR was carried out with an initial denaturation at 94**°**C for 5 min followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 55° C for 30 sec, extension at 72°C for 1 min with a final extension at 72°C for 5 min (Bharathy *et al*. (2015).

Table 1: Primers used for the detection of *nuc* and *mecA* gene of *S. aureus*

Gene	Primer	Sequence	Fragment size	Reference
nuc	nuc-F nuc-R	GCG ATT GAT GGT GAT ACG GTT AGC CAA GCC TTG ACG AAC TAA AGC	270bp	Bharathy *et al*. (2015)
mecA	mecA-F mecA-R	ACT GCT ATC CAC CCT CAA AC CTG GTG AAG TTG TAA TCT GG	163 bp	Mehrotra *et al*. (2000)

The *mecA* gene (Table 1) was used for the identification of MRSA isolates. For the amplification of the *mecA* gene by PCR the thermal cycling conditions were used: an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55° for 30 s, extension at 72°C for 1 min, with a final extension at 72°C for 5 min.

Antimicrobial Susceptibility Testing

The PCR confirmed isolates (n=21) were tested for susceptibility to a panel of 10 standard antibiotics on Mueller-Hinton Agar by the Disc diffusion method according to the CLSI standards. Five discs were placed on each plate to avoid overlapping of zones and antibiotic discs and the plates were incubated at 37°c for 16-18 h. Antibiotic sensitivity patterns were interpreted according to CLSI guidelines (CLSI, 2020). Isolates with intermediate susceptibility to the tested antibiotics were considered susceptible for analysis purposes. An isolate was considered resistant when it was resistant to one or more antibiotics: it was considered multidrug resistant when it was resistant to three or more classes of antibiotics (Schwarz *et al*., 2010).

Results and Discussion

Total of 120 samples were screened for the presence of *S. aureus* and MRSA. Out of 120 samples, 38 (31.6%) samples were confirmed as *S. aureus* by visualising golden-yellow colonies on Mannitol Salt Agar (Fig.1) and grape like clusters under the microscope. All the 38 isolates were further confirmed by biochemical test such as the catalase test. An almost similar incidence of 31.14% and 34.3% was reported from Nigeria **(**Mohammed *et al.,* 2020) and Iran (Arfatahery *et al*., 2016). A markedly higher incidence of 87% and 93% were documented, respectively, from different geographical locations like Japan (Hammad *et al*., 2012) and India (Visnuvinayagam *et al*., 2015) and the researchers opined that the contamination may be due to the use of polluted water to wash fish after taking them from the sea.

Molecular Detection of Methicillin Resistant Gene

All the culturally confirmed isolates were subjected to PCR targeting *nuc* gene, and out of 38 isolates, 21 (17.5%) isolates were confirmed as *S. aureus* (Fig. 2). A higher incidence of 26% and 35.2% from fresh fish than the present study was reported by Rashid *et al.* (2023) and Derke *et al.* (2025), respectively, and

they noticed that fresh fish are more exposed to microbial contamination during handling and transportation. Slightly lower incidence of 15% was reported by Sivaraman *et al.* (2022).

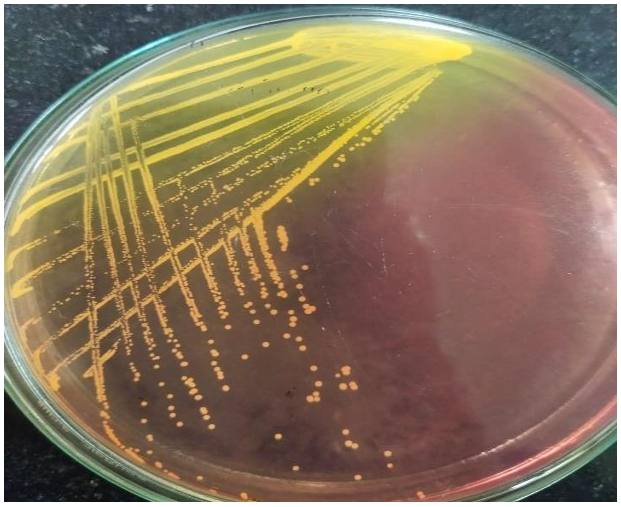

Fig. 1: Golden yellow colonies of *S. aureus*

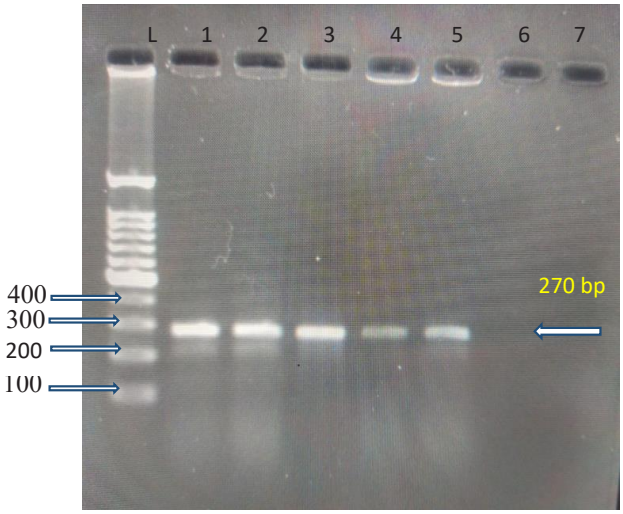

Fig. 2: Detection of the *nuc* gene among *S.aureus*; Lane L:DNA Ladder; Lane 2, 3, 4, 5: positive samples with amplification of 270 bp; Lane 1: Positive control; Lane 6: Negative control

Twenty one PCR confirmed isolates were subjected to PCR targeting *mecA* gene. Out of 21, 16 (76.2%) isolates have shown the presence of *mecA* gene (Fig.3). The significant presence of resistance genes must be acknowledged as a potential health hazard for both humans and sea food (Shanehbandi *et al.,* 2014). An incidence of 100% *mecA* was reported among the isolates of Assam retail fish markets which might be due to discharge of hospital effluents into the water bodies (Sivaraman *et al*., 2021). A lower incidence of 16.00% to 57.36% was reported by Costa *et al*. (2015), Mirani *et al*. (2017), and Derke *et al.* (2025). In contrary, a very low incidence (3%) of *mecA* among *S. aureus* isolates was reported by Sivaraman *et al*. (2022), where they found the poor management of the fish market as a reservoirs for the spread of MRSA. In contrast to the studies, that reported presence of *mecA* among the oxacillin (methicillin) resistant isolates, Arslan and Özdemir (2017) reported the absence of *mecA* gene among the oxacillin resistant isolates. Despite the lack of *mecA* gene the cause of higher phenotypic oxacillin resistance may be due to overproduction of beta-lactamase enzyme by bacteria which inactivates oxacillin (Felten *et al*., 2002).

Taha *et al*. (2024) reported almost similar findings to the present study, *i.e.* an incidence of 15.0% and 66.7% of *nuc* and *mecA* genes, respectively, among the *S. aureus* isolates in fish, which may be due to inadequate cold chain management while selling of fish and poor packaging of fish prior to retail sale, whereas Obaidat *et al.* (2015) reported 7.2% and 13.5% of *nuc* and *mecA* genes, respectively. Microbial interference might occur while preserving fish with ice and salt (Choudhary *et al*., 2022). The possibility of cross-contamination while handling fish with bare hands without wearing gloves (Kukułowicz *et al*., 2021). Fly infestation at the fish market also contributes to the contamination of fish meat (Bujjamma and Padmavathi, 2015).

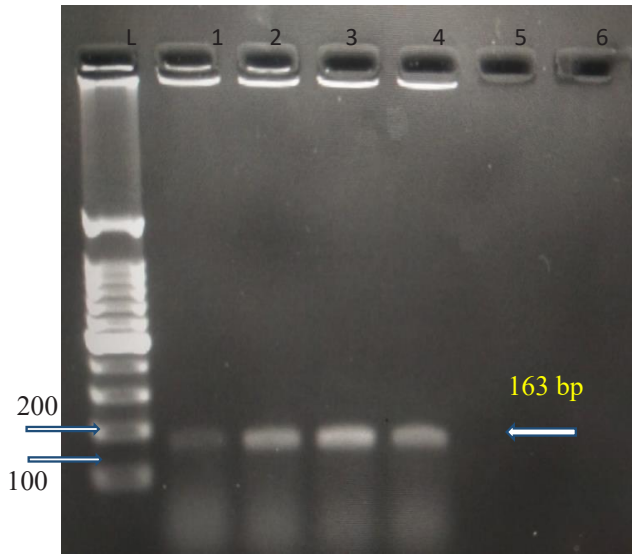

Fig. 3: Detection of the *mec* A gene among *S. aureus*; Lane L: DNA Ladder; Lane 1, 2, 4: positive samples with amplification of 163 bp; Lane 3: Positive control; Lane 5: Negative control

Antimicrobial Susceptibility Pattern

Twenty one PCR confirmed isolates were subjected to ABST to a panel of 10 antibiotics. All the isolates showed higher resistance to oxacillin (100%) followed by ciprofloxacin (86%), tetracycline (81%), erythromycin (68%), vancomycin (56%), trimethoprim (31%) and lower resistance for ceftriaxone (25%), ampicillin (18%), chloramphenicol (18%) and no resistance to gentamicin. In a similar study, Egege *et al.* (2020) reported 100% resistance for oxacillin among the isolates of shell fish, which may be due to overuse of unprescribed antibiotics which are readily available to the public. Further, Inuwa *et al*. (2025) reported 81.9% resistance to oxacillin among the isolates from ready to eat foods in Nigeria. In contrast, Arfatahery *et al*. (2016) reported 23.8% of resistance to oxacillin among the isolates of fish products.

Conclusion

The current finding revealed an incidence of 17.5% of *S. aureus* in retail fish meat samples of Tirupati town by PCR with antibiotic resistance towards most commonly used antibiotics especially oxacillin (methicillin), which alarms indiscriminate use of antibiotics. This study also concluded with distribution of resistance genes among the isolates which indicates the possibility of cross-contamination while handling fish with bare hands without wearing gloves. Fish retailers should be therefore educated regarding the post-harvest management of fishes, and continuous monitoring of these AMRS will help minimize the future occurrence and protect public health.

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